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(54) Title: CD8-SPECIFIC BINDING PROTEINS AND METHODS OF USING THE SAME

(57) Abstract: Binding agents (e.g., antibodies or antigen-binding fragments thereof, binding proteins) that specifically bind to CD8, and their use in the treatment of diseases or disorders, such as an inflammatory disease or an autoimmune disease, are provided.

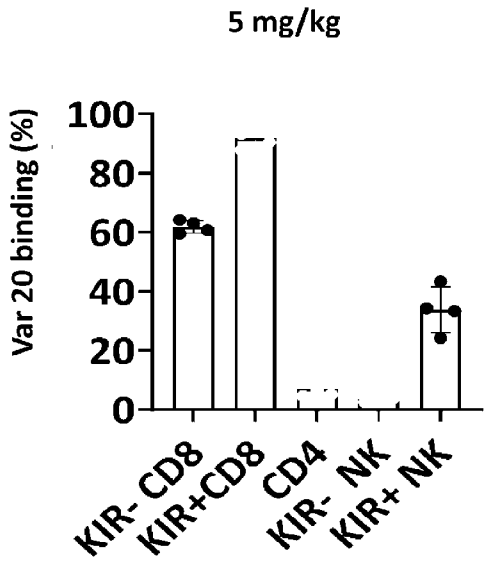

FIG. 14I

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[Continued on next page]

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CD8-SPECIFIC BINDING PROTEINS AND METHODS OF USING THE SAME

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0001] This application contains a Sequence Listing which has been submitted electronically in xml format and is hereby incorporated by reference in its entirety. Said xml copy, created on May 20, 2024, is named SeqList-368576-41002.xml and is 166,696 bytes in size.

BACKGROUND

[0002] CD8 is a protein expressed on the surface of many different types of immune cells, including about 90% of cytotoxic T lymphocytes (Cole et al., Imunology 137:139–148, 2012). It functions as a co-receptor during antigen engagement by the T-cell receptor (TCR), stabilizing the interaction between the TCR and peptide-major histocompatibility complex (pMHC) molecule and providing a co-activation signal (Cole et al., 2012, supra).

[0003] Antibodies and similar binding proteins targeting CD8 can be used as immune system modulators. For example, activating anti-CD8 antibodies can be used to trigger CD8+ T cell effector function (Clement et al., J Immunol 187(2):654–663, 2011), while blocking anti-CD8 antibodies can be used to suppress autoreactive CD8+ T cells (Clement et al., Scientific Reports Sci Rep 6:35332, 2016).

BRIEF SUMMARY

[0004] In some embodiments, the present disclosure provides binding domains and binding proteins that target CD8a.

[0005] In some embodiments, the present disclosure provides a binding protein comprising: a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSR FSGSGSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGXGTKVEIK (SEQ ID NO:133), wherein: X = G or C.

[0006] In some embodiments, the present disclosure provides a binding protein comprising: a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to

EVQLVESGGGLVQPGGSLRLSCAASGFNX$_1$KDTYIHFVRQAPGKX$_2$LEWIGRIDPANDNT LYASKX$_3$QGKX$_4$TISX$_5$DTSKNTAYLQMNSLRAEDTAVYYCX$_6$RGYGYYVFDHWGQGTL VTVSS (SEQ ID NO:134),

wherein: (a) X_1 = I or F; (b) X_2= G or C; (c) X_3 = F or V; (d) X_4 = A or F; (e) X_5 = A or R; and (f) X_6 = G or A.

[0007] In some embodiments, the present disclosure provides a binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:133, wherein: X = G or C; and (b) a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:144, wherein: (1) X_1 = I or F; (2) X_2= G or C; (3) X_3 = F or V; (4) X_4 = A or F; (5) X_5 = A or R; and X_6 = G or A.

[0008] In some embodiments, the present disclosures provides a binding protein comprising: (a) CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively; and (b) one or more of the following framework regions: (i) a light chain FR4 according to SEQ ID NO:126; (ii) a heavy chain FR2 according to SEQ ID NO:128; (iii) a heavy chain FR3 according to SEQ ID NO:129; (iv) a heavy chain FR3 according to SEQ ID NO:130; (v) a heavy chain FR3 according to SEQ ID NO:131; (vi) a heavy chain FR3 according to SEQ ID NO:132; and (vii) a heavy chain FR3 according to SEQ ID NO:16 with one or more of the following substitutions: F6V, A10F, A14R, and G39A, according to the position of the amino acid within SEQ ID NO:16.

[0009] In some embodiments, the present disclosure provides a binding protein comprising: (a) CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:127, SEQ ID NO:5, and SEQ ID NO:6, respectively; and (b) one or more of the following framework regions: (i) a light chain FR4 according to SEQ ID NO:126; (ii) a heavy chain FR2 according to SEQ ID NO:128; (iii) a heavy chain FR3 according to SEQ ID NO:129; (iv) a heavy chain FR3 according to SEQ ID NO:130; (v) a heavy chain FR3 according to SEQ ID NO:131; (vi) a heavy chain FR3 according to SEQ ID NO:132; (vii) a heavy chain FR3 according to SEQ ID NO:16 with one or more of the following substitutions: F6V, A10F, A14R, and G39A, according to the position of the amino acid within SEQ ID NO:16; (viii) light chain framework regions of the VL according to SEQ ID NO:7; and (ix) heavy chain framework regions of the VH according to SEQ ID NO:8.

[0010] In some embodiments, the present disclosure provides a binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:56; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:56; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:56.

[0011] In some aspects, pharmaceutical compositions comprising a binding domain or binding protein disclosed herein are provided.

[0012] In some additional aspects, the present disclosure provides methods of using a binding protein or pharmaceutical composition as disclosed herein are provided, such as methods of activating CD8+ regulatory T cells (CD8+ Treg cells), and methods of treating or preventing a disease (e.g., an autoimmune disease).

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 shows a "bottle-opener" structure of a bispecific binding protein.

[0014] FIG. 2 shows anti-KIR2DL/anti-CD8a parental and variant 20 binding to CD8 SKW cells.

[0015] FIG. 3 shows anti-KIR2DL/anti-CD8a parental and variant 20 binding to primary CD8 T cells.

[0016] FIG. 4 shows a single dose PK/tolerability study design in which variant 20 was administered to humanized CD34+ NSG-Tg(Hu-IL15) mice. Mice exhibiting >25% hCD45, >3% hCD3, and >2% hCD56 were subsequently shipped to the testing facility.

[0017] FIGS. 5A-5D show binding of variant 20 to KIR+ CD8 cells and NK cells and associated activation levels for KIR+ CD8 cells and elimination of CD4+ cells. A nonblocking anti-KIR antibody was used to selectively gate on the KIR+ CD8s within the total PBMCs, while an anti-NKp46 antibody was used to define NK cells. Binding to different cell populations was detected using an anti-human IgG1 Fc secondary antibody. Intracellular Granzyme B was detected using an anti-Granzyme B antibody following intracellular staining. FIG. 5A shows binding of variant 20 to KIR+ CD8 cells in PBMCs from a Celiac donor. FIG. 5B shows a reduction in KIR+ CD8 cells in PBMCs from a Celiac donor incubated with variant 20,

compared to cells incubated with control monospecific antibodies. FIG. 5C shows binding of variant 20 to NK cells in PBMCs from a normal donor. FIG. 5D shows elimination of gliadin-responsive Celiac donor CD4+ T cells as detected by increase in Annexin V+ CD4+ T cells at 48 hrs following addition of variant 20. In FIGS. 5A-5C, circles, inverted triangles, and diamonds represent results for variant 20, CD8 monospecific antibody, and KIR2DL monospecific antibody, respectively.

[0018] FIGS. 5E-5F show cytokine release in primary PBMCs derived from ten healthy human donors in the wet-coated presentation formats. A range of concentrations of variant 20 ("Var 20") (0.032 μg/mL, 0.16 μg/mL, 0.8 μg/mL, 4 μg/mL, 20 μg/mL, and 100 μg/mL) was evaluated in the assays. Anti-CD3 antibody (OKT3 clone; positive control) treatment, human IgG1 antibody (negative control) treatment, and a "no treatment" control were also included in the assay. All treatments were evaluated in triplicate in both the soluble and wet-coated plate formats. Tissue culture supernatants from treated PBMC samples were collected after 24 hours of treatment. The levels of IL-2, IL-6, IL-8, IL-10, TNF-a, and IFN-g were measured using MSD platform. All donors were responsive to positive control anti-CD3 (FIG. 5E for IL-6, FIG. 5F for TNF-a), demonstrating that these donors have the capacity to release cytokines in response to an immunomodulatory stimulus. In both treatment formats, there was no dose-responsive cytokine release (IL-2, IL-6, IL-8, IL-10, and TNF-a) stimulated by variant 20 above the level of isotype or untreated controls, for all donors (*e.g.*, FIG. 5E for IL-6, FIG. 5F for TNF-a).

[0019] FIGS. 6A-6F show the frequencies of human immune cells in peripheral blood of huCD34+ NSG-Tg(Hu-IL15) mice following a single dose of variant 20. Frequencies of immune cell subsets, including CD4+ or CD8+ T-cell subsets (FIGS. 6A-6D) and NK cells (FIGS. 6E-6F), were assessed by flow cytometry at baseline, Day 14, and Day 28 in peripheral blood following IV administration of variant 20. Enrolled study mice at baseline had a mean of 62% hCD45, 5% CD3, and 7% CD56. FIG. 6A shows the frequency of KIR+ hCD8 T cells (cells/μL). FIG. 6B shows the frequency of hCD4 T cells (cells/μL). FIG. 6C shows the frequency of hCD8 T cells (cells/μL). FIG. 6D shows the ratio of CD4 T cells to CD8 T cells. FIG. 6E shows the frequency of hCD56+ NK cells (cells/μL). FIG. 6F shows the frequency of KIR+ hCD56+ NK cells (cells/μL).

[0020] FIGS. 7A-7D show binding of variant 20 to peripheral blood cells and spleen in huCD34+ NSG-Tg(Hu-IL15) mice. Upper panels indicate prevalence (Fc detection, as %Fc positive) and lower panels show MFI of %Fc positive cells. Data are presented for individuals with mean bars ± SD. FIG. 7A shows binding of variant 20 to total CD8 cells in peripheral blood.

FIG. 7B shows binding of variant 20 to KIR+ CD8 Treg cells in peripheral blood. FIG. 7C shows binding of variant 20 to NK cells in peripheral blood. FIG. 7D shows a summary of prevalence and MFI of binding of variant 20 for total CD8 T cells, KIR+CD8, and NK cells in spleen at the terminal timepoint.

[0021] FIGS. 8A-8D show the pharmacologic impact following a single dose of variant 20 in huCD34+ NSG(IL-15Tg) mice. FIG. 8A shows Ki67 MFI in total CD8 T cells. FIG. 8B shows Ki67 MFI in KIR+ CD8 T cells. FIG. 8C shows the percentage of CD69+CD25+ cells in total CD8 T cells. FIG. 8D shows the percentage of CD69+CD25+ cells in KIR+ CD8 T cells.

[0022] FIGS. 9A-9B show loss of CD69+ CD4 T cells after a single dose of variant 20. FIG. 9A shows the percentage of total CD4 T cells. FIG. 9B shows the percentage of activated (CD69+) CD4 T cells.

[0023] FIG. 10 shows concentration of variant 20 (ng/mL) over time in blood samples from humanized CD34+ NSG-Tg(Hu-IL15) mice. Variant 20 was detectable through 672-hr of CD34+ NSG-Tg(Hu-IL15) mice at 10 or 1 mg/kg (serial microsample) and in BALB/cJ mice at 5 mg/kg (serum) and was consistent between donors and strains. Blood collection for serum in BALB/cJ mice was staggered as n=3 per timepoint. Data are presented as mean +/- SD of individuals at individual time points shown in graph. Squares=5 mg/kg BALB/cJ; inverted triangles=10 mg/kg CD34+ NSG-Tg(Hu-IL15); triangles=1 mg/kg CD34+ NSG-Tg(Hu-IL15).

[0024] FIG. 11 shows the frequency of CD8 Tregs as a proportion of total CD8 T cells in the peripheral blood of healthy donors and donors with rheumatologic autoimmune disorders, wherein CD8 Tregs are defined as KIR2DL1/2/3-expressing (KIR+) CD8 Tregs. The frequency of CD8 Tregs is shown in healthy donors and patients with psoriasis (left), systemic lupus erythematosus (SLE; center), psoriatic arthritis (PsA; center), ankylosing spondylitis (ASp; right), and Sjögren's syndrome (SS; right).

[0025] FIGS. 12A-12C show upregulation of granzyme B in CD8 Tregs (CD8 Treg expressing KIR2DL1/2/3) from donors with rheumatologic autoimmune disorders following activation with anti-CD3 antibody. PBMCs from healthy donors or patients with rheumatologic autoimmune disorders were activated with anti-CD3 antibody (OKT3), after which, expression of intracellular granzyme B was assessed by intracellular staining. PBMCs rested overnight, but not activated, were used as a control. The frequency of granzyme B-expressing CD8 Tregs as a proportion of total CD8 Tregs is shown for healthy donors and patients with psoriasis (FIG. 12A), systemic lupus erythematosus (SLE; FIG. 12B), psoriatic arthritis (PsA; FIG. 12B),

ankylosing spondylitis (ASp; FIG. 12C), and Sjögren's syndrome (SS; FIG. 12C) in unstimulated and stimulated cells.

[0026] FIGS. 13A-13O show the design and results of a multidose tolerability study design in which variant 20 was administered to humanized CD34+ NSG-Tg(Hu-IL15) mice.

[0027] In the repeat dose toxicity study, shown in FIG. 13A, human CD34+ cord blood cells from two independent donors were engrafted into female NSG-Tg(Hu-IL15) mice and screened at 12 weeks for inclusion in the study. FIG. 13B depicts the percentages of hCD45, CD3 T, and CD56 NK cells at 12 weeks post engraftment in the mice enrolled for the study. CD34+ cells from two donors were engrafted into NSG-Tg(Hu-IL15) mice aged 4 weeks, followed by a 12-week post engraftment assessment for hCD45+, CD19+, CD3+, CD33+, and CD56+ cells. Mice with >25% hCD45, >3% hCD3 and >2% hCD56 were accepted for the study. After shipment and acclimation, animals received weekly IV dose at 5 or 50 mg/kg of variant 20 or vehicle (~17-18 weeks post engraftment). Blood samples were collected from subsets of animals for PK (n=3/dose/donor), serum cytokine analysis (n=3/dose/donor), and immunophenotyping (n=5/dose of one donor) at specific time points following dose. PK time points were collected pre-dose and following dosing on Day 1 and 22 at 0.5, 2, 24, 96, and 168 hours; time points were also collected post-dose on Day 8, 15, and 29. Flow cytometry time points were pre-dose on Day 1 and Day 15 and post-dose on Day 29. Serum cytokine time points were pre-dose on Day 1 and at 8 and 24 hr post-dose. Terminal blood and spleen were collected on Day 29.

[0028] Body weight was measured over the course of the study (FIG. 13C).

[0029] Frequency of human immune cell subsets were assessed at baseline, Day 15, and Day 29 in peripheral blood and spleen following weekly IV administration of vehicle (FIGS. 13D-13O, left circles for each day), variant 20 at 50 mg/kg (FIGS. 13D-13O, center circles for each day), or variant 20 at 5 mg/kg (FIGS. 13D-13O, right circles for each day). Over the course of the study the percentage of total hCD45, CD3, CD4, CD8, NK (CD56+CD8-), KIR+ CD8+, and KIR+ NK cells in blood and spleen remained similar between 0 mg/kg (vehicle), 5 mg/kg variant 20, and 50 mg/kg variant 20 (FIG. 13D-FIG. 13I, for blood; FIG. 13J-FIG. 13O, for spleen; data are presented for individuals with mean bars ± SD).

[0030] FIGS. 14A-14I show binding of variant 20 to cells in humanized CD34+ NSG-Tg(Hu-IL15) mice from the toxicity study shown in FIG. 13A. Binding of variant 20 to CD8 Tregs (FIG. 14A) and CD8 T cells (FIG. 14B) from the peripheral blood of the humanized CD34+ NSG-Tg(Hu-IL15) mice pre-dose on Day 15 (7 days after Day 8 dosing) and 30 min

post-dosing on Day 29 was assessed via Fc detection using an anti-human IgG1 Fc secondary antibody (vehicle=left circles for each day; variant 20 at 50 mg/kg =center circles for each day; variant 20 at 5 mg/kg= right circles for each day). Binding of variant 20 to (from left to right on each day) CD8 Treg, CD8 T cells, KIR+ NK cells, and NK cells from the spleen of humanized CD34+ NSG-Tg(Hu-IL15) mice at day 29 (30 min post-dosing) (FIG. 14C) was also assessed via Fc detection using an anti-human IgG1 Fc secondary antibody. Further illustrations of variant 20 binding are shown in FIGS. 14D-14G (peripheral blood; vehicle=left circles for each day; variant 20 at 50 mg/kg =center circles for each day; variant 20 at 5 mg/kg= right circles for each day) and FIGS. 14H-14I (spleen). Data are presented for individuals with mean bars ± SD.

[0031] FIGS. 15A-15G show the effect of variant 20 on activation and proliferation of cells from humanized CD34+ NSG-Tg(Hu-IL15) mice from the toxicity study shown in FIGS 13A-13B. FIG. 15A shows the frequency of IFNγ+ CD8 Treg cells, and FIG. 15B shows the frequency of IFNγ+ NK cells (vehicle=left circles for each day; variant 20 at 50 mg/kg =center circles for each day; variant 20 at 5 mg/kg= right circles for each day). FIG. 15C shows the frequency of Helios+ CD8 Treg cells (left circles) and NK cells (right circles). FIG. 15D shows the frequency of Ki67+ CD8 Treg cells (left circles) and NK cells (right circles). FIG. 15E shows CD69+CD25+ activation in peripheral blood, in KIR+ CD8 T cells (upper panel) and in KIR+ NK cells (lower panel) (vehicle=left circles for each day; variant 20 at 50 mg/kg =center circles for each day; variant 20 at 5 mg/kg= right circles for each day). FIG. 15F shows CD69+CD25+ activation in spleen cells (left to right: CD8 Treg, Total CD8, KIR+ NK cells, NK cells). FIG. 15G shows CD69+ activation in total CD4 T cells (vehicle=left circles for each day; variant 20 at 50 mg/kg =center circles for each day; variant 20 at 5 mg/kg= right circles for each day).

[0032] FIG. 16 shows pro-inflammatory serum cytokines after a single dose of 5 or 50 mg/kg variant 20, at 8 and 24 hr; data are presented as mean bars ± SD. Variant 20 did not increase the expression of pro-inflammatory serum cytokines.

[0033] FIGS. 17A-17D show pharmacokinetic characterization of variant 20 in Cynomolgus monkeys. Animals were injected with a single dose of 5, 0.5, or 0.05 mg/kg of variant 20. Blood was collected at multiple time points and processed into serum. FIG. 17A shows serum levels of variant 20. FIG. 17B shows pharmacokinetic parameters calculated from concentration versus time data. The relationship between dose and C_{max} and dose and AUC are shown in FIGS. 17C and 17D, respectively.

[0034] FIGS. 18A-18I describe features of CD8 Tregs and methods of their modulation. KIR CD8 Tregs express the surface markers NKG2C, Helios, and KLRG1 and when enriched

from Celiac donors PBMCs specifically eliminate activated gliadin-responsive cell line in a dose-dependent manner. FIGS. 18A and 18B show CD8 Treg phenotypic markers NKG2C, Helios, and KLRG1 (FIG. 18A) and the cytolytic marker Granzyme B (FIG. 18B) in healthy donor PBMCs plated overnight in the presence and absence of 1 ug/mL anti-CD3 and then stained with KIR antibodies. The presence of each of these markers is shown for the KIR+ and KIR- CD8 T cell populations from 7 healthy donors. FIG. 18C shows CD8 Treg expansion. KIR+ CD8 Tregs were isolated from celiac PBMCs following expansion with IL-7 and IL-15 and gliadin peptides. Sorted CD8 Tregs were then placed with gliadin expanded CD4 T cells and autologous APCs with or without (unstimulated) additional gliadin peptide stimulation for three days and end point CD8 Treg expansion is shown. Results are representative of 2 independent experiments across 3 different celiac donors. For FIG. 18D, CD8 Tregs were enriched from celiac donor PBMCs following expansion in IL-7 and IL-15 for seven days and sorting. CD8 Treg enriched cells were then placed at increasing numbers with the GFP+ activated LS2.8 SKW CD4+ target cells. Percent change in GFP+ objects from the 8 hour timepoint is shown in the graphs for target cells in the absence of CD8 Tregs and with increasing CD8 Treg number. Results are representative of 3 independent experiments. FIG. 18E shows the percent change in GFP+ object relative to the 8 hour time point (n=2 independent experiments) for CD8 Treg enriched cells that were placed with either activated or unactivated parental SKW or alpha gliadin peptide specific SKW target cells for 48 hours. FIG. 18F-FIG.18H show that CD8 Treg enriched CD8 cells eliminate activated gliadin-responsive CD4 targets. CD8 T cells were enriched from celiac donor PBMCs following expansion in IL-7 and IL-15 for seven days and sorting on the surface markers KLRG1+CD244+CD28- (CD8 Treg enriched effectors) or KLRG1-CD244-CD28+ (non-CD8 Treg effectors) and placed with activated gliadin-responsive GFP+ SKW CD4 target cell line at a 2 to 1 ratio. Percent change in GFP+ objects from an 8 hour time point is shown for both CD8 Treg and non-CD8 Treg effectors over 48 hours and targets only in the absence of addition of CD8 effectors (FIG. 18G, 18H). Incucyte-generated movies show the decrease in masked GFP+ gliadin-responsive SKW target cells over 48 hours for both CD8 Treg enriched (FIG. 18G) and Non-CD8 Treg effector cells (FIG. 18H). FIG.18I shows a schematic for modulation of CD8 Treg activity.

[0035] FIGS. 19A-19E show bispecific antibody binding to target surface receptors on CD8 Treg cells, and that binding is specific and dose-dependent. FIG. 19A shows binding affinity for human KIR2Dl1/2/3 and CD8α. The table shows the association and dissociation rate constants measured for each binding interaction, and KD binding affinity calculated from the

measured rate constants. FIG. 19B shows dose-dependent binding curves for binding to KIR2DL1/2/3 and CD8α target antigens along with association and dissociation curves as detected by Bio-Layer Interferometry. FIG. 19C shows bispecific antibody dose-response curves to stably expressed KIR2DL1 and CD8α SKW cell lines. EC50 values were calculated for each cell line using Prism Graphpad software. Binding curves are representative of 14 independent experiments for the KIR2DL1 line and 10 for the CD8 line. FIG. 19D shows Ab00 binding to KIR3DL1 transfected HEK 293 or untransfected cells. Dose-dependent binding of a KIR3DL1 targeted bispecific antibody is shown as a positive control on the KIR3DL1 transfected cell line. FIG. 19E shows Retrogenix live cell microarray technology study evaluated surface protein library transfected 293T cell binding of Ab20 and single arm controls. A summary report of target binding is shown.

[0036] FIGS. 20A-20D show specific binding by bispecific antibodies to target surface receptors on CD8 Tregs. FIG. 20A shows simultaneous co-binding of antigens to bispecific antibody as determined by Bio-Layer Interferometry. The bispecific antibody was captured with either KIR2DL or CD8α and then additional antigen binding was detected with CD8α or KIR2DL, respectively. FIG. 20B shows dose-dependent bispecific antibody binding to immune cell populations within total human PBMCs is shown as detected with an anti-human Fc secondary antibody. Data are representative of 7 independent experiments. FIG. 20C shows quantification of CD8 and KIR binding sites per CD8 Treg target cell as determined based on receptor MFI and quantitative beads. Results are shown for PBMCs from 12 healthy donors, 3 celiac, and 5 Crohn's donor CD8 Tregs. FIG. 20D shows detection of unbound KIR sites with a saturating dose of fluorescently labeled single arm KIR-Fc antibody on CD8 Tregs in total PBMCs following incubation with increasing doses of the bispecific antibody. IC50 values were calculated for KIR-Fc binding using Prism Graphpad software and nonlinear fit log (inhibitor) vs response with variable slope and 4 parameters. The graph is representative of binding data across 3 independent healthy donors.

[0037] FIGS. 21A-21D show bispecific antibody activation of CD8 Tregs in Celiac and Healthy PBMCs. FIG. 21A and FIG. 21B shows percentage of CD8 Tregs detected in healthy (n=2) and celiac (n=3) PBMCs (FIG. 21A) and associated antibody dose dependent CD8 Treg binding (FIG. 21B) across the same donors in total PBMCs. FIG. 21C shows antibody dose dependent activation of CD8 Tregs in celiac donor PBMCs across 3 different celiac donors as determined by the percentage of CD69 and ICOS positive CD8 Tregs following 48 hours of incubation. FIG. 21D shows that CD8 Treg activation is not induced with control anti-RSV

antibody. Healthy donor PBMCs were thawed overnight and plated the next day with 100nM Ab20 or anti-RSV control antibody for 48 hours. The graph depicts the percentage of CD69 positive CD8 Tregs as detected following incubation with no antibody, anti-RSV, or Ab20 across two different healthy donors.

[0038] FIGS. 22A- 22G show induction of lysis of gliadin-specific targets cells in Celiac T cell co-culture assay. FIG. 22A shows CD8 Treg prevalence, Granzyme B, and degranulation following incubation of celiac donor CD8 Tregs with CD4 target cells (1:5) and APCs in the presence or absence of 0.02 μg/ml anti-CD3 and Ab00 (100nM) for three days. FIG. 22B shows IFNγ, TNFα, and GMCSF concentrations detected following the three day CD8 Treg and CD4 target co-culture as in B in the presence of anti-CD3 with and without addition of Ab00. FIG. 22C and FIG. 22D show concentration of cytokines in PBMCs from two celiac donors. The PBMCs were incubated with increasing concentrations of Ab00 (1, 10, or 100nM) in the presence of low dose anti-CD3 (0.1 ug/mL) for 48 hours (left) and endpoint Annexin+ CD4s are shown. Concentrations of the pro-inflammatory cytokines (IL-2, TNFα, IFNγ, IL-6, and IL-17a) detected in the supernatant after incubation with low dose anti-CD3 in the presence or absence of Ab00 for 48 hours across two different celiac donors is shown in respective graphs. FIG. 22E shows percentage of CD8 Tregs and CD8 Treg Granzyme B and CD107a expression shown following bispecific antibody treatment. FIG. 22F shows CD4 readouts including number of total T cells, percentage of necrotic and apoptotic (dead) cells, CD25 MFI, and production of IFNγ by gliadin-stimulated CD4s following a three-day incubation with bispecific antibody, relative to untreated controls. FIG. 22G shows percentage decrease in IFNγ, TNFα, and GMCSF concentrations detected in the supernatant of co-cultures stimulated with gliadin peptides in the presence of Ab00 relative to untreated controls. For FIGS. 22E-22G, results are representative of 2 independent experiments including 3 different celiac donors.

[0039] FIGS. 23A-23D show that Ab20 restores CD8 Treg functions and reduces antigen induced pro-inflammatory responses in Crohn's donor PBMCs and organoids. FIG. 23A shows that CD8 Treg are present in the peripheral blood of healthy and Crohn's disease donors. For FIG. 23B, CD8 Tregs were stained in the PBMCs derived from 6 healthy and 6 Crohn's donors for the intracellular cytolytic marker Granzyme B and the transcription factor Helios. Baseline expression of CD161, CXCR3, and CD39+ on CD4 T cells in PBMCs from 8 Healthy and 11 Crohn's donors. For FIG. 23C, Crohn's PBMCs were incubated for 7 days with a mixture of bacterial flagellin and OmpC peptide and IL-7 and IL-15 cytokines in the presence and absence of Ab20 at 100nM. The percentage increase in concentration of Granzyme B as detected in the

supernatant on study day 5 and decrease in INFγ and TNFα at day 7 is shown for Ab20- treated donors relative to untreated for both responders and non-responders in the assay. The percentage decrease in proliferation as determined by diluted CFSE is also shown on day 7 for both responders and non-responders relative to untreated controls. FIG. 23D shows Helios expression on CD4 T cells in PBMCs from Crohn's donors stimulated with anti-CD3 (1ug/mL) overnight in the presence and absence of Ab20 (100nM). Paired two-tailed t test of untreated and Ab20-treated samples show no statistical significance between the values across 6 Crohn's donors. FIG. 23D also shows percentage reduction in antigen-induced epithelial cell death in both celiac and Crohn's donor derived organoids following incubation with 100nM Ab20. The results represent organoids generated from intestinal tissue for 3 independent celiac donors and two Crohn's donors.

[0040] FIGS. 24A-24F show effects of Ab20 on organoids derived from Crohn's donors. FIGS. 24A-24C show that Crohn's organoids maintain tissue architecture, and tissue contains relevant immune cells. FIG. 24A shows a representative image of Crohn's intestinal tissue derived organoids. FIG. 24B shows percentage of epithelial (EpCAM+), T, B, and NK cells present in both blood and primary intestinal tissue (colon) of 7 different Crohn's donors at baseline. The T cell graph shows the percentage of CD4 and CD8 T cells detected in the blood and colon tissue at baseline. FIG. 24C shows the KIR2DL and CD8 receptor quantification per CD8 Treg in the blood and colon as determined based on receptor MFI and quantitative beads. Results are representative of 3 independent Crohn's donors. FIG. 24D shows Ab20 binding as detected with an anti-human IgG Fc antibody for an Ab20-treated well relative to an organoid well that did not receive Ab ("Ag") (left). The percentage increase in CD8 Treg activation (CD25) is show as determined relative to the untreated control at study endpoint. n=3 independent experiments. The reduction in antigen induced CD4 expansion following treatment with Ab20 is also shown across celiac and Crohn's organoid cultures n=4 independent experiments. FIG. 24E shows reduction in antigen-induced epithelial cell death and CD4 T cell expansion across celiac and Crohn's organoid cultures following Ab20 treatment. FIG. 24F shows the percentage reduction in IL-17a and GMCSF detected in organoid supernatant following Ab20 treatment for 2 independent celiac donors.

[0041] FIGS. 25A-25B show that Ab00 does not activate CD8 and NK cells, and microbial and viral immune responses are maintained. For FIG. 25A, IFNg was measured on Day 5 in the supernatants of PBMC cultures from 3 healthy and 3 celiac donors restimulated following an initial 13 day expansion with CEFT, Influenza HA, SARS-CoV-2, and AVV5,6,8

peptide (0, 0.1, and 1 ug/mL) and tetanus toxoid (5ug/mL) in the presence and absence of Ab00. For FIG. 25B, IFNg levels following a five day PBMC culture in the presence of SEB (100ng/mL) are shown as a positive, polyclonal stimulation control. For both figures, graphs show IFNg levels for all 6 donors and each point represents the mean of 2 technical replicates. Values untreated and with Ab00 were compared using one-way ANOVA and a statistically significant results considered as $p < 0.05$.

[0042] FIGS. 26A-26B show activation of CD8 T cells (FIG. 26A) or NK (FIG. 26B) cells (as determined by percentage CD25 positive) for 2 healthy donor PBMCs following a two-day incubation in the presence and absence of increasing concentrations of KIR single arm antibody, CD8 single arm antibody, and Ab20. Resting graphs indicate readouts in the absence of additional anti-CD3 stimulation (0.1 ug/mL), while activated graphs show results in the presence of additional anti-CD3 stimulation over the 48-hour incubation. FIGS. 26C-26G show that Ab20 binds target cells in terminal tissues and does not result in the production of pro-inflammatory cytokines. FIG. 26C shows the percentage of T cells (CD3+) and NK cells (CD56+) in CD34+ NSG-Tg(Hu-IL-15) mice following 12 weeks of initial engraftment. Each symbol represents an individual mouse engrafted for a total of 24 mice per donor. FIG. 26D shows Ab20 is detected in the serum with antibody-like half-like and on CD8 Tregs and 5mg/kg does not induce immune cell activation in human engrafted mice that are transgenic for IL-15. Ab20 binding as detected at each of the blood draw time points by an anti-human Fc secondary antibody. The percentage of Fc positive cells is shown for CD8 Tregs, total CD8, and NK cells following treatment with 5 mg/kg Ab20, single arm KIR, single arm CD8, or KIR bivalent antibodies or saline control. Data are representative of two samples of pooled blood from 3 mice that received CD34 cells from two different donors at baseline, 3, 24, and 72 hours and all 6 total mice at 168 hours. FIG. 26E shows the percentage of total Fc positive CD8 Treg, total CD8, or NK cells detected in the terminal spleens of Ab20, vehicle, single arm KIR or CD8, and bivalent KIR antibody or OKT 3 (0.5 mg/kg) at the terminal time point (168 hrs). Each symbol in the graph represents a single mouse and each donor (n=2) is represented across 3 mice in each treatment group. FIG. 26F shows activation of CD8 Treg, total CD8, and NK cells as determined by the percentage positive for CD69 and CD25 over the course of the study for 6 mice at each time point following injection with 5 mg/kg Ab20, single arm KIR or CD8 antibody, or bivalent KIR antibody along with anti-CD3 (OKT3) control antibody at 0.5 mg/kg. FIG. 26G shows serum levels of proinflammatory cytokines is graphed for each of the mice in the study (n=6) for the time points and treatment

groups listed. Anti-CD3 (OKT3) is shown as a positive control for proinflammatory cytokine production.

[0043] FIGS. 27A-27K show effects of bispecific antibody binding, such as selective binding, efficacy, mechanism of action, and dose titration, in an acute human PBMC engrafted NSG mouse model. FIG. 27A shows a survival study experimental design. NSG mice were irradiated (0.75 Gy) and injected intravenously with 1e7 human PBMCS from healthy donor 3578 (Haplotype HLA-DQ2.5). Antibody (2 mg/kg) or Saline were injected every 7 days to study day 28. Low dose IL-2 (25,000 IU) was injected every other day from study day 0 to study day 10, n=20/cohort. FIG. 27B shows percentage of human CD45 as detected in the lymphocytes in the blood of mice on study day 14 across saline, antibody (Ab00), and IL-2 treatment groups in the survival study. FIG. 27C shows antibody binding to CD4, CD8, and CD8 Treg populations as detected by MFI of anti-human Fc antibody in the blood on study day 20, spleen for mice taken down early across treatment groups on study day 15 (Ab and saline; n=3 mice, IL-2; n=2 mice), and following study termination in the frozen splenocytes stained post-thaw for mice removed due to weight loss or clinical endpoints (Ab and saline; n=6 mice). Study day 20 time point is representative of 8 mice remaining in the antibody treatment group and the 6 remaining mice in the low dose IL-2 group. FIG. 27D shows percentage of CD25 and ICOS positive CD8 and CD8 Tregs within the peripheral blood as detected in the peripheral blood on study day 9 and 15 across saline, Ab, and low dose IL-2 treatment groups. Data is representative of 10 individual mice from each treatment group of study day 9 and mice taken down early on study day 15. Granzyme B MFI is shown for remaining mice across antibody and low dose IL-2 treatment groups on study day 20 for CD8 T cells and CD8 Tregs. FIG. 27E shows percentage and MFI of Granzyme in CD8 Tregs in the peripheral blood (left) or splenocytes of mice terminated early due to clinical endpoints (right) in the antibody dose titration study. FIG. 27F shows absolute counts of CD25+ CD4 T cells/uL of blood are shown for mice taken down early on study day 15 (Ab and saline; n=3 mice, IL-2; n=2 mice) across saline, Ab, and IL-2 treatment groups. MFI of the Ki67 is also shown for early take down mice across treatment groups on study day 15 within the peripheral blood. FIG. 27G shows the survival curve for mice across each of the three treatment groups (saline, Ab, and low dose IL-2) is shown. Data are representative of 18 mice per treatment group. FIG. 27H shows serum concentrations of IFNγ in the survival study on day 14 (left) or with increasing dose of antibody on study day 42 (right) in mice with clinical disease scores 3 or greater. Symbols in each of the graphs represents measurements from a single mouse. FIG. 27I shows H&E stain of disease affected intestinal tissue in anti-CD3 stimulated control

(left) or Ab (right) treated mice. Clinical disease scores of Ab (green) relative to Saline (black) or abatacept technical control (red) groups (n=6/cohort). FIGS. 27J-27K show results of studies using human NSG mice engrafted with 1e7 human PBMCs following irradiation (0.5 Gy) from a donor and dosed with increasing concentrations of antibody intravenously every seven days from day 0-21. FIG. 27J shows binding of antibody as detected by MFI of an anti-human Fc secondary antibody is shown for CD4, CD8, and CD8 Treg (KLRG1+ CD8 T cells) on study day 14 in the blood across increasing doses of antibody in each of the treatment groups. The percentage of CD25 positive KLRG1+ CD8 T cells and Helios positive CD8 T cells is shown with increasing dose of antibody in the blood at study days 14 and 42. Study day 14 shows data from 8 mice per group, while study day 42 shows the mice remaining at this terminal time point. Granzyme B (MFI and percentage) on total CD8 and KLRG1+ CD8 T cell populations in the peripheral blood and spleens from mice that were terminated on study day 11 (n=3 mice total per group) is shown with increasing dose of antibody. FIG. 27K shows percentage of CD25 and Annexin positive CD4 T cells is shown on study day 42 for remaining mice in both endpoint blood and splenocytes with increasing dose of antibody. Each symbol in graphs represents an individual mouse in each of the saline and antibody groups.

[0044] FIGS. 28A-28H show results of additional studies confirming that a 2mg/kg dose of bispecific antibody Ab00 enhances survival via the postulated mechanism of action, and support long term target engagement. FIGS. 28A-28D show results of a small cohort survival study (n=4 mice/group), using human NSG mice engrafted with 1e7 human PBMCs following irradiation (0.5 Gy) from a donor LZ0007 and dosed with 2 mg/kg Ab00 or saline intravenously every seven days from day 0-21. FIG. 28A shows engraftment as measured by the percentage of human CD45 detected for surviving mice in the blood across both saline and Ab00 treatment groups. FIG. 28B shows Ab00 binding as detected by MFI with an anti-human secondary Fc and MFI of Granzyme on CD8 Tregs on study day 14 in the blood, 2 hours post-dosing. The percentage of CD25 positive CD8 Tregs is also shown for Saline and Ab00 treatment groups in the blood on study day 14 (post-Ab00 injections on study day 0 and 7) at 2 hours post-dose. FIG. 28C shows percentage of Annexin+ and CD25+ CD4 T cells is shown for remaining mice in both saline and Ab00 treatment groups. FIG. 28D shows a survival curve for mice for the duration of the study and out to the end point of study day 42 for mice in each of the treatment groups (n=4). FIG. 28E shows an in vivo study experimental design. Human NSG mice engrafted with 1e7 human PBMCs following irradiation (0.75 Gy) from donor AC3004 and dosed with 2mg/kg Ab00 or saline every 7 days starting on day 0 out to study day 70. Each cohort consisted of 8

mice. FIG. 28F shows engraftment levels as determined by the percentage of human CD45 immune cells in the peripheral blood on study day 14 for both saline and Ab00 treated mice. FIG. 28G shows Ab00 binding as determined by anti-human Fc secondary antibody MFI on CD4, CD8, and CD8 Treg subsets on study day 14, pre-dose. FIG. 28H shows Ab00 binding for the remaining mice in Ab00 treatment group on CD4, CD8 and CD8 Treg immune cell subsets on study day 49 and 70 in the blood. For all graphs each symbol represents an individual surviving mouse at that time point.

DETAILED DESCRIPTION

I. Glossary

[0045] The following sections provide a detailed description of binding domains and binding proteins, including antibodies or antigen-binding fragments thereof, that target CD8, and related pharmaceutical compositions, methods of activating CD8+ T cells (including CD8+ regulatory T cells, "CD8+ Treg cells"), and methods of treating or preventing a disease (e.g., an autoimmune disease). Prior to setting forth this disclosure in more detail, definitions of certain terms to be used herein are provided. Additional definitions are set forth throughout this disclosure.

[0046] Unless the context requires otherwise, throughout the present specification and claims, the word "comprise" and variations thereof, such as "comprises" and "comprising," are to be construed in an open, inclusive sense, that is, as "including, but not limited to". "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps disclosed herein, and in the case of an amino acid or nucleic acid sequence, excluding additional amino acids or nucleotides, respectively. The term "consisting essentially of" limits the scope of a claim to the specified materials or steps, or to those that do not materially affect the basic characteristics of a claimed invention. For example, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from an isolation and purification method and would not exclude pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. Similarly, a protein consists essentially of a particular amino acid sequence when the protein includes additional amino acids that contribute to at most 20% of the length of the protein and do not substantially affect the activity of the protein (e.g., alters the activity of the protein by no more than 50%). Embodiments defined by each of these transitional terms are within the scope of this invention.

[0047] In the present description, the term "about" means + 20% of the indicated range, value, or structure, unless otherwise indicated.

[0048] It should be understood that the terms "a" and "an" as used herein includes "one" or "one or more" of the enumerated components unless stated otherwise.

[0049] The use of the alternative (e.g., "or") should be understood to mean either one, both, or any combination thereof of the alternatives, and may be used synonymously with "and/or".

[0050] As used herein, the terms "include" and "have" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

[0051] The word "substantially" does not exclude "completely"; e.g., a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from definitions provided herein.

[0052] "Optional" or "optionally" means that the subsequently described element, component, event, or circumstance may or may not occur, and that the description includes instances in which the element, component, event, or circumstance occurs and instances in which they do not.

[0053] As used herein, "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refer to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

[0054] As used herein, the terms "peptide", "polypeptide", and "protein", and variations of these terms, refer to a molecule that comprises at least two amino acids joined to each other by a (normal or modified) peptide bond. For example, a peptide, polypeptide, or protein may comprise or be composed of a plurality of amino acids selected from the 20 amino acids defined by the genetic code or an amino acid analog or mimetic, each being linked to at least one other by

a peptide bond. A peptide, polypeptide, or protein can comprise or be composed of L-amino acids and/or D-amino acids (or analogs or mimetics thereof). The terms "peptide", "polypeptide", and "protein" also include "peptidomimetics" which are defined as peptide analogs containing non-peptidic structural elements, which peptides are capable of mimicking or antagonizing the biological action(s) of a natural parent peptide. In certain embodiments, a peptidomimetic lacks characteristics such as enzymatically scissile peptide bonds.

[0055] A peptide, polypeptide, or protein may comprise amino acids other than the 20 amino acids defined by the genetic code in addition to these amino acids, or it can be composed of amino acids other than the 20 amino acids defined by the genetic code. In certain embodiments, a peptide, polypeptide, or protein in the context of the present disclosure can comprise amino acids that are modified by natural processes, such as post-translational maturation processes, or by chemical processes (e.g., synthetic processes), which are known in the art and include those described herein. Such modifications can appear anywhere in the polypeptide; e.g., in the peptide skeleton; in the amino acid chain; or at the carboxy- or amino-terminal ends. A peptide or polypeptide can be branched, such as following an ubiquitination, or may be cyclic, with or without branching. The terms "peptide", "polypeptide", and "protein" also include modified peptides, polypeptides and proteins. For example, peptide, polypeptide, or protein modifications can include acetylation, acylation, ADP-ribosylation, amidation, covalent fixation of a nucleotide or of a nucleotide derivative, covalent fixation of a lipid or of a lipidic derivative, the covalent fixation of a phosphatidylinositol, covalent or non-covalent cross-linking, cyclization, disulfide bond formation, demethylation, glycosylation including pegylation, hydroxylation, iodization, methylation, myristoylation, oxidation, proteolytic processes, phosphorylation, prenylation, racemization, seneloylation, sulfatation, or amino acid addition such as arginylation or ubiquitination. Such modifications have been described in the literature (see Proteins Structure and Molecular Properties (1993) 2nd Ed., T. E. Creighton, New York; Post-translational Covalent Modifications of Proteins (1983) B. C. Johnson, Ed., Academic Press, New York; Seifter et al., Meth. Enzymol. 182: 626-646, 1990; Rattan et al., Ann NY Acad Sci 663:48-62, 1992). Accordingly, the terms "peptide", "polypeptide", "protein" can include, for example, lipopeptides, lipoproteins, glycopeptides, glycoproteins, and the like. Variants of proteins, peptides, and polypeptides of this disclosure are also contemplated. In certain embodiments, variant proteins, peptides, and polypeptides comprise or consist of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, or 99.9% identical to an amino acid sequence of a defined or reference amino acid sequence as described herein.

[0056] "Protein" and "polypeptide" are often used in reference to relatively large polypeptides, whereas the term "peptide" is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms "protein" and "polypeptide" are used interchangeably herein when referring to an encoded gene product and fragments thereof. Additionally, as used herein, "(poly)peptide" and "protein" may be used interchangeably in reference to a polymer of amino acid residues, such as a plurality of amino acid monomers linked by peptide bonds.

[0057] "Nucleic acid molecule" or "polynucleotide" or "nucleic acid" refers to a polymeric compound including covalently linked nucleotides, which can be made up of natural subunits (e.g., purine or pyrimidine bases) or non-natural subunits (e.g., morpholine ring). Purine bases include adenine, guanine, hypoxanthine, and xanthine, and pyrimidine bases include uracil, thymine, and cytosine. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like.

[0058] Nucleic acid molecules include polyribonucleic acid (RNA), polydeoxyribonucleic acid (DNA), which includes cDNA, genomic DNA, and synthetic DNA, any of which may be single or double-stranded. If single-stranded, the nucleic acid molecule may be the coding strand or non-coding (anti-sense strand). Polynucleotides (including oligonucleotides), and fragments thereof may be generated, for example, by polymerase chain reaction (PCR) or by in vitro translation, or generated by any of ligation, scission, endonuclease action, or exonuclease action.

[0059] A nucleic acid molecule encoding an amino acid sequence includes all nucleotide sequences that encode the same amino acid sequence. Some versions of the nucleotide sequences may also include intron(s) to the extent that the intron(s) may be removed through co- or post-transcriptional mechanisms. Different nucleotide sequences may encode the same amino acid sequence as the result of the redundancy or degeneracy of the genetic code, or by splicing, or both.

[0060] Variants of nucleic acid molecules of this disclosure are also contemplated. Variant nucleic acid molecules are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical a nucleic acid molecule of a defined or reference polynucleotide as

described herein, or that hybridize to a polynucleotide under stringent hybridization conditions of 0.015M sodium chloride, 0.0015M sodium citrate at about 65-68°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at about 42°C. Nucleic acid molecule variants retain the capacity to encode a binding domain having a functionality described herein, such as specifically binding a target molecule.

[0061] As used herein, the term "sequence variant" refers to any sequence having one or more alterations in comparison to a reference sequence, whereby a reference sequence is any published sequence and/or any of the sequences disclosed herein, i.e., SEQ ID NO:1 to SEQ ID NO:137. Thus, the term "sequence variant" includes nucleotide sequence variants and amino acid sequence variants. In certain embodiments, a sequence variant in the context of a nucleotide sequence, the reference sequence is also a nucleotide sequence, whereas in certain embodiments for a sequence variant in the context of an amino acid sequence, the reference sequence is also an amino acid sequence. A "sequence variant" as used herein can be at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the reference sequence.

[0062] "Percent sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. Methods to determine sequence identity can be designed to give the best match between the sequences being compared. For example, the sequences may be aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment). Further, non-homologous sequences may be disregarded for comparison purposes. The percent sequence identity referenced herein is calculated over the length of the reference sequence, unless indicated otherwise. Methods to determine sequence identity and similarity can be found in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using a BLAST program (e.g., BLAST 2.0, BLASTP, BLASTN, or BLASTX). The mathematical algorithm used in the BLAST programs can be found in Altschul et al. (Nucleic Acids Res. 25:3389-3402, 1997). Within the context of this disclosure, it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default values" of the program referenced. "Default values" mean any set of values or parameters that originally load with the software when first initialized.

[0063] A "sequence variant" in the context of a nucleic acid (nucleotide) sequence has an altered sequence in which one or more of the nucleotides in the reference sequence is deleted or substituted, or one or more nucleotides are inserted into the sequence of the reference nucleotide

sequence. Nucleotides are referred to herein by the standard one-letter designation (A, C, G, or T). Due to the degeneracy of the genetic code, a "sequence variant" of a nucleotide sequence can either result in a change in the respective reference amino acid sequence, i.e., in an amino acid "sequence variant" or not. In certain embodiments, a nucleotide sequence variant does not result in an amino acid sequence variant (e.g., a silent mutation). In some embodiments, a nucleotide sequence variant that results in one or more "non-silent" mutation is contemplated. In some embodiments, a nucleotide sequence variant of the present disclosure encodes an amino acid sequence that is at least 80%, at least 85 %, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a reference amino acid sequence. Nucleotide and amino sequences as disclosed herein refer also to codon-optimized versions of a reference or wild-type nucleotide or amino acid sequence. In any of the embodiments described herein, a polynucleotide of the present disclosure may be codon-optimized for a host cell containing the polynucleotide (see, e.g., Scholten et al., Clin. Immunol. 119:135-145, 2006). Codon optimization can be performed using known techniques and tools, e.g., using the GenScript® OptimumGeneTM tool, or the GeneArt Gene Synthesis Tool (Thermo Fisher Scientific). Codon-optimized sequences include sequences that are partially codon-optimized (i.e., at least one codon is optimized for expression in the host cell) and those that are fully codon-optimized.

[0064] A "sequence variant" in the context of an amino acid sequence has an altered sequence in which one or more of the amino acids is deleted, substituted, or inserted in comparison to a reference amino acid sequence. As a result of the alterations, such a sequence variant has an amino acid sequence which is at least 80%, at least 85 %, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the reference amino acid sequence. For example, per 100 amino acids of the reference sequence a variant sequence that has no more than 10 alterations, i.e., any combination of deletions, insertions, or substitutions, is "at least 90% identical" to the reference sequence.

[0065] A "conservative substitution" refers to amino acid substitutions that do not significantly affect or alter binding characteristics of a particular protein. Generally, conservative substitutions are ones in which a substituted amino acid residue is replaced with an amino acid residue having a similar side chain. Conservative substitutions include a substitution found in one of the following groups: Group 1: Alanine (Ala or A), Glycine (Gly or G), Serine (Ser or S), Threonine (Thr or T); Group 2: Aspartic acid (Asp or D), Glutamic acid (Glu or Z); Group 3:

Asparagine (Asn or N), Glutamine (Gln or Q); Group 4: Arginine (Arg or R), Lysine (Lys or K), Histidine (His or H); Group 5: Isoleucine (Ile or I), Leucine (Leu or L), Methionine (Met or M), Valine (Val or V); and Group 6: Phenylalanine (Phe or F), Tyrosine (Tyr or Y), Tryptophan (Trp or W). Additionally or alternatively, amino acids can be grouped into conservative substitution groups by similar function, chemical structure, or composition (e.g., acidic, basic, aliphatic, aromatic, or sulfur-containing). For example, an aliphatic grouping may include, for purposes of substitution, Gly, Ala, Val, Leu, and Ile. Other conservative substitutions groups include: sulfur-containing: Met and Cysteine (Cys or C); acidic: Asp, Glu, Asn, and Gln; small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, and Gly; polar, negatively charged residues and their amides: Asp, Asn, Glu, and Gln; polar, positively charged residues: His, Arg, and Lys; large aliphatic, nonpolar residues: Met, Leu, Ile, Val, and Cys; and large aromatic residues: Phe, Tyr, and Trp. Additional information can be found in Creighton (1984) Proteins, W.H. Freeman and Company.

[0066] Amino acid sequence insertions can include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include the fusion to the N- or C-terminus of an amino acid sequence to a reporter molecule or an enzyme.

[0067] In general, alterations in the sequence variants do not abolish or significantly reduce a desired functionality of the respective reference sequence. For example, it is preferred that a variant sequence of the present disclosure does not significantly reduce or completely abrogate the functionality of a sequence of an antibody, or antigen binding fragment thereof, to bind to the same epitope as compared to antibody or antigen binding fragment having (or encoded by) the reference sequence. Guidance in determining which nucleotides and amino acid residues, respectively, may be substituted, inserted, or deleted without abolishing a desired structure or functionality can be found by using, e.g., known computer programs.

[0068] As used herein, a nucleic acid sequence or an amino acid sequence "derived from" a designated nucleic acid, peptide, polypeptide, or protein refers to the origin of the nucleic acid, peptide, polypeptide, or protein. A nucleic acid sequence or amino acid sequence that is derived from a particular sequence may have an amino acid sequence that is essentially identical to that sequence or a portion thereof, from which it is derived, whereby "essentially identical" includes sequence variants as defined above. A nucleic acid sequence or amino acid sequence that is derived from a particular peptide or protein, may be derived from the corresponding domain in

the particular peptide or protein. In this context, "corresponding" refers to possession of a same functionality or characteristic of interest. For example, an "extracellular domain" corresponds to another "extracellular domain" (of another protein), or a "transmembrane domain" corresponds to another “transmembrane domain” (of another protein). "Corresponding" parts of peptides, proteins, and nucleic acids are thus easily identifiable to one of ordinary skill in the art. Likewise, a sequence "derived from" another (e.g., "source") sequence can be identified by one of ordinary skill in the art as having its origin in the source sequence.

[0069] A nucleic acid sequence or an amino acid sequence derived from another nucleic acid, peptide, polypeptide, or protein may be identical to the starting nucleic acid, peptide, polypeptide, or protein (from which it is derived). However, a nucleic acid sequence or an amino acid sequence derived from another nucleic acid, peptide, polypeptide, or protein may also have one or more mutations relative to the starting nucleic acid, peptide, polypeptide, or protein (from which it is derived), in particular a nucleic acid sequence or an amino acid sequence derived from another nucleic acid, peptide, polypeptide, or protein may be a functional sequence variant as described above of the starting nucleic acid, peptide, polypeptide, or protein (from which it is derived). For example, in a peptide/protein, one or more amino acid residues may be substituted with other amino acid residues, or one or more amino acid residue insertions or deletions may occur.

[0070] As used herein, the term "mutation" relates to a change in a nucleic acid sequence and/or in an amino acid sequence in comparison to a reference sequence, e.g., a corresponding genomic, wild type, or reference sequence. A mutation, e.g., in comparison to a reference genomic sequence, may be, for example, a (naturally occurring) somatic mutation, a spontaneous mutation, an induced mutation, e.g., induced by enzymes, chemicals or radiation, or a mutation obtained by site-directed mutagenesis (molecular biology methods for making specific and intentional changes in the nucleic acid sequence and/or in the amino acid sequence). Thus, the terms "mutation" or "mutating" shall be understood to also include physically making or inducing a mutation, e.g., in a nucleic acid sequence or in an amino acid sequence. A mutation includes substitution, deletion, and insertion of one or more nucleotides or amino acids as well as inversion of several successive nucleotides or amino acids. To achieve a mutation in an amino acid sequence, a mutation may be introduced into the nucleotide sequence encoding said amino acid sequence in order to express a (recombinant) mutated polypeptide. A mutation may be achieved, for example, by altering (e.g., by site-directed mutagenesis) a codon (e.g., by altering one, two, or three nucleotide bases therein) of a nucleic acid molecule encoding one amino acid

to provide a codon that encodes a different amino acid, or that encodes a same amino acid, or by synthesizing a sequence variant.

[0071] The term "introduced" in the context of inserting a nucleic acid molecule into a cell, means "transfection", or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid molecule into a eukaryotic or prokaryotic cell wherein the nucleic acid molecule may be incorporated into the genome of a cell (e.g., chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

[0072] The term "recombinant", as used herein (e.g., a recombinant antibody, a recombinant protein, a recombinant nucleic acid, or the like, refers to any molecule (antibody, protein, nucleic acid, or the like) which is prepared, expressed, created, or isolated by recombinant means, and which is not naturally occurring. "Recombinant" can be used synonymously with "engineered" or "non-natural" and can refer to an organism, microorganism, cell, nucleic acid molecule, or vector that includes at least one genetic alteration or has been modified by introduction of an exogenous nucleic acid molecule, wherein such alterations or modifications are introduced by genetic engineering (i.e., human intervention). Genetic alterations include, for example, modifications introducing expressible nucleic acid molecules encoding proteins, fusion proteins, or enzymes, or other nucleic acid molecule additions, deletions, or substitutions or other functional disruption of a cell's genetic material. Additional modifications include, for example, non-coding regulatory regions in which the modifications alter expression of a polynucleotide, gene or operon.

[0073] As used herein, "heterologous" or "non-endogenous" or "exogenous" refers to any gene, protein, compound, nucleic acid molecule, or activity that is not native to a host cell or a subject, or any gene, protein, compound, nucleic acid molecule, or activity native to a host cell or a subject that has been altered. Heterologous, non-endogenous, or exogenous includes genes, proteins, compounds, or nucleic acid molecules that have been mutated or otherwise altered such that the structure, activity, or both is different as between the native and altered genes, proteins, compounds, or nucleic acid molecules. In certain embodiments, heterologous, non-endogenous, or exogenous genes, proteins, or nucleic acid molecules may not be endogenous to a host cell or a subject, but instead nucleic acids encoding such genes, proteins, or nucleic acid molecules may have been added to a host cell by conjugation, transformation, transfection, electroporation, or the like, wherein the added nucleic acid molecule may integrate into a host cell genome or can exist as extra-chromosomal genetic material (e.g., as a plasmid or other self-replicating vector).

The term "homologous" or "homolog" refers to a gene, protein, compound, nucleic acid molecule, or activity found in or derived from a host cell, species, or strain. For example, a heterologous or exogenous polynucleotide or gene encoding a polypeptide may be homologous to a native polynucleotide or gene and encode a homologous polypeptide or activity, but the polynucleotide or polypeptide may have an altered structure, sequence, expression level, or any combination thereof. A non-endogenous polynucleotide or gene, as well as the encoded polypeptide or activity, may be from the same species, a different species, or a combination thereof.

[0074] As used herein, the term "endogenous" or "native" refers to a polynucleotide, gene, protein, compound, molecule, or activity that is normally present in a host cell or a subject.

[0075] As used herein, the terms "cell," "cell line", and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same or substantially the same function, phenotype, or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0076] The terms "isolated" or "partially purified" as used herein refer in the case of a nucleic acid, polypeptide, or protein, to a nucleic acid, polypeptide, or protein separated from at least one other component (e.g., nucleic acid or polypeptide or protein) that is present with the nucleic acid, polypeptide, or protein as found in its natural source and/or that would be present with the nucleic acid, polypeptide, or protein when expressed by a cell, or secreted in the case of secreted polypeptides and proteins. A chemically synthesized nucleic acid, polypeptide, or protein, or one synthesized using in vitro transcription/translation, is considered "isolated." The terms "purified" or "substantially purified" refer to an isolated nucleic acid, polypeptide, or protein that is at least 95% by weight the subject nucleic acid, polypeptide, or protein, including, for example, at least 96%, at least 97%, at least 98%, at least 99%, or more.

[0077] CD8alpha (or CD8a) is a protein that is expressed on T cells, including regulatory T cells. CD8alpha polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_001759.3, NP001139345.1, NP_741969.1, NP_001369627.1, NP_757362.1, NP_001171571.1, NP_742100.1, NP_742099.1, and NP_004922; these sequences are incorporated by reference herein.

[0078] KIR (Killer Immunoglobulin-Like Receptor) proteins are cell surface molecules expressed on natural killer (NK) cells and on some T cells. The KIR gene family includes several gene loci, associated with different protein structures having two or three domains and short or long cytoplasmic tails. Some KIR receptors are inhibitory while others are activating, and one KIR receptor, KIR2DL4, can conduct both inhibitory and activating signals (see Dębska-Zielkowska et al., Cells 10(7):1777, 2021).

[0079] KIR3DL1 is a protein expressed on NK cells and on some T cells. It is also known as CD158E1, KIR, KIR2DL5B, KIR3DL1/S1, NKAT-3, NKAT3, NKB1, and NKB1B. KIR3DL1 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_037421.2 and NP_001309097.1; these sequences are incorporated by reference herein.

[0080] KIR3DL2 is a protein expressed on NK cells and on some T cells. It is also known as 3DL2, CD158K, KIR-3DL2, NKAT-4, NKAT4, NKAT4B, and p140. KIR3DL2 polypeptides include, but are not limited to, those having the amino acid sequences set forth in NP_006728.2 and NP_001229796.1; these sequences are incorporated by reference herein.

[0081] KIR2DL1 is a protein expressed on NK cells and on some T cells. It is also known as CD158A, KIR-K64, KIR221, KIR2DL3, NKAT, NKAT-1, NKAT1, and p58.1. KIR2DL1 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_055033.2; this sequence is incorporated by reference herein.

[0082] KIR2DL2 is a protein expressed on NK cells and on some T cells. It is also known as CD158B1, CD158b, NKAT-6, NKAT6, and p58.2. KIR2DL2 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_055034.2; this sequence is incorporated by reference herein.

[0083] KIR2DL3 is a protein expressed on NK cells and on some T cells. It is also known as CD158B2, CD158b, GL183, KIR-023GB, KIR-K7b, KIR-K7c, KIR2DL, KIR2DS5, KIRCL23, NKAT, NKAT2, NKAT2A, NKAT2B, and p58. KIR2DL3 polypeptides include, but are not limited to, those having the amino acid sequence set forth in NP_056952.2; this sequence is incorporated by reference herein.

II. Antibodies and Antigen-Binding Fragments

[0084] In one aspect, the present disclosure provides an antibody, or an antigen binding fragment thereof, that is capable of binding to CD8. In some embodiments, such antibodies, or

antigen binding fragments thereof, can bind to and modulate the activity of CD8+ regulatory T cells (Tregs). In some embodiments, the CD8+ Tregs are also KIR+.

[0085] Antibodies generally are comprised of a heavy chain and a light chain. Each heavy chain is composed of a variable region (abbreviated as VH) and a constant region. The heavy chain constant region may include three domains CH1, CH2, and CH3 and optionally a fourth domain, CH4. Each of these domains is referred to as an "Fc domain". As used herein, when a binding agent includes an Fc domain, it can include one or more Fc domains, or an entire Fc region, unless otherwise specified by context. Each light chain is composed of a variable region (abbreviated as VL) and a constant region or constant domain. The light chain constant region is a CL domain. The VH and VL regions may be further divided into hypervariable regions referred to as complementarity-determining regions (CDRs) and interspersed with conserved regions referred to as framework regions (FR). Each VH and VL region thus consists of three CDRs and four FRs that are arranged from the N terminus to the C terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. This structure is well known to those skilled in the art.

[0086] As used herein, and unless the context clearly indicates otherwise, "antibody" refers to an intact antibody comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (though it will be understood that heavy chain antibodies, which lack light chains, are still encompassed by the term "antibody"), as well as any antigen-binding portion or fragment of an intact antibody that has or retains the ability to bind to the antigen target molecule recognized by the intact antibody, such as, for example, a scFv, Fab, or F(ab')2 fragment. Thus, the term "antibody" herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments thereof, including fragment antigen-binding (Fab) fragments, F(ab')2 fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (e.g., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, e.g., bispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length

antibodies, including antibodies of any class or sub-class thereof, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0087] As used herein, the terms "antigen binding fragment", "fragment", and "antibody fragment" are used interchangeably to refer to any fragment of an antibody of the disclosure that retains the antigen-binding activity of the antibody. Examples of antibody fragments include, but are not limited to, a single chain antibody, Fab, Fab', $F(ab')_2$, Fv, and scFv.

[0088] Human antibodies are known (e.g., van Dijk and van de Winkel, Curr. Opin. Chem. Biol. 5:368-374, 2001). Human antibodies can be produced in transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire or a selection of human antibodies in the absence of endogenous immunoglobulin production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA 90:2551-2555, 1993; Jakobovits et al., Nature 362:255-258, 1993; Bruggemann et al., Year Immunol. 7:3340, 1993). Human antibodies can also be produced in phage display libraries (Hoogenboom and Winter, J. Mol. Biol. 227:381-388, 1992; Marks et al., J. Mol. Biol. 222:581-597, 1991). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); and Boerner et al., J. Immunol. 147:86-95, 1991). Human monoclonal antibodies may be prepared by using improved EBV-B cell immortalization as described in Traggiai et al. (Nat Med. 10(8):871-875, 2004). The term "human antibody" as used herein also comprises such antibodies which are modified, e.g., in the variable region, to generate properties according to the antibodies and antibody fragments of the present disclosure.

[0089] Antibodies according to the present disclosure can be of any isotype (e.g., IgA, IgG, IgM, IgE, IgD; i.e., comprising a α, γ, μ, ε, or δ heavy chain). Within the IgG isotype, for example, antibodies may be IgG1, IgG2, IgG3, or IgG4 subclass. In specific embodiments, an antibody of the present disclosure is an IgG1 antibody. Antibodies or antigen binding fragments provided herein may include a κ or a λ light chain.

[0090] As used herein, the term "variable region" (variable region of a light chain (VL), variable region of a heavy chain (VH)) denotes each variable region polypeptide of the pair of light and heavy chains, which, in most instances, is involved directly in binding the antibody to the antigen. The terms "VL" and "VH" refer to the variable binding region from an antibody light and heavy chain, respectively. The variable binding regions are made up of discrete, well-defined sub-regions known as "complementarity-determining regions" (CDRs) and "framework regions"

(FRs). The terms "complementarity-determining region" and "CDR" are synonymous with "hypervariable region" or "HVR," and are known in the art to refer to sequences of amino acids within TCR or antibody variable regions, which confer antigen specificity and/or binding affinity and are separated by framework sequence. In general, there are three CDRs in each variable region of an immunoglobulin binding protein; e.g., for antibodies, the VH and VL regions comprise six CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, LCDR3 (also referred to herein as CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively). As used herein, a "variant" of a CDR refers to a functional variant of a CDR sequence having up to 1-3 amino acid substitutions, deletions, or combinations thereof.

[0091] It will be understood that in certain embodiments, an antibody or antigen binding fragment of the present disclosure can comprise all or part of a heavy chain (HC), a light chain (LC), or both. For example, a full-length intact IgG antibody monomer typically includes a VH, a CH1, a CH2, a CH3, a VL, and a CL. Fc components are described further herein. In certain embodiments, an antibody or antigen binding fragment of the present disclosure comprises a CDRH1, a CDRH2, a CDRH3, a CDRL1, a CDRL2, and a CDRL3 according to any one of the presently disclosed VH and VL sequences, respectively.

[0092] Fragments of the antibodies described herein can be obtained from the antibodies by methods that include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, fragments of the antibodies can be obtained by cloning and expression of part of the sequences of the heavy or light chains. The present disclosure encompasses single-chain Fv fragments (scFv) derived from the heavy and light chains of an antibody as described herein, including, for example, an scFv comprising the CDRs from an antibody according to the present description, heavy or light chain monomers and dimers, single domain heavy chain antibodies, single domain light chain antibodies, as well as single chain antibodies, in which the heavy and light chain variable domains are joined by a peptide linker.

[0093] In certain embodiments, an antibody according to the present disclosure, or an antigen binding fragment thereof, comprises a purified antibody, a monoclonal antibody, a single chain antibody, Fab, Fab', $F(ab')_2$, Fv, or scFv.

[0094] Throughout this disclosure, antibodies, antigen binding fragments thereof, and fusion proteins may individually or collectively (e.g., in any combination) be referred to as "binding proteins".

[0095] Binding proteins according to the present disclosure may be provided in purified form. For example, an antibody may be present in a composition that is substantially free of other polypeptides, e.g., where less than 90% (by weight), usually less than 60% and more usually less than 50% of the composition is made up of other polypeptides.

[0096] Binding proteins according to the present disclosure may be immunogenic in human and/or in non-human (or heterologous) hosts; e.g., in mice. For example, an antibody may have an idiotope that is immunogenic in non-human hosts, but not in a human host. Antibodies of the disclosure for human use include those that are not typically isolated from hosts such as mice, goats, rabbits, rats, non-primate mammals, or the like, and in some instances are not obtained by humanization or from xeno-mice. Also contemplated herein are variant forms of the disclosed antibodies, which are engineered so as to reduce known or potential immunogenicity and/or other potential liabilities, or to confer a desired structure and/or functionality of the antibody in a non-human animal, such as a mouse (e.g., a "murinized " antibody wherein one or more human amino acid residue, sequence, or motif is replaced by a residue, sequence, or motif that has reduced or abrogated immunogenicity or other liability, or has a desired structure and/or function, in a mouse; e.g., for model studies using a mouse).

[0097] Antibodies or antigen-binding fragments thereof such as those described herein, including but not limited to scFv, may, in certain embodiments, be comprised in a fusion protein that is capable of specifically binding to an antigen as described herein. As used herein, "fusion protein" refers to a protein that, in a single chain, has at least two distinct domains or motifs, wherein the domains or motifs are not naturally found together, or in the given arrangement, in a protein. A polynucleotide encoding a fusion protein may be constructed using PCR, recombinantly engineered, or the like, or such fusion proteins can be synthesized.

[0098] Immunoglobulin sequences can be aligned to a numbering scheme (e.g., Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo), which can allow equivalent residue positions to be annotated and for different molecules to be compared using Antigen receptor Numbering And Receptor Classification (ANARCI) software tool (Bioinformatics 15:298-300, 2016; see also Dondelinger et al., Front. Immunol. 9:2278, 2018).

[0099] As used herein, "specifically binds" or "specific for" refers to an association or union of a binding protein (e.g., an antibody or antigen binding fragment thereof) or a binding domain to a target molecule with an affinity or Ka (i.e., an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1} (which equals the ratio of the on-rate [K_{on}] to the off rate [K_{off}] for this association reaction), while not

significantly associating or uniting with any other molecules or components in a sample. Binding proteins or binding domains may be classified as "high-affinity" binding proteins or binding domains or as "low-affinity" binding proteins or binding domains. "High-affinity" binding proteins or binding domains refer to those binding proteins or binding domains having a Ka of at least 10^7 M^{-1}, at least 10^8 M^{-1}, at least 10^9 M^{-1}, at least 10^{10} M^{-1}, at least 10^{11} M^{-1}, at least 10^{12} M^{-1}, or at least 10^{13} M^{-1}. "Low-affinity" binding proteins or binding domains refer to those binding proteins or binding domains having a Ka of up to 10^7 M^{-1}, up to 10^6 M^{-1}, or up to 10^5 M^{-1}. Alternatively, affinity may be defined as an equilibrium dissociation constant (Kd) of a particular binding interaction with units of M (e.g., 10^{-5} M to 10^{-13} M). The terms "binding" and "specifically binding" and similar references do not encompass non-specific sticking.

[0100] Binding of a binding protein can be determined or assessed using an appropriate assay, such as, for example, Surface Plasmon Resonance (SPR) methods, e.g., a Biacore™ system; kinetic exclusion assays such as KinExA®; and BioLayer interferometry (e.g., using the ForteBio® Octet platform); isothermal titration calorimetry (ITC), or the like, an antigen-binding ELISA (e.g., direct or indirect) with imaging by, e.g., optical density at 450nm, or by flow cytometry, or the like.

[0101] The term "epitope" or "antigenic epitope" includes any molecule, structure, amino acid sequence, or protein determinant that is recognized and specifically bound by a cognate binding molecule, such as an immunoglobulin or other binding molecule, domain, or protein. Epitopic determinants generally contain chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope to which binding protein binds may be linear (continuous) or conformational (discontinuous). A linear or a sequential epitope is an epitope that is recognized by an antibody according to its linear sequence of amino acids, or primary structure. A conformational epitope may be recognized according to a three-dimensional shape and protein structure. In the case of a conformational epitope (3D structure), the amino acid sequence typically forms a 3D structure as epitope and, thus, the amino acids forming the epitope may be or may be not located in adjacent positions of the primary structure (i.e., maybe or may be not consecutive amino acids in the amino acid sequence).

Multispecific Antibodies and Binding Proteins

[0102] Antibodies and antigen binding fragments of the present disclosure may, in embodiments, be multispecific (e.g., bispecific, trispecific, tetraspecific, or the like), and may be

provided in any multispecific format, as disclosed herein. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites or antigens. In certain embodiments, an antibody or antigen binding fragment of the present disclosure is a multispecific antibody, such as a bispecific or trispecific antibody. Formats for bispecific antibodies are disclosed in, for example, Spiess et al. (Mol. Immunol. 67(2):95, 2015), and in Brinkmann and Kontermann, (mAbs 9(2):182-212, 2017), which bispecific formats and methods of making the same are incorporated herein by reference and include, for example, Bispecific T cell Engagers (BiTEs), DARTs, Knobs-Into-Holes (KIH) assemblies, scFv-CH3-KIH assemblies, KIH Common Light-Chain antibodies, TandAbs, Triple Bodies, TriBi Minibodies, Fab-scFv, scFv-CH-CL-scFv, $F(ab')_2$-scFv2, tetravalent HCabs, Intrabodies, CrossMabs, Dual Action Fabs (DAFs) (two-in-one or four-in-one), DutaMabs, DT-IgG, Charge Pairs, Fab-arm Exchange, SEEDbodies, Triomabs, LUZ-Y assemblies, Fcabs, κλ-bodies, orthogonal Fabs, DVD-IgGs, IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zybody, and DVI-IgG (four-in-one). Bispecific and multi-specific antibodies include the following: an scFv1-ScFv2, an ScFv12-Fc-scFv22, an IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a scFv2-Fc, a TandAb, an scFv-HSA-scFv, an scFv-VHH, a Fab-scFv-Fc, a Fab-VHH-Fc, a dAb-IgG, an IgG-VHH, a Tandem scFv-Fc, a (scFv1)2-Fc-(VHH)2, a scFv-Fc, a one-armed tandem scFv-Fc, and a DART-Fc. An IgG-scFv may be an IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, svFc-(L)IgG, 2scFV-IgG, or IgG-2scFv.

[0103] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305:537, 1983; WO 93/08829; Traunecker et al., EMBO J. 10:3655, 1991), and "knob-in-hole" engineering (see, e.g., U.S. Pat. No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking of two or more antibodies or fragments (see, e.g., U.S. Pat. No. 4,676,980; Brennan et al., Science 229:81, 1985); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., J. Immunol. 148(5):1547-1553, 1992); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448, 1993); using single-chain Fv (scFv) dimers (see, e.g., Gruber et al., J. Immunol. 152:5368, 1994); and preparing trispecific antibodies (e.g., as described in Tutt et al., J. Immunol. 147:60, 1991).

[0104] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies", are also included herein (see, e.g., US 2006/0025576A1).

[0105] Antibodies or antigen binding fragments disclosed herein also include a "Dual Acting Fab" or "DAF" comprising an antigen binding site that binds to two different antigens (see, e.g., US 2008/0069820; Bostrom et al., Science 323:1610-1614, 2009).

[0106] "CrossMab" antibodies are also included herein (see, e.g., WO 2009/080251; WO 2009/080252; WO2009/080253; WO2009/080254; WO2013/026833).

[0107] In some embodiments, the antibodies or antigen binding fragments disclosed herein comprise different antigen-binding sites, fused to one or the other of the two subunits of the Fc domain; thus, the two subunits of the Fc domain may be comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the bispecific molecules in recombinant production, it is advantageous to introduce in the Fc domain of the binding agent a modification promoting the association of the desired polypeptides.

[0108] Accordingly, in particular aspects relates to a binding agent (e.g., an antibody or antigen binding fragment thereof) comprising (a) at least a first binding domain, (b) a second binding domain, and (c) a Fc domain composed of a first and a second subunit capable of stable association, wherein the Fc domain comprises a modification promoting the association of the first and second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one aspect said modification is in the CH3 domain of the Fc domain.

[0109] In a specific aspect, the Fc modification is a so-called "knob-into-hole" modification, comprising a "knob" modification in one of the two subunits of the Fc domain and a "hole" modification in the other one of the two subunits of the Fc domain. In a particular aspect, the first subunit of the Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second subunit of the Fc domain comprises the amino acid substitutions Y349C, T366S, and Y407V (numbering according to Kabat EU index). The knob-into-hole technology is described in, e.g., U.S. Pat. Nos. 5,731,168; 7,695,936; Ridgway et al. (Prot Eng 9:617-621, 1996) and Carter (J Immunol Meth 248:7-15, 2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer

formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g., tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine).

[0110] Accordingly, in some embodiments, in a CH3 domain of an Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain which is positionable in a cavity within a CH3 domain of a second Fc domain, and in the CH3 domain of the second Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second Fc domain within which the protuberance within the CH3 domain of the first Fc domain is positionable. The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g., by site-specific mutagenesis, or by peptide synthesis. In a specific embodiment, in the CH3 domain of the first Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V). In another embodiment, in the second Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A).

[0111] In some embodiments, in the first Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C), and in the second Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C). Introduction of these two cysteine residues results in the formation of a disulfide bridge between the two Fc domains that further stabilizes the dimer (Carter, J Immunol Methods 248:7-15, 2009). In some embodiments, the first Fc domain comprises the amino acid substitutions S354C and T366W (EU numbering) and the second Fc domain comprises the amino acid substitutions Y349C, T366S, and Y407V (numbering according to Kabat EU index).

[0112] In some embodiments, a modification promoting association of the first and the second Fc domains comprises a modification mediating electrostatic steering effects, for example, as described in PCT publication WO 2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two Fc domains by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

[0113] In some embodiments, a binding agent (e.g., an antibody or antigen binding fragment thereof) comprises one or more scFvs or "single-chain variable fragments". An scFv is a fusion protein of the variable regions of the heavy (VH) and light chain (VL) variable regions of an antibody, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original antibody, despite removal of the constant regions and the introduction of the linker. scFv antibodies are, described in, e.g., Houston (Methods in Enzymol. 203: 46-96, 1991). Methods for making scFv molecules and designing suitable peptide linkers are described in, for example, U.S. Pat. No. 4,704,692; U.S. Pat. No. 4,946,778; Raag and Whitlow (FASEB 9:73-80, 1995); and Bird and Walker (TIBTECH 9:132-137, 1991).

[0114] Binding agents (e.g., an antibody or antigen binding fragment thereof) that are scFv-Fcs have been described by Sokolowska-Wedzina et al. (Mol. Cancer Res. 15(8):1040-1050, 2017).

[0115] In some embodiments, a binding agent (e.g., an antibody or antigen binding fragment thereof) is a "bispecific T cell engager" or BiTE (see, e.g., WO2004/106381; WO2005/061547; WO2007/042261; WO2008/119567). This approach utilizes two antibody variable domains arranged on a single polypeptide. For example, a single polypeptide chain can include two single chain Fv (scFv) fragments, each having a variable heavy chain (VH) and a variable light chain (VL) domain separated by a polypeptide linker of a length sufficient to allow intramolecular association between the two domains. This single polypeptide further includes a polypeptide spacer sequence between the two scFv fragments. Each scFv recognizes a different epitope, and these epitopes may be specific for different proteins, such that both proteins are bound by the BiTE. As it is a single polypeptide, the bispecific T cell engager may be expressed using any prokaryotic or eukaryotic cell expression system known in the art, e.g., a CHO cell line. However, specific purification techniques (see, e.g., EP1691833) may be necessary to separate monomeric bispecific T cell engagers from other multimeric species, which may have biological activities other than the intended activity of the monomer. In one exemplary purification scheme, a solution containing secreted polypeptides is first subjected to a metal affinity chromatography, and polypeptides are eluted with a gradient of imidazole concentrations. This eluate is further purified using anion exchange chromatography, and polypeptides are eluted using with a gradient of sodium chloride concentrations. Finally, this eluate is subjected to size exclusion chromatography to separate monomers from multimeric species. In some

embodiments, a binding agent that is a bispecific antibody is composed of a single polypeptide chain comprising two single chain FV fragments (scFV) fused to each other by a peptide linker.

[0116] A single-domain antibody is an antibody fragment consisting of a single monomeric variable antibody domain. Single-domain antibodies can be derived from the variable domain of the antibody heavy chain from camelids (e.g., nanobodies or VHH fragments). Furthermore, the term single-domain antibody includes an autonomous human heavy chain variable domain (aVH) or VNAR fragments derived from sharks (see, e.g., Hasler et al., Mol. Immunol. 75:28-37, 2016). Techniques for producing single-domain antibodies (DABs or VHH) are known in the art, as disclosed in, for example, Cossins et al. (Prot Express Purif 51:253-259, 2006) and Li et al. (Immunol. Lett. 188:89-95, 2017). Single-domain antibodies may be obtained, for example, from camels, alpacas or llamas by standard immunization techniques (see, e.g., Muyldermans et al., TIBS 26:230-235, 2001; Yau et al., J Immunol Methods 281:161-75, 2003; and Maass et al., J Immunol Methods 324:13-25, 2007). A VHH may have potent antigen-binding capacity and can interact with novel epitopes that are inacessible to conventional VH-VL pairs (see, e.g., Muyldermans et al., 2001, supra). Alpaca serum IgG contains about 50% camelid heavy chain only IgG antibodies (HCAbs) (see, e.g., Maass et al., 2007, supra). Alpacas may be immunized with antigens and VHHs can be isolated that bind to and neutralize the target antigen (see, e.g., Maass et al., 2007, supra). PCR primers that amplify alpaca VHH coding sequences have been identified and may be used to construct alpaca VHH phage display libraries, which can be used for antibody fragment isolation by standard biopanning techniques well known in the art (see, e.g., Maass et al., 2007, supra).

[0117] In some embodiments, a binding agent (e.g., an antibody or antigen binding fragment thereof) is a IgG-scFV. IgG-scFv formats include IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, svFc-(L)IgG, 2scFV-IgG, and IgG-2scFv. These and other bispecific antibody formats and methods of making them have been described in for example, Brinkmann and Kontermann (MAbs 9(2):182-212, 2017); Wang et al. (Antibodies 8:43, 2019); Dong et al. (MAbs 3:273-88, 2011); Natsume et al. (J. Biochem. 140(3):359-368, 2006); Cheal et al. (Mol. Cancer Ther. 13(7):1803-1812, 2014); and Bates and Power (Antibodies 8:28, 2019).

[0118] Igg-like dual-variable domain antibodies (DVD-Ig) have been described by Wu et al. (Nat Biotechnol 25:1290-97, 2007); by Hasler et al. (Mol. Immunol. 75:28-37, 2016); and in WO 08/024188 and WO 07/024715.

[0119] Triomabs have been described by Chelius et al. (MAbs 2(3):309-319, 2010). 2-in-1-IgGs have been described by Kontermann et al. (Drug Discovery Today 20(7):838-847, 2015).

[0120] Tandem antibody or TandAb have been described by Kontermann et al. (Drug Discovery Today 20(7):838-847, 2015).

[0121] ScFv-HSA-scFv antibodies have also been described by Kontermann et al. (Drug Discovery Today 20(7):838-847, 2015).

[0122] In some embodiments, the binding agent (e.g., an antibody or antigen binding fragment thereof) is a scaffold antigen binding protein, such as for example, fibronectin and designed ankyrin repeat proteins (DARPins) which have been used as alternative scaffolds for antigen-binding domains (see, e.g., Gebauer and Skerra Curr Opin Chem Biol 13:245-255, 2009; Stumpp et al., Drug Discovery Today 13:695-701, 2008). In some embodiments, a scaffold antigen binding protein is selected from the group consisting of Lipocalins (Anticalin), a Protein A-derived molecule such as Z-domain of Protein A (Affibody), an A-domain (Avimer/Maxibody), a serum transferrin (trans-body); a designed ankyrin repeat protein (DARPin), a fibronectin (AdNectin), a C-type lectin domain (Tetranectin); a variable domain of a new antigen receptor beta-lactamase (VNAR fragments), a human gamma-crystallin or ubiquitin (Affilin molecules); a kunitz type domain of human protease inhibitors, and microbodies such as the proteins from the knottin family, peptide aptamers, and fibronectin (adnectin). Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids, and lipids. They have a rigid beta-sheet secondary structure with a number of loops at the open end of the conical structure which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. (For further details, see Biochim Biophys Acta 1482:337-350, 2000; U.S. Pat. No. 7,250,297B1; US20070224633.)

[0123] Designed Ankyrin Repeat Proteins (DARPins) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33-residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different target antigens by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). (For further details, see J. Mol. Biol. 332:489-503, 2003; PNAS 100(4):1700-1705, 2003; J. Mol. Biol. 3691015-1028; 2007; US20040132028A1).

Fc Domain Modifications

[0124] In some embodiments, a binding protein (e.g., antibody or an antigen binding fragment thereof) of the present disclosure comprises an Fc moiety. In certain embodiments, the Fc moiety may be derived from human origin, e.g., from human IgG1, IgG2, IgG3, and/or IgG4, or from another Ig class or isotype. In specific embodiments, an antibody or antigen binding fragments can comprise an Fc moiety derived from human IgG1.

[0125] As used herein, the term "Fc moiety" refers to a sequence comprising, consisting, consisting essentially of, or derived from a portion of an immunoglobulin heavy chain beginning in the hinge region just upstream of the papain cleavage site (e.g., residue 216 in native IgG, taking the first residue of heavy chain constant region to be 114) and ending at the C-terminus of the immunoglobulin heavy chain. Accordingly, an Fc moiety may be a complete Fc moiety or a portion (e.g., a domain) thereof. In certain embodiments, a complete Fc moiety comprises a hinge domain, a CH2 domain, and a CH3 domain (e.g., EU amino acid positions 216-446). An additional lysine residue (K) is sometimes present at the extreme C-terminus of the Fc moiety, but is often cleaved from a mature antibody. Amino acid positions within an Fc moiety can be numbered according to the EU numbering system of Kabat (see, e.g., Kabat et al., "Sequences of Proteins of Immunological Interest", U.S. Dept. Health and Human Services, 1983 and 1987). Amino acid positions of an Fc moiety can also be numbered according to the IMGT numbering system (including unique numbering for the C-domain and exon numbering) and the Kabat numbering system.

[0126] In some embodiments, an Fc moiety comprises at least one of: a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant, portion, or fragment thereof. In some embodiments, an Fc moiety comprises at least a hinge domain, a CH2 domain or a CH3 domain. In further embodiments, the Fc moiety is a complete Fc moiety. The Fc moiety may also comprise one or more amino acid insertions, deletions, or substitutions relative to a naturally occurring Fc moiety. For example, at least one of a hinge domain, CH2 domain, or CH3 domain, or a portion thereof, may be deleted. For example, an Fc moiety may comprise or consist of: (i) hinge domain (or a portion thereof) fused to a CH2 domain (or a portion thereof), (ii) a hinge domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), (iii) a CH2 domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), (iv) a hinge domain (or a portion thereof), (v) a CH2 domain (or a portion thereof), or (vi) a CH3 domain or a portion thereof.

[0127] An Fc moiety of the present disclosure may be modified such that it varies in amino acid sequence from the complete Fc moiety of a naturally occurring immunoglobulin

molecule, while retaining or enhancing at least one desirable function conferred by the naturally occurring Fc moiety, and/or reducing an undesired function of a naturally occurring Fc moiety. Such functions include, for example, Fc receptor (FcR) binding, antibody half-life modulation (e.g., by binding to FcRn), ADCC function, protein A binding, protein G binding, and complement binding. Portions of naturally occurring Fc moieties which are involved with such functions have been described in the art.

[0128] In some embodiments, an Fc region or Fc domain has substantially no binding to at least one Fc receptor selected from FcγRI (CD64), FcγRIIA (CD32a), FcγRIIB (CD32b), FcγRIIIA (CD16a), and FcγRIIIB (CD16b). In some embodiments, an Fc region or domain exhibits substantially no binding to any of the Fc receptors selected from FcγRI (CD64), FcγRIIA (CD32a), FcγRIIB (CD32b), FcγRIIIA (CD16a), and FcγRIIIB (CD16b). As used herein, "substantially no binding" refers to weak to no binding to a selected Fcgamma receptor or receptors. In some embodiments, "substantially no binding" refers to a reduction in binding affinity (e.g., increase in Kd) to a Fc gamma receptor of at least 1000-fold. In some embodiments, an Fc domain or region is an Fc null. As used herein, an "Fc null" refers to an Fc region or Fc domain that exhibits weak to no binding to any of the Fcgamma receptors. In some embodiments, an Fc null domain or region exhibits a reduction in binding affinity (e.g., increase in Kd) to Fc gamma receptors of at least 1000-fold.

[0129] In some embodiments, an Fc domain has reduced or substantially no effector function activity. As used herein, "effector function activity" refers to antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), and/or complement dependent cytotoxicity (CDC). In some embodiments, an Fc domain exhibits reduced ADCC, ADCP, or CDC activity, as compared to a wildtype Fc domain. In some embodiments, an Fc domain exhibits a reduction in ADCC, ADCP, and CDC, as compared to a wildtype Fc domain. In some embodiments, an Fc domain exhibits substantially no effector function (i.e., the ability to stimulate ADCC, ADCP, or CDC). As used herein, "substantially no effector function" refers to a reduction in effector function activity of at least 1000-fold, as compared to a wildtype Fc domain.

[0130] In some embodiments, an Fc domain has reduced or no ADCC activity. As used herein reduced or no ADCC activity refers to a decrease in ADCC activity of an Fc domain by of a factor of at least 10, at least 20, at least 30, at least 50, at least 100, or at least 500.

[0131] In some embodiments, an Fc domain has reduced or no CDC activity. As used herein reduced or no CDC activity refers to a decrease in CDC activity of an Fc domain by of a factor of at least 10, at least 20, at least 30, at least 50, at least 100, or at least 500.

[0132] In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of ADCC and/or CDC activity. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fcgamma receptor (hence likely lacking ADCC activity). The primary cells for mediating ADCC, NK cells, express FcgammaRIII only, whereas monocytes express FcgammaRI, FcgammaRII, and FcgammaRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet (Annu. Rev. Immunol. 9:457-492, 1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362 (see, e.g., Hellstrom et al., Proc. Nat'l Acad. Sci. USA 83:7059-7063, 1986; Hellstrom et al., Proc. Nat'l Acad. Sci. USA 82:1499-1502, 1985; U.S. Pat. No. 5,821,337; Bruggemann et al., J. Exp. Med. 166:1351-1361, 1987). Alternatively, non-radioactive assays methods may be employed (see, e.g., ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96TM non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. (Proc. Nat'l Acad. Sci. USA 95:652-656, 1998).

[0133] C1q binding assays may also be carried out to confirm that an antibody or Fc domain or region is unable to bind C1q and hence lacks CDC activity or has reduced CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163, 1996; Cragg et al., Blood 101:1045-1052, 2003; Cragg and Glennie, Blood 103:2738-2743, 2004).

[0134] In some embodiments, an Fc domain has reduced or no ADCP activity. As used herein reduced or no ADCP activity refers to a decrease in ADCP activity of an Fc domain by of a factor of at least 10, at least 20, at least 30, at least 50, at least 100, or at least 500.

[0135] ADCP binding assays may also be carried out to confirm that an antibody or Fc domain or region lacks ADCP activity or has reduced ADCP activity (see, e.g., US20190079077 and US20190048078 and the references disclosed therein).

[0136] Antibodies with reduced effector function activity include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327, and 329 (see U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297, and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (see U.S. Pat. No. 7,332,581). Certain antibody variants with diminished binding to FcRs are also known (see, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312; Shields et al., J. Biol. Chem. 9(2):6591-6604, 2001).

[0137] In certain embodiments, a binding agent comprises an Fc domain or region with one or more amino acid substitutions which diminish FcgammaR binding, e.g., substitutions at positions 234 and 235 of the Fc region (EU numbering of residues). In some embodiments, the substitutions are L234A and L235A (LALA). In some embodiments, the Fc domain further comprises D265A and/or P329G in an Fc region derived from a human IgG1 Fc region. In some embodiments, the substitutions are L234A, L235A, and P329G (LALA-PG) in an Fc region derived from a human IgG1 Fc region (see, e.g., WO 2012/130831). In some embodiments, the substitutions are L234A, L235A, and D265A (LALA-DA) in an Fc region derived from a human IgG1 Fc region.

[0138] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. (J. Immunol. 164:4178-4184, 2000).

Production of Binding Proteins

[0139] In various embodiments, binding proteins (e.g., antibody or an antigen binding fragment thereof) can be produced in human, murine, or other animal-derived cells lines. Recombinant DNA expression can be used to produce the binding agents. This allows the production of antibodies as well as a spectrum of antigen binding portions and other binding agents (including fusion proteins) in a host species of choice. The production of antibodies, antigen binding portions thereof and other binding agents in bacteria, yeast, transgenic animals, and chicken eggs are also alternatives for cell-based production systems. The main advantages of transgenic animals are potential high yields from renewable sources.

[0140] Nucleic acid molecules encoding the amino acid sequence of an antibody, or antigen binding portion thereof, as well as other binding agents can be prepared by a variety of methods known in the art. These methods include, but are not limited to, preparation of synthetic

nucleotide sequences encoding of an antibody, antigen binding portion or other binding agent(s). In addition, oligonucleotide-mediated (or site-directed) mutagenesis, PCR-mediated mutagenesis, and cassette mutagenesis can be used to prepare nucleotide sequences encoding an antibody or antigen binding portion as well as other binding agents. A nucleic acid sequence encoding at least an antibody, antigen binding portion thereof, binding agent, or a polypeptide thereof, as described herein, can be recombined with vector DNA in accordance with conventional techniques, such as, for example, blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, e.g., by Maniatis et al. (Molecular Cloning, Lab. Manual (Cold Spring Harbor Lab. Press, NY, 1982 and 1989)), and Ausubel et al. (Current Protocols in Molecular Biology (John Wiley & Sons), 1987-1993), and can be used to construct nucleic acid sequences and vectors that encode an antibody or antigen binding portion thereof or a VH and/or VL polypeptide thereof. Where the binding agent comprises antibodies or antigen binding portions thereof, in some embodiments, a VH polypeptide is encoded by a first nucleic acid. In some embodiments, a VL polypeptide is encoded by a second nucleic acid. In some embodiments, the VH and VL polypeptides are encoded by one nucleic acid.

[0141] A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences that contain transcriptional and translational regulatory information, and such sequences are "operably linked" to nucleotide sequences that encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed (e.g., an antibody or antigen binding portion thereof) are connected in such a way as to permit gene expression of a polypeptide(s) or antigen binding portions in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art (see, e.g., Sambrook et al., 1989; Ausubel et al., 1987-1993, supra).

[0142] Accordingly, the expression of an antibody or antigen-binding portion thereof or other binding agent as described herein can occur in either prokaryotic or eukaryotic cells. Suitable hosts include bacterial or eukaryotic hosts, including yeast, insects, fungi, bird, and mammalian cells either in vivo or in situ, or host cells of mammalian, insect, bird, or yeast origin. The mammalian cell or tissue can be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog, or cat origin, but any other mammalian cell may be used. Further, by use of, for example, the yeast ubiquitin hydrolase system, in vivo synthesis of ubiquitin-transmembrane

polypeptide fusion proteins can be accomplished. The fusion proteins so produced can be processed in vivo or purified and processed in vitro, allowing synthesis of an antibody or antigen binding portion thereof as described herein with a specified amino terminus sequence. Moreover, problems associated with retention of initiation codon-derived methionine residues in direct yeast (or bacterial) expression may be avoided (see, e.g., Sabin et al., 7 Bio/Technol. 705, 1989); Miller et al., 7 Bio/Technol. 698, 1989) Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeast are grown in medium rich in glucose can be utilized to obtain recombinant antibodies or antigen-binding portions thereof or other binding agents. Known glycolytic genes can also provide very efficient transcriptional control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase gene can be utilized.

[0143] Production of antibodies or antigen-binding portions thereof and other binding agents in insects can be achieved, for example, by infecting an insect host with a baculovirus engineered to express a polypeptide by methods known to those of ordinary skill in the art. See Ausubel et al., 1987-1993.

[0144] In some embodiments, the introduced nucleic acid sequence (encoding an antibody or antigen binding portion thereof or a polypeptide thereof or other binding agent) is incorporated into a plasmid or viral vector capable of autonomous replication in a recipient host cell. Any of a wide variety of vectors can be employed for this purpose and are known and available to those of ordinary skill in the art (see, e.g., Ausubel et al., 1987-1993, supra). Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

[0145] Exemplary prokaryotic vectors known in the art include plasmids such as those capable of replication in E. coli. Other gene expression elements useful for the expression of DNA encoding antibodies or antigen-binding portions thereof and other binding agents include, but are not limited to (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter (Okayama et al., 3 Mol. Cell. Biol. 280, 1983), Rous sarcoma virus LTR (Gorman et al., 79 PNAS 6777, 1982), and Moloney murine leukemia virus LTR (Grosschedl et al., 41 Cell 885, 1985); (b) splice regions and polyadenylation sites such as those derived from

the SV40 late region (Okayarea et al., 1983, supra); and (c) polyadenylation sites such as in SV40 (Okayama et al., 1983, supra). Immunoglobulin-encoding DNA genes can be expressed as described by Liu et al. and Weidle et al. (51 Gene 21, 1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements.

[0146] For immunoglobulin encoding nucleotide sequences, the transcriptional promoter can be, for example, human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin.

[0147] In some embodiments, for expression of DNA coding regions in rodent cells, the transcriptional promoter can be a viral LTR sequence, the transcriptional promoter enhancers can be either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, and the polyadenylation and transcription termination regions. In other embodiments, DNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

[0148] Each coding region or gene fusion is assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the variable region(s) or antigen binding portions thereof are then transfected singly with nucleotides encoding an antibody or an antibody polypeptide or antigen-binding portion thereof, or are co-transfected with a polynucleotide(s) encoding VH and a VL chain coding regions. The transfected recipient cells are cultured under conditions that permit expression of the incorporated coding regions and the expressed antibody chains or intact antibodies or antigen binding portions are recovered from the culture.

[0149] In some embodiments, the nucleic acids containing the coding regions encoding an antibody or antigen-binding portion thereof are assembled in separate expression vectors that are then used to co-transfect a recipient host cell. Each vector can contain one or more selectable genes. For example, in some embodiments, two selectable genes are used, a first selectable gene designed for selection in a bacterial system and a second selectable gene designed for selection in a eukaryotic system, wherein each vector has a set of coding regions. This strategy results in vectors which first direct the production, and permit amplification, of the nucleotide sequences in a bacterial system. The DNA vectors so produced and amplified in a bacterial host are subsequently used to co-transfect a eukaryotic cell, and allow selection of a co-transfected cell carrying the desired transfected nucleic acids (e.g., encoding antibody heavy and light chains). Non-limiting examples of selectable genes for use in a bacterial system are the gene that confers

resistance to ampicillin and the gene that confers resistance to chloramphenicol. Selectable genes for use in eukaryotic transfectants include the xanthine guanine phosphoribosyl transferase gene (designated gpt) and the phosphotransferase gene from Tn5 (designated neo). Alternatively, the fused nucleotide sequences encoding VH and VL chains can be assembled on the same expression vector.

[0150] For transfection of the expression vectors and production of the antibodies or antigen binding portions thereof or other binding agents, the recipient cell line can be a Chinese Hamster ovary cell line (e.g., DG44) or a myeloma cell. Myeloma cells can synthesize, assemble, and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. For example, in some embodiments, the recipient cell is the recombinant Ig-producing myeloma cell SP2/0. SP2/0 cells only produce immunoglobulins encoded by the transfected genes. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites fluid.

[0151] An expression vector encoding an antibody or antigen-binding portion thereof or other binding agent can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection and microprojectile bombardment (Johnston et al., Science 240:1538, 1988), as known to one of ordinary skill in the art.

[0152] Yeast provides certain advantages over bacteria for the production of immunoglobulin heavy and light chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies exist that utilize strong promoter sequences and high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned mammalian gene products and secretes polypeptides bearing leader sequences (i.e., pre-polypeptides) (see, e.g., Hitzman et al., 11th Intl. Conf. Yeast, Genetics & Molec. Biol. (Montpelier, France, 1982)).

[0153] Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of antibodies, and assembled antibodies and antigen binding portions thereof. Various yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known

glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase (PGK) gene can be utilized. Another example is the translational elongation factor 1alpha promoter. A number of approaches can be taken for evaluating optimal expression plasmids for the expression of immunoglobulins in yeast (see, e.g., II DNA Cloning 45, (Glover, ed., IRL Press, 1985); U.S. Patent Publication No. US 2006/0270045 A1).

[0154] Bacterial strains can also be utilized as hosts for the production of the antibody molecules or antigen binding portions thereof or other binding agents described herein. E. coli K12 strains such as E. coli W3110, Bacillus species, enterobacteria such as Salmonella typhimurium or Serratia marcescens, and various Pseudomonas species can be used. Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches can be taken for evaluating the expression plasmids for the production of antibodies and antigen binding portions thereof in bacteria (see Glover, 1985, supra; Ausubel, 1987, 1993, supra; Sambrook, 1989; Colligan, 1992-1996).

[0155] Host mammalian cells can be grown in vitro or in vivo. Mammalian cells provide post-translational modifications to immunoglobulin molecules including leader peptide removal, folding and assembly of VH and VL chains, glycosylation of the antibody molecules, and secretion of functional antibody and/or antigen binding portions thereof.

[0156] Mammalian cells that can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero or CHO-K1 cells. Exemplary eukaryotic cells that can be used to express immunoglobulin polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, CHO-K1, and DG44 cells; PERC6TM cells (Crucell); and NSO cells. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the heavy chains and/or light chains. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0157] In some embodiments, one or more antibodies or antigen-binding portions thereof or other binding agents can be produced in vivo in an animal that has been engineered or transfected with one or more nucleic acid molecules encoding the polypeptides, according to any suitable method.

[0158] In some embodiments, an antibody or antigen-binding portion thereof is produced in a cell-free system. Non-limiting exemplary cell-free systems are described, e.g., in Sitaraman et al. (Methods Mol. Biol. 498:229-244, 2009); Spirin (Trends Biotechnol. 22:538-545, 2004); and Endo et al. (Biotechnol. Adv. 21:695-713, 2003).

[0159] Many vector systems are available for the expression of the VH and VL chains in mammalian cells (see Glover, 1985, supra). Various approaches can be followed to obtain intact antibodies. As discussed above, it is possible to co-express VH and VL chains and optionally the associated constant regions in the same cells to achieve intracellular association and linkage of VH and VL chains into complete tetrameric H2L2 antibodies or antigen-binding portions thereof. The co-expression can occur by using either the same or different plasmids in the same host. Nucleic acids encoding the VH and VL chains or antigen binding portions thereof can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the VL chain, followed by transfection of the resulting cell line with a VH chain plasmid containing a second selectable marker. Cell lines producing antibodies, antigen-binding portions thereof or other binding agents via either route could be transfected with plasmids encoding additional copies of peptides, VH, VL, or VH plus VL chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled antibodies or antigen binding portions thereof or enhanced stability of the transfected cell lines.

[0160] Additionally, plants have emerged as a convenient, safe, and economical alternative expression system for recombinant antibody production, which are based on large scale culture of microbes or animal cells. Antibodies or antigen binding portions can be expressed in plant cell culture, or plants grown conventionally. The expression in plants may be systemic, limited to sub-cellular plastids, or limited to seeds (endosperms) (see, e.g., U.S. Patent Pub. No. 2003/0167531; U.S. Pat. No. 6,080,560; U.S. Pat. No. 6,512,162; WO 0129242). Several plant-derived antibodies have reached advanced stages of development, including clinical trials (see, e.g., Biolex, N.C.).

[0161] For intact antibodies, the variable regions (VH and VL) of the antibodies are typically linked to at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well-known procedures from a variety of human cells, such as immortalized B-cells (see, e.g., WO 87/02671; which is incorporated by reference herein in its entirety). An antibody can

contain both light chain and heavy chain constant regions. The heavy chain constant region can include CH1, hinge, CH2, CH3, and, sometimes, CH4 regions. In some embodiments, the CH2 domain can be deleted or omitted.

[0162] Alternatively, techniques described for the production of single chain antibodies (see, e.g., U.S. Pat. No. 4,946,778; Bird, Science 242:423-42, 1988; Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883, 1988); Ward et al., Nature 334:544-554,1989; which are incorporated by reference herein in their entireties) can be adapted to produce single chain antibodies that specifically bind to the desired antigen. Single chain antibodies are formed by linking the heavy and light chain variable regions of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli can also be used (see, e.g., Skerra et al., Science 242:1038-1041, 1988; which is incorporated by reference herein in its entirety).

[0163] Intact (e.g., whole) antibodies, their dimers, individual light and heavy chains, or antigen binding portions thereof can be recovered and purified by known techniques, e.g., immunoadsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), ammonium sulfate precipitation, gel electrophoresis, or any combination of these (see generally, Scopes, Protein Purification (Springer-Verlag, N.Y., 1982)). Substantially pure antibodies or antigen binding portions thereof of at least about 90% to 95% homogeneity are advantageous, as are those with 98% to 99% or more homogeneity, particularly for pharmaceutical uses. Once purified, partially or to homogeneity as desired, an intact antibody or antigen binding portions thereof can then be used therapeutically or in developing and performing assay procedures, immunofluorescent staining, and the like (see generally, Vols. I & II Immunol. Meth. (Lefkovits & Pernis, eds., Acad. Press, NY, 1979 and 1981)).

Anti-CD8 Antibodies and Antigen-Binding Fragments Thereof, and Binding Proteins

[0164] In some embodiments, an anti-CD8 antibody, antigent-binding fragment thereof, or binding protein of the present disclosure comprises any of the substitutions in Table 1 or Table 2. In some embodiments, an anti-CD8 antibody, antigen-binding fragment, or binding protein comprising or consisting of one or more of SEQ ID NOs:17-60 is provided. In some embodiments, a binding protein having the sequences of Table 3 is provided.

III. Pharmaceutical Compositions or Formulations

[0165] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof) disclosed herein relate to compositions comprising active ingredients (i.e., including a binding agent as described herein or a nucleic acid encoding an antibody or antigen-binding portion thereof or other binding agent as described herein). In some embodiments, the composition is a pharmaceutical composition. As used herein, the term "pharmaceutical composition" refers to the active agent in combination with a pharmaceutically acceptable carrier accepted for use in the pharmaceutical industry. The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0166] The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on any particular formulation. Typically, such compositions are prepared as injectable either as liquid solutions or suspensions; however, solid forms suitable for rehydration, or suspensions, in liquid prior to use can also be prepared. A preparation can also be emulsified or presented as a liposome composition. An antibody or antigen binding portion thereof or other binding agent can be mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, a pharmaceutical composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance or maintain the effectiveness of the active ingredient (e.g., an antibody or antigen binding portion thereof or other binding agent). The pharmaceutical compositions as described herein can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of a polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile

aqueous solutions that contain the active ingredients (e.g., an antibody and/or antigen binding portions thereof or other binding agent) and water, and may contain a buffer such as sodium phosphate at physiological pH value, physiological saline, or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions. The amount of an active agent that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques.

[0167] In some embodiments, a pharmaceutical composition comprising an antibody or antigen-binding portion thereof or other binding agent or a nucleic acid encoding an antibody or antigen-binding portion thereof or other binding agent as described herein can be a lyophilisate.

[0168] In some embodiments, a syringe comprising a therapeutically effective amount of a binding agent, or a pharmaceutical composition described herein is provided.

IV. Methods of Treatment and Related Uses

[0169] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) as described herein can be used in a method(s) comprising administering a binding agent or a pharmaceutical composition as described herein to a subject having an inflammatory disease.

[0170] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) as described herein can be used in a method comprising administering a binding agent or a pharmaceutical composition as described herein to a subject having an autoimmune disease. In some embodiments, the autoimmune disease is a rheumatological disorder, fibrotic disorder, gastrointestinal disorder, endocrinological disorder, neurological disorder, or skin disorder. In some embodiments, the autoimmune disease is autoimmune-induced hepatitis, Addison's Disease, Alopecia Areata, Alport's Syndrome, Ankylosing Spondylitis, Anti-phospholipid Syndrome, Arthritis, Ascariasis, Aspergillosis Atopic Allergy, Atopic Dermatitis, Atopic Rhinitis, Autoimmune Hemolytic Anemia, Autoimmune Hepatitis, Autoimmune Myositis, Behcet's Disease, Bird-Fancier's Lung, Bronchial Asthma, Caplan's Syndrome, Cardiomyopathy, Celiac Disease, Chagas' Disease, Chronic Glomerulonephritis, Chronic Graft versus Host Disease, Cogan's Syndrome, Cold Agglutinin

Disease, CREST Syndrome, Crohn's Disease, Cryoglobulinemia, Cushing's Syndrome, Dermatomyositis, Discoid Lupus, Dressier's Syndrome, Eaton-Lambert Syndrome, Encephalomyelitis, Endocrine ophthalmopathy, Erythematosus, Evan's Syndrome, Felty's Syndrome, Fibromyalgia, Fuch's Cyclitis, Gastric Atrophy, Gastrointestinal Allergy, Giant Cell Arteritis, Glomerulonephritis, Goodpasture's Syndrome, Graft v. Host Disease, Graves' Disease, Guillain-Barre Disease (Syndrome), Hashimoto's Thyroiditis, Hemolytic Anemia, Henoch-Schonlein Purpura, Hyperviscosity Syndrome, Idiopathic Adrenal Atrophy, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenic Purpura, IgA Nephropathy, Inflammatory Bowel Disease (Syndrome), Insulin-Dependent Diabetes Mellitus (IDDM or Type I), Juvenile Arthritis, Juvenile Idiopathic Arthritis, Juvenile Diabetes Mellitus (Type I), Lambert-Eaton Syndrome Laminitis, Lichen Planus, Lupoid Hepatitis, Lupus, Lupus Nephritis, Lymphopenia, Macroglobulinemia, Meniere's Disease, Mixed Connective Tissue Disease, Monoclonal Gammopathy of Undermined Origin, Multiple Sclerosis, Amyotrophic Lateral Sclerosis (ALS), Myasthenia Gravis, Myocarditis, Pemphigus/Pemphigoid, Pernicious Anemia, POEMS syndrome, Polyglandular Syndromes, Polyarteritis Nodosa, Polymyositis, Presenile Dementia, Primary Agammaglobulinemia, Primary Biliary Cirrhosis/Cholangitis, Psoriasis, Psoriatic Arthritis, Raynauds Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sampter's Syndrome, Schmidt's Syndrome, Scleroderma/Systemic Sclerosis, Shulman's Syndrome, Sjörgen's Syndrome, Stiff-Man Syndrome, Sympathetic Ophthalmia, Systemic Lupus Erythematosus, Takayasu's Arteritis, Temporal Arteritis, Thyroiditis, Thrombocytopenia, Thyrotoxicosis, Toxic Epidermal Necrolysis, Type B Insulin Resistance, Type I Diabetes Mellitus, Ulcerative Colitis, Uveitis, Vitiligo, Waldenstrom's Macroglobulinemia, and/or Wegener's Granulomatosis. In some embodiments the autoimmune disease is autoimmune hepatitis, celiac disease, Crohn's disease, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), lupus nephritis, myasthenia gravis, myocarditis, multiple sclerosis (MS), pemphigus/pemphigoid, primary biliary cirrhosis/cholangitis, rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), or ulcerative colitis. In some embodiments, the autoimmune disease is selected from autoimmune hepatitis, celiac disease, Crohn's disease, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), multiple sclerosis (MS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or ulcerative colitis.

[0171] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) as described herein can be used in a method comprising administering a binding agent or a pharmaceutical composition as described herein to a subject having celiac disease.

[0172] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) as described herein can be used in a method comprising administering a binding agent or a pharmaceutical composition as described herein to a subject having type 1 diabetes.

[0173] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) as described herein can be used in a method of treating complications of a transplant associated with graft versus host disease (GVHD), comprising administering a binding agent or a pharmaceutical composition as described herein to a subject.

[0174] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) as described herein can be used in a method(s) comprising administering a binding agent or a pharmaceutical composition as described herein to a subject to modulate an immune response to a virus in a subject. In some embodiments, the binding agent or pharmaceutical composition is administered to suppress, reduce, or prevent an immune response to a virus. In some embodiments, the immune response that is suppressed, reduced, or prevented is an immune response to a virus, or antigenic portions thereof. In some embodiments, the virus is a viral vector, and the administration of the binding agent suppresses, reduces, or prevents the induction of undesired immune responses associated with vector-mediated delivery of genetic material. The use of viral vectors, such as adeno-associated virus (AAV) vectors, to deliver genes of interest is currently an important tool for therapeutic approaches involving gene replacement, gene silencing, gene addition, and gene editing. However, host immune responses can limit the effectiveness of these approaches (Wang et al., Nat Rev Drug Discov 18, 358–378, 2019, https://doi.org/10.1038/s41573-019-0012-9). For example, the host may produce neutralizing antibodies against the vector capsids based on exposure to the wild-type virus, blocking gene delivery. The host may also produce neutralizing antibodies against the vector capsid that limit the effectiveness of re-administration of the vector in therapies requiring repeated dosing. Additionally, hosts can mount a cytotoxic T lymphocyte (CTL)-mediated cytotoxicity that clears transduced cells. In a subset of 'reactive patients', AAV mediated gene delivery can be associated with inflammatory side effects and toxicities mediated by pathogenic CD4+ T cells.

[0175] Accordingly, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) as described herein can be used in a method(s) comprising administering a binding agent or a pharmaceutical composition as described herein to a subject to suppress, reduce, or prevent an immune response to a viral vector. As used herein, "an immune response to a virus" or "an immune response to a viral vector" may refer to any immune response to a virus, a viral vector, or antigenic portions thereof, e.g., viral proteins or fragments thereof. In some embodiments, the immune response may be activation or proliferation of CD4+ T cells. The immune response may be characterized by, for example, pro-inflammatory cytokine (e.g., IFN-ɣ) production by CD4+ T cells. In some embodiments, the viral vector has been, is, or will be administered to the subject. In some embodiments, the immune response to the viral vector is induced by administration of a viral vector to the subject. In some embodiments, the virus or viral vector is a retrovirus, adenovirus, parvovirus, coronavirus, ortho-myxovirus, rhabdovirus, paramyxovirus, picornavirus, alphavirus, herpesvirus, poxvirus, Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, or hepatitis virus. In some embodiments, the virus or viral vector is an adeno-associated virus (AAV) vector, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh.10, AAVrh.43, AAVrh.74, or AAVhu.37, or a variant thereof. In some embodiments, the virus or viral vector is an adenovirus vector. In some embodiments, the virus or viral vector is a lentivirus vector.

[0176] As used herein, to "activate or stimulate" CD8+ Tregs, or activated CD8+ Tregs, refers to an increase of the regulatory T cell functions of such cells, such as the ability to suppress an immune response. Activation or stimulation of CD8+ Tregs may include removal of a suppressive effect on such cells, so as to restore the CD8+ Tregs (e.g., restore balance to the immune system or restore balanced immune activity in the subject prior to receiving a viral vector). Activation or stimulation of CD8+ Tregs may also include results of such activation or stimulation, including removal of a CD4+ cells, B cells, or other cells mediating an immune response, such as by elimination, for example, cytolysis, of such cells.

[0177] In some embodiments, the CD8+ Tregs are contacted with the binding agent in vivo. In some embodiments, the CD8+ Tregs are contacted with the binding agent ex vivo. The activated CD8+ Tregs can then be administered in an effective amount to a subject in need thereof.

[0178] In some embodiments, the activated CD8+ Tregs exert a suppressive effect on other immune cells, such as CD4+ T cells, antibody producing B cells, antigen presenting dendritic cells, or antigen presenting cells. In some embodiments, the activated CD8+ Tregs exert

a suppressive effect on other immune cells, such as CD4+ T cells, antibody producing B cells, and antigen presenting dendritic cells. In some embodiments, the activated CD8+ Tregs deplete other immune cells, such as CD4 T cells, antibody producing B cells, and antigen presenting dendritic cells. In some embodiments, the activated CD8+ Tregs modulate the activity of undesired immune cells and decrease the titer of antibodies in the subject. In some embodiments, the activated CD8+ Tregs decrease the titer of antibodies in the subject. In some embodiments, the CD8+ Tregs are CD39+ and KIR+.

[0179] In some embodiments, a binding agent or a pharmaceutical composition comprising any of the binding agents described herein, is administered with an immunosuppressive agent, such as a corticosteroid. In some embodiments, the immunosuppressive agent is one or more of: a calcineurin inhibitor, e.g., a cyclosporin or an ascomycin, e.g., cyclosporin A (NEORAL®), FK506 (tacrolimus), pimecrolimus, an mTOR inhibitor, e.g., rapamycin or a derivative thereof, e.g., sirolimus (RAPAMUNE®), everolimus (Certican®), temsirolimus, zotarolimus, biolimus-7, biolimus-9, a rapalog, e.g., ridaforolimus, azathioprine, campath 1H, a S1P receptor modulator, e.g., fingolimod or an analogue thereof, an anti-IL-8 antibody, mycophenolic acid or a salt thereof, e.g., sodium salt, e.g., mycophenolate mofetil (CELLCEPT®), OKT3 (ORTHOCLONE OKT3®), Prednisone, ATGAM®, THYMOGLOBULIN®, Brequinar Sodium, OKT4, T10B9.A-3A, 33B3.1, 15-deoxyspergualine, tresperimus, leflunomide ARAVA®, CTLAI-Ig, anti-CD25, anti-IL2R , basiliximab (SIMULECT®), Daclizumab (ZENAPAX®), mizorbine, methotrexate, dexamethasone, ISAtx-247, SDZ ASM 981 (pimec rolimus, Elidel®), CTLA4lg (Abatacept), belatacept, LFA3lg, etanercept (sold as Enbrel® by Immunex), adalimumab (Humira®), infliximab (Remicade®), an anti-LFA-1 antibody, natalizumab (Antegren®), Enlimomab, gavilimomab, antithymocyte immunoglobulin, siplizumab, alefacept efalizumab, pentase, mesalazine, asacol, codeine phosphate, benorylate, fenbufen, naprosin, diclofenac, etodolac, and indomethacin, tocilizumab (Actemra), siltuximab (Sylvant), secukibumab (Cosentyx), ustekinumab (Stelara), risankizumab, sifalimumab, aspirin, ibuprofen, imlifidase, a proteasome inhibitor, arsenic trioxide, and rabbit anti-thymocyte globulin (see, e.g., Chu et al., Frontiers in Immunology 12:658038, 2021).

[0180] In some embodiments, a binding agent (e.g., antibodies and antigen binding fragments thereof, binding proteins) or a pharmaceutical composition of any of the binding agents described herein, is administered with an anti-inflammatory agent, such as a corticosteroid. In some embodiments, the anti-inflammatory agent is one or more of: methotrexate, dexamethasone, dexamethasone alcohol, dexamethasone sodium phosphate,

fluromethalone acetate, fluromethalone alcohol, lotoprendol etabonate, medrisone, prednisolone acetate, prednisolone sodium phosphate, difluprednate, rimexolone, hydrocortisone, hydrocortisone, lodoxamide tromethamine, aspirin, ibuprofen, suprofen, piroxicam, meloxicam, flubiprofen, naproxan, ketoprofen, tenoxicam, diclofenac sodium, ketotifen fumarate, diclofenac sodium, nepafenac, bromfenac, flurbiprofen sodium, suprofen, celecoxib, naproxen, rofecoxib, glucocorticoids, diclofenac, and any combination thereof. In some embodiments, the anti-inflammatory agent is one or more nonsteroidal anti-inflammatory drugs (NSAIDs), such as naproxen sodium (Anaprox), celecoxib (Celebrex), sulindac (Clinoril), oxaprozin (Daypro), salsalate (Disalcid), diflunisal (Dolobid), piroxicam (Feldene), indomethacin (Indocin), etodolac (Lodine), meloxicam (Mobic), naproxen (Naprosyn), nabumetone (Relafen), ketorolac tromethamine (Toradol), naproxen/ esomeprazole (Vimovo), and diclofenac (Voltaren), and combinations thereof.

[0181] In some embodiments of the aforementioned methods, the binding agent (e.g., an antibody or antigen binding fragment thereof, a binding protein) is administered in a dose of from about 0.01 mg/kg to about 20 mg/kg (i.e., about 0.01 mg to about 20 mg of binding agent per kg of body weight). In some embodiments, the binding agent is administered in a dose of from about 0.5 mg/kg to about 15 mg/kg. In some embodiments, the binding agent is administered in a dose of from about 0.5 mg/kg to about 5 mg/kg. In some embodiments, the binding agent is administered in a dose of from about 0.01 mg/kg to about 10 mg/kg. In some embodiments, the binding agent is administered in a dose of from about 0.1 mg/kg to about 10 mg/kg. In some embodiments, the binding agent is administered in a dose of from about 0.5 mg/kg to about 10 mg/kg. In some embodiments, the binding agent is administered in a dose of from about 0.05 mg/kg to about 0.1 mg/kg. In some embodiments, the binding agent is administered in a dose of from about 0.5 mg/kg to about 1.0 mg/kg. In some embodiments, the binding agent is administered in a dose of up to about 10 mg/kg. In some embodiments, the binding agent is administered in a dose of about 10 mg/kg. In some embodiments, the binding agent is administered in a dose of from 0.01 mg/kg to 20 mg/kg. In some embodiments, the binding agent is administered in a dose of from 0.5 mg/kg to 15 mg/kg. In some embodiments, the binding agent is administered in a dose of from 0.5 mg/kg to 5 mg/kg. In some embodiments, the binding agent is administered in a dose of from 0.01 mg/kg to 10 mg/kg. In some embodiments, the binding agent is administered in a dose of from 0.1 mg/kg to 10 mg/kg. In some embodiments, the binding agent is administered in a dose of from 0.5 mg/kg to 10 mg/kg. In some embodiments, the binding agent is administered in a dose of from 0.05 mg/kg to 0.1 mg/kg. In

some embodiments, the binding agent is administered in a dose of from 0.5 mg/kg to 1.0 mg/kg. In some embodiments, the binding agent is administered in a dose of up to 10 mg/kg. In some embodiments, the binding agent is administered in a dose of 10 mg/kg. In some embodiments, the binding agent is administered in a dose of about 50 mg/kg. In some embodiments, the binding agent is administered in a dose of 50 mg/kg. In some embodiments, the binding agent is administered in a dose of 0.01 mg/kg. In some embodiments, the binding agent is administered in a dose of up to 0.1 mg/kg. In some embodiments, the binding agent is administered in a dose of 0.1 mg/kg. In some embodiments, the binding agent is administered in a dose of up to 0.5 mg/kg. In some embodiments, the binding agent is administered in a dose of 0.5 mg/kg. In some embodiments, the binding agent is administered in a dose of up to 2.0 mg/kg. In some embodiments, the binding agent is administered in a dose of 2.0 mg/kg. In some embodiments, the binding agent is administered in a dose of up to 8.0 mg/kg. In some embodiments, the binding agent is administered in a dose of 8.0 mg/kg. In some embodiments, the binding agent is administered multiple doses, e.g., two doses. In some embodiments, the dose range can be titrated to maintain serum levels between 0.1 ug/mL and 1000 ug/mL.

[0182] In some embodiments, the binding agent is administered via intravenous infusion.

[0183] In some aspects, the binding agents (e.g., antibodies and antigen binding fragments thereof, binding proteins) disclosed herein are for use in the aforementioned methods, or are used in manufacture of a medicament for use in the aforementioned methods.

V. Example Embodiments

[0184] Example embodiments include, but are not limited to, the following:

[0185] 1. A binding protein comprising: a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGXGTKVEIK (SEQ ID NO:133), wherein: X = G or C.

[0186] 2. The binding protein of embodiment 1, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

[0187] 3. The binding protein of embodiment 1, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

[0188] 4. A binding protein comprising: a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to EVQLVESGGGLVQPGGSLRLSCAASGFNX$_1$KDTYIHFVRQAPGKX$_2$LEWIGRIDPANDNTLYASK X$_3$QGKX$_4$TISX$_5$DTSKNTAYLQMNSLRAEDTAVYYCX$_6$RGYGYYVFDHWGQGTLVTVSS (SEQ ID NO:134), wherein: (a) X_1 = I or F; (b) X_2= G or C; (c) X_3 = F or V; (d) X_4 = A or F; (e) X_5 = A or R; and (f) X_6 = G or A.

[0189] 5. The binding protein of embodiment 4, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.

[0190] 6. The binding protein of embodiment 4, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

[0191] 7. A binding protein comprising:

[0192] (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGS GSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGXGTKVEIK (SEQ ID NO:133), wherein: X = G or C; and

[0193] (b) a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to EVQLVESGGGLVQPGGSLRLSCAASGFNX$_1$KDTYIHFVRQAPGKX$_2$LEWIGRIDPANDNTLYASK X$_3$QGKX$_4$TISX$_5$DTSKNTAYLQMNSLRAEDTAVYYCX$_6$RGYGYYVFDHWGQGTLVTVSS (SEQ ID NO:144), wherein: (1) X_1 = I or F; X_2= G or C; (3) X_3 = F or V; (4) X_4 = A or F; (5) X_5 = A or R; and (6) X_6 = G or A.

[0194] 8. The binding protein of embodiment 7, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

[0195] 9. The binding protein of embodiment 7, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

[0196] 10. The binding protein of any one of embodiments 7-9, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.

[0197] 11. The binding protein of any one of embodiments 7-9, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

[0198] 12. A binding protein comprising: (a) CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively; and (b) one or more of the following framework regions: (i) a light chain FR4 according to SEQ ID NO:126; (ii) a heavy chain FR2 according to SEQ ID NO:128; (iii) a heavy chain FR3 according to SEQ ID NO:129; (iv) a heavy chain FR3 according to SEQ ID NO:130; (v) a heavy chain FR3 according to SEQ ID NO:131; (vi) a heavy chain FR3 according to SEQ ID NO:132; and (vii) a heavy chain FR3 according to SEQ ID NO:16 with one or more of the following substitutions: F6V, A10F, A14R, and G39A, according to the position of the amino acid within SEQ ID NO:16.

[0199] 13. A binding protein comprising: (a) CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:127, SEQ ID NO:5, and SEQ ID NO:6, respectively; and (b) one or more of the following framework regions: (i) a light chain FR4 according to SEQ ID NO:126; (ii) a heavy chain FR2 according to SEQ ID NO:128; (iii) a heavy chain FR3 according to SEQ ID NO:129; (iv) a heavy chain FR3 according to SEQ ID NO:130; (v) a heavy chain FR3 according to SEQ ID NO:131; (vi) a heavy chain FR3 according to SEQ ID NO:132; (vii) a heavy chain FR3 according to SEQ ID NO:16 with one or more of the following substitutions: F6V, A10F, A14R, and G39A, according to the position of the amino acid within SEQ ID NO:16; (viii) light chain framework regions of the VL according to SEQ ID NO:7; and (ix) heavy chain framework regions of the VH according to SEQ ID NO:8.

[0200] 14. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:56; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:56; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:56.

[0201] 15. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:17; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:18; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:17; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:18; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:17; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:18.

[0202] 16. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:19; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:20; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:19; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:20; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:19; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:20.

[0203] 17. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:21; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:22; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:21; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:22; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:21; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:22.

[0204] 18. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:23; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:24; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:23; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:24; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:23; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:24.

[0205] 19. A binding protein comprising:

[0206] (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:25; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:26; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:25; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:26; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:25; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:26.

[0207] 20. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:27; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:28; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:27; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:28; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:27; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:28.

[0208] 21. A binding protein comprising:(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:29; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:30; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:29; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:30; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:29; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:30.

[0209] 22. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:31; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:32; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:31; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:32; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:31; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:32.

[0210] 23. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:33; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:34; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:33; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:34; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:33; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:34.

[0211] 24. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:35; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:36; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:35; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:36; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:35; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:36.

[0212] 25. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:37; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:38; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:37; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:38; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:37; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:38.

[0213] 26. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:39; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:40; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:39; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:40; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:39; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:40.

[0214] 27. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:41; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:42; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:41; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:42; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:41; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:42.

[0215] 28. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:43; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:44; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:43; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:44; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:43; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:44.

[0216] 29. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:45; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:46; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:45; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:46; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:45; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:46.

[0217] 30. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:47; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:48; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:47; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:48; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:47; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:48.

[0218] 31. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:49; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:50; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:49; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:50; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:49; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:50.

[0219] 32. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:51; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:52; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:51; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:52; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:51; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:52.

[0220] 33. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:53; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:54; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:53; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:54; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:53; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:54.

[0221] 34. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:57; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:58; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:57; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:58; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:57; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:58.

[0222] 35. A binding protein comprising: (a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:59; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:60; (b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:59; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:60; or (c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:59; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:60.

[0223] 36. A binding protein comprising: a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:56.

[0224] 37. A binding protein comprising: a light chain variable region (VL) comprising at least 95% identity to the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising at least 95% identity to the amino acid sequence according to SEQ ID NO:56.

[0225] 38. A binding protein comprising: a light chain variable region (VL) comprising at least 99% identity to the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising at least 99% identity to the amino acid sequence according to SEQ ID NO:56.

[0226] 39. A binding protein comprising: a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:56.

[0227] 40. A binding protein comprising: a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:56.

[0228] 41. The binding protein of any one of embodiments 1-40, wherein the binding protein is an scFv.

[0229] 42. The binding protein of any one of embodiments 1-41, wherein the VH and VL are connected by a linker polypeptide.

[0230] 43. The binding protein of embodiment 42, wherein the linker polypeptide comprises or consists of the amino acid sequence according to SEQ ID NO:61.

[0231] 44. The binding protein of any one of embodiments 41-43, further comprising a constant domain comprising the sequence according to SEQ ID NO:62.

[0232] 45. The binding protein of any one of embodiments 1-44, further comprising a polypeptide that binds to a KIR protein.

[0233] 46. The binding protein of any one of embodiments 1-44, further comprising a second polypeptide that comprises: CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, respectively.

[0234] 47. The binding protein of embodiment 46, further comprising a third polypeptide that comprises: CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, respectively.

[0235] 48. The binding protein of any one of embodiments 1-44, further comprising: (a) a second polypeptide comprising a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:69; and a third polypeptide comprising a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:70; (b) a second polypeptide comprising a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:69; and third polypeptide comprising a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:70; or(c) a second polypeptide comprising a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:69; and third polypeptide comprising a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:70.

[0236] 49. The binding protein of any one of embodiments 1-44, further comprising: a second polypeptide comprising a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:69; and a third polypeptide comprising a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:70.

[0237] 50. The binding protein of any one of embodiments 1-44, further comprising: a second polypeptide comprising a light chain variable region (VL) comprising at least 95% identity to the amino acid sequence according to SEQ ID NO:69; and a third polypeptide comprising a heavy chain variable region (VH) comprising at least 95% identity to the amino acid sequence according to SEQ ID NO:70.

[0238] 51. The binding protein of any one of embodiments 1-44, further comprising: a second polypeptide comprising a light chain variable region (VL) comprising at least 99%

identity to the amino acid sequence according to SEQ ID NO:69; and a third polypeptide comprising a heavy chain variable region (VH) comprising at least 99% identity to the amino acid sequence according to SEQ ID NO:70.

[0239] 52. The binding protein of any one of embodiments 1-44, further comprising: a second polypeptide comprising a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:69; and third polypeptide comprising a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:70.

[0240] 53. The binding protein of any one of embodiments 1-44, further comprising: a second polypeptide comprising a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:69; and third polypeptide comprising a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:70.

[0241] 54. The binding protein of any one of embodiments 48-53, wherein the VL of the second polypeptide has CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, respectively; and the VH of the third polypeptide has CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, respectively.

[0242] 55. The binding protein of any one of embodiments 48-53, wherein the VL of the second polypeptide has CDRL1, CDRL2, and CDRL3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

[0243] 56. The binding protein of any one of embodiments 48-53, wherein the VH of the third polypeptide has CDRH1, CDRH2, and CDRH3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

[0244] 57. A binding protein according to Table 3.

[0245] 58. A binding protein comprising: (a) a polypeptide sequence comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:120; (b) a polypeptide sequence comprising the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising the amino acid sequence according to SEQ ID NO:120; or (c) a polypeptide sequence consisting of the amino acid sequence according to SEQ ID NO:78, a

polypeptide consisting of the amino acid sequence according to SEQ ID NO:80, and a polypeptide consisting of the amino acid sequence according to SEQ ID NO:120.

[0246] 59. A binding protein comprising: a polypeptide sequence comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:120.

[0247] 60. A binding protein comprising: a polypeptide sequence comprising at least 95% identity to the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising at least 95% identity to the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising at least 95% identity to the amino acid sequence according to SEQ ID NO:120.

[0248] 61. A binding protein comprising: a polypeptide sequence comprising at least 99% identity to the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising at least 99% identity to the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising at least 99% identity to the amino acid sequence according to SEQ ID NO:120.

[0249] 62. A binding protein comprising: a polypeptide sequence comprising the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising the amino acid sequence according to SEQ ID NO:120.

[0250] 63. A binding protein comprising: a polypeptide sequence consisting of the amino acid sequence according to SEQ ID NO:78, a polypeptide consisting of the amino acid sequence according to SEQ ID NO:80, and a polypeptide consisting of the amino acid sequence according to SEQ ID NO:120.

[0251] 64. A pharmaceutical composition comprising the binding protein of any one of embodiments 1-63 and a pharmaceutically acceptable carrier.

[0252] 65. A nucleic acid encoding the binding protein of any one of embodiments 1-63.

[0253] 66. A nucleic acid molecule comprising SEQ ID NOs:139, 141, and 141.

[0254] 67. A nucleic acid molecule comprising SEQ ID NOs:138, 140, and 142.

[0255] 68. A vector comprising the nucleic acid of embodiment 65.

[0256] 69. A vector comprising the nucleic acid of embodiment 66.

[0257] 70. A vector comprising the nucleic acid of embodiment 67.

[0258] 71. A cell line comprising the vector of embodiment 68.

[0259] 72. A cell line comprising the vector of embodiment 69.

[0260] 73. A cell line comprising the vector of embodiment 70.

[0261] 74. A method of treating a disease, comprising administering the binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64 to a subject in need thereof.

[0262] 75. A method of preventing a disease, comprising administering the binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64 to a subject in need thereof.

[0263] 76. The method of embodiment 74 or embodiment 75, wherein the disease is an inflammatory disease or an autoimmune disease.

[0264] 77. The method of embodiment 74 or embodiment 75, wherein the disease is a CD4+ T cell-driven inflammatory disease or autoimmune disease.

[0265] 78. The method of embodiment 76 or embodiment 77, wherein the number or activity of pathogenic immune cells in the subject is decreased.

[0266] 79. The method of any one of embodiments 74-78, wherein the disease is a rheumatological disorder, fibrotic disorder, gastrointestinal disorder, endocrinological disorder, neurological disorder, or skin disorder.

[0267] 80. The method of any one of embodiments 76-78, wherein the autoimmune disease is celiac disease, Crohn's disease, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), lupus, lupus nephritis, cutaneous lupus, discoid lupus, myasthenia gravis, myocarditis, multiple sclerosis (MS), pemphigus/pemphigoid, rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), or ulcerative colitis.

[0268] 81. The method of any one of embodiments 76-78, wherein the autoimmune disease is celiac disease.

[0269] 82. The method of any one of embodiments 76-78, wherein the autoimmune disease is Crohn's disease.

[0270] 83. The method of any one of embodiments 76-78, wherein the autoimmune disease is inflammatory bowel disease (IBD).

[0271] 84. The method of any one of embodiments 76-78, wherein the autoimmune disease is ulcerative colitis.

[0272] 85. The method of any one of embodiments 74-78, wherein the disease is graft versus host disease (GVHD).

[0273] 86. The method of any one of embodiments 74-78, wherein the disease is type 1 diabetes.

[0274] 87. A method of suppressing an immune response mediated by pathogenic immune cells, comprising contacting CD8+ T regulatory cells (Tregs) with the binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64, thereby activating or stimulating CD8+ Tregs.

[0275] 88. A method of suppressing an immune response to an antigen, such as an autoantigen, comprising administering to a subject in need thereof the binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64, thereby activating or stimulating CD8+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the antigen or autoantigen is decreased.

[0276] 89. The method of embodiment 87 or embodiment 88, wherein the pathogenic immune cells are autoreactive CD4+ T cells, autoantibody producing B cells, or self antigen presenting dendritic cells.

[0277] 90. The method of embodiment 87 or embodiment 88, wherein the pathogenic immune cells are autoreactive CD4+ T cells.

[0278] 91. The method of any one of embodiments 87-90, wherein the CD8+ Tregs are CD8+KIR+ Tregs.

[0279] 92. The method of any one of embodiments 87-91, wherein the activated CD8+ Tregs are administered to the subject or to a second subject.

[0280] 93. The method of any one of embodiments 87-92, wherein the subject and/or the second subject has an inflammatory or autoimmune disease.

[0281] 94. A method of suppressing, reducing, or preventing an immune response to a viral vector in a subject, the method comprising administering to the subject the binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64.

[0282] 95. The method of embodiment 94, wherein the viral vector has been, is, or will be administered to the subject and the immune response to the viral vector is induced by administration of the viral vector to the subject.

[0283] 96. The method of any one of embodiments 74-95, wherein the binding protein is administered in a dose of from about 0.01 mg/kg to about 20 mg/kg.

[0284] 97. The method of embodiment 96, wherein the binding protein is administered in a dose of from about 0.01 mg/kg to about 10 mg/kg.

[0285] 98. The method of embodiment 96, wherein the binding protein is administered in a dose of from about 0.05 mg/kg to about 0.1 mg/kg.

[0286] 99. The method of embodiment 96, wherein the binding protein is administered in a dose of from about 0.5 mg/kg to about 1.0 mg/kg.

[0287] 100. The method of embodiment 96, wherein the binding protein is administered in a dose of from 0.05 mg/kg to 0.1 mg/kg.

[0288] 101. The method of embodiment 96, wherein the binding protein is administered in a dose of from 0.5 mg/kg to 1.0 mg/kg.

[0289] 102. The method of embodiment 96, wherein the binding protein is administered in a dose up to about 10 mg/kg.

[0290] 103. The method of embodiment 96, wherein the binding protein is administered in a dose up to 10 mg/kg.

[0291] 104. The method of embodiment 96, wherein the binding protein is administered in a dose of about 10 mg/kg.

[0292] 105. The method of embodiment 96, wherein the binding protein is administered in a dose of 10 mg/kg.

[0293] 106. Use of the binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64 in the method of any one of embodiments 74-105.

[0294] 107. The binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64 for use in the method of any one of embodiments 74-105.

[0295] 108. Use of the binding protein of any one of embodiments 1-63 or the pharmaceutical composition of embodiment 64 in the manufacture of a medicament for use in the method of any one of embodiments 74-105.

[0296] 109. A method of treating celiac disease in a subject in need thereof, comprising administering the binding protein of embodiment 58 to the subject.

[0297] 110. A method of preventing celiac disease in a subject in need thereof, comprising administering the binding protein of embodiment 58 to the subject.

[0298] 111. A method of treating celiac disease in a subject in need thereof, comprising administering the pharmaceutical composition of embodiment 64 to the subject.

[0299] 112. A method of preventing celiac disease in a subject in need thereof, comprising administering the pharmaceutical composition of embodiment 64 to the subject.

[0300] 113. A method of treating type 1 diabetes in a subject in need thereof, comprising administering the binding protein of embodiment 58 to the subject.

[0301] 114. A method of preventing type 1 diabetes in a subject in need thereof, comprising administering the binding protein of embodiment 58 to the subject.

[0302] 115. A method of treating type 1 diabetes in a subject in need thereof, comprising administering the pharmaceutical composition of embodiment 64 to the subject.

[0303] 116. A method of preventing type 1 diabetes in a subject in need thereof, comprising administering the pharmaceutical composition of embodiment 64 to the subject.

[0304] 117. Use of the binding protein of embodiment 58 or the pharmaceutical composition of embodiment 64 in the method of any one of embodiments 109-116.

[0305] 118. The binding protein of embodiment 58 or the pharmaceutical composition of embodiment 64 for use in the method of any one of embodiments 109-116.

[0306] 119. Use of the binding protein of embodiment 58 or the pharmaceutical composition of embodiment 64 in the manufacture of a medicament for use in the method of any one of embodiments 109-116.

EXAMPLES

Example 1: CD8-Targeting Binding Proteins

[0307] Variant CD8-specific binding domains were developed, starting with a parental VL amino acid sequence according to SEQ ID NO:7 and a parental VH amino acid sequence according to a SEQ ID NO:8. Substitutions were made at one or more amino acid positions in framework regions, as shown in Table 1.

Table 1. Variant substitutions, with numbering relative to the parental VL or VH sequence.

Domain	Region	Position	Substituted Residue	Features
VL – SEQ ID NO:7	FR4	G100	C	Stabilizing disulfide bond when paired with G44C
VH – SEQ ID NO:8	HCDR1/ FR1	I29	F	Close to or within HCDR1, depending on numbering scheme; may provide improved stability; may be paired with A72R

VH – SEQ ID NO:8	FR2	G44	C	Stabilizing disulfide bond when paired with G100C
VH – SEQ ID NO:8	FR3	F64	V	May reduce steric clashes in the region where A68F has been introduced and provide improved stability
VH – SEQ ID NO:8	FR3	A68	F	Distant from CDRs; may provide improved stability; may be paired with F64A
VH – SEQ ID NO:8	FR3	A72	R	Close to DE loop of heavy chain; may provide improved stability and solubility; may be paired with I29F
VH – SEQ ID NO:8	FR3	G97	A	Close to HCDR3; may provide improved stability

[0308] Variant binding proteins were designed to incorporate one or more of the substitutions, as shown in Table 2.

Table 2. CD8-binding variant designs.

No.	Substitutions						
	VL G100	VH I29	VH G44	VH F64	VH A68	VH A72	VH G97
1		F		V	F	R	A
2		F		V	F	R	
3		F		V	F		A
4		F				R	A
5				V	F	R	A
6		F		V	F		
7		F				R	
8		F					A
9				V	F	R	
10				V	F		A
11						R	A
12	C	F	C	V	F	R	
13	C	F	C	V	F		A
14	C	F	C			R	A
15	C		C	V	F	R	A
16	C	F	C	V	F		
17	C	F	C			R	
18	C	F	C				A
19	C		C	V	F	R	
20	C		C	V	F		A
21	C		C			R	A
22	C		C				

Example 2: CD8-KIR-Targeting Binding Proteins

[0309] CD8+ T regulatory cells (Tregs) with immunosuppressive characteristics in inflammatory disease settings have been previously identified. See, e.g., WO2022/169825A1. These cells have been observed in samples from patients having celiac disease (CeD), inflammatory bowel disease (IBD), rheumatoid arthritis (RA), or systemic lupus (SLE), where they may be hampered in their ability to eliminate disease-causing CD4+ T cells. Bispecific antibodies have been developed to target specific cell surface receptors on the surface of CD8+ Treg cells to modulate the function of these cells, with the aim of activating oligoclonal expansion and perforin-dependent elimination of pathogenic CD4+ T cells in auto immune and inflammatory disease indications. See WO2022/169825A1. For example, a bispecific antibody or binding protein with a binding domain that targets the KIR2DL cell surface protein and a binding domain that targets CD8a has been developed (i.e., an anti-KIR2DL/anti-CD8a bispecific) to modulate these CD8+ Treg cells.

[0310] Here, bispecific binding molecules that target and bind CD8 and KIR2DL were developed that incorporated the variant CD8-binding domains of Table 2. The bispecific binding molecules had an asymmetric monovalent bispecific antibody-like structure, with a Fab targeting KIR2DL for one "arm" and an scFv targeting CD8a on the other arm (FIG. 1). This structure is referred to as a "bottle-opener" structure. The heterodimerization of the heavy chains was achieved by knobs-into-holes (KiH) mutations on the CH3 portion of the IgG that sterically favor the formation of specific heteropairs. The anti-KIR2DL variable heavy (VH) binding domain was designed to directly adjoin to the human IgG1 CH1-CH2-CH3 domains to form an intact IgG heavy chain. The anti-KIR2DL variable light (VL) binding domain likewise adjoins to the human kappa constant region, thereby forming an intact kappa light chain. The anti-CD8a scFv arms were comprised of the VL binding domain, followed by an 18-mer Gly-Ser linker, followed by the VH binding domain. This scFv configuration can be referred to as the 'LH' (Light-Heavy) orientation. The anti-CD8a LH scFv is directly attached to the IgG1 hinge region, followed by the hIgG1 CH2 and CH3 domains, thereby forming a scFv-Fc molecule.

[0311] The hIgG1 Fc utilized in the anti-KIR2DL/anti-CD8a bispecific construct contained a subset of mutations in the CH2 domain to reduce effector function. Three amino acid substitutions were introduced to reduce Fc ɣ receptor I (Fc ɣ RI) binding, corresponding to substitutions at EU index positions L234A, L235E, and G237A of human IgG1. These amino acid substitutions are reported to also reduce FcγRIIa binding as well as FcγRIII binding. Two

amino acid substitutions in the complement C1q binding site, corresponding to substitutions at EU index positions A330S and P331S of hIgG1, were introduced to reduce complement fixation.

[0312] The anti-CD8a LH scFv-IgG1 Fc heavy chain contained a mutation at IgG EU index position C220S in the hinge region, because of the lack of a covalent light chain partner. The Cys to Ser residue substitution prevents deleterious effects due to the potential presence of an unpaired sulfhydral group.

[0313] The knobs-into-holes mutations were in the hIgG1 CH3 domain. The anti-KIR2DL heavy chain knob substitutions were at EU index positions S354C and T366W. The anti-CD8a scFv-Fc heavy chain hole substitutions were at EU index positions Y349C, T366S, L368A, and Y407V.

[0314] The variant binding proteins had amino acid sequences as set forth in Table 3. The nucleic acid sequences encoding the protein sequences included a signal sequence, encoding the amino acid sequence of SEQ ID NO:71, which was cleaved post-translationally during production.

Table 3. Variant anti-CD8/anti-KIR bottle-opener molecules.

No.	Anti-KIR Arm Light Chain SEQ ID NO	Anti-KIR Arm Heavy Chain SEQ ID NO	Anti-CD8 Arm scFv-Constant SEQ ID NO
1	78	80	82
2	78	80	84
3	78	80	86
4	78	80	88
5	78	80	90
6	78	80	92
7	78	80	94
8	78	80	96
9	78	80	98
10	78	80	100
11	78	80	102
12	78	80	104
13	78	80	106
14	78	80	108
15	78	80	110
16	78	80	112
17	78	80	114
18	78	80	116
19	78	80	118
20	78	80	120
21	78	80	122
22	78	80	124

Methods

[0315] The nucleic acid molecules encoding the anti-KIR2DL/anti-CD8a parental sequences (SEQ ID NOs:135-137) and variant bispecific sequences from Table 3 (see SEQ ID NOs: 78-125) were cloned into Horizon vectors and transfected into the HD-BIOP3 GS Null CHO K1 cell line. Once the pools had recovered in selective media to > 95% viability, 10-day fed-batch production was initiated at small scale. Upon harvest at day 10, the conditioned media from each CHO pool underwent one-step Protein A purification. Recovery for all samples was > 80%. The Protein A eluate material was used for all biophysical characterization assays listed in Table 4.

The variants were analyzed according to the assays in Table 4.

Table 4. Assays performed for parental and variant binding proteins.

Characteristic	**Assay**
Conformational Stability	Titer and specific productivity (Qp)
	Protein A recovery
	Size Exclusion Chromatography (SEC-HPLC)
	Non-reduced Capillary Electrophoresis-Sodium Dodecyl Sulfate (CE-SDS)
	Differential Scanning Fluorimetry (DSF)
	Chemical unfolding
	Low pH hold
	Thermal hold
Colloidal Stability	Self-Interaction Nanoparticle Spectroscopy (SINS)
	Relative Solubility Analysis (RSA)
	Zenix column chromatography
	Polyspecificity assays
Binding	Octet binding
	Cell-based binding

Results – Conformational Stability

[0316] Expression titer for the anti-KIR2DL/anti-CD8a parental CHO pool was 1.25g/L. Anti-KIR2DL/anti-CD8a variant CHO pool titers ranged from 0.27 – 2.09g/L. Specific

productivity (Qp) measures the amount of target protein produced per cell for a specified time unit, reported as picograms per cell per day (pg/c/d, or pcd). Specific productivity for the anti-KIR2DL/anti-CD8a parental CHO pool was 11.26 pcd. Anti-KIR2DL/anti-CD8a variant CHO pool specific productivities ranged from 2.91 – 18.23 pcd. In general, specific productivity measurements correlated with pool titer.

[0317] Size exclusion chromatography (SE-HPLC) is an analytical protein technique that separates molecules in a solution by size. Resulting peaks have an associated column retention time that can be compared against known protein standards, where each peak is reported as a percentage of the species in the sample. Typically, % high molecular weight (HMW), % main peak, and % low molecular weight (LMW) species are reported. HMW species are an indication of protein aggregation. SE-HPLC can be used to assess the aggregation propensity that amino acid substitutions may have on a protein, and thus % HMW can be used as an indicator of protein instability. The anti-KIR2DL/anti-CD8a parental molecule displayed the following SE-HPLC profile: 4.97% high molecular weight (HMW) species, 90.48% main peak, 2.86% low molecular weight (LMW) species. The anti-KIR2DL/anti-CD8a variants had SE-HPLC profiles that ranged from 1.81 – 8.41% HMW, 82.91 – 91.51% main peak and 1.24 – 5.25% LMW.

[0318] Non-reduced capillary electrophoresis sodium docecyl sulfate (nrCE-SDS) is an analytical protein technique that separates molecules in a solution by size and charge and is used to assess the homogeneity of a protein sample. A typical nrCE-SDS readout will report % pre-peaks, % main peak and % post-peak species. Elevated % pre-peak and/or % post-peak species are an indication of protein heterogeneity. CE-SDS can be used to assess the impact that amino acid substitutions may have on the resulting purity of a protein, and thus can be used as an indicator of protein stability. The anti-KIR2DL/anti-CD8a parental molecule displayed the following nrCE-SDS profile: 14.26% pre-peak, 82.38% main peak, 3.36% post-peak species. The anti-KIR2DL/anti-CD8a variants had nrCE-SDS profiles that ranged from 7.54 – 27.63% pre-peaks, 70.0 – 87.63% main peak and 2.26 – 6.73% post-peak species.

[0319] Differential Scanning Fluorimetry (DSF) is another biophysical characterization technique that measures conformational changes, or unfolding, of a protein when subjected to a gradual increase in temperature. As a protein unfolds, hydrophobic core residues become accessible to bind a fluorescent dye. The binding emits a fluorescence that can be measured and reported as a melting temperature, or Tm, where each domain/binder has a unique Tm. In general, the higher the Tm the more stable the protein. Lower Tms are indicative of decreased conformational stability. A weighted shoulder score (WSS) takes the peak size of the second

thermal transition temperature into account, which is an indicator of greater conformational stability (a WSS < 20 indicates instability). Therefore, DSF can be used to assess the impact the variant sequences have on that molecule's conformational stability. The anti-KIR2DL/anti-CD8a parental molecule displayed the following DSF thermogram profile: Tm1 = 63.8oC , Tm2 = 77.1oC, WSS = 18.2. The anti-KIR2DL/anti-CD8a variants had DSF profiles that ranged from Tm1 = 60.4 – 65.2oC, Tm2 = 72.6 – 77.1oC and WSS = 16.8 – 62.9.

[0320] Thermal hold is used to assess conformational stability by measuring protein aggregation that forms as a protein is held constant at selected elevated temperatures for a defined incubation period. This analysis defines conditions for precipitation relating to the potential destabilization of a protein during room temperature incubation. The thermal hold readout is reported as an A350nm, which measures the protein aggregation a sample contains at a specified temperature post-incubation. An A350nm > 0.5 indicates protein instability. In general, the less aggregation-prone a protein is during elevated temperature holds, the more stable the protein. Thermal hold can be used to assess the impact the variant sequences have on that molecule's temperature-induced protein instability and is therefore an indicator of conformational stability. Table 5 below provides the results for the anti-KIR2DL/anti-CD8a parental and variant bispecifics. The variant data are supplied as a range that captures the aggregated data for all variant sequences.

Table 5. Comparison of anti-KIR2DL/anti-CD8a parental and variant thermal-induced aggregation.

Molecule	**69°C hold**	**69.4°C hold**	**70°C hold**	**71°C hold**	**72.2°C hold**	**73.2°C hold**	**73.7°C hold**	**74°C hold**
anti-KIR2DL/ anti-CD8a parental	0.37	0.56	0.77	1.25	1.68	1.94	1.98	1.88
anti-KIR2DL/ anti-CD8a variants	0.20 – 1.37	0.19 – 1.69	0.20 – 1.88	0.22 – 2.06	0.61 – 2.16	1.16 – 2.21	1.28 – 2.11	1.43 – 2.13

[0321] Low pH aggregation is used to assess conformational stability by measuring protein aggregation that may form following neutralization after low pH exposure, thereby replicating the viral inactivation low pH hold. Low pH-induced aggregation can correlate with the potential destabilization of a protein during the viral inactivation step operation of downstream purification. The low pH aggregation readout is reported as % HMW measured by

SE-HPLC. A measurement of > 10% HMW after low pH exposure indicates protein instability. In general, the less aggregation-prone a protein is following low pH exposure and neutralization, the more stable the protein. As such, low pH aggregation can be used to assess the impact the variant sequences have on that molecule's ability to withstand a routine purification step and is therefore an indicator of conformational stability. The anti-KIR2DL/anti-CD8a parental molecule percent aggregation following low pH hold was 5.37% HMW. The anti-KIR2DL/anti-CD8a variants percent aggregation following low pH hold ranged from 2.74 – 11.19% HMW.

[0322] Chemical unfolding is used to assess conformational stability by measuring the inflection point of a protein when exposed to a chemical denaturant, e.g., guanidine hydrochloride. As a protein unfolds in the presence of the chaotropic agent, denaturation will proceed until the protein achieves an unfolded state. The first unfolding transition of a protein from native to denatured can be measured via its 'inflection point', which is defined as the point where 50% of the protein is folded, and 50% is denatured. Molecules with higher inflection points may display lower rates of aggregation during storage and are thus more conformationally stable. Protein with an inflection point < 2.1M may experience instability during long-term storage conditions. No significant changes were observed between the parental and variant inflection points. The anti-KIR2DL/anti-CD8a parental molecule had an inflection point of 1.99M. The anti-KIR2DL/anti-CD8a variants had inflection points ranging from 1.94 – 2.02M.

Results - KIRxCD8 Parental and Variant Octet Binding Affinities

[0323] Binding affinities of the anti-KIR2DL/anti-CD8a parental and variant bispecifics were determined by Bio-Layer Interferometry (BLI) utilizing an Octet Red 384 instrument (Sartorius). The anti-KIR2DL/anti-CD8a molecules were captured onto an AHC (anti-human IgG Fc) sensor tip (Sartorius, P/N 18-5060), then a dilution series of either KIR2DL1 (SinoBiological, P/N 13145-H08H) or CD8a (SinoBiological, P/N 10980-H08H) was allowed to bind to determine affinity. Binding was performed at 25°C in PBS buffer pH 7.4, containing 0.1% BSA and 0.02% Tween-20. Ten minutes of association followed by 15 minutes of dissociation were utilized for these measurements. Buffer reference subtraction was applied to the binding curves. Association and dissociation rate constants were globally fit to a 1:1 Langmuir binding model, and KD binding affinity was calculated. The data fit well to the binding model.

[0324] Octet affinities of anti-KIR2DL/anti-CD8a parental and variant molecules are shown in Table 6. Similar affinities were obtained for the anti-KIR2DL/anti-CD8a parental

variant bispecifics binding to KIR2DL1. In contrast, the binding to CD8a was variable, with KD's ranging from 4 nM to total loss in binding activity.

Table 6. Octet affinities of anti-KIR2DL/anti-CD8a parental and variant molecules. Content in parentheses indicate the expression system used to produce the anti-KIR2DL/anti-CD8a bispecific.

Variant Number	KIR2DL1 (K_D)	CD8a (k_a)	CD8a (k_d)	CD8a (K_D)
Parental – HEK 293	2.5nM	5.33E+04	1.51E-04	2.8nM
Parental - CHO	2.6nM	1.50E+05	7.58E-04	5.1nM
1	2.9nM	4.73E+04	7.84E-03	166nM
2	3.7nM	no binding	no binding	no binding
3	3.6nM	6.05E+04	9.57E-03	158nM
4	3.1nM	5.28E+04	5.87E-03	111nM
5	3.9nM	6.17E+04	6.65E-03	108nM
6	3.1nM	3.94E+04	8.23E-03	210nM
7	3.4nM	4.20E+02	6.59E-02	157,000nM
8	3.3nM	8.97E+04	4.51E-03	50.3nM
9	3.9nM	6.84E+04	4.69E-03	68.5nM
10	3.4nM	1.87E+05	8.67E-04	4.6nM
11	4.0nM	6.74E+04	3.49E-03	51.8nM
12	3.7nM	6.97E+04	1.50E-02	215nM
13	3.2nM	1.12E+05	3.05E-03	27.3nM
14	3.7nM	7.43E+04	4.53E-03	61.0nM
15	3.2nM	8.26E+04	2.73E-03	33.1nM
16	3.3nM	1.06E+05	4.43E-03	41.8nM
17	3.4nM	3.62E+04	9.80E-03	271nM
18	3.7nM	1.06E+05	1.88E-03	17.7nM
19	3.6nM	7.51E+04	4.01E-03	53.4nM
20	3.3nM	1.78E+05	7.03E-04	3.9nM
21	3.5nM	8.22E+04	1.67E-03	20.3nM
22	3.7nM	1.39E+05	6.57E-04	4.7nM

KIRxCD8 Parental and Variant Cell-Based Binding Affinities

[0325] The CD8a SKW target cell line was generated by transduction of Lenti ORF particles, CD8A (Myc-DDK tagged) - Human CD8a molecule (CD8A), transcript variant 1 (Origene) into the parental SKW line. CD8a SKW were then used as targets for anti-KIR2DL/anti-CD8a parental and variant molecules to assess relative CD8 binding. CD8a SKW were stained with live/dead zombie dye for the exclusion of dead cells during analysis and then plated with increasing molar concentrations (0.003-1600nM) of anti-KIR2DL/anti-CD8a parental or variant bispecific antibody. Cells were incubated with anti-KIR2DL/anti-CD8a parental or

variant antibody for 30 minutes on ice and then washed. Bispecific bound cells were detected by incubation with Allophycocyanin (APC) AffiniPure F(ab')$_2$ Fragment Goat Anti-Human IgG, Fcγ fragment specific secondary antibody (Jackson ImmunoResearch) at 4°C for 30 minutes. Cells were then washed, fixed, and run on Symphony A1. For each molar concentration of anti-KIR2DL/anti-CD8a sample tested, the amount of bound drug was determined by the geometric mean of anti-Fc APC on the live, single cells. Geometric mean values were graphed for each of the molar drug concentrations and values were fit to a nonlinear curve in GraphPad Prism. EC50 best fit values were determined for each anti-KIR2DL/anti-CD8a molecule tested, as calculated with the asymmetrical sigmoidal curve, 5PL, X is concentration analysis (Table 7 and FIG. 2). Parental and variant cell binding was comparable.

Table 7. EC50 for binding proteins in an assay using CD8 SKW cells.

Binding Protein	**EC50**
Parental (HEK 293)	4.154
17	194.7
18	9.822
19	59.12
20	3.171
Parental (CHO)	1.673

[0326] Primary CD8 T cells were negatively selected from cryopreserved healthy human PBMCs as described in the the StemCell CD8 T cell isolation kit (17953) to preserve the CD8a binding site on the T cell surface. Isolated CD8+ T cells were then used as targets for each of the anti-KIR2DL/anti-CD8a binding proteins: parental expressed from HEK 293 cells, parental expressed from CHO cells, and anti-KIR2DL/anti-CD8a variant 20. CD8+ T cells were stained with live/dead zombie dye for the exclusion of dead cells during analysis and then plated with increasing molar concentrations of each of the anti-KIR2DL/anti-CD8a bispecific antibodies. Cells were incubated with anti-KIR2DL/anti-CD8a antibody (0.78-3200nM) for 30 minutes on ice and then washed. Bispecific bound cells were detected by incubation with Allophycocyanin (APC) AffiniPure F(ab')$_2$ Fragment Goat Anti-Human IgG, Fcɣ fragment specific secondary antibody (Jackson ImmunoResearch) at 4°C. Cells were then washed, fixed, and run on

Symphony A1. For each different molar concentration of anti-KIR2DL/anti-CD8a molecule tested, the amount of bound drug was determined by the geometric mean of anti-Fc APC on the live, single cells. Geometric mean values were graphed for each of the molar drug concentrations and fit to a nonlinear curve in GraphPad Prism. EC50, Hill Slope, Top, Bottom and logEC50 best fit values were determined for each anti-KIR2DL/anti-CD8a sample as calculated with the asymmetrical sigmoidal curve, 5PL, X is concentration analysis (Table 8 and FIG. 3). Parental and variant cell binding was comparable.

Table 8. EC50 for binding proteins in an assay using CD8+ T cells.

Best Fit Values	**Binding Protein**		
	Parental (HEK 293)	Parental (CHO)	20
EC50	NA (3179)	NA (1892)	4.748
Hill Slope	0.185	0.2561	0.403
Top	52402	80036	28599
Bottom	1212	1037	1594
logEC50	3.502	3.277	0.6765

Summary of Results

[0327] The variants showed a range of biophysical properties and binding affinities. Framework substitutions ranked from improved binding and/or stability, to little impact, to reduced binding were as follows: G100C/G44C (disulfide addition) > G97A > F64V/A68F >> I29F > A72R.

[0328] No changes in colloidal stability relative to the parental molecule were observed for any of the anti-KIR2DL/anti-CD8a bispecific variants (data not shown).

[0329] Variant 20 retained comparable binding to the parental molecule but had an improved stability profile. This variant contains the following mutations in the anti-CD8a scFv sequences: VH F64V, A68F, and G97A plus disulfide mutations at VH G44C + VL G100C relative to SEQ ID NOs.:7 and 8 (equivalent to VH F74V, A78F, G107A plus disulfide mutations at VH G51C + VL G141C in AHo numbering). Variant 20 was the only variant that displayed the desired phenotypic characteristics. Improvement was observed in expression titer, specific productivity, non-reduced CE-SDS % pre-peaks and % main peak, Tm, and thermal-induced aggregation, as illustrated in Tables 9, 10, 11, and 12. No significant differences were observed

between the parental and variant 20 bispecifics for SE-HPLC, low pH aggregation, and chemical unfolding (data not shown).

Table 9. Comparison of anti-KIR2DL/anti-CD8a parental and variant 20 titer and specific productivity.

Variant Number	Titer (g/L)	Qp (pg/c/d)
Parental (CHO)	1.25	11.26
20	1.77	16.06

Table 10. Comparison of anti-KIR2DL/anti-CD8a parental and variant 20 nrCE-SDS.

Variant Number	Pre-peaks (%)	Main peak (%)	Post-peaks (%)
Parental (CHO)	14.26	82.38	3.36
20	9.73	84.86	5.41

Table 11. Comparison of anti-KIR2DL/anti-CD8a parental and variant 20 DSF.

Variant Number	WSS (°C)	Tm1 (°C)	Tm2 (°C)
Parental (CHO)	18.2	63.8	77.1
20	62.9	64.2	75.3

Table 12. Comparison of anti-KIR2DL/anti-CD8a parental and variant 20 thermal-induced aggregation.

Variant Number	Hold Avg	69°C hold	69.4°C hold	70°C hold	71°C hold	72.2°C hold	73.2°C hold	73.7°C hold	74°C hold
Parental (CHO)	1.30	0.37	0.56	0.77	1.25	1.68	1.94	1.98	1.88
20	0.88	0.41	0.63	0.29	0.52	0.61	1.27	1.67	1.67

[0330] The anti-KIR2DL/anti-CD8a variant 20 has an improved conformational stability profile while retaining comparable binding to the anti-KIR2DL/anti-CD8a parental bispecific. Additionally, CHO pool expression titer and specific productivity for the variant was higher than the parental, which may be attributed to the variant sequence being a more stable configuration.

Example 3: Pre-clinical Pharmacologic and Tolerability Characterization of a KIRxCD8-Targeting Bispecific CD8+ Treg Modulator

[0331] In healthy individuals, CD8+ Treg activation leads to selective elimination of self-reactive CD4+ T cells. The CD8+ Treg network appears to be dysfunctional in autoimmune

diseases and insufficient to kill self-reactive CD4+ T cells, in part due to expression of inhibitory KIR2DL1/2/3 molecules (see, e.g., Li et al., Science 376(6590), 2022, doi: 10.1126/science.abi9591).

[0332] Bispecific CD8 Treg modulator targeting CD8 and KIR2DL(1/2/3) molecules have been developed that target CD8+ Treg, activating the cells, thereby reducing inflammation without increasing unwanted immune cell activation or pro-inflammatory cytokines. This enhancement of CD8+ Treg function is a broadly applicable and promising CD8+ Treg-specific therapeutic that may be used to restore immune balance for the treatment of autoimmune diseases, including gastrointestinal indications (e.g., celiac disease, Crohn's, and ulcerative colitis).

[0333] Previously, binding and specificity profiles of a bispecific binding molecule targeting CD8 and KIR2DL(1/2/3) were assessed in vitro and in vivo (see WO2022/169825A1).

[0334] In this example, a bottle-opener bispecific molecule comprising SEQ ID NOs:78, 80, and 120 (variant 20 in Examples 1 and 2) was used in binding and activation assays in primary human peripheral blood mononuclear cells (PBMCs), and was also administered to humanized CD34+-engrafted NSG(IL-15Tg) mice, where binding, impact on immune cell phenotypes, pharmacology, and early tolerability of the binding protein were assessed. PK profiles were assessed following single doses (up to 10 mg/kg variant 20) in female BALB/cJ and healthy CD34+ NSG-Tg(Hu-IL15) mice (Abeynaike et al., Viruses 15(2):365, 2023, doi.org/10.3390/v15020365; Aryee et al., FASEB 36:e22476, 2022, doi.org/10.1096/fj.202200045R). It was found that the pharmacokinetic profile was consistent with antibody-like molecules, and detectable binding on immune cells in peripheral blood and terminal tissues was observed, with no resulting activation. No induction of proinflammatory cytokines in the serum was observed following a single dose of the molecule.

Methods

[0335] Variant 20, a bispecific molecule comprising SEQ ID NOs: 78, 80, and 120, was produced as described in Example 2.

[0336] Binding and activation assays for variant 20 were conducted using PBMCs from healthy human donors or from a Celiac human donor. PBMCs from a Celiac donor were thawed, rested, and incubated with variant 20 or a monospecific control molecules. A nonblocking anti-KIR antibody was used to selectively gate on the KIR+ CD8s within the total PBMCs, while an anti-NKp46 antibody was used to define NK cells. The binding of variant 20 to different cell populations was detected using an anti-human IgG1 Fc secondary antibody. Activation of KIR+

CD8s from Celiac donor PBMCs was detected using anti-Granzyme B following intracellular staining.

[0337] Variant 20 was tested in healthy humanized CD34+ NSG-Tg(Hu-IL15) mice at approximately 17-18 weeks post engraftment with two independent human donor cells (FIG. 4). CD34+ Cord Blood cells from two independent donors were engrafted into NSG-Tg(IL-15) mice aged 4 weeks, and mice were screened for inclusion on study at 12 weeks. Mice with >25% hCD45, >3% hCD3 and >2% hCD56 were accepted for the study. After shipment and acclimation, animals received a single IV dose at 1 or 10 mg/kg of variant 20 (~17-18 weeks post engraftment). Blood samples were collected from subsets of animals for PK (n=4/dose/donor) and immunophenotyping (n=5/dose/donor) at specific time points following dose, and terminal blood and spleen were collected on Day 28.

[0338] The *in vivo* pharmacological impact of variant 20 was evaluated using flow cytometry and human U-plex Meso Scale Discovery (MSD) assays. For PK analyses, quantitation of variant 20 in BALB/cJ and humanized CD34+ NSG-Tg(Hu-IL15) mice was performed using a sandwich ELISA using samples collected with a microsampling procedure. Concentrations achieved with microsamples yielded about 45% of value expected from serum collections from mice and allowed serial sampling of small volumes from all individual mice following dosing.

Results

[0339] In the binding and activation study using PBMCs obtained from a Celiac donor or a healthy donor, variant 20 modulated function of CD8+ Treg cells. Variant 20 was observed to bind to KIR+CD8+ T cells (CD8+ Tregs) (FIG. 5A) causing a reduction in intracellular Granzyme B compared to control monospecific mAb (FIG. 5B). Variant 20 was also observed to bind to NK cells (FIG. 5C). Elimination of gliadin-responsive Celiac donor CD4+ T cells was observed, as detected by an increase in Annexin V+ CD4_ T cells at 48hrs following addition of variant 20 (FIG 5D).

[0340] The potential for variant 20 to trigger cytokine release in whole blood or PBMCs derived from ten healthy human donors was assessed in the soluble and wet-coated presentation formats. A range of concentrations of variant 20 (0.032 µg/mL, 0.16 µg/mL, 0.8 µg/mL, 4 µg/mL, 20 µg/mL, and 100 µg/mL) was evaluated in the assays. Anti-CD3 antibody (OKT3 clone; positive control) treatment, human IgG1 antibody (negative control) treatment, and a no treatment control were also included in the assay. All treatments were evaluated in triplicate in both the soluble and wet-coated plate formats. Tissue culture supernatants from treated PBMC

samples were collected after 24 hours of treatment. The levels of IL-2, IL-6, IL-8, IL-10, TNF-a, and IFN-g were measured using MSD platform. All donors were responsive to positive control anti-CD3 (FIG. 5E, FIG. 5F for wet-coated assay), demonstrating that these donors had the capacity to release cytokines in response to an immunomodulatory stimulus. In both treatment formats, there was no dose-responsive cytokine release (IL-2, IL-6, IL-8, IL-10, and TNF-a) stimulated by variant 20 above the level of isotype or untreated controls, for all donors (*see* FIG. 5E, FIG. 5F for wet-coated assay).

[0341] After a single dose of variant 20 was administered to huCD34+ NSG-Tg(Hu-IL15) mice, the frequency of human immune cells in peripheral blood remained steady. The frequency of immune cell subsets, including CD4+ or CD8+ T-cell subsets (FIGS. 6A-6D) and NK cells (FIGS. 6E-6F), were assessed by flow cytometry at baseline, Day 14, and Day 28 in peripheral blood following IV administration of variant 20. Enrolled study mice at baseline had a mean of 62% hCD45, 5% CD3, and 7% CD56. An increase in the number of CD8 Treg cells in peripheral blood was observed after a single dose of variant 20 (FIGS. 6A) without a change in the CD4/CD8 ratio (FIG. 6D). An increase KIR2D+ NK cells was also observed at late time points relative to baseline levels (FIG. 6F). Notable changes also occurred in total CD4 and CD8 T cells (FIGS. 6B-6C), which may be indicative of a still evolving immune system in these mice following engraftment (see Abeynaike et al., Viruses 15:365, 2023, doi: 10.3390/v15020365).

[0342] Binding of variant 20 to total CD8, CD8 Treg, NK, and panKIR2D+NK cells were found on cells from blood and spleen. Sustained binding was observed to cells expressing both targets in peripheral blood (FIGS. 7A-7C). Variant 20 was detectable on CD8 Treg cells in the spleen (FIG. 7D). As expected, no binding was observed to CD4 T cells either in blood or spleen (data not shown).

[0343] In mice treated with variant 20, a slight increase in Ki67 MFI and CD69+CD25+ Treg cells were observed over the course of the study (relative to total CD8 population) (FIGS. 8A-8D). FIGS. 8A and 8B show Ki67 MFI in total CD8 T cells and KIR+ CD8 T cells, respectively. FIGS. 8C and 8D show percentage of CD69+CD25+ cells in total CD8 T cells and KIR+ CD8 T cells, respectively.

[0344] After a single dose of variant 20, no change in total CD4+ T cells was detected (FIG. 9A) but a decrease in %CD69+ CD4 cells was observed (FIG. 9B).

[0345] FIG. 10 and Table 13 show quantification of variant 20 concentration over time in huCD34+ NSG-Tg(Hu-IL15) mice. A single dose of 10 or 1 mg/kg variant 20 was detectable through 672 hr in blood (serial microsample collection) of humanized CD34+ NSG-Tg(Hu-IL15)

mice at 10 mg/kg or 1 mg/kg and in serum of BALB/cJ mice at 5 mg/kg through and was consistent between donors and strains.

Table 13. Quantification of variant 20 concentration over time in huCD34+ NSG-Tg(Hu-IL15) mice and BALB/cJ mice[a].

Parameter	Units	CD34+ NSG-Tg(Hu-IL15)		BALB/cJ
Dose	mg/kg	10	1	5
Sample type	n/a	Microsample		Serum
Tmax	hr	0.5	0.8	0.5
Cmax	µg/mL	75	7.68	106
AUCinf	hr*µg/mL	7824	1431.8	15500
t1/2	day	11.1	10.5	11.1
CL	mL/hr/kg	1.275	0.763	0.323
Vz	mL/kg	490	264	124
Number evaluated per Study Group/ dose level		1	5	9

[a] Only mice with AUC%Extrap <20% were used to calculate PK parameters shown in table (hu CD34+ NSG(IL-15Tg): n=5, 1mg/kg; n=1, 10 mg/kg; BALB/cJ: n=9).

Summary

[0346] Variant 20 was well-tolerated following a single dose in humanized mice and did not increase activation of CD4 T cells or CD8 T cells. Following a single dose of variant 20, an increase in CD8 Treg activation and proliferation at later time points was observed. The data suggest that variant 20 may selectively increase expression of Granzyme B in CD8 Treg cells at early time points, suggesting an impact on their cytolytic capacity. Additionally, following a single dose of variant 20, a decrease of activated CD4 T cells was observed at late timepoints, supporting the postulated mechanism of action and proposed therapeutic uses.

[0347] The binding of variant 20 to CD8 Treg cells, total CD8 T cells, and KIR+NK cells was measurable in peripheral blood and spleen of humanized mice, with sustained binding to cells expressing both targets. The concentration of variant 20 indicated high exposure in humanized mice, with a $T_{1/2}$ of about 11.5 days and antibody-like PK parameters.

[0348] The data also support the use of the humanized mouse model for safety assessment and understanding of a PK/PD relationship, and to inform clinical development. Using humanized mice as a relevant toxicology species has the potential to reduce the number of toxicology studies for IND-enabling assessments that will inform safety and PK/PD assessments to support dosing in humans in clinical trials. The humanized mouse model can also be valuable

for testing targets with insufficient cross-reactivity to other species and non-human primates (NHP). Variant 20 was well-tolerated following multiple doses in CD34+ NSG-Tg(Hu-IL15) mice. Data derived from the CD34+ NSG-Tg(Hu-IL15) mouse model align with *in vitro* and *in vivo* findings for variant 20, highlighting the utility of this model for non-clinical safety assessment. The data underline the use of the CD34+ NSG-Tg(Hu-IL15) mouse to assess non-clinical safety of development candidates targeting human immune system receptors with limited or restricted cross-reactivity in conventional toxicology species. The CD34+ NSG-Tg(Hu-IL15) mouse model has physiological levels of human IL-15 and supports long-term engraftment of human CD45+ immune cells, including NK cells and KIR expressing CD8 Treg. Compared to other humanized models, acute macrophage activation is not observed in CD34+ NSG-Tg(Hu-IL15) mice, making this model ideal for long-term toxicology studies.

Example 4: Prevalence and Activation Status of KIR2DL1/2/3-expressing CD8 Tregs in Patients with Rheumatologic Autoimmune Disorders

[0349] To determine the potential therapeutic applicability of variant 20 in rheumatologic autoimmune disorders, the prevalence and activation status of KIR2DL1/2/3 expressing (KIR+) CD8 Tregs (herein referred to as CD8 Tregs) were evaluated in PBMCs derived from patients with rheumatologic autoimmune disorders.

[0350] The frequency of CD8 Tregs as a proportion of total CD8 T cells in the peripheral blood of healthy donors and donors with psoriasis, systemic lupus erythematosus (SLE), psoriatic arthritis (PsA), ankylosing spondylitis (ASp), and Sjögren's syndrome (SS) was assessed (FIG. 11). CD8 Tregs were shown to be present in the peripheral blood of patients in all indications tested and in all patients, regardless of disease status.

[0351] To assess the activation status of CD8 Tregs in patients with rheumatologic autoimmune disorders, PBMCs were activated with anti-CD3 antibody (OKT3), after which, expression of intracellular granzyme B was assessed by intracellular staining. PBMCs rested overnight, but not activated, were used as a control. FIGS. 12A-12C show the frequency of granzyme B-expressing CD8 Tregs relative to a negative control as a proportion of total CD8 Tregs from healthy donors and donors with psoriasis (FIG. 12A), systemic lupus erythematosus (SLE; FIG. 12B), psoriatic arthritis (PsA; FIG. 12B), ankylosing spondylitis (ASp; FIG. 12C), and Sjögren's syndrome (SS; FIG. 12C) in unstimulated and stimulated cells. These assays suggest that CD8 Tregs are impaired in patients with rheumatologic autoimmune disorders, showing reduced responsiveness to stimulation and expression of proteins critical to CD8 Treg

functions relative to CD8 Tregs from healthy donors. While CD8 Tregs appear to be dysfunctional in patients with rheumatologic autoimmune disorders, defects in the cytotoxic function of CD8 Tregs can be reversed by their activation. By disrupting inhibitory KIR, an autoimmune checkpoint, it is expected that variant 20 can restore CD8 Treg function.

Example 5: Multidose Pre-clinical Tolerability Characterization of a KIRxCD8-Targeting Bispecific CD8+ Treg Modulator in Humanized CD34+ NSG-Tg(Hu-IL15) mice

[0352] Variant 20 was tested for tolerability (toxicity, immunotoxity, and PK/PD parameters) in healthy humanized CD34+ NSG-Tg(Hu-IL15) mice at least 15 weeks post engraftment using cells from two independent human donors (FIG. 13A). CD34+ cord blood cells from two independent donors were engrafted into NSG-Tg(IL-15) mice and screened for inclusion in the study at 12 weeks. FIG. 13B depicts the percentages of hCD45+ cells, CD3+ T cells, and CD56+ NK cells at 12 weeks post engraftment in the mice enrolled for the study. Mice with >25% hCD45, >3% hCD3 and >2% hCD56 were accepted for the study. Mice were administered 5 or 50 mg/kg of variant 20 or vehicle intravenously via the tail vein, weekly on Days 1, 8, 15, 22, and 29, over 4 weeks at 5 mL/kg. Blood samples were collected for pharmacokinetic analysis (n=3/dose/donor), serum cytokine analysis (n=3/dose/donor), and immunophenotyping (n=5/dose/donor) at specific time points following dosing. For pharmacokinetic analysis, blood samples were collected pre-dose on Day 0 and at 0.5, 2, 24, 96, and 168 hours following dosing on Days 1 and 22; additional samples were collected on Day 15 and 29 at 0.5 hour post-dose. Mice (n=6)were also assessed for toxicity readouts at terminal time points. For immunophenotyping, blood samples were collected pre-dose on Day 0 and Day 15 and post-dose on Day 29. Terminal blood and spleen samples were collected on Day 29. Variant 20 was well-tolerated following repeated doses of up to 50 mg/kg. Body weight was also measured the course of the study and no difference in body weight was observed for either the 50 mg/kg or 5 mg/kg dose as compared to dosing with vehicle control (FIG. 13C). No differences in the body weight were observed among the treatment groups or donors 1029 and 1200.

[0353] After multiple doses of variant 20, the presence of human immune cells in peripheral blood of CD34+ NSG-Tg(Hu-IL-15) mice remained consistent with vehicle group (FIGS. 13D-13O).

[0354] Blood and spleen samples taken from the mice engrafted with human CD34+ cord blood cells from the study described in FIG. 13A were used to examine target binding and activation of variant 20 *in vivo*. Binding of variant 20 to CD8 Tregs (FIG. 14A) and CD8 T cells

(FIG. 14B) from the peripheral blood of the humanized CD34+ NSG-Tg(Hu-IL15) mice pre-dose on Day 15 (7 days after Day 8 dosing) and 30 min post-dosing on Day 29, and binding of variant 20 to CD8 Treg, CD8 T cells, and NK cells from the spleen of humanized CD34+ NSG-Tg(Hu-IL15) mice at day 29 (30 min post-dosing) (FIG. 14C) was assessed via Fc detection using an anti-human IgG1 Fc secondary antibody. Variant 20 was found to selectively bind CD8 Tregs in humanized CD34+ NSG-Tg(Hu-IL15) mice (FIGS. 14A-14C). Further illustrations of variant 20 binding in peripheral blood and spleen are shown in FIGS. 14D-14G and FIGS. 14H-14I, respectively. Sustained binding was observed to cells expressing both targets in peripheral blood (FIG. 14A, FIG. 14G). No binding to CD4+ T cells was observed in blood or in spleen (FIG. 14E, FIG. 14H, FIG. 14I).

[0355] The effect of variant 20 on activation and proliferation of CD8 Treg and NK cells was also assessed by measuring the frequency of IFNγ+ CD8 Treg cells (FIG. 15A), IFNγ+ NK cells (FIG. 15B), Helios+ CD8 Treg cells and NK cells (FIG. 15C), Ki67+ CD8 Treg (KIR+) cells and NK cells (FIG. 15D), CD69+CD25+ KIR+ CD8 T cells and NK cells (in blood, FIG. 15E; in spleen, FIG. 15F), and CD69+ CD4 T cells (FIG 15G). Variant 20 induced IFNγ production by CD8 Treg cells as measured by intracellular cytokine production (FIG. 15A). Variant 20 induced IFNγ production in NK cells slightly on Day 15 for the high dose, but it decreased back to baseline by Day 29 (FIG. 15B). Variant 20 also increased the proportion of Helios+ cells in the CD8 Treg population but not in NK cells (FIG 15C). Variant 20 induced proliferation of CD8 Treg but not NK cells (FIG. 15D). No increase of CD69+CD25+ in total CD8, KIR+ CD8+, CD4+, NK, and KIR+ NK cells was detectable after multiple doses of variant 20 (FIG. 15E, FIG. 15F). At Day 29, a reduction of CD69+ in total CD4 T cells was observed (FIG. 15G).

[0356] Variant 20 did not increase the expression of pro-inflammatory serum cytokines after a single dose of 5 or 50 mg/kg variant 20, at 8 and 24 hr (FIG. 16).

[0357] As shown previously (Gardell et al. JI, 2022, 2023) and here, variant 20 increases cytolytic capacity, activation, and prevalence in healthy donor and autoimmune patient-derived CD8 Treg. Tolerability studies performed using NSG-Tg(IL-15) mice engrafted with CD34+ cells, in which human lymphocytes engraft at physiologic ratios, showed selective CD8 Treg binding, without unwanted immune cell activation or body weight loss in animals treated with doses up to 50 mg/kg of variant 20. Treatment with variant 20 selectively increased the Helios content and proliferation of the CD8 Treg population, but not of NK cells, which may serve as clinical biomarkers.

[0358] The data also support the use of the humanized mouse model for safety assessment and understanding of a PK/PD relationship, and to inform clinical development. Using humanized mice as a relevant toxicology species has the potential to reduce the number of toxicology studies for IND-enabling assessments that will inform safety and PK/PD assessments to support dosing in humans in clinical trials. The humanized mouse model can also be valuable for testing targets with insufficient cross-reactivity to other species and non-human primates (NHP). Variant 20 was well-tolerated following multiple doses in CD34+ NSG-Tg(Hu-IL15) mice. Data derived from the CD34+ NSG-Tg(Hu-IL15) mouse model align with *in vitro* and *in vivo* findings for variant 20, highlighting the utility of this model for non-clinical safety assessment. The data underline the use of the CD34+ NSG-Tg(Hu-IL15) mouse to assess non-clinical safety of development candidates targeting human immune system receptors with limited or restricted cross-reactivity in conventional toxicology species. The CD34+ NSG-Tg(Hu-IL15) mouse model has physiological levels of human IL-15 and supports long-term engraftment of human CD45+ immune cells, including NK cells and KIR expressing CD8 Treg. Compared to other humanized models, acute macrophage activation is not observed in CD34+ NSG-Tg(Hu-IL15) mice, making this model ideal for long-term toxicology studies.

Example 6: Single Dose Pre-clinical Pharmacokinetic Characterization of a KIRxCD8-Targeting Bispecific CD8+ Treg Modulator in Cynomolgus Monkeys

[0359] The pharmacokinetics of variant 20 were characterized in Cynomolgus monkeys. Animals were injected with a single dose of 5, 0.5, or 0.05 mg/kg of variant 20. Blood was collected at multiple time points and processed into serum to determine serum levels of variant 20 using a human Meso Scale Discovery (MSD) assay (FIG. 17A). Pharmacokinetic parameters were calculated from concentration versus time data using Phoenix WinNonLin (Certara), and analyzed using an IV bolus administration model (FIG. 17B). Variant 20 showed a linear relationship between dose and C_{max} (FIG. 17C) and dose and AUC (FIG. 17D). Variant 20 demonstrated a half-life of ~11 days following a single dose; the half-life was similar in wild type mice (Balb/c) and humanized mice as described above in Example 3.

Example 7: Clinical Study to Assess Safety, Pharmacokinetics, and Pharmacodynamics

[0360] Safety, pharmacokinetics, and pharmacodynamics for a bispecific CD8 Treg modulator (an anti-KIRxanti-CD8 binding protein) are assessed in healthy adults and patients

having celiac disease or type 1 diabetes, in a prospective, multi-center, randomized, double-blind, placebo-controlled single ascending dose (SAD) and multiple ascending dose (MAD) study.

[0361] The study is an intervential crossover assignment study with an estimated enrollment of 96 participants.

[0362] Part A enrolls Healthy Adult Volunteers (HA) in a randomized, double-blind, placebo-controlled, single ascending dose (SAD) study, randomized to receive the anti-KIRxanti-CD8 binding protein or placebo in a 1:1 ratio for the first 2 participants (sentinel dosing) and a 3:1 ratio thereafter. Participants in the multiple ascending dose (MAD) are randomized in a 3:1 ratio and be dosed on Days 1 and 22 for a total of 2 doses.

[0363] Part B enrolls at least 24 (up to 40) participants with Celiac Disease (CeD) or Type 1 Diabetes (T1D), randomized in a 1:1 ratio, in a crossover design, dosed with the anti-KIRxanti-CD8 binding protein or placebo on Days 1 and 22 for a total of 2 doses.

[0364] The anti-KIRxanti-CD8 binding protein is variant 20 ("V20") as described in Examples 1-6 above. It is stored in a frozen liquid solution (25 mg/mL) in 1.4 mL in 2 mL vials, suitable for injection or intravenous infusion. It is administered to participants via intravenous infusion. It is formulated as shown in Table 14.

Table 14. Formulation for variant 20.

Ingredient	Quantity	Unit
V20	25	Microgram/millilitre
Sodium Acetate	10	Microgram/millilitre
Sucrose	9	Percent weight/volume
Polysorbate 80	0.015	Percent weight/volume

[0365] Placebo is 0.9% sodium chloride for intravenous infusion.

[0366] Inclusion criteria include the following:

[0367] 1. Adults, age $\geq$ 18 and $\leq$ 65 years at the time of anticipated dosing (Day 1).

[0368] 2. Healthy individuals without known current or chronic medical conditions, including no history of any autoimmune diseases, in the opinion of the Investigator.

[0369] 3. Body mass index (BMI) $\geq$ 18 kg/m2 and $\leq$ 32 kg/m2.

[0370] 4. Body weight $\geq$ 50 and $\leq$ 90 kg.

[0371] 5. Negative Coronavirus Disease 2019 (COVID-19) test within 24 hours prior to each dose.

[0372] 6. Persons of child-bearing potential must have a negative pregnancy test and either abstain from sex or use highly effective method(s) of birth control from Day 1 through the duration of the study.

[0373] Exclusion criteria include the following:

[0374] 1. Clinically significant findings in physical examination (PE), vital signs (blood pressure, heart rate, and body temperature), electrocardiogram (ECG), and safety laboratory parameters at Screening in the opinion of the Investigator

[0375] 2. Renal function calculated by the Chronic Kidney Disease-Epidemiology (CKD-EPI) equation with estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m2 or abnormal level of proteinuria detected by dipstick at the time of Screening.

[0376] 3. Any disease or condition that, in the opinion of the Investigator, might significantly compromise the cardiovascular, hematological, renal, hepatic, pulmonary (including chronic asthma), endocrine (e.g., diabetes), central nervous, or gastrointestinal (including an ulcer) systems.

[0377] 4. History of seizures or history of epilepsy (excluding a history of pediatric febrile seizures).

[0378] 5. History of serious mental illness that, in the opinion of the Investigator, would impact participant safety or study compliance.

[0379] 6. Participation in a clinical study within 28 days or 5 half-lives (whichever is longer) of the investigational drug(s) prior to Day 1.

[0380] 7. Positive serology for human immunodeficiency virus (HIV) type 1 or 2, hepatitis (Hep) B surface antigen, or Hep C.

[0381] 8. Positive test results for urine drug screen at the time of Screening or on Day 1 prior to randomization.

[0382] 9. History of alcohol abuse that, in the opinion of the Investigator, would impact participant safety or study compliance.

[0383] 10. History of drug abuse that, in the opinion of the Investigator, would impact participant safety or study compliance.

[0384] The arms and interventions are shown in Table 15. Outcome measures are shown in Table 16.

Table 15. Arms and interventions.

Arm	Intervention/Treatment

Placebo Comparator: Cohort A1S - Healthy Volunteers • Cohort A1S (n = 6): V20, 0.01 mg/kg IV, or Placebo IV; single dose	Drug: Placebo Drug: V20
Placebo Comparator: Cohort A2S - Healthy Volunteers • Cohort A2S (n = 6): V20, up to 0.1 mg/kg IV, or Placebo IV; single dose	Drug: Placebo Drug: V20
Placebo Comparator: Cohort A3S - Healthy Vounteers • Cohort A3S (n = 6): V20, up to 0.5 mg/kg IV, or Placebo IV; single dose	Drug: Placebo Drug: V20
Placebo Comparator: Cohort A4S - Healthy Volunteers • Cohort A4S (n =6): V20, up to 2.0 mg/kg IV, or Placebo IV; single dose	Drug: Placebo Drug: V20
Placebo Comparator: Cohort A4Sa - Healthy Volunteers • Optional Cohort A4Sa (n = 8): V20, up to 8.0 mg/kg IV, or Placebo IV; single dose	Drug: Placebo Drug: V20
Placebo Comparator: Cohort A5M - Healthy Volunteers • Cohort A5M (n=8): V20, up to 0.5 mg/kg IV, or Placebo IV; dosed on Days 1 and 22 for a total of 2 doses	Drug: Placebo Drug: V20
Placebo Comparator: Cohort A6M - Healthy Volunteers • Cohort A6M (n = 8): V20, up to 2.0 mg/kg IV, or Placebo IV; dosed on Days 1 and 22 for a total of 2 doses	Drug: Placebo Drug: V20
Placebo Comparator: Cohort A6Ma - Healthy Volunteers • Optional Cohort A6Ma (n = 8): V20, up to 8.0 mg/kg IV, or Placebo IV; dosed on Days 1 and 22 for a total of 2 doses	Drug: Placebo Drug: V20
Placebo Comparator: Cohort B7 - Celiac Disease Patients, Type 1 Diabetes Patients • Dose Group 1 (n = 6): V20 up to 0.5 mg/kg IV Day 1, placebo IV Day 22 • Dose Group 2 (n = 6): Placebo IV Day 1, V20 up to 0.5 mg/kg IV Day 22	Drug: Placebo Drug: V20

Placebo Comparator: Cohort B8 - Celiac Disease Patients, Type 1 Diabetes Patients • Dose Group 1 (n = 6): V20 up to 2.0 mg/kg IV Day 1, V20 up to 2.0 mg/kg IV Day 2 • Dose Group 2 (n = 6): Placebo IV Day 1, V20 up to 2.0 mg/kg IV (or the maximum tolerated dose in Part A MAD) Day 22	Drug: Placebo Drug: V20

Table 16. Outcome measures.

Outcome Measure	**Time Frame**	
Primary		
Safety of single, ascending dose levels of V20	Enrollment to 8 weeks post dose	Assess the safety of single, ascending dose levels of V20
Safety of multiple, ascending dose levels of V20	Enrollment to 11 weeks following the last dose	Assess the safety of multiple, ascending dose levels of V20
Secondary		
pharmacokinetics (PK) of V20	Enrollment to 11 weeks following the last dose	Characterize the pharmacokinetics (PK) of V20
anti-drug antibody (ADA) formation	Enrollment to 11 weeks following the last dose	Evaluate incidence of anti-drug antibody (ADA) formation
Other		
pharmacodynamics (PD) of V20	Enrollment up to 11 weeks following the last dose	Evaluate the pharmacodynamics (PD) of V20
receptor occupancy of V20	Enrollment up to 11 weeks following the last dose	Evaluate the receptor occupancy of V20 binding to KIR+ CD8 T cells

Example 8: Pre-Clinical Assessment of Bispecific Antibodies Targeting Inhibitory KIR and CD8

[0385] *Summary*

[0386] Regulatory CD8 T cells (CD8 Treg) are responsible for the selective killing of self-reactive and pathogenic CD4 T cells. In autoimmune disease, CD8 Treg may accumulate in the peripheral blood but they fail to control the expansion of pathogenic CD4 T cells that subsequently cause tissue destruction. This CD8 Treg dysfunction is due in part to the expression of inhibitory killer immunoglobulin-like receptors (KIR; specifically KIR2DL, which has three main isoforms [KIR2DL1, KIR2DL2, and KIR2DL3]); these molecules serve as autoimmune checkpoints and limit CD8 Treg activation. Bispecific antibodies targeting inhibitory KIR and

CD8, both of which are expressed on CD8 Treg, were shown to bind to KIR2DL, and demonstrated that inhibiting KIR signaling can restore CD8 Treg ability to eliminate pathogenic CD4 T cells.

[0387] These bispecific antibodies bound and activated CD8 Treg in human peripheral blood mononuclear cells (PBMC), resulting in increased CD8 Treg cytolytic capacity, activation, and prevalence. Enhancing CD8 Treg function with the bispecific antibodies reduced pathogenic CD4 T cell expansion and inflammation, without increasing pro-inflammatory cytokines or the activation of immune cells that express either target alone. The bispecific antibodies reduced antigen induced epithelial cell death in disease affected tissues, including in tissue biopsies from individuals with autoimmune disease (i.e., celiac disease, Crohn's disease). The effects of the bispecific antibodies were specific to autoreactive CD4 T cells and not broadly immunosuppressive in that responses to viral and bacterial antigens were maintained.

[0388] In a human PBMC engrafted Graft vs Host Disease (GvHD) mouse model of acute inflammation, bispecific antibody binding to CD8 Treg delayed onset of disease in mice. Dose dependent binding, increased prevalence, and cytolytic capacity of CD8 Treg, as well as increased CD4 T cell death, were observed. The bispecific antibody selectively bound CD8 Treg without unwanted immune cell activation or increase of pro-inflammatory serum cytokines and exhibited an antibody-like half-life in pharmacokinetic and exploratory tolerability studies performed using IL-15 transgenic humanized mice with engrafted human lymphocytes, including CD8 Treg at physiologic ratios. Collectively, these data support the development of these anti-KIR/anti-CD8 antibodies for the treatment of autoimmune diseases.

[0389] *Background*

[0390] In autoimmune disease, the immune system lacks the ability to distinguish "self" from "non-self" proteins, and self-antigens can initiate a proinflammatory immune response that expands self-reactive CD4 T cells. Although the exact causes of autoimmune disease are not completely understood, microbial or viral insults in combination with genetic predisposition and additional environmental factors contribute to the onset of autoimmune disease or to trigger disease flares (Li et al., 2022; Bjornevik et al., 2022). In the context of an antimicrobial or antiviral immune response, expansion of CD4 T cells specific for the pathogen may cross-react with self-antigens, which are thought to be retained during thymic selection to support antiviral and antimicrobial responses. If left unrestrained, self-cross-reactive CD4 T cells can become pathogenic and attack host-derived tissues. Regulatory T cells ("Treg") (e.g., CD4 Treg, CD8 Treg) suppress inflammatory responses and are important for maintaining homeostasis and

tolerance to self-antigens. Specifically, CD8 Treg are a population of cytotoxic CD8 T cells with oligoclonal T cell receptors ("TCR") that are specific for potentially pathogenic CD4 T cells.

[0391] Since the discovery of Treg in 1972, several studies have described a subpopulation of CD8 Treg with ameliorating effect on inflammatory responses in murine models of disease (Keino et al., 2006; Ho et al., 2008; Ortega et al., 2013; Itani et al., 2017; Saligrama et al., 2019), as well as in human autoimmune disease (Herold et al., 2019; Diggins et al., 2021; Houston et al., 2023; Zheng et al., 2013). CD8 Treg are distinct from, and likely work in concert with, CD4 Treg in both primary mechanisms of action and impact to inflammatory cascade pathology. CD4 Treg primarily function during active inflammation through a variety of described mechanisms, including production of anti-inflammatory cytokines and adenosine, depletion of IL-2, and tolerization of antigen presenting cells (Shevyrev et al., 2020). CD8 Treg can act upstream of the autoimmune inflammatory cascade, preventing pathogenic T cell expansion and consequently reducing self-reactive antibody production and pro-inflammatory cytokines in the periphery and in tissue (Li et al., 2022). Therefore, in autoimmune disease, the elimination of pathogenic cells prior to multi-cellular and robust inflammation may have a more durable impact on the establishment and maintenance of immune balance.

[0392] Recent data have defined a CD8 Treg phenotypic signature that is conserved in healthy individuals and individuals with autoimmune disease. CD8 Treg display an expression profile of effector cytotoxic cells and require direct cell-to-cell contact, leading to elimination of pathogenic CD4 T cells. Consistent with this observation, the increased prevalence of functional CD8 Treg is associated with the delayed onset of type I diabetes (T1D) in at-risk populations (Herold et al., 2019; Diggins et al., 2021) and improved outcomes in individuals with multiple sclerosis (MS) (Houston et al., 2023). Despite increased CD8 Treg numbers in populations with CD4-driven autoimmune diseases, the expansion of pathogenic CD4 T cells is not sufficiently inhibited to prevent pathology and/or disease progression. The lack of productive engagement with pathogenic CD4 T cells may be due partly to the presence of inhibitory receptors on CD8 Treg cell surface that regulate their threshold for activation, including inhibitory KIRs which engage major histocompatibility complex (MHC) class I on target cells (reviewed in Djaoud and Parham, 2020). Inhibitory KIR compete with the TCR for MHC class I/peptide binding and send inhibitory signals that counteract activating stimuli.

[0393] It is believed that restoration of TCR/MHC/peptide interactions and elimination of KIR inhibitory signaling would restore the elimination of pathogenic CD4 T cells via functional CD8 Treg. Bispecific antibodies that targets both CD8 and the inhibitory immune checkpoint

KIR2DL, which are co-expressed on the cell surface of CD8 Treg, were developed, engineered to include a non-blocking, non-signaling scFv binding domain directed to CD8 to promote interactions with cells expressing both inhibitory KIR and CD8.

[0394] In this study, the proposed mechanism of action of the anti-KIR/anti-CD8 bispecific antibodies, by which blocking inhibitory KIR relieves suppression of CD8 Treg function, allowing for productive engagement with MHC class I and elimination of pathogenic CD4 T cells, was investigated. Assessments in vitro and in vivo in a mouse model of inflammation provide compelling evidence to support these molecules as a new and selective approach to restoring CD8 Treg network functions in the setting of CD4 T cell- driven autoimmune disease. The bispecific antibodies were found to specifically bind to and enhance CD8 Treg activity in vitro and in highly inflammatory in vivo models, restoring their functionality in healthy and autoimmune patient PBMC and ex vivo disease affected tissue cultures derived from autoimmune patients with Celiac or Crohn's Disease. The binding of the bispecific antibodies selectively increases intracellular Granzyme B in CD8 Treg, indicative of enhanced cytolytic capacity, and decreases autoimmune peptide expanded CD4 T cell activation and proinflammatory cytokine production. In contrast, polyclonal, viral, and microbial responses were not impacted, suggesting that the effects of the bispecific antibodies on pathogenic CD4 T cells is selective for the CD8 Treg network and will not result in broad immunosuppression. When evaluated at high doses using mice engrafted with a full complement of immune cells, including NK cells, the bispecific antibodies appear safe, with no detrimental activation of immune cells expressing either CD8 or KIR. Collectively, these findings suggest that the bispecific antibodies may be a selective approach to restore CD8 Treg network functions in patients with CD4 driven autoimmune diseases.

[0395] *Methods*

[0396] *Antibodies:* Two bottle-opener bispecific antibody molecules targeting KIR and CD8 were developed. The first comprised SEQ ID NOs:135, 136, and 137 ("parental" in Examples 1 and 2, referred to as "Ab00" in this Example); the second comprised SEQ ID NOs:78, 80, and 120 ("variant 20" in Examples 1 and 2, referred to as "Ab20" in this Example). Ab00 and Ab20 share the same amino acid sequences except Ab20 contains the anti-CD8 VL and VH "variant 20" substitutions described in Examples 1 and 2.

[0397] *Surface staining of human donor PBMC:* PBMC from healthy donors (Bloodworks Northwest, Seattle, WA and StemCell Technologies, Vancouver, BC, Canada) were thawed and rested overnight at 37° C in human T cell media (huTCM; X-VIVO® 15, 5% human

AB serum, 1x penicillin/streptomycin, 1x GlutaMax™ supplement). Cells were then plated at 3e5 cells/well in 96-well plates and stimulated with or without 1 µg/mL anti-CD3 (OKT3, BioLegend) for 24 hours before staining for phenotypic markers. Antibodies against KIR2DL1, KIR2DL2/3, and KIR3DL1 were allophycocyanin (APC)-labeled and together that population was considered "KIR+". Prior to staining, the cells were treated with Fc block and Zombie Aqua™ (BioLegend) for 15 minutes at room temperature. Extracellular staining was performed at 4° C for 20 minutes before fixing and permeabilizing cells. Intracellular staining was performed using eBioscience™ Intracellular Fixation and Permeabilization Buffer Set (Thermo Fisher), and intranuclear staining was performed using the True-Nuclear™ Transcription Factor Buffer Set (BioLegend). Cells were analyzed on a FACSymphony™ A1 cell analyzer (BD Biosciences), and data was analyzed using FlowJo™ software (version 10.5 or later, BD).

[0398] *Restimulation assay:* PBMC derived from donors with celiac disease (Sanguine Biosciences, Woburn, MA) were plated at 5e6 cells/mL in human T cell media (huTCM; X-VIVO® 15 with 5% human AB serum, 1x GlutaMax™ supplement and 1x penicillin/streptomycin) in 6-well plates and pulsed with gliadin peptides (Elim BioPharmaceuticals, 12.5 µg/mL of each of 7 peptides). huTCM containing 5 ng/mL each IL-2 and IL-15 was added 4 days post peptide addition and every 3-4 days thereafter for 3 weeks. On day 21, autologous PBMC were thawed, and antigen presenting cells (APCs; $CD3^+$- and $CD56^+$-depleted cell subsets, Easy Sep™ Human CD3 Positive Selection Kit II cat#17851 and Human CD56 Positive Selection Kit II cat#17855) were plated at 50,000 cells/well and stimulated with 5 µg/mL of each of 7 gliadin peptides, 0.02 µg/mL anti-CD3 (OKT3) antibody or media alone. On day 22, expanded PBMC were sorted into CD5+CD4+IntB7+ and CD5+CD4-KIR2D+ populations using a FACSAria™ Fusion flow cytometer (BD). IntB7+CD4+ targets (100,000 cells/well) and KIR2D+CD8+ Treg (20,000 cells/well) were added to 96-well plates with autologous stimulated APCs. Cultures were treated with 100 nM Ab00 or Ab20 or media alone. Supernatants were collected at 3 days post-restimulation for cytokine analysis, and cells were stained on day 3 for flow cytometry.

[0399] *Incucyte assay cell preparation and killing assay:* To generate effector cells, PBMC derived from a donor with celiac disease (Sanguine Biosciences, Woburn, MA) were thawed and plated in huTCM with 5 ng/mL IL-7 (BioLegend) and 2.5 ng/mL IL-15 (BioLegend) for 7 days, including a complete change in media and addition of cytokines on day 2 and day 5. On day 7, cells were harvested and stained for sorting using the following strategy: CD5+/NKp46-/CD4-/CCR7-/CD28-. An aliquot of cells were stained for CD8 and

KIR2DL1/CD158 expression to determine the percentage of enriched CD8+KIR+ cells resulting from sorting and used to calculate the number of Treg present at each effector-to-target ratio. GFP+ SKW CD4 cells engineered to express a TCR responsive to gliadin peptide alpha-1a (NPL001, Elim BioPharmaceuticals) (GFP+ SKW-LS2.8, ImmunsanT) or parental SKWs (pSKW) were stimulated using CD14+ cells isolated from PBMC derived from a healthy donor (Bloodworks Northwest, Seattle, WA) with an HLA-DQ2.5 haplotype. PBMC plated in huTCM with 10 µg/mL NPL001 and 5 ng/mL IFNg (BioLegend). Unactivated targets did not receive NPL001 or IFNg stimulation. Equal numbers of SKW-LS2.8 or pSKW cells were plated on top of CD14 cells and allowed to incubate overnight prior to use in the assay. Activated or unactivated SKW-LS2.8 or pSKW target cells were plated in poly-L-lysine coated 384-well, optically clear plates at 20,000 cells/well in 50 µL huTCM and allowed to settle for 30-60 minutes at room temperature before placing in the incubator. Effector cells were plated on top in 50 µL at various ratios to achieve the number of Treg per well as calculated using the %KIR2DL1/CD158+CD8+ in the sorted population. The plate was read in IncuCyte® cell analysis system (Sartorius) every 4 hours for GFP signal. The percent change in GFP+ objects from the 8-hour timepoint was graphed.

[0400] *Binding affinity by Octet®*: The binding affinity of Ab00 or Ab20 to human KIR and human CD8α was determined by Bio-Layer Interferometry utilizing an Octet® Red 384 instrument (Sartorius). After Ab00 or AB20 was captured onto an anti-human IgG Fc Capture (AHC) sensor tip, a dilution series of the monomeric target analyte (KIR2DL1, KIR2DL2, KIR2DL3, or CD8α) was allowed to bind to determine affinity. Ten minutes of association followed by 15 minutes of dissociation were utilized for these measurements. Buffer reference subtraction was applied to the binding curves. Association and dissociation rate constants were globally fit to a 1:1 Langmuir binding model, and K_D binding affinity was calculated using Octet Analysis Studio. Co-binding experiments were performed by coupling KIR2DL2-Fc and CD8α to separate AR2G (amine reactive) sensor tips via amine chemistry (EDC/NHS). Ab00 or Ab20 was allowed to bind to the immobilized antigen, followed by further binding of antigen (either KIR2DL2-Fc or CD8α-His) to the captured bispecific molecule. Simultaneous co-binding of both antigens to Ab00 or Ab20 was verified with an increase in mass on the sensor surface.

[0401] *Generation of cell lines stably expressing target protein:* SKW cells were resuspended at $1e^6$/ml in pre-warmed target media. Lentiviral particles for CD8 and KIR2DL1 lentiviruses (Origene) were added according to the desired multiplicity of infection (MOI) along with polybrene (8 µg/mL Sigma Aldrich) in a 1.5 mL microcentrifuge tube. Lentivirus cells were

incubated for 20 minutes at room temperature prior to spinoculation for 30 minutes at 800 x gravity at 32° C. Following centrifugation, virus containing cells were incubated for 48 hours at 37 ° C in a 48-well plate and expanded under antibiotic selection to generate a polyclonal stable cell line.

[0402] *On Cell and PBMC Binding:* After thawing, transduced cells or donor PBMC were harvested, and the cell count was determined based on the viability measured using the Countess™ cell counter (Thermo Fisher). Per standard procedures, the cells were plated with live/dead Zombie viability dye (Invitrogen) for 20 minutes at room temperature, then the cells were plated 2e5 cells/well in 96-well plate for drug binding. The cells were incubated with increasing molar concentrations of Ab00 or Ab20 according to a dilution series for 30 minutes on ice in the dark. After which, the cells were incubated with anti-human Fc secondary antibody for 30 minutes at 4° C in the dark followed by staining for extracellular markers. Cells were then fixed with intracellular fixative for 20 minutes at room temperature and then the samples were analyzed on a FACSymphony™ A1 cell analyzer (BD Biosciences).

[0403] *Transfection of Expi293 cells for off-target binding assay:* Fifty mL of Expi293 cells (Thermo Scientific) were cultured to a density of 1.5e6 cells/mL in 125 mL flasks (HST Labs). Cells were allowed to grow overnight at 37° C, 8% CO_2, 150 RPM in Expi293 Expression Medium (Thermo Scientific). The following day, the cells were counted (between 2.7 to 3e6 cells/mL, > 90% viable) and transfected using 1 mg/mL polyethyleinimine (PEI, Polysciences) as the complexing reagent at a concentration of 3.5 mg/L with a DNA concentration of 1 mg/L. Then 16.66 µg of each plasmid was combined with 175 µL of 1 mg/mL PEI using 2.5 mL Opti-MEM™ Reduced Serum Medium (Thermo Scientific) for both PEI and DNA. These solutions were then combined to make 5 mL DNA/PEI complexes. The DNA/PEI mixtures were allowed to incubate for 30 minutes at room temperature with periodic mixing. After 30 minutes, transfection was performed by adding the PEI/DNA complexes to the cells and cultures were then placed in the incubator. Eighteen hours after transfection, cells were fed with 2.5 mL CHO Efficient Feed B Nutrient Supplement (Thermo Scientific cat.# A1024001), and 400 µL 500mM Valproic acid (Fisher Cat.# 501786601) to aid transfection efficiency. The cells were allowed to grow in a Kuhner Climo-Shaker ISF1-XC Incubator @ 150RPM 37 ° C, 8% CO_2 for another 48 hours prior to performing a binding assay with Ab00, Ab20, or KIR3DL1 targeted antibodies.

[0404] *KIR and CD8 receptor quantification:* KIR and CD8 receptors were quantified on CD8 Treg from PBMC derived from healthy donors, or donors with celiac or Crohn's disease using quantification beads (Quantum Simply Cellular, Bangs Laboratories Inc. cat#815). Four

sets of beads with known numbers of anti-murine IgG binding sites were used to generate a standard binding curve that is then used to calculate antibody binding sites per individual cell. Quantification beads were mixed together and stained following standard procedures and then samples were analyzed on a FACSymphony™ A1 cell analyzer (BD Biosciences). KIR and CD8 gMFI data was evaluated for each antibody and used for the calculation of KIR and CD8 antibody binding sites on CD8 Treg.

[0405] *Bound KIR receptor measurement:* PBMC from healthy donors were thawed and rested overnight in huTCM in a 50 mL conical tubes. CD3 T cells were isolated from rested PBMC (StemCell negative selection kit, cat#17951). 2e5 T cells were plated per well and a dose titration of Ab00 or Ab20 up to 1600 nM was incubated for 30 minutes at 4° C. Cells were then washed and incubated with 800 nM of KIR-Fc for 20 minutes at 4° C. Extracellular immune cell phenotyping antibodies were added for an additional 30 minutes at 4° C, and cells were then washed and samples were samples were analyzed on a FACSymphony™ A1 cell analyzer (BD Biosciences).

[0406] *Crohn's Flagellin and OmpC antigen stimulation:* Frozen PBMC derived from donors with Crohn's disease or healthy donors were thawed and rested overnight in huTCM media. Rested cells were counted, labeled with a carboxyfluorescein succinimidyl ester (CFSE, Invitrogen), and plated in a 96-well, round-bottom plate at 2e5 cells/well in huTCM media. 100 ng/mL bacterial flagellin (Invivogen) and 1 μg/mL OmpC $_{321\text{-}340}$ peptide (Genscript) was added for stimulation. Ab00 or Ab20 (100 nM) was added to the treated wells. Half media changes with the cytokines IL-7 and IL-15 (final concentration 10 ng/mL) were performed on day 3 and day 5 of the PBMC culture. Supernatants collected on day 5 and day 7 were placed at -20° C for future cytokine analysis by Meso Scale Discovery (MSD) following the manufacturer's instructions. Cells at day 7 were harvested and stained for flow cytometry.

[0407] *Primary intestinal tissue organoid culturing:* Duodenal and colonic tissue was received from Celiac and Crohn's donors (Lord laboratory, Benoroya Research Institute) and processed for organoid generation following the digestion protocol. The tissue was resuspended in collagen matrix and divided into 6 trans-well membranes for organoid generation with appropriate supplemental growth factors. The organoids were allowed to establish growth for at least 9 days, and then media was replaced with media including antigenic stimulation (Celiac; gliadin peptides and Crohn's; flagellin and OmpC peptides) with or without addition of Ab00 or Ab20 for three to four days. Following incubation organoids were harvested and collagenase was

used to digest the Matrigel and immune and epithelial cell were stained following standard procedures.

[0408] *Activation of immune cell subsets in PBMC culture+/- anti-CD3:* Healthy, celiac, and Crohn's donor PBMCs were plated at 2e5/well in the presence of increasing doses of Ab00 or Ab20 with and without additional anti-CD3 (Ultra-LEAF purified anti-human CD3, clone OKT3, Biolegend) stimulation for 24 or 48 hours at concentrations as specified. Ab00- or Ab20-treated cells were then stained with anti-human Fc antibody (Jackson Immunoresearch Laboratories) to detect Ab00 or Ab20 binding to CD8 Treg or other immune cell populations following standard binding protocol procedures. Cells were also stained for surface phenotyping and activation (CD69 and ICOS) markers following incubations.

[0409] *Immune response to common pathogens:* PBMCs from 3 healthy donors (StemCell Technologies, Vancouver, BC, Canada) and 3 celiac donors (Sanguine Biosciences, Woburn, MA) were plated at 0.5e6 cells/well in 48-well plates and pulsed with 1 ug/ml pooled peptides from JPT (CEFT, Influenza A, SARS-CoV-2 or AAV5/6/8), 5 ug/ml Tetanus toxoid, 37.5 ug/ml gliadin peptides (12.5 ug/ml of each of 3 peptides), or DMSO alone. Cultures were fed fresh media with 5 ng/ml IL-2 on days 7 and 10 post peptide addition. On day 13, expanded PBMCs were combined in 96-well plates at a 1:1 ratio with newly thawed autologous PBMCs. Cultures were untreated, restimulated with the same antigen PBMCs were exposed to during the expansion phase, or stimulated with 100 ng/mL SEB (Fisher Scientific) as a positive control. Additionally, cultures were treated with 10 ug/ml (80.4 nM) Ab00 or Ab20 or media alone. Supernatants were collected at 5 days post-restimulation for cytokine analysis. Supernatants were diluted at 1:50. IFNγ levels were analyzed using 96-well single-spot plates from MSD. Standards and samples were run in duplicate following the manufacturer's instructions with the exception that the standard curve was adjusted to cover a broader range of concentrations (10-153500 pg/ml IFNγ). Plates were visualized on the MESO QuickPlex SQ 120 imager (MSD) and exported data was analyzed using MSD Discovery Workbench.

[0410] *Acute GVHD model:* Human NSG mice were randomized into groups by weight and engrafted with 1e7 healthy human donor PBMCs 4 hours post-irradiation at the dose as specified. One day post-engraftment, mice were treated with Saline or Ab00 or Ab20 intravenously and every 7 days for the duration as specified in study designs. Mice in the IL-2 control group were injected every other day intravenously with 25,000 IU IL-2 (R&D Systems) from Day 0 to Day 10. Abatacept (Jackson Laboratories) control mice received 5 mg/kg intraperitoneally every 48 hours starting on study day 0 to study day 26 for a total of 14 doses.

Whole blood was collected at the time points shown in the graphs for flow cytometry and stained for surface and extracellular markers following standard protocols. At clinical and study terminal endpoints spleen samples were also collected for flow cytometry.

[0411] *Exploratory Tolerability Study and Pharmacokinetic Assessment:* Female BALB/cJ (Stock number: 000651) aged 7-weeks (N=9) and NOD.Cg-Prkdc Il2rg Tg(IL15) /SzJ (CD34+ NSG-Tg(Hu-IL15)) (Stock number: 703089) at 12 weeks post engraftment (N=48) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Forty-eight NSG-Tg(Hu-IL15) mice at 4 weeks of age were engrafted with CD34+ cells from 2 donors (24 mice per donor; Donor 0818 and 0894). Twelve weeks post engraftment CD34+ NSG-Tg(Hu-IL15) were evaluated for human CD45 (hCD45) cells, CD3+ T cells, and CD56+ NK cells in the peripheral blood. The cut-off for hCD45 was 25%, 3% for CD3+ T cells, and 2% for CD56+ NK cells for enrolled mice. Predose bleed for CD34+ NSG-Tg(Hu-IL15) mice was performed at 15 weeks after engraftment. On the day of dosing, CD34+ NSG-Tg(Hu-IL15) animals were 16 weeks post engraftment. The two donor cohorts of female CD34+ NSG-Tg(Hu-IL15) mice were administered via tail-vein one dose of vehicle, Ab20, single arm KIR, or single arm CD8 as 5 mg/kg or 0.5 mg/kg OKT3 (n=3 per dosing group and donor). Mice were injected at a volume was 5 mL/kg. All animals were retro-orbitally bled under anesthesia at pre-dose (-336-hrs) and post-dose at 2, 24, 72-hrs. At terminal timepoint of 168-hrs, blood via cardiac puncture was collected from each animal under anesthesia. Samples were evaluated for PK, cytokine, and immunophenotyping. On the day of dosing, BALB/cJ mice were 8 weeks old. The female BALB/cJ (n=9) mice received a retro-orbital dose of 5 mg/kg Ab20 with injection volume of 100 µL (a volume was 5 mL/kg). Mice were bled sub-mentally post-dose at 0.5, 2, 6, 24, 48 and 96-hrs and terminally at study days (SD) 8, 14, 21, and 28. For timepoints nine mice were staggered for blood collection across groups of n=3. All blood samples were processed to serum and used for Pharmacokinetic (PK) analysis.

[0412] *MSD Cytokine Analysis:* Serum cytokine analysis was carried out by using human 6-spots U-PLEX MSD assay platform in assessing for IFN-γ, IL-2, IL-6, IL-10, MCP-1, and TNF-α according to manufacturer's instructions. The plate was read on Meso QuickPlex SQ 120MM and analyzed using MSD Discovery Workbench. Due to limitation in serum volume availability, each timepoint was conducted as single measurements.

[0413] *Results*

[0414] *CD8 Treg Expression and Activity*

[0415] In addition to KIR expression, CD8 Treg displayed higher expression of NKG2C, KLRG-1, and the transcription factor Helios relative to KIR negative CD8 T cells, supporting the identity of a distinct subset of CD8 T cells (FIG. 18A; see also Choi et al., 2023). A higher percentage of CD8 Tregs from healthy donor PBMCs were also positive for the cytolytic protein Granzyme B compared to KIR-negative non Treg CD8 T cells in the absence of stimulation (FIG. 18B), indicative of a high baseline cytolytic capacity and supportive of CD8 Treg functions previously observed (Li et al., 2022). Select gliadin peptide restimulation of CD4 T cells derived from human donors with celiac disease induced a corresponding expansion of purified CD8 Treg when cultured with CD4 T cells (FIG. 18C), consistent with data illustrating the expansion of CD8 Treg in response to high dose deamidated gluten (Li et al, 2022). The use of immunodominant gliadin epitopes supports that CD8 Treg can respond to oligoclonal pathogenic CD4 T cell responses in the absence of inflammatory responses derived from other cell types (Voisine and Abadie, 2021).

[0416] To confirm that CD8 Treg eliminate pathogenic CD4 T cells by direct killing as described (see, e.g., Li et al., 2022), a green fluorescent protein (GFP)-labeled CD4 target cell line engineered to express an alpha-gliadin peptide specific TCR was used. CD8 Treg suppressed gliadin-stimulated CD4 T cell expansion in a dose-dependent manner (FIG. 18D). CD8 Treg killing was specific to activated, gliadin-responsive CD4 T cells, as parental cells lacking the gliadin-responsive TCR were not eliminated in the presence of CD8 Treg even when activated (FIG. 18E). Confirming the phenotypic signature of CD8 Treg, cultures depleted of CD8 Treg did not eliminate gliadin-responsive CD4 target cells (FIG. 18F-18H). Together, these results illustrate that CD8 Treg derived from donors with autoimmune disease can directly and selectively kill pathogenic CD4 T cells in a dose-dependent fashion.

[0417] Bispecific Treg modulators could be used to target CD8 and KIR2DL1/2/3 on CD8 Treg cells, and increase cytolytic activity of dysfunctional cells (FIG. 18I).

[0418] *Ab20 Binds to KIR and CD8*

[0419] Ab20 was developed as a bispecific antibody containing a Fab that targets inhibitory KIR and a non-signaling, non-blocking scFv binding domain that targets CD8a (herein referred to as CD8). The binding, affinity, and kinetic rate constants of Ab20 were evaluated. Single target binding affinities to human CD8 or the three targeted isoforms of KIR2DL (KIR2DL1, KIR2DL2, and KIR2DL3) were found to be similar (FIG. 19A-B). Association and dissociation rate constants were measured for each binding interaction, and K_D binding affinity was calculated from the measured rate constants (FIG. 19A). As further support for the binding

of Ab20, the binding of Ab00 to inhibitory KIR and CD8 was tested in the context of on-cell binding using SKW cell lines stably transduced to overexpress KIR2DL (EC_{50} = 4.8nM) or CD8 (EC_{50}=4.1nM) (FIG. 19C). Binding of Ab00 to inhibitory KIR was specific to the intended KIR family members, as binding to unmodified or KIR3DL1 expressing HEK 293 cells was not detected (FIG. 19D). Restricted KIR binding by Ab20 was confirmed using a cell-based binding assay that screened Ab20 interactions with over 6500 surface proteins (FIG. 19E).

[0420] Sequential binding to CD8 and inhibitory KIR was verified to ensure the order of binding did not impact targeting. KIR2DL2 was utilized as the representative inhibitory KIR. In the first orientation, Ab20 was captured onto immobilized KIR2DL2, followed by CD8 antigen binding (FIG. 20A, left). For testing in the reverse orientation, Ab20 was captured onto immobilized CD8 then followed by KIR2DL2 antigen binding (FIG. 20A, right). These results demonstrated that both binding arms of Ab20 are functional and that Ab20 can simultaneously bind both inhibitory KIR and CD8 antigens. Co-binding of inhibitory KIR and CD8 by Ab00 was also observed in PBMC binding assays where Ab00 preferentially bound CD8 Treg relative to single target expressing NK or non-Treg CD8 T cells (FIG. 20B). As anticipated, no binding to CD4 T cells was observed at any concentration tested.

[0421] Ab00 binding measured by geometric mean fluorescence intensity (gMFI) on CD8 Treg is driven by the number of CD8 receptors (FIG. 20B), due to the significantly higher number of CD8 receptors on the cell surface relative to KIR in both cells derived from healthy and disease state donors (i.e., celiac disease; Crohn's disease) (FIG. 20C). Given that the proposed mechanism of action of Ab20 is dependent on blocking inhibitory KIR, the saturating concentration for KIR on CD8 Treg was determined. Using a labeled single arm KIR-Fc molecule to probe for unbound KIR following binding of increasing concentrations of Ab20, KIR saturation at a concentration near 100 nM was observed, with an IC50 of 5.5 nM (FIG. 20D).

[0422] *in vitro Assessments of Ab20*

[0423] To determine the functional consequences of Ab20 binding, PBMC derived from healthy donors and donors with celiac disease were treated with Ab20. In donors used for binding studies, PBMC had similar percentages of CD8 Treg (FIG. 21A), and Ab20 binding was similar in healthy donors and celiac donors who were following a gluten-free diet (FIG. 21B). Ab20 treatment increased CD8 Treg activation in celiac donor-derived PBMC, as measured by ICOS and CD69 expression (FIG. 21C). CD8 Treg activation was Ab20-dependent, as activation was not seen with the addition of an anti-RSV control antibody (FIG. 21D; see also Weisser et al., 2023).

[0424] The possibility of additional functional outcomes of antibody binding was assessed using Ab00. Ab00-mediated activation was found to be limited to CD8 Treg surface receptor modulation (FIG. 22A-22B) and killing of antigen-responsive CD4 T cells (FIG. 22C), as proinflammatory cytokines, IL-2, IFNg, and TNFa, IL-6, and IL-17, were not enhanced following low dose polyclonal T cell stimulation (FIG. 22D). Functional consequences of Ab00 binding were tested using PBMC derived from donors with celiac disease that were restimulated with gliadin peptides as in Figure 18. Ab00 increased the prevalence of CD8 Treg, as well as CD8 Treg cytolytic capacity and degranulation (FIG. 22E). Consistent with enhanced cytolysis of pathogenic CD4 T cell targets, Ab00-treated cocultures increased pathogenic CD4 T cell death and reduced markers of pathogenic CD4 T cell function, including markers of their activation (CD25) and production of pro-inflammatory cytokines, i.e., IFNg (FIG. 22F-22G).

[0425] The functional characterization of Ab20 was also tested using PBMC derived from donors with Crohn's disease. CD8 Treg are present in the peripheral blood of healthy and Crohn's disease donors (FIG. 23A). At baseline, Granzyme B and Helios were reduced in CD8 Treg derived from donors with Crohn's disease when compared to healthy donors (FIG. 23B), suggesting impaired cytolytic functions, with an increased CD4 T cell subset expressing the surface markers CXCR3, CD39, and CD161 (FIG. 23B, right), which are co-expressed on autoimmune pathogenic CD4 T cells (Christophersen et al., 2022). Pathogenic CD4 T cells were activated in a subset of PBMC derived from donors with Crohn's disease using a mixture of flagellin and OmpC peptides as antigens (Caderon-Gomez et al., 2016; Lodes et al., 2024; Morgan et al., 2021; Uchida et al., 2019). In antigenic peptide responders, Ab20 increased Granzyme B secretion, which was associated with a selective reduction of pathogenic CD4 T cell expansion and proinflammatory cytokines IFNg and TNFa (FIG. 23C). In contrast, polyclonal CD4 T cell responses were not inhibited (FIG. 23D, left). These in vitro data support a specific and selective Ab20 mechanism of action in pathogenic CD4 T cells derived from donors with autoimmune disease (i.e., celiac disease; Crohn's disease) when stimulated with their respective antigens.

[0426] To test the effects of Ab00 and Ab20 on tissue resident CD8 Treg, organoids derived from colonic tissue biopsies from patients with Crohn's disease and from duodenal tissue biopsies from patients with celiac disease were used. Organoids expand from primary intestinal tissue (FIG. 24A) and contain NK, B, and T cell populations (FIG. 24B), including CD8 Treg with phenotypes like those observed in peripheral blood (FIG. 24C). Epithelial cell death can be induced in tissues derived from patients without active inflammation using antigens that activate

CD4 T cells (Santos et al., under review; Dieterich et al., 2020). Upon antigenic peptide stimulation in both celiac and Crohn's patient-derived organoids, Ab20 bound and activated CD8 Treg (FIG. 24D), and reduced CD4 T cells and antigen-induced intestinal epithelial cell death (FIGS. 24D-24E). Additionally, Ab00 reduced proinflammatory cytokines (FIG. 24F). These results suggest that Ab20 can impact tissue resident CD8 Treg functions.

[0427] The observation that polyclonal CD4 T cell responses were unaffected in the presence of Ab20 (FIG. 23D) is consistent with a previous report demonstrating that mice deficient in CD8 Treg did not have impaired antiviral responses (Li et al., 2022). To extend these findings to human CD8 Treg responses, the effect of Ab20 on PBMC responses to viral and bacterial pathogens was examined. PBMC cytokine responses to a CEFT peptide cocktail (including immunodominant epitopes derived from cytomegalovirus [CMV], Epstein-Barr virus [EBV], influenza, and tetanus toxoid), influenza HA, Sars-Co-V-2, and adenoviral vectors (FIG. 25A), as well as to bacterial antigens derived from tetanus and Staphylococcus enterotoxin B (FIG. 25B) were unaltered in the presence of Ab20. Collectively, these data suggest that anti-inflammatory effects of Ab20 are specific for activated pathogenic CD4 T cells in autoimmune indications and are not expected to be broadly immunosuppressive.

[0428] *in vivo Assessment of Ab20 in a Humanized Transgenic Mouse Model*

[0429] Given that select NK cells express KIR and non-Treg CD8 T cells express CD8, the effect of single arm binding and the functional impact of Ab20 to CD8 and NK cells were tested in PBMC. While single arm binding was observed, no increase of CD8 T cell (FIG. 26A) or NK cell (FIG. 26B) activation relative to the untreated controls was observed in either resting or activated PBMC over a wide dose range of Ab20. These findings were confirmed in vivo using Ab20 in humanized NSG mice engrafted with CD34+ cells derived from human umbilical cord blood that are transgenic for human IL-15 cytokine (CD34+ NSG-Tg(Hu-IL15)) (Ayree et al., 2022). These mice have physiologic quantities of serum IL-15, which supports engraftment of a broad range of human immune cell subsets, including NK cells (FIG. 26C).

[0430] As observed in vitro, Ab20 had sustained binding to CD8 Tregs relative to single arm KIR and CD8 controls (FIG. 26D). Ab20 also bound total CD8 T cells and NK cells, which declined rapidly in comparison to CD8 Treg binding (FIG. 26D). Ab20 binding to CD8 Treg was greater than that detected on total CD8 T cells within terminal splenocyte samples (FIG. 26E), suggesting that engagement of both antibody targets supported sustained CD8 Treg binding. Despite binding to total CD8 T cells and NK cells, no increase in activation (FIG. 26F) or pro-inflammatory cytokines was detected (FIG. 26G).

[0431] *Ab20 Activity in a Mouse Model of Inflammation*

[0432] To evaluate antibody effects in vivo, Ab00 and Ab20 were tested in an aggressive, systemic inflammatory murine model of acute graft-versus-host disease (GvHD) that engrafts human T cells including CD8 Treg (FIG. 27A; see also King et al., 2009). In contrast to abatacept, which is FDA-approved for the treatment of rheumatoid arthritis and ablates lymphocyte engraftment (Blazer et al., 1994), Ab00 treatment did not reduce human PBMC engraftment in this model (FIG. 27B), making the humanized GvHD model ideal for evaluation of the impact of the bispecific antibodies on onset, progression, and severity over the course of disease.

[0433] Similar to observations in healthy donor engrafted (CD34+ NSG-Tg(Hu-IL15) mice, when administered intravenously weekly, Ab20 selectively bound to CD8 Treg in the peripheral blood and spleen, and binding was sustained between doses (FIG. 27C). Additionally, in this model of inflammation, binding of Ab20 or Ab00 resulted in the increase of CD8 Treg activation and cytolytic capacity (FIGS. 27D-27E), as well as the reduced proliferation and prevalence of activated CD4 T cells (FIG. 27F). Weekly Ab20 administration through day 28 caused a statistically significant delay of disease progression and extension of survival (FIG. 27G). In a separate study in the same GvHD mouse model, a reduction in serum concentrations of the proinflammatory cytokine, IFNγ (Ab00, FIG. 27H), clinical disease scores, and pathology in disease affected tissue were observed (Ab00, FIG. 27I). Results supporting the mechanism of action (including binding to CD8 Treg but not CD4 T cells; increase in CD8 Treg intracellular Granzyme B; increase in CD8 Treg prevalence, activation, and cytolytic capacity) were confirmed to be dose-dependent (Ab00, FIG. 27J). These observations corresponded to a dose-dependent reduction of activated CD4 and increase in CD4 T cell death in both the peripheral blood and spleens of Ab00-treated mice that persisted until study termination (FIG. 27K). Efficacy and mechanistic readouts were consistent across four repeat studies using three different donors (Ab00, FIGS. 28A-28H), including detection of Ab00 binding to CD8 Treg up to 7 days after the last 2 mg/kg dose. Collectively, these data support the anti-inflammatory effect and proposed mechanism of action for the antibodies that was observed in vitro.

[0434] *Discussion*

[0435] Pre-clinical characterization of Ab20, a CD8 Treg modulator that is a bispecific antibody directed toward CD8 and inhibitory KIR, was described. CD8 and KIR are co-expressed by CD8 Treg, a distinct CD8 cell subset, which recognize and specifically eliminate pathogenic CD4 T cells that drive pathogenic inflammatory responses. CD8 Treg exhibit specific

cytotoxic mechanisms, such as the release of cytotoxic molecules and induction of death in target CD4 T cells and maintain their selectivity for pathogenic CD4 T cells through oligoclonal TCR specificity and MHC class I/peptide interactions. The results extend previous work illustrating the dependence on CD8 Treg to control of EAE in a syngeneic model, and elimination of pathogenic gluten-responsive CD4 T cells in PBMC derived from donors with celiac disease (Saligrama et al., 2019; Li et al., 2022). In this example, a phenotypic signature (FIG. 18A) was linked to functional elimination using Incucyte live cell imaging (FIGS. 18D, 18F-18H), confirming direct pathogenic CD4 T cell lysis without elimination of polyclonally stimulated CD4 T cells (FIG. 18E).

[0436] Ab20-mediated inhibition of inhibitory KIR on CD8 Treg enhances their activation, Granzyme B content, and ability to eliminate activated pathogenic CD4 T cells derived from individuals with autoimmune disorders (FIGS. 21A-21C, 22E, 22F, 22G, 23B, 23C). This enhanced elimination of autoreactive CD4 T cells results in reduced antigen induced CD4 T cell activation and healthy epithelial cell death in tissues affected by autoimmune diseases (FIG. 23D) and in a persistently inflammatory mouse model (FIGS. 27A-27K). The mechanism of action of Ab20 aligns with the known biology of the CD8 Treg network (Li et al., 2022), suggesting that Ab20 effectively restores the primary function of CD8 Treg to eliminate potentially pathogenic cells.

[0437] The results also illustrate unique properties of Ab00 and Ab20 of selectively targeting a relatively rare population of CD8 T cells expressing inhibitory KIR, despite the presence of an abundance of single target-expressing non-Treg CD8 T and NK cells. By incorporating a non-functional CD8 targeting arm, a preference for binding to and activating CD8 Treg over non-Treg CD8 T and NK cells is observed (FIG. 20B), distinguishing it from the bivalent monoclonal antibody lirilumab, which elicits NK cell activation through preferential NK cell binding (Vey et al., 2018). Ab20 testing in humanized mouse models exhibited a favorable PK profile which is similar to what has been observed for monoclonal humanized antibodies (see Example 3), with no adverse activation of immune cells nor induction of pro-inflammatory serum cytokines (FIGS. 26D, 26F). The safety of inhibitory KIR blockade observed with Ab20 is consistent with the clinical testing of an inhibitory bivalent KIR monoclonal antibody tested in an oncology setting, in which there were no dose limiting toxicities observed following treatment (Vey et al., 2018).

[0438] The presence of detectable CD8 Treg populations in healthy donors implies the critical role of CD8 Treg functions in controlling the expansion of potentially harmful CD4 T

cells, a mechanism that if dysregulated could contribute to the onset and lack of control of autoimmune disease pathology as observed in mice deficient in CD8 Treg (Li et al., 2022). In individuals with autoimmune conditions, sustained or repeated expansion and elimination of pathogenic CD4 T cells during the disease may further impair CD8 Treg function. In addition, the inadequate functioning of CD8 Treg cells seems to be different from functional exhaustion, as the failure to control pathogenic CD4 T cells persists despite the cytolytic capacity retained by autoimmune patient CD8 Treg and their ability to regain cytolytic functions upon activation.

[0439] The association of genomic expression of KIR with various autoimmune diseases (Schweiger et al., 2014; Diaz-Pena et al., 2015), coupled with the expression of these receptors by CD8 Treg, indicates a potential role of KIR to regulate CD8 Treg functions in certain contexts. KIR, which are known to maintain and regulate NK cell self-tolerance (reviewed in Pende et al., 2019), function by competing with the TCR for binding to the MHC class I/peptide complex, as well as initiating an inhibitory signaling cascade that counteracts activating stimuli (Djaoud and Parham, 2020). In CD8 Treg, KIR may function as an autoimmune checkpoint that can prevent appropriate TCR signaling, activation, and cytotoxicity.

[0440] The presence or expansion of functional CD8 Treg has been linked to the reduction of inflammation and amelioration of disease severity in various experimental models, as evidenced by studies that selectively deplete (Li et al., 2022) or adoptively transfer CD8 Treg (Saligrama et al., 2019). An increased prevalence of CD8 Treg has been described in patients with autoimmune disease that recover functions when activated ex vivo (Li et al., 2022), and increases of functional CD8 Treg are associated with improved outcomes in patients treated with immune system-targeted therapies such as teplizumab (Herold et al., 2019), fingolimod (Houston et al., 2023), and low-dose IL-2 (La Cava et al., 2023).

[0441] Targeting a dysfunctional CD8 Treg compartment represents a promising therapeutic strategy with a sustained and durable impact on inflammation in patients with autoimmune disease. This approach has the potential to restore CD8 Treg function to selectively reduce pathogenic CD4 T cells without the broad immune suppression or off-target toxicities that can be associated with the use of steroids or other non-specific immune-suppressive interventions. Collectively, these findings support Ab20 as a promising therapeutic agent for restoring immune balance and targeting autoreactive cells in autoimmune diseases.

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SEQUENCES

[0443] Underlined portions of sequences are complementarity-determining regions (CDRs) according to Chothia numbering

Table 17. Sequences.

SEQ ID NO:	Description	Sequence
1	Anti-CD8 CDRL1	RTSRSISQYLA
2	Anti-CD8 CDRL2	SGSTLQS
3	Anti-CD8 CDRL3	QQHNENPLT
4	Anti-CD8 CDRH1	GFNIKDT
5	Anti-CD8 CDRH2	RIDPANDNT
6	Anti-CD8 CDRH3	GYYVFDH
7	Anti-CD8 VL, parental	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
8	Anti-CD8 VH, parental	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
9	Anti-CD8 VL G100C (AHo G141C) substituted	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
10	Anti-CD8 VH I29F (AHo I31F) substituted	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
11	Anti-CD8 VH G44C (AHo G51C) substituted	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
12	Anti-CD8 VH F64V (AHo F74V) substituted	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGKATISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
13	Anti-CD8 VH A68F (AHo A78F) substituted	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGK**F**TISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
14	Anti-CD8 VH A72R (AHo A82R) substituted	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS

15	Anti-CD8 VH G97A (AHo G107A) substituted	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
16	Anti-CD8 VH FR3, parental	LYASK**F**QGK**A**TISADTSKNTAYLQMNSLRAEDTAVYYC**G**RGY
17	Anti-CD8 VL, variant 1	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
18	Anti-CD8 VH, variant 1	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
19	Anti-CD8 VL, variant 2	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
20	Anti-CD8 VH, variant 2	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
21	Anti-CD8 VL, variant 3	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
22	Anti-CD8 VH, variant 3	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
23	Anti-CD8 VL, variant 4	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
24	Anti-CD8 VH, variant 4	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
25	Anti-CD8 VL, variant 5	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
26	Anti-CD8 VH, variant 5	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
27	Anti-CD8 VL, variant 6	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
28	Anti-CD8 VH, variant 6	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
29	Anti-CD8 VL, variant 7	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
30	Anti-CD8 VH, variant 7	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS

31	Anti-CD8 VL, variant 8	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
32	Anti-CD8 VH, variant 8	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
33	Anti-CD8 VL, variant 9	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
34	Anti-CD8 VH, variant 9	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
35	Anti-CD8 VL, variant 10	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
36	Anti-CD8 VH, variant 10	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
37	Anti-CD8 VL, variant 11	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIK
38	Anti-CD8 VH, variant 11	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKG LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
39	Anti-CD8 VL, variant 12	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
40	Anti-CD8 VH, variant 12	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
41	Anti-CD8 VL, variant 13	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
42	Anti-CD8 VH, variant 13	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
43	Anti-CD8 VL, variant 14	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
44	Anti-CD8 VH, variant 14	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
45	Anti-CD8 VL, variant 15	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
46	Anti-CD8 VH, variant 15	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
47	Anti-CD8 VL, variant 16	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK

48	Anti-CD8 VH, variant 16	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
49	Anti-CD8 VL, variant 17	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
50	Anti-CD8 VH, variant 17	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
51	Anti-CD8 VL, variant 18	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
52	Anti-CD8 VH, variant 18	EVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
53	Anti-CD8 VL, variant 19	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPE DVATYYCQQHNENPLTFG**C**GTKVEIK
54	Anti-CD8 VH, variant 19	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
55	Anti-CD8 VL, variant 20	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
56	Anti-CD8 VH, variant 20	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
57	Anti-CD8 VL, variant 21	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIK
58	Anti-CD8 VH, variant 21	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRA EDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSS
59	Anti-CD8 VL, variant 22	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPE DVATYYCQQHNENPLTFG**C**GTKVEIK
60	Anti-CD8 VH, variant 22	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSS
61	Linker	GSTSGGGSGGGSGGGGSS
62	Anti-CD8 Constant	EPKSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQ VCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
63	Anti-KIR CDRL1	RASQSVSSYLA
64	Anti-KIR CDRL2	DASNRAT

65	Anti-KIR CDRL3	QQRSNWMYTF
66	Anti-KIR CDRH1	FYAIS
67	Anti-KIR CDRH2	GFIPIFGAANYAQKF
68	Anti-KIR CDRH3	IPSGSYYYDYDMDV
69	Anti-KIR VL	EIVLTQSPVTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQRSNWMYTFGQGTKLEIKR
70	Anti-KIR VH	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSFYAISWVRQAPGQG LEWMGGFIPIFGAANYAQKFQGRVTITADESTSTAYMELSSLRS DDTAVYYCARIPSGSYYYDYDMDVWGQGTTVTVSS
71	Signal Sequence	MGWSCIILFLVATATGVHS
72	Parental Anti-KIR/Anti-CD8 KIR Arm Light Chain	EIVLTQSPVTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQRSNWMYTFGQGTKLEIKRTVAAPSVFIFPPSDEKLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
73	Parental Anti-KIR/Anti-CD8 KIR Arm Light Chain with Signal	MGWSCIILFLVATATGVHSEIVLTQSPVTLSLSPGERATLSCRA SQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGT DFTLTISSLEPEDFAVYYCQQRSNWMYTFGQGTKLEIKRTVAAP SVFIFPPSDEKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
74	Parental Anti-KIR/Anti-CD8 KIR Arm Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSFYAISWVRQAPGQG LEWMGGFIPIFGAANYAQKFQGRVTITADESTSTAYMELSSLRS DDTAVYYCARIPSGSYYYDYDMDVWGQGTTVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREP QVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
75	Parental Anti-KIR/Anti-CD8 KIR Arm Heavy Chain with Signal	MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGSSVKVSCKAS GGTFSFYAISWVRQAPGQGLEWMGGFIPIFGAANYAQKFQGRVT ITADESTSTAYMELSSLRSDDTAVYYCARIPSGSYYYDYDMDVW GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK

76	Parental Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVE SGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGR IDPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVY YCGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAE GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
77	Parental Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
78	Variant Anti-KIR/Anti-CD8 KIR Arm Light Chain	EIVLTQSPVTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQRSNWMYTFGQGTKLEIKRTVAAPSVFIFPPSDEKLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
79	Variant Anti-KIR/Anti-CD8 KIR Arm Light Chain with Signal	MGWSCIILFLVATATGVHSEIVLTQSPVTLSLSPGERATLSCRA SQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGT DFTLTISSLEPEDFAVYYCQQRSNWMYTFGQGTKLEIKRTVAAP SVFIFPPSDEKLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
80	Variant Anti-KIR/Anti-CD8 KIR Arm Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSFYAISWVRQAPGQG LEWMGGFIPIFGAANYAQKFQGRVTITADESTSTAYMELSSLRS DDTAVYYCARIPSGSYYYDYDMDVWGQGTTVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVEDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREP QVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
81	Variant Anti-KIR/Anti-CD8 KIR Arm Heavy Chain with Signal	MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGSSVKVSCKAS GGTFSFYAISWVRQAPGQGLEWMGGFIPIFGAANYAQKFQGRVT ITADESTSTAYMELSSLRSDDTAVYYCARIPSGSYYYDYDMDVW GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVEDYFP EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAEGAPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE

		VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPSSIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
82	Variant 1 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVE SGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKGLEWIGR IDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRAEDTAVY YC**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAE GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
83	Variant 1 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
84	Variant 2 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
85	Variant 2 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
86	Variant 3 Anti-	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC

	KIR/Anti-CD8 CD8 Arm	QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
87	Variant 3 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
88	Variant 4 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
89	Variant 4 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATIS**R**DTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
90	Variant 5 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS

		NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
91	Variant 5 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
92	Variant 6 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
93	Variant 6 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
94	Variant 7 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
95	Variant 7 Anti-KIR/Anti-CD8	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG

	CD8 Arm with Signal	GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATIS**R**DTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
96	Variant 8 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
97	Variant 8 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATISADTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
98	Variant 9 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
99	Variant 9 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC

		TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
100	Variant 10 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
101	Variant 10 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
102	Variant 11 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
103	Variant 11 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFGGGTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGKGLEWIGRIDPANDNTLYASKFQGKATIS**R**DTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
104	Variant 12 Anti-	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVE

	KIR/Anti-CD8 CD8 Arm	SGGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C**LEWIGR IDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRAEDTAVY YCGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAE GAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
105	Variant 12 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
106	Variant 13 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
107	Variant 13 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
108	Variant 14 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA

		VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
109	Variant 14 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
110	Variant 15 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
111	Variant 15 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
112	Variant 16 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
113	Variant 16 Anti-KIR/Anti-CD8	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT

	CD8 Arm with Signal	YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
114	Variant 17 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
115	Variant 17 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
116	Variant 18 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFN**F**KDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
117	Variant 18 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFN**F**KDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASKFQGKATISADTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT

		TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
118	Variant 19 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQ YLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGTDFTLTI SSLQPE DVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSS EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C** LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSKNTAYLQMNSLRA EDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPC PAPEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
119	Variant 19 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASK**V**QGK**F**TIS**R**DTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
120	Variant 20 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASK**V**QGK**F**TISADTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
121	Variant 20 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASK**V**QGK**F**TISADTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
122	Variant 21 Anti-	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES

	KIR/Anti-CD8 CD8 Arm	GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASKFQGKATIS**R**DTSKNTAYLQMNSLRAEDTAVYY C**A**RGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
123	Variant 21 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASKFQGKATIS**R**DTSK NTAYLQMNSLRAEDTAVYYC**A**RGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
124	Variant 22 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFG**C**GTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGK**C**LEWIGRI DPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
125	Variant 22 Anti-KIR/Anti-CD8 CD8 Arm with Signal	MGWSCIILFLVATATGVHSDVQITQSPSSLSASVGDRVTITCRT SRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGSGSGT DFTLTISSLQPEDVATYYCQQHNENPLTFG**C**GTKVEIKGSTSGG GSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASGFNIKDT YIHFVRQAPGK**C**LEWIGRIDPANDNTLYASKFQGKATISADTSK NTAYLQMNSLRAEDTAVYYCGRGYGYYVFDHWGQGTLVTVSSEP KSSDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
126	Anti-CD8 VL FR4, G100C substituted	FG**C**GTKVEIK
127	Anti-CD8 VH CDR1, I29F substituted	GFN**F**KDT
128	Anti-CD8 VH FR2, G44C substituted	YIHFVRQAPGK**C**LEWIG

129	Anti-CD8 VH FR3, F64V substituted	LYASK**V**QGKATISADTSKNTAYLQMNSLRAEDTAVYYCGRGY
130	Anti-CD8 VH FR3, A68F substituted	LYASKFQGK**F**TISADTSKNTAYLQMNSLRAEDTAVYYCGRGY
131	Anti-CD8 VH FR3, A72R substituted	LYASKFQGKATIS**R**DTSKNTAYLQMNSLRAEDTAVYYCGRGY
132	Anti-CD8 VH FR3, G97A substituted	LYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYYC**A**RGY
133	ANTI-CD8 VL, PARENTAL WITH SUBSTITUTION	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGXGTKVEIK wherein X = G or C
134	Anti-CD8 VH, parental with Substitution	EVQLVESGGGLVQPGGSLRLSCAASGFNX$_1$KDTYIHFVRQAPGK X$_2$LEWIGRIDPANDNTLYASKX$_3$QGKX$_4$TISX$_5$DTSKNTAYLQMN SLRAEDTAVYYCX$_6$RGYGYYVFDHWGQGTLVTVSS wherein (a) X$_1$ = I or F; (b) X$_2$= G or C; (c) X$_3$ = F or V; (d) X$_4$ = A or F; (e) X$_5$ = A or R; and (f) X$_6$ = G or A
135	Parental 2 Anti-KIR/Anti-CD8 KIR Arm Light Chain	EIVLTQSPVTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQRSNWMYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
136	Parental 2 Anti-KIR/Anti-CD8 KIR Arm Heavy Chain	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSFYAISWVRQAPGQG LEWMGGFIPIFGAANYAQKFQGRVTITADESTSTAYMELSSLRS DDTAVYYCARIPSGSYYYDYDMDVWGQGTTVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPEAEGAPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQPREP QVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
137	Parental 2 Anti-KIR/Anti-CD8 CD8 Arm	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVP KLLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQHNENPLTFGGGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVES GGGLVQPGGSLRLSCAASGFNIKDTYIHFVRQAPGKGLEWIGRI DPANDNTLYASKFQGKATISADTSKNTAYLQMNSLRAEDTAVYY CGRGYGYYVFDHWGQGTLVTVSSEPKSSDKTHTCPPCPAPEAEG APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPSSIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

138	Anti-KIR arm light chain DNA sequence, with signal	ATGGGTTGGAGTTGCATCATCCTATTTCTAGTCGCTACCGCTAC AGGCGTGCATTCTGAAATCGTGCTGACCCAGTCCCCCGTGACAC TGAGCCTGTCTCCCGGAGAACGCGCTACCCTCAGCTGCAGAGCT TCTCAGTCTGTGTCCTCCTATCTGGCCTGGTACCAGCAGAAGCC TGGCCAGGCCCCTCGGCTGCTGATCTACGACGCCTCTAATCGGG CCACCGGCATCCCTGCTAGATTCTCCGGCTCTGGCTCCGGCACC GACTTCACCCTGACCATCTCCTCTCTGGAACCTGAGGATTTTGC CGTGTACTACTGCCAGCAGAGATCCAACTGGATGTACACCTTTG GCCAAGGCACAAAACTGGAGATCAAGCGGACCGTGGCTGCTCCT TCCGTGTTCATCTTCCCTCCATCTGATGAAAAGCTGAAGTCCGG AACCGCCTCCGTGGTGTGCCTGCTGAACAACTTCTACCCCAGAG AGGCCAAAGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGC AACTCCCAAGAGTCCGTCACCGAGCAGGACTCTAAGGACTCCAC CTACTCCCTGAGCAGCACACTGACCCTGTCCAAGGCCGACTACG AGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGCCTG TCTTCTCCTGTGACCAAGTCCTTCAACAGAGGCGAGTGTTGA
139	Anti-KIR arm light chain DNA sequence, without signal	GAAATCGTGCTGACCCAGTCCCCCGTGACACTGAGCCTGTCTCC CGGAGAACGCGCTACCCTCAGCTGCAGAGCTTCTCAGTCTGTGT CCTCCTATCTGGCCTGGTACCAGCAGAAGCCTGGCCAGGCCCCT CGGCTGCTGATCTACGACGCCTCTAATCGGGCCACCGGCATCCC TGCTAGATTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGA CCATCTCCTCTCTGGAACCTGAGGATTTTGCCGTGTACTACTGC CAGCAGAGATCCAACTGGATGTACACCTTTGGCCAAGGCACAAA ACTGGAGATCAAGCGGACCGTGGCTGCTCCTTCCGTGTTCATCT TCCCTCCATCTGATGAAAAGCTGAAGTCCGGAACCGCCTCCGTG GTGTGCCTGCTGAACAACTTCTACCCCAGAGAGGCCAAAGTGCA GTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACTCCCAAGAGT CCGTCACCGAGCAGGACTCTAAGGACTCCACCTACTCCCTGAGC AGCACACTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGT GTACGCCTGCGAGGTGACCCACCAGGGCCTGTCTTCTCCTGTGA CCAAGTCCTTCAACAGAGGCGAGTGTTGA
140	Anti-KIR arm heavy chain DNA sequence, with signal	ATGGGTTGGAGTTGCATCATCCTATTTCTAGTCGCGACCGCTAC CGGCGTGCACTCTCAAGTGCAGCTGGTGCAGTCTGGAGCTGAGG TGAAGAAACCCGGCTCCTCCGTGAAAGTTTCCTGTAAGGCCTCC GGCGGCACTTTCAGCTTCTACGCCATCAGCTGGGTGCGGCAGGC CCCAGGCCAAGGACTGGAATGGATGGGCGGCTTCATCCCCATCT TCGGCGCCGCCAATTATGCTCAGAAGTTCCAGGGCAGAGTGACC ATTACCGCCGACGAATCCACCAGCACAGCCTACATGGAACTGTC TTCTCTGAGATCCGACGACACCGCTGTGTACTACTGCGCCAGAA TCCCTTCTGGCAGCTACTACTACGACTACGATATGGACGTGTGG GGCCAGGGCACCACCGTTACCGTGTCCTCCGCCTCTACCAAGGG CCCTTCTGTGTTTCCTCTGGCCCCTAGTTCCAAATCCACCTCTG GCGGAACAGCTGCTCTCGGTTGCCTGGTGGAAGATTACTTCCCA GAGCCTGTGACCGTGTCTTGGAACTCCGGAGCTCTGACCTCCGG CGTGCATACCTTTCCCGCCGTGCTGCAGTCTTCCGGCCTGTACT CTCTGTCCAGCGTGGTCACAGTGCCTAGCAGCTCTCTGGGCACA CAGACCTACATCTGCAACGTGAACCACAAGCCTTCCAACACCAA GGTGGACAAGAAGGTGGAGCCCAAATCCTGCGATAAGACCCACA CCTGTCCTCCTTGTCCTGCCCCCGAGGCTGAGGGCGCTCCTTCC GTGTTTCTGTTCCCTCCTAAGCCCAAGGACACCCTGATGATCTC TCGGACCCCTGAGGTGACATGTGTGGTGGTGGATGTCTCTCACG AGGACCCAGAGGTGAAGTTCAACTGGTACGTAGACGGAGTGGAA

		GTGCACAATGCTAAGACCAAGCCTAGAGAGGAACAGTACAACTC AACCTACCGGGTGGTGTCTGTCCTGACAGTGCTGCACCAGGACT GGCTGAACGGCAAAGAGTACAAGTGCAAAGTCTCGAACAAGGCT CTGCCTTCCTCCATCGAGAAGACCATCTCCAAGGCCAAGGGCCA GCCTCGCGAACCTCAAGTGTACACACTGCCCCCTTGCCGGGACG AGCTGACCAAGAACCAGGTGAGCCTGTGGTGCCTCGTCAAGGGC TTCTACCCTTCTGACATCGCCGTGGAATGGGAGTCTAACGGCCA GCCTGAGAACAACTATAAGACTACCCCACCAGTGCTGGACTCCG ACGGCAGCTTCTTCCTGTACTCCAAGCTGACCGTGGATAAGTCC AGATGGCAGCAGGGCAACGTGTTCTCTTGCTCCGTGATGCACGA GGCCCTGCATAATCACTACACCCAGAAGTCCCTGTCCCTGTCCC CTGGCAAGTGA
141	Anti-KIR arm heavy chain DNA sequence, without signal	CAAGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAACCCGG CTCCTCCGTGAAAGTTTCCTGTAAGGCCTCCGGCGGCACTTTCA GCTTCTACGCCATCAGCTGGGTGCGGCAGGCCCCAGGCCAAGGA CTGGAATGGATGGGCGGCTTCATCCCCATCTTCGGCGCCGCCAA TTATGCTCAGAAGTTCCAGGGCAGAGTGACCATTACCGCCGACG AATCCACCAGCACAGCCTACATGGAACTGTCTTCTCTGAGATCC GACGACACCGCTGTGTACTACTGCGCCAGAATCCCTTCTGGCAG CTACTACTACGACTACGATATGGACGTGTGGGGCCAGGGCACCA CCGTTACCGTGTCCTCCGCCTCTACCAAGGGCCCTTCTGTGTTT CCTCTGGCCCCTAGTTCCAAATCCACCTCTGGCGGAACAGCTGC TCTCGGTTGCCTGGTGGAAGATTACTTCCCAGAGCCTGTGACCG TGTCTTGGAACTCCGGAGCTCTGACCTCCGGCGTGCATACCTTT CCCGCCGTGCTGCAGTCTTCCGGCCTGTACTCTCTGTCCAGCGT GGTCACAGTGCCTAGCAGCTCTCTGGGCACACAGACCTACATCT GCAACGTGAACCACAAGCCTTCCAACACCAAGGTGGACAAGAAG GTGGAGCCCAAATCCTGCGATAAGACCCACACCTGTCCTCCTTG TCCTGCCCCCGAGGCTGAGGGCGCTCCTTCCGTGTTTCTGTTCC CTCCTAAGCCCAAGGACACCCTGATGATCTCTCGGACCCCTGAG GTGACATGTGTGGTGGTGGATGTCTCTCACGAGGACCCAGAGGT GAAGTTCAACTGGTACGTAGACGGAGTGGAAGTGCACAATGCTA AGACCAAGCCTAGAGAGGAACAGTACAACTCAACCTACCGGGTG GTGTCTGTCCTGACAGTGCTGCACCAGGACTGGCTGAACGGCAA AGAGTACAAGTGCAAAGTCTCGAACAAGGCTCTGCCTTCCTCCA TCGAGAAGACCATCTCCAAGGCCAAGGGCCAGCCTCGCGAACCT CAAGTGTACACACTGCCCCCTTGCCGGGACGAGCTGACCAAGAA CCAGGTGAGCCTGTGGTGCCTCGTCAAGGGCTTCTACCCTTCTG ACATCGCCGTGGAATGGGAGTCTAACGGCCAGCCTGAGAACAAC TATAAGACTACCCCACCAGTGCTGGACTCCGACGGCAGCTTCTT CCTGTACTCCAAGCTGACCGTGGATAAGTCCAGATGGCAGCAGG GCAACGTGTTCTCTTGCTCCGTGATGCACGAGGCCCTGCATAAT CACTACACCCAGAAGTCCCTGTCCCTGTCCCCTGGCAAGTGA
142	Anti-CD8a arm DNA sequence, with signal	ATGGGTTGGAGTTGCATCATCCTATTTCTAGTCGCTACCGCTAC CGGCGTGCATTCTGACGTGCAGATCACCCAGTCTCCTTCCAGCC TGTCTGCTTCTGTGGGCGACAGAGTGACAATCACCTGCCGGACC TCTCGCAGCATTAGCCAGTACCTGGCCTGGTACCAGCAAAAGCC CGGAAAGGTGCCTAAGCTGCTGATCTACTCCGGCTCTACCCTGC AGTCCGGCGTTCCTTCACGGTTCTCTGGCTCCGGCTCCGGCACC GACTTCACCCTGACCATCTCCTCTCTGCAGCCTGAGGACGTGGC CACCTATTACTGCCAGCAGCACAACGAGAATCCTCTGACCTTCG GCTGTGGCACAAAAGTGGAAATCAAGGGCTCCACATCCGGCGGC

		GGCTCTGGCGGAGGATCTGGCGGAGGCGGCTCGTCGGAAGTGCA GCTGGTGGAATCTGGCGGCGGCCTGGTGCAGCCTGGCGGTTCTC TAAGACTGTCCTGTGCCGCCTCCGGCTTTAACATCAAGGACACC TACATCCACTTCGTGCGGCAGGCCCCTGGAAAGTGCCTGGAATG GATCGGCAGAATCGACCCTGCCAACGACAACACCCTGTACGCTA GCAAGGTGCAGGGAAAGTTTACCATCTCTGCCGACACCTCCAAA AACACCGCCTACCTGCAGATGAACTCCCTGCGGGCCGAGGATAC CGCCGTGTACTACTGCGCCAGAGGCTACGGCTACTACGTGTTCG ACCACTGGGGCCAGGGCACCTTGGTGACCGTGTCTTCTGAGCCC AAGAGCTCCGACAAGACCCACACCTGTCCTCCTTGTCCCGCTCC AGAAGCTGAGGGCGCTCCTTCCGTGTTTCTGTTCCCTCCAAAAC CTAAGGATACTCTGATGATCAGTAGAACCCCTGAAGTCACATGC GTGGTGGTGGATGTGTCCCACGAGGATCCTGAGGTCAAGTTCAA CTGGTATGTGGACGGCGTGGAAGTGCACAATGCCAAGACCAAGC CAAGAGAAGAGCAGTACAACTCCACCTACCGGGTGGTATCCGTG CTGACCGTCCTGCACCAAGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTGTCCAACAAGGCTCTGCCCAGCTCTATCGAGAAGA CCATCTCCAAGGCCAAGGGACAACCTAGAGAGCCTCAGGTGTGC ACACTGCCTCCGTCTAGGGATGAGCTGACCAAGAACCAGGTGTC TCTGAGCTGCGCTGTGAAGGGCTTCTACCCCTCCGACATCGCCG TCGAGTGGGAGTCCAATGGCCAGCCAGAGAACAACTACAAAACC ACCCCTCCTGTGCTGGACTCCGATGGCTCCTTCTTCCTGGTGTC CAAGCTGACAGTGGACAAGTCTAGATGGCAGCAGGGCAACGTGT TCTCCTGCTCCGTGATGCACGAGGCCCTGCATAACCACTACACC CAGAAGTCTCTGTCTCTCAGCCCCGGCAAGTGA
143	Anti-CD8a arm DNA sequence, without signal	GACGTGCAGATCACCCAGTCTCCTTCCAGCCTGTCTGCTTCTGT GGGCGACAGAGTGACAATCACCTGCCGGACCTCTCGCAGCATTA GCCAGTACCTGGCCTGGTACCAGCAAAAGCCCGGAAAGGTGCCT AAGCTGCTGATCTACTCCGGCTCTACCCTGCAGTCCGGCGTTCC TTCACGGTTCTCTGGCTCCGGCTCCGGCACCGACTTCACCCTGA CCATCTCCTCTCTGCAGCCTGAGGACGTGGCCACCTATTACTGC CAGCAGCACAACGAGAATCCTCTGACCTTCGGCTGTGGCACAAA AGTGGAAATCAAGGGCTCCACATCCGGCGGCGGCTCTGGCGGAG GATCTGGCGGAGGCGGCTCGTCGGAAGTGCAGCTGGTGGAATCT GGCGGCGGCCTGGTGCAGCCTGGCGGTTCTCTAAGACTGTCCTG TGCCGCCTCCGGCTTTAACATCAAGGACACCTACATCCACTTCG TGCGGCAGGCCCCTGGAAAGTGCCTGGAATGGATCGGCAGAATC GACCCTGCCAACGACAACACCCTGTACGCTAGCAAGGTGCAGGG AAAGTTTACCATCTCTGCCGACACCTCCAAAAACACCGCCTACC TGCAGATGAACTCCCTGCGGGCCGAGGATACCGCCGTGTACTAC TGCGCCAGAGGCTACGGCTACTACGTGTTCGACCACTGGGGCCA GGGCACCTTGGTGACCGTGTCTTCTGAGCCCAAGAGCTCCGACA AGACCCACACCTGTCCTCCTTGTCCCGCTCCAGAAGCTGAGGGC GCTCCTTCCGTGTTTCTGTTCCCTCCAAAACCTAAGGATACTCT GATGATCAGTAGAACCCCTGAAGTCACATGCGTGGTGGTGGATG TGTCCCACGAGGATCCTGAGGTCAAGTTCAACTGGTATGTGGAC GGCGTGGAAGTGCACAATGCCAAGACCAAGCCAAGAGAAGAGCA GTACAACTCCACCTACCGGGTGGTATCCGTGCTGACCGTCCTGC ACCAAGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCC AACAAGGCTCTGCCCAGCTCTATCGAGAAGACCATCTCCAAGGC CAAGGGACAACCTAGAGAGCCTCAGGTGTGCACACTGCCTCCGT CTAGGGATGAGCTGACCAAGAACCAGGTGTCTCTGAGCTGCGCT

		GTGAAGGGCTTCTACCCCTCCGACATCGCCGTCGAGTGGGAGTC CAATGGCCAGCCAGAGAACAACTACAAAACCACCCCTCCTGTGC TGGACTCCGATGGCTCCTTCTTCCTGGTGTCCAAGCTGACAGTG GACAAGTCTAGATGGCAGCAGGGCAACGTGTTCTCCTGCTCCGT GATGCACGAGGCCCTGCATAACCACTACACCCAGAAGTCTCTGT CTCTCAGCCCCGGCAAGTGA

[0444] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications, and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including U.S. Provisional Patent Application Nos. 63/504,168 filed May 24, 2023, 63/507,332 filed June 9, 2023, 63/596,911 filed November 7, 2023, 63/560,531 filed March 1, 2024, and 63/645,271 filed May 10, 2024, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications, and publications to provide yet further embodiments.

[0446] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:56;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:56; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:55; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:56.

2. A binding protein comprising: a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGS GSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGXGTKVEIK (SEQ ID NO:133), wherein: X = G or C.

3. The binding protein of claim 2, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to RTSRSISQYLA (SEQ ID NO:1), SGSTLQS (SEQ ID NO:2), and QQHNENPLT (SEQ ID NO:3).

4. The binding protein of claim 2, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

5. A binding protein comprising: a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to EVQLVESGGGLVQPGGSLRLSCAASGFNX$_1$KDTYIHFVRQAPGKX$_2$LEWIGRIDPANDNTLYASK X$_3$QGKX$_4$TISX$_5$DTSKNTAYLQMNSLRAEDTAVYYCX$_6$RGYGYYVFDHWGQGTLVTVSS (SEQ ID NO:134), wherein:

(a) X_1 = I or F;

(b) X_2= G or C;

(c) X_3 = F or V;

(d) X_4 = A or F;

(e) X_5 = A or R; and

(f) X_6 = G or A.

6. The binding protein of claim 5, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to GFNIKDT (SEQ ID NO:4), RIDPANDNT (SEQ ID NO:5), and GYYVFDH (SEQ ID NO:6), respectively.

7. The binding protein of claim 5, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

8. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to

DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKVPKLLIYSGSTLQSGVPSRFSGS GSGTDFTLTISSLQPEDVATYYCQQHNENPLTFGXGTKVEIK (SEQ ID NO:133),

wherein: X = G or C; and

(b) a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to

EVQLVESGGGLVQPGGSLRLSCAASGFNX$_1$KDTYIHFVRQAPGKX$_2$LEWIGRIDPANDNTLYASK X$_3$QGKX$_4$TISX$_5$DTSKNTAYLQMNSLRAEDTAVYYCX$_6$RGYGYYVFDHWGQGTLVTVSS (SEQ ID NO:144),

wherein:

(1) X_1 = I or F;

(2) X_2= G or C;

(3) X_3 = F or V;

(4) X_4 = A or F;

(5) X_5 = A or R; and

(6) X_6 = G or A.

9. The binding protein of claim 8, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to RTSRSISQYLA (SEQ ID NO:1), SGSTLQS (SEQ ID NO:2), and (QQHNENPLT) SEQ ID NO:3.

10. The binding protein of claim 8, wherein the VL has CDRL1, CDRL2, and CDRL3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

11. The binding protein of any one of claims 8-10, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to GFNIKDT (SEQ ID NO:4), RIDPANDNT (SEQ ID NO:5), and GYYVFDH (SEQ ID NO:6), respectively.

12. The binding protein of any one of claims 8-10, wherein the VH has CDRH1, CDRH2, and CDRH3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

13. A binding protein comprising:

(a) CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively; and

(b) one or more of the following framework regions:

(i) a light chain FR4 according to SEQ ID NO:126;

(ii) a heavy chain FR2 according to SEQ ID NO:128;

(iii) a heavy chain FR3 according to SEQ ID NO:129;

(iv) a heavy chain FR3 according to SEQ ID NO:130;

(v) a heavy chain FR3 according to SEQ ID NO:131;

(vi) a heavy chain FR3 according to SEQ ID NO:132; and

(vii) a heavy chain FR3 according to SEQ ID NO:16 with one or more of the following substitutions: F6V, A10F, A14R, and G39A, according to the position of the amino acid within SEQ ID NO:16.

14. A binding protein comprising:

(a) CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:127, SEQ ID NO:5, and SEQ ID NO:6, respectively; and

(b) one or more of the following framework regions:

(i) a light chain FR4 according to SEQ ID NO:126;

(ii) a heavy chain FR2 according to SEQ ID NO:128;

(iii) a heavy chain FR3 according to SEQ ID NO:129;

(iv) a heavy chain FR3 according to SEQ ID NO:130;

(v) a heavy chain FR3 according to SEQ ID NO:131;

(vi) a heavy chain FR3 according to SEQ ID NO:132;

(vii) a heavy chain FR3 according to SEQ ID NO:16 with one or more of the following substitutions: F6V, A10F, A14R, and G39A, according to the position of the amino acid within SEQ ID NO:16;

(viii) light chain framework regions of the VL according to SEQ ID NO:7; and

(ix) heavy chain framework regions of the VH according to SEQ ID NO:8.

15. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:17; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:18;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:17; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:18; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:17; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:18.

16. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:19; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:20;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:19; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:20; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:19; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:20.

17. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:21; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:22;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:21; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:22; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:21; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:22.

18. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:23; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:24;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:23; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:24; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:23; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:24.

19. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:25; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:26;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:25; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:26; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:25; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:26.

20. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:27; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:28;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:27; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:28; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:27; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:28.

21. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:29; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:30;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:29; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:30; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:29; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:30.

22. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:31; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:32;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:31; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:32; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:31; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:32.

23. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:33; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:34;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:33; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:34; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:33; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:34.

24. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:35; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:36;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:35; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:36; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:35; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:36.

25. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:37; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:38;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:37; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:38; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:37; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:38.

26. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:39; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:40;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:39; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:40; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:39; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:40.

27. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:41; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:42;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:41; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:42; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:41; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:42.

28. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:43; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:44;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:43; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:44; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:43; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:44.

29. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:45; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:46;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:45; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:46; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:45; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:46.

30. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:47; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:48;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:47; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:48; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:47; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:48.

31. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:49; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:50;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:49; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:50; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:49; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:50.

32. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:51; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:52;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:51; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:52; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:51; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:52.

33. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:53; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:54;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:53; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:54; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:53; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:54.

34. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:57; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:58;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:57; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:58; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:57; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:58.

35. A binding protein comprising:

(a) a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:59; and a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:60;

(b) a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:59; and a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:60; or

(c) a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:59; and a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:60.

36. The binding protein of any one of claims 1-35, wherein the binding protein is an scFv.

37. The binding protein of any one of claims 1-36, wherein the VH and VL are connected by a linker polypeptide.

38. The binding protein of claim 37, wherein the linker polypeptide comprises or consists of the amino acid sequence according to SEQ ID NO:61.

39. The binding protein of any one of claims 36-38, further comprising a constant domain comprising the sequence according to SEQ ID NO:62.

40. The binding protein of any one of claims 1-39, further comprising a polypeptide that binds to a KIR protein.

41. The binding protein of any one of claims 1-39, further comprising a second polypeptide that comprises: CDRL1, CDRL2, and CDRL3 amino acid sequences according to RASQSVSSYLA (SEQ ID NO:63), DASNRAT (SEQ ID NO:64), and QQRSNWMYTF (SEQ ID NO:65), respectively.

42. The binding protein of claim 41, further comprising a third polypeptide that comprises: CDRH1, CDRH2, and CDRH3 amino acid sequences according to FYAIS (SEQ ID NO:66), GFIPIFGAANYAQKF (SEQ ID NO:67), and IPSGSYYYDYDMDV (SEQ ID NO:68), respectively.

43. The binding protein of any one of claims 1-39, further comprising:

(a) a second polypeptide comprising a light chain variable region (VL) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:69; and a third polypeptide comprising a heavy chain variable region (VH) comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:70;

(b) a second polypeptide comprising a light chain variable region (VL) comprising the amino acid sequence according to SEQ ID NO:69; and third polypeptide comprising a heavy chain variable region (VH) comprising the amino acid sequence according to SEQ ID NO:70; or

(c) a second polypeptide comprising a light chain variable region (VL) consisting of the amino acid sequence according to SEQ ID NO:69; and third polypeptide comprising a heavy chain variable region (VH) consisting of the amino acid sequence according to SEQ ID NO:70.

44. The binding protein of claim 43, wherein the VL of the second polypeptide has CDRL1, CDRL2, and CDRL3 amino acid sequences according to SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65, respectively; and the VH of the third polypeptide has CDRH1, CDRH2, and CDRH3 amino acid sequences according to SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:68, respectively.

45. The binding protein of claim 43, wherein the VL of the second polypeptide has CDRL1, CDRL2, and CDRL3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

46. The binding protein of claim 43 or claim 45, wherein the VH of the third polypeptide has CDRH1, CDRH2, and CDRH3 amino acid sequences according to any one of Kabat, Chothia, EU, International Immunogenetics Information System (IMGT), and AHo.

47. A binding protein according to Table 3.

48. A binding protein comprising:

(a) a polypeptide sequence comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising at least 90% identity to the amino acid sequence according to SEQ ID NO:120;

(b) a polypeptide sequence comprising the amino acid sequence according to SEQ ID NO:78, a polypeptide comprising the amino acid sequence according to SEQ ID NO:80, and a polypeptide comprising the amino acid sequence according to SEQ ID NO:120; or

(c) a polypeptide sequence consisting of the amino acid sequence according to SEQ ID NO:78, a polypeptide consisting of the amino acid sequence according to SEQ ID NO:80, and a polypeptide consisting of the amino acid sequence according to SEQ ID NO:120.

49. A pharmaceutical composition comprising the binding protein of any one of claims 1-48 and a pharmaceutically acceptable carrier.

50. A nucleic acid encoding the binding protein of any one of claims 1-48.

51. A nucleic acid molecule comprising SEQ ID NOs:139, 141, and 141.

52. A nucleic acid molecule comprising SEQ ID NOs:138, 140, and 142.

53. A vector comprising the nucleic acid of any one of claims claim 50-52.

54. A cell line comprising the vector of claim 53.

55. A method of treating a disease, comprising administering the binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49 to a subject in need thereof.

56. A method of preventing a disease, comprising administering the binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49 to a subject in need thereof.

57. The method of claim 55 or claim 56, wherein the disease is an inflammatory disease or an autoimmune disease.

58. The method of claim 55 or claim 66, wherein the disease is a CD4+ T cell-driven inflammatory disease or autoimmune disease.

59. The method of claim 57 or claim 58, wherein the number or activity of pathogenic immune cells in the subject is decreased.

60. The method of any one of claims 55-59, wherein the disease is a rheumatological disorder, fibrotic disorder, gastrointestinal disorder, endocrinological disorder, neurological disorder, or skin disorder.

61. The method of any one of claims 57-59, wherein the autoimmune disease is celiac disease, Crohn's disease, juvenile idiopathic arthritis, inflammatory bowel disease (IBD), insulin-dependent diabetes mellitus (IDDM or type 1 diabetes), lupus, lupus nephritis, cutaneous lupus, discoid lupus, myasthenia gravis, myocarditis, multiple sclerosis (MS), pemphigus/pemphigoid, rheumatoid arthritis (RA), scleroderma/systemic sclerosis, Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), or ulcerative colitis.

62. The method of any one of claims 57-59, wherein the autoimmune disease is celiac disease.

63. The method of any one of claims 57-59, wherein the autoimmune disease is Crohn's disease.

64. The method of any one of claims 57-59, wherein the autoimmune disease is inflammatory bowel disease (IBD).

65. The method of any one of claims 57-59, wherein the autoimmune disease is ulcerative colitis.

66. The method of any one of claims 55-59, wherein the disease is graft versus host disease (GVHD).

67. The method of any one of claims 55-59, wherein the disease is type 1 diabetes.

68. A method of suppressing an immune response mediated by pathogenic immune cells, comprising contacting CD8+ T regulatory cells (Tregs) with the binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49, thereby activating or stimulating CD8+ Tregs.

69. A method of suppressing an immune response to an antigen, such as an autoantigen, comprising administering to a subject in need thereof the binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49, thereby activating or stimulating CD8+ Tregs, whereby the number or activity of pathogenic immune cells that are responsive to the antigen or autoantigen is decreased.

70. The method of claim 68 or claim 69, wherein the pathogenic immune cells are autoreactive CD4+ T cells, autoantibody producing B cells, or self antigen presenting dendritic cells.

71. The method of claim 68 or claim 69, wherein the pathogenic immune cells are autoreactive CD4+ T cells.

72. The method of any one of claims 68-71, wherein the CD8+ Tregs are CD8+KIR+ Tregs.

73. The method of any one of claims 68-72, wherein the activated CD8+ Tregs are administered to the subject or to a second subject.

74. The method of any one of claims 68-73, wherein the subject and/or the second subject has an inflammatory or autoimmune disease.

75. A method of suppressing, reducing, or preventing an immune response to a viral vector in a subject, the method comprising administering to the subject the binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49.

76. The method of claim 75, wherein the viral vector has been, is, or will be administered to the subject and the immune response to the viral vector is induced by administration of the viral vector to the subject.

77. The method of any one of claims 55-76, wherein the binding protein is administered in a dose of from about 0.01 mg/kg to about 20 mg/kg.

78. The method of claim 77, wherein the binding protein is administered in a dose of from about 0.01 mg/kg to about 10 mg/kg.

79. The method of claim 77, wherein the binding protein is administered in a dose of from about 0.05 mg/kg to about 0.1 mg/kg.

80. The method of claim 77, wherein the binding protein is administered in a dose of from about 0.5 mg/kg to about 1.0 mg/kg.

81. The method of claim 77, wherein the binding protein is administered in a dose of from 0.05 mg/kg to 0.1 mg/kg.

82. The method of claim 77, wherein the binding protein is administered in a dose of from 0.5 mg/kg to 1.0 mg/kg.

83. The method of claim 77, wherein the binding protein is administered in a dose up to about 10 mg/kg.

84. The method of claim 77, wherein the binding protein is administered in a dose up to 10 mg/kg.

85. The method of claim 77, wherein the binding protein is administered in a dose of about 10 mg/kg.

86. The method of claim 77, wherein the binding protein is administered in a dose of 10 mg/kg.

87. Use of the binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49 in the method of any one of claims 55-86.

88. The binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49 for use in the method of any one of claims 55-86.

89. Use of the binding protein of any one of claims 1-48 or the pharmaceutical composition of claim 49 in the manufacture of a medicament for use in the method of any one of claims 55-86.

90. A method of treating celiac disease in a subject in need thereof, comprising administering the binding protein of claim 48 to the subject.

91. A method of preventing celiac disease in a subject in need thereof, comprising administering the binding protein of claim 48 to the subject.

92. A method of treating celiac disease in a subject in need thereof, comprising administering the pharmaceutical composition of claim 49 to the subject.

93. A method of preventing celiac disease in a subject in need thereof, comprising administering the pharmaceutical composition of claim 49 to the subject.

94. A method of treating type 1 diabetes in a subject in need thereof, comprising administering the binding protein of claim 48 to the subject.

95. A method of preventing type 1 diabetes in a subject in need thereof, comprising administering the binding protein of claim 48 to the subject.

96. A method of treating type 1 diabetes in a subject in need thereof, comprising administering the pharmaceutical composition of claim 49 to the subject.

97. A method of preventing type 1 diabetes in a subject in need thereof, comprising administering the pharmaceutical composition of claim 49 to the subject.

98. Use of the binding protein of claim 48 or the pharmaceutical composition of claim 49 in the method of any one of claims 90-97.

99. The binding protein of claim 48 or the pharmaceutical composition of claim 49 for use in the method of any one of claims 90-97.

100. Use of the binding protein of claim 48 or the pharmaceutical composition of claim 49 in the manufacture of a medicament for use in the method of any one of claims 90-97.

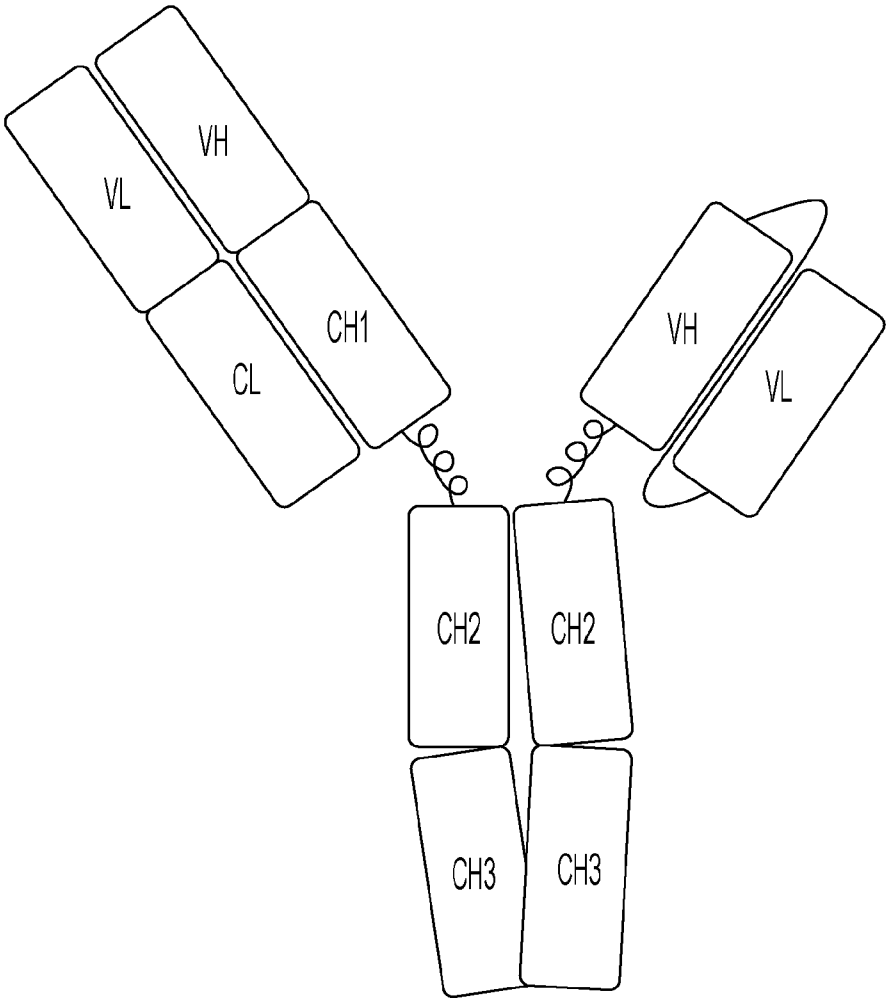
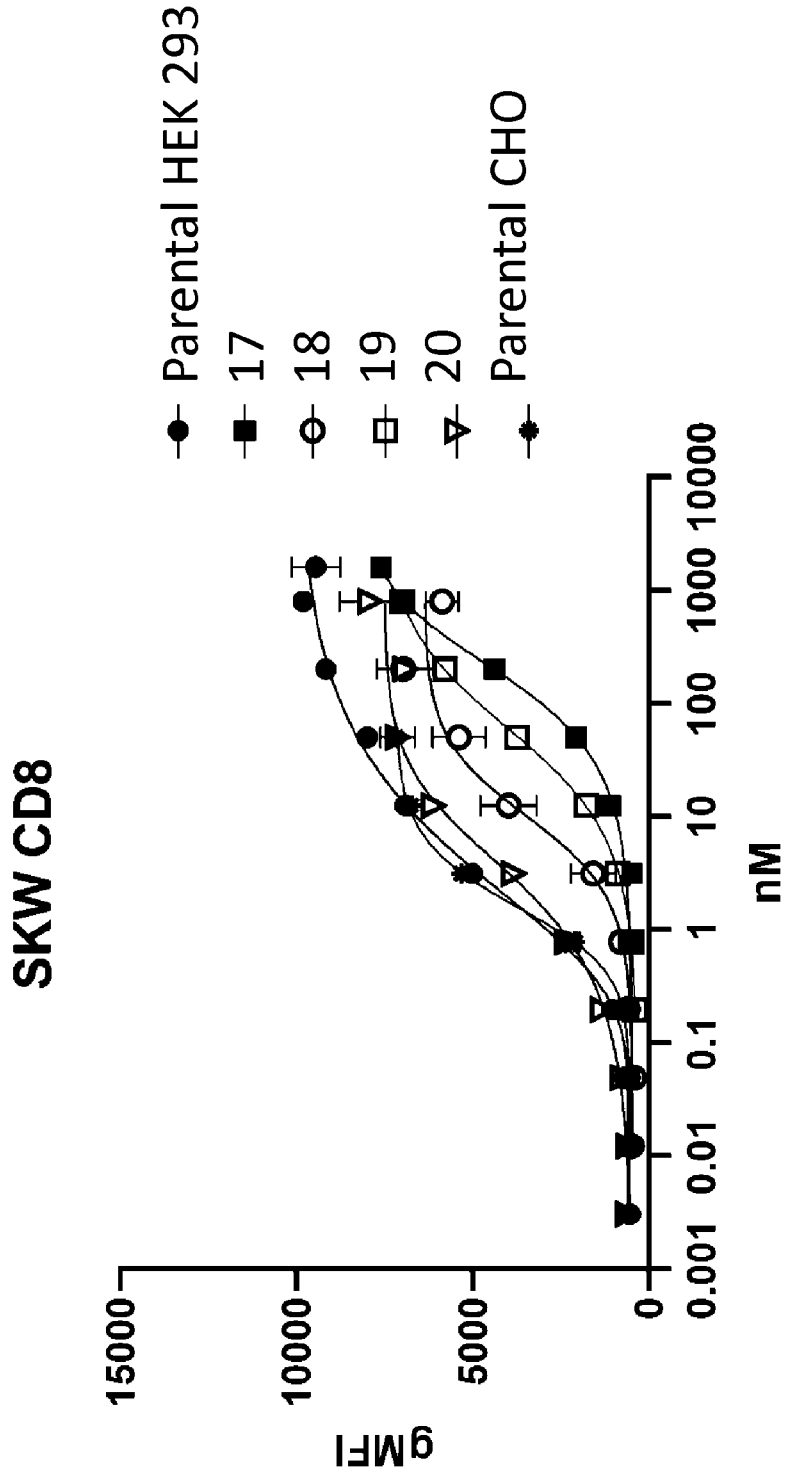


FIG. 1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18
19
20
Parental CHO

FIG. 2

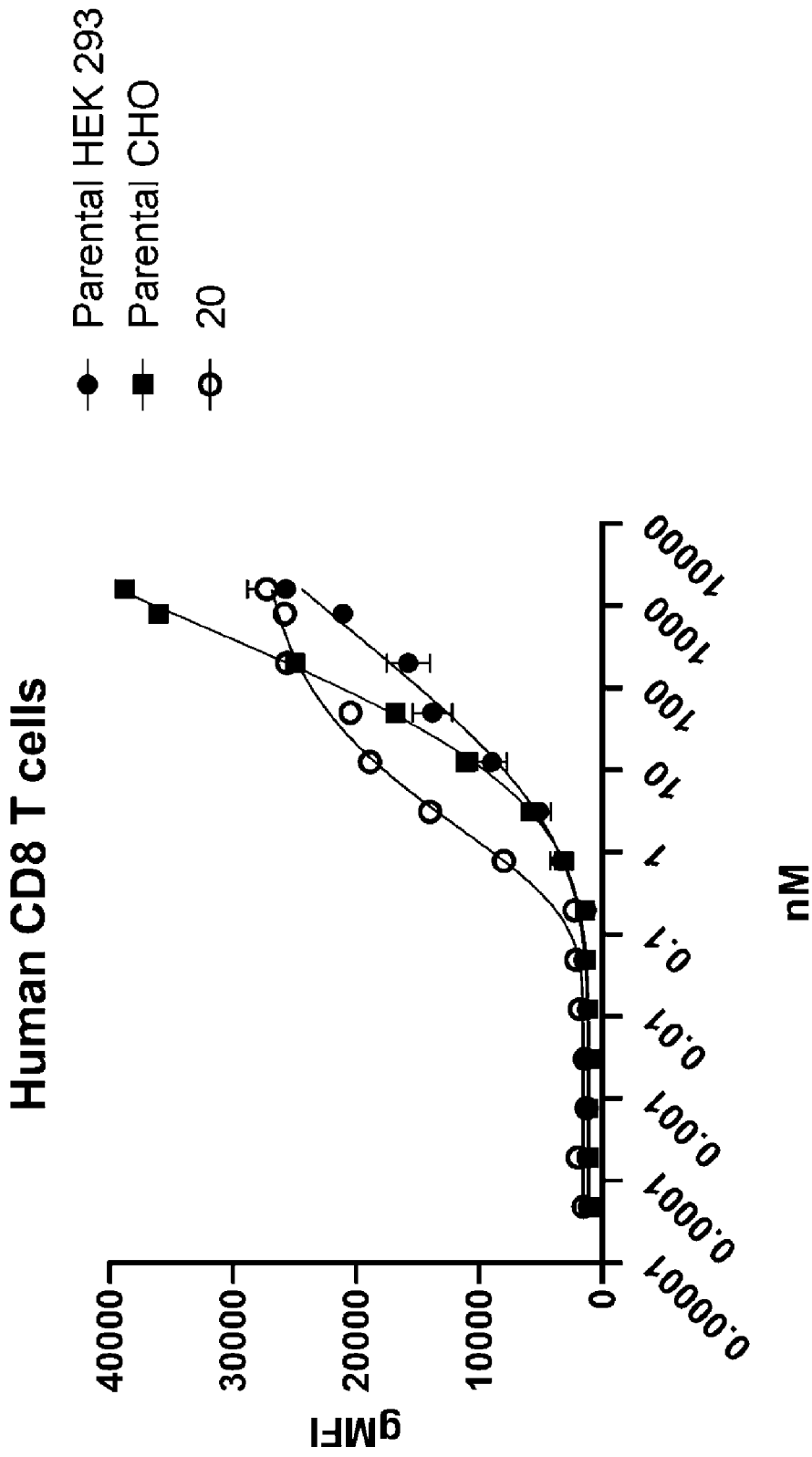
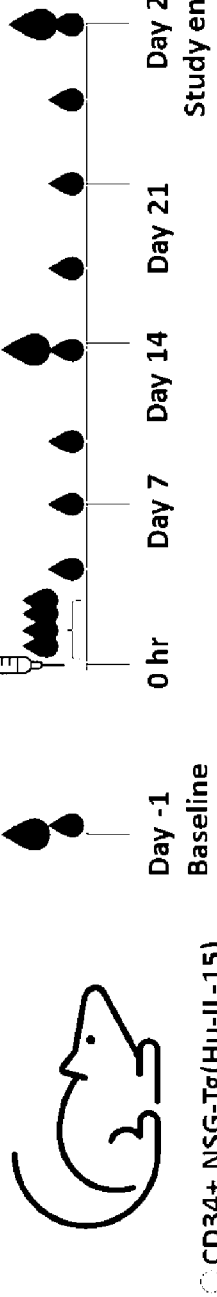

Parental HEK 293
Parental CHO
20
Human CD8 T cells
gMFI
40000
30000
20000
10000
0
nM
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001

FIG. 3



- PK time points (💧): pre-dose and at 0.5, 2, 8, 24, 96, 168, 252, 336, 420, 504, 558, and 696 hrs post-dose
- Flow cytometry time points (💧): pre-dose on day 0 and on Day 14 and 28

FIG. 4

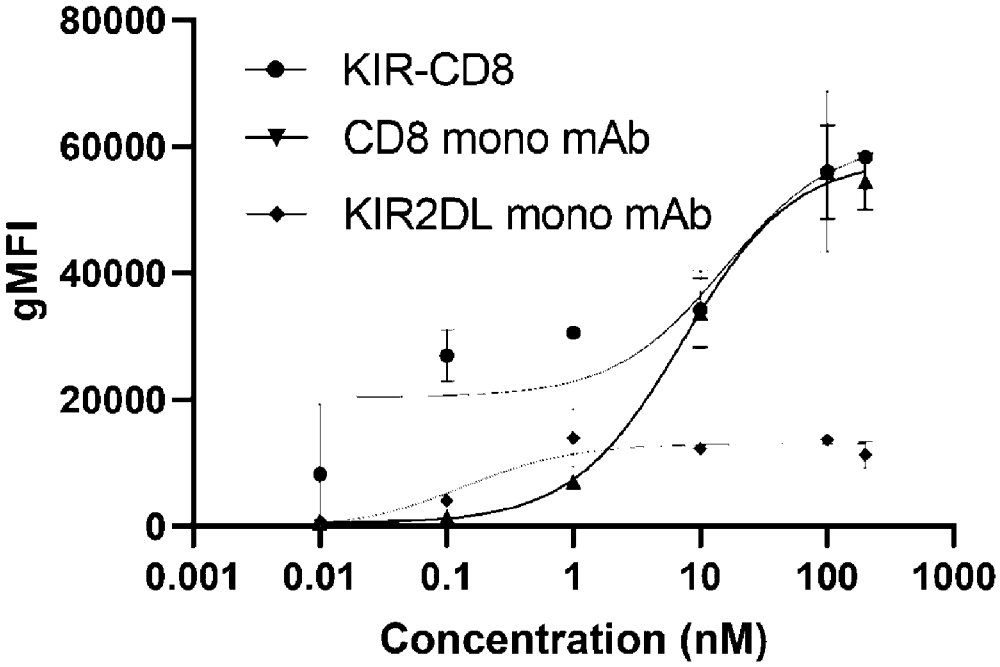

80000
60000
40000
20000
0
gMFI
KIR-CD8
CD8 mono mAb
KIR2DL mono mAb
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 5A

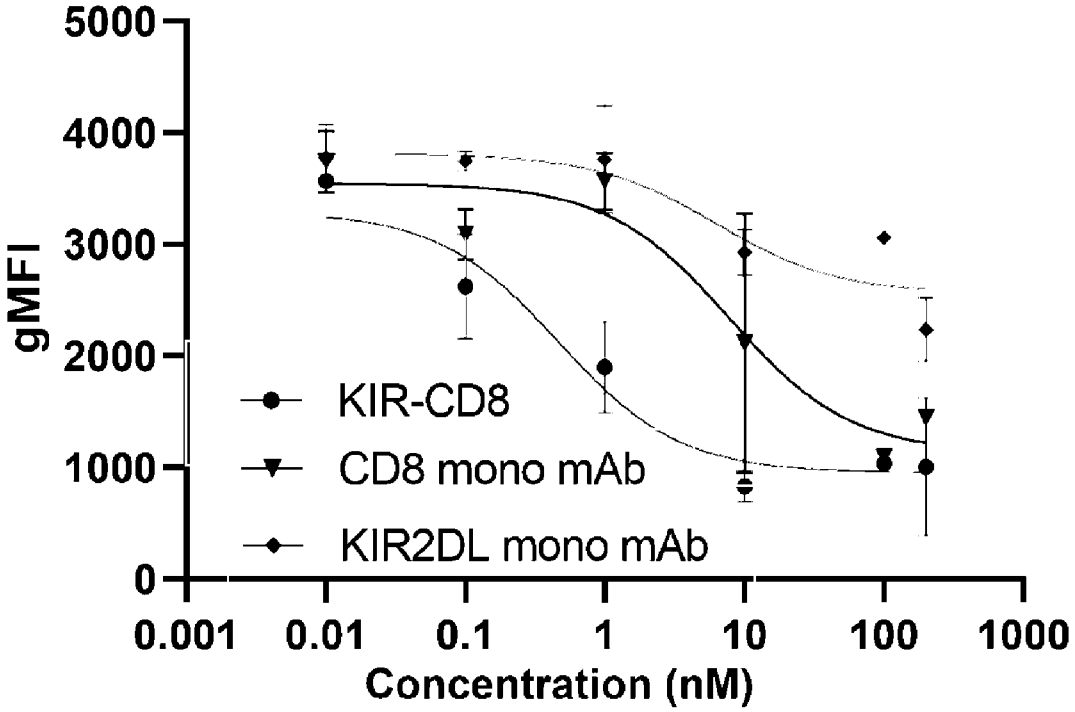

5000
4000
3000
2000
1000
0
gMFI
KIR-CD8
CD8 mono mAb
KIR2DL mono mAb
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 5B

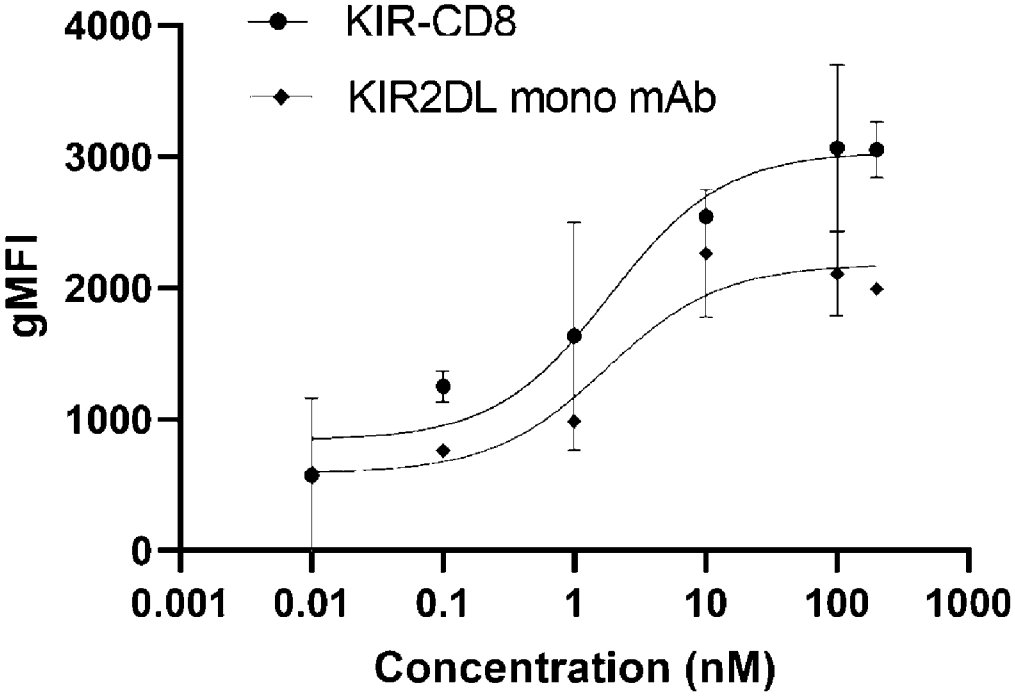

KIR-CD8
KIR2DL mono mAb
gMFI
4000
3000
2000
1000
0
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 5C

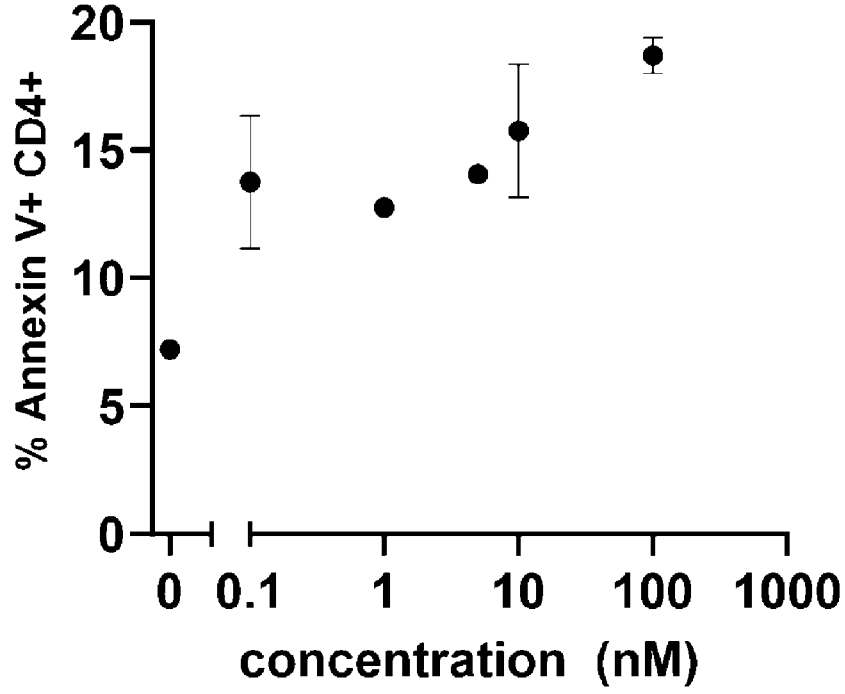

% Annexin V+ CD4+
20
15
10
5
0
0
0.1
1
10
100
1000
concentration (nM)

FIG. 5D

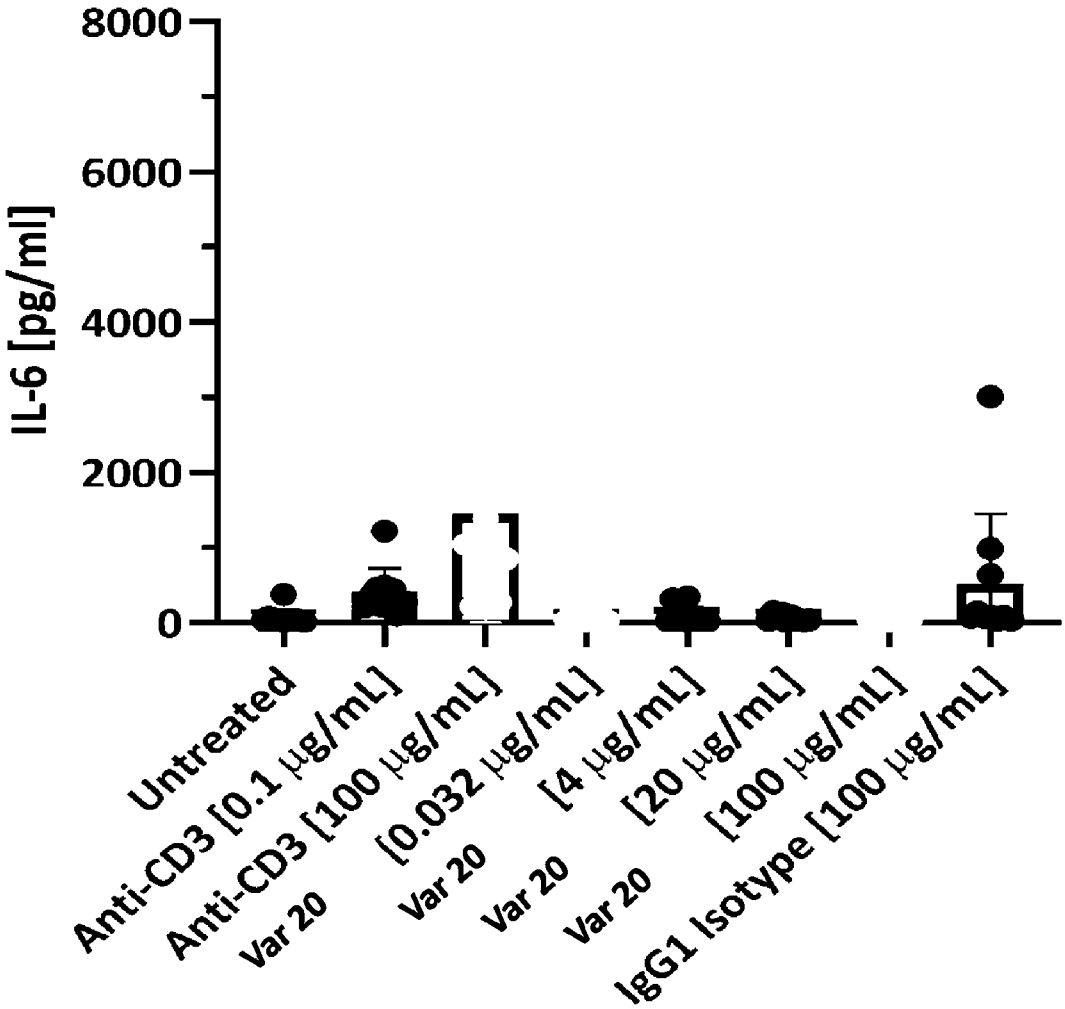

8000
6000
4000
2000
0
IL-6 [pg/ml]
Untreated
Anti-CD3 [0.1 µg/mL]
Anti-CD3 [100 µg/mL]
[0.032 µg/mL] Var 20
[4 µg/mL] Var 20
[20 µg/mL] Var 20
[100 µg/mL] IgG1 Isotype
[100 µg/mL]

FIG. 5E

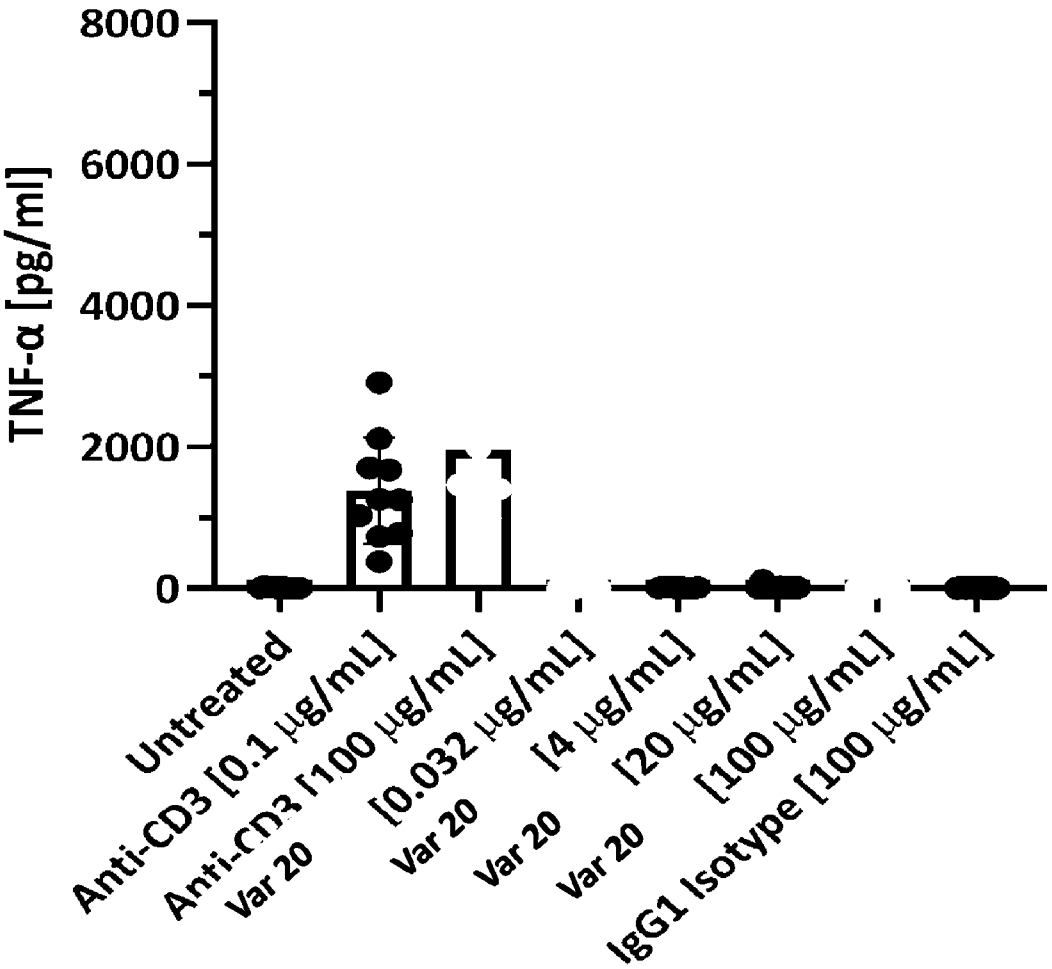

8000
6000
4000
2000
0
TNF-α [pg/ml]
Untreated
Anti-CD3 [0.1 μg/mL]
Anti-CD3 [100 μg/mL]
[0.032 μg/mL] Var 20
[4 μg/mL] Var 20
[20 μg/mL] Var 20
[100 μg/mL] IgG1 Isotype
[100 μg/mL]

FIG. 5F

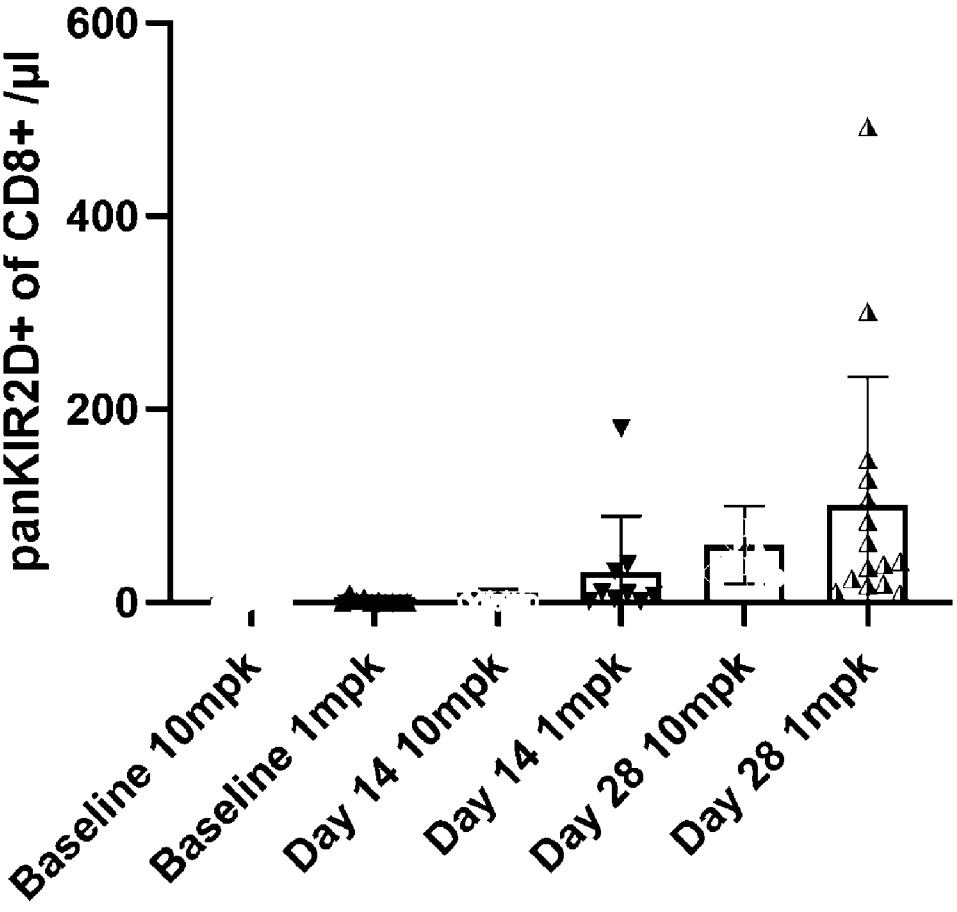

600
400
200
0
panKIR2D+ of CD8+ /µl
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 6A

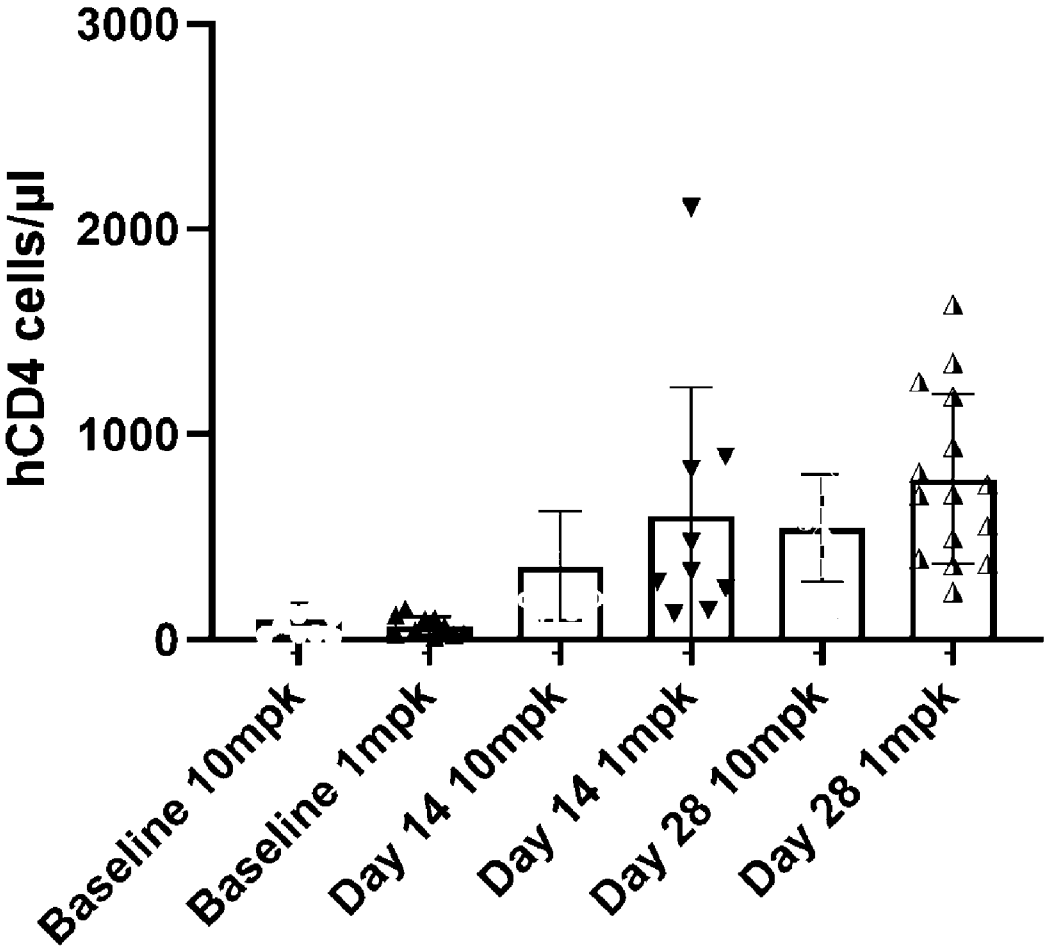

3000
2000
1000
0
hCD4 cells/µl
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 6B

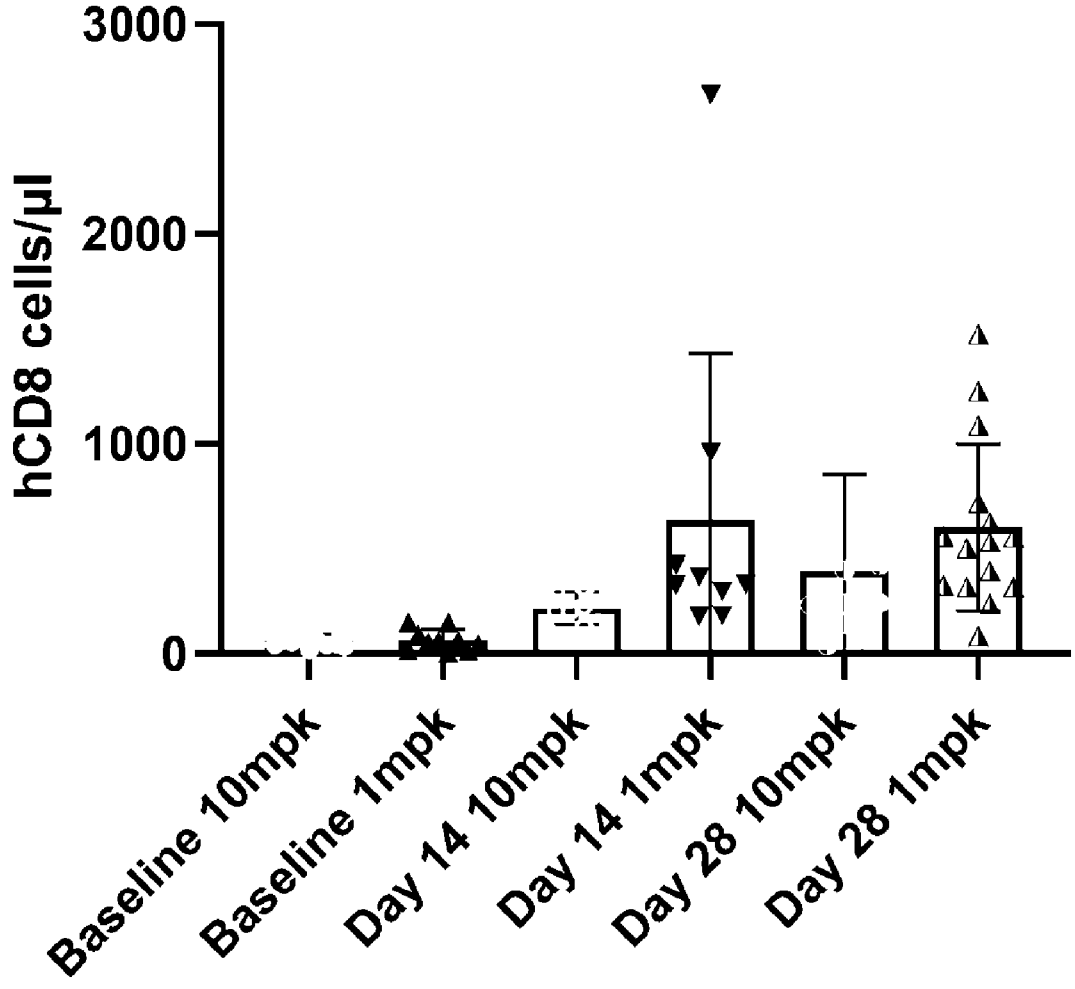

3000
2000
1000
0
hCD8 cells/µl
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 6C

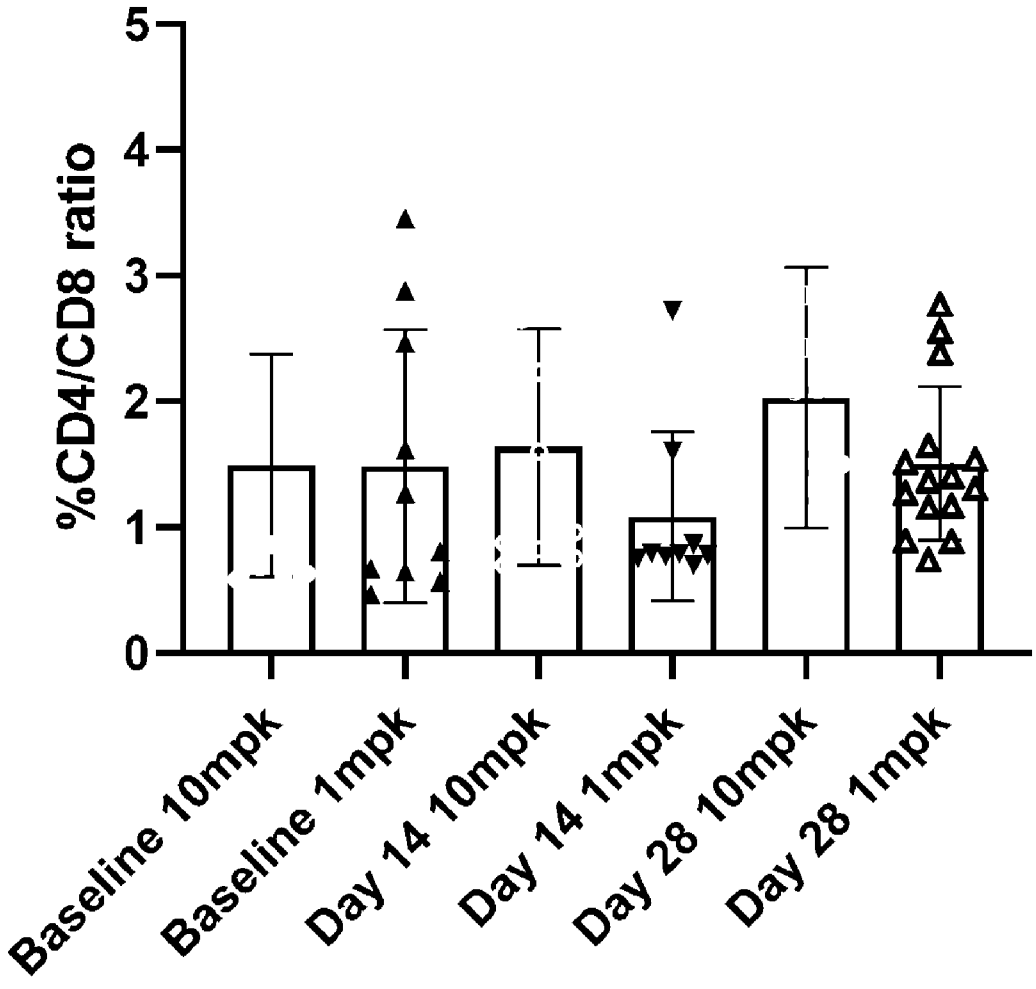

%CD4/CD8 ratio
5
4
3
2
1
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 6D

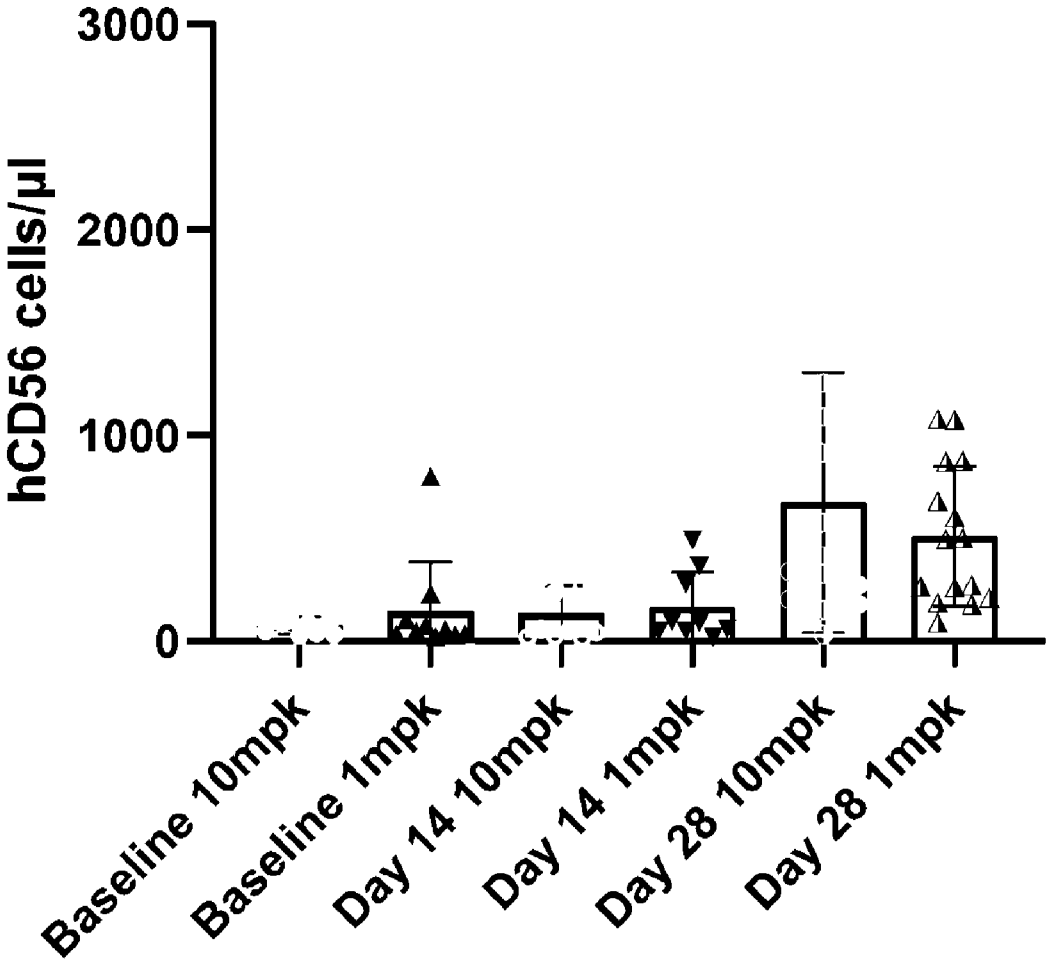

3000
2000
1000
0
hCD56 cells/µl
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 6E

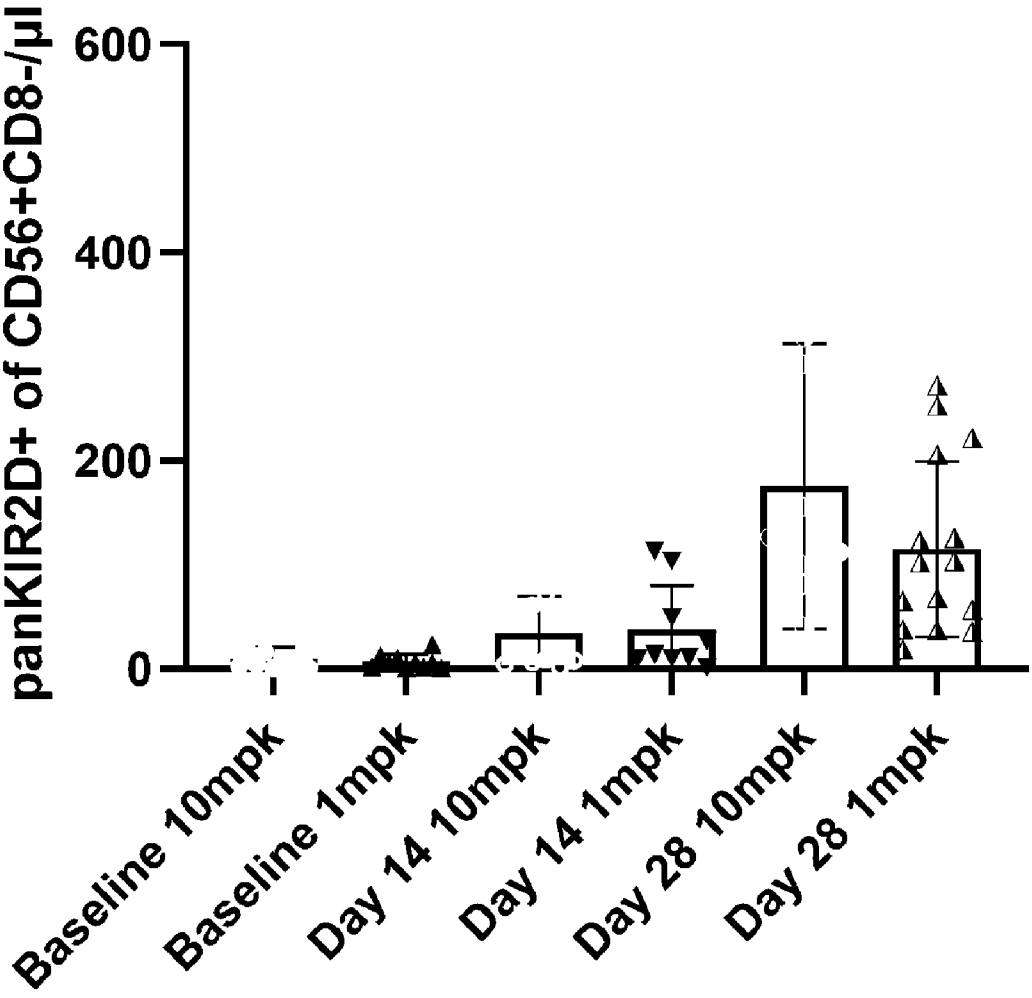

panKIR2D+ of CD56+CD8-/µl
600
400
200
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 6F

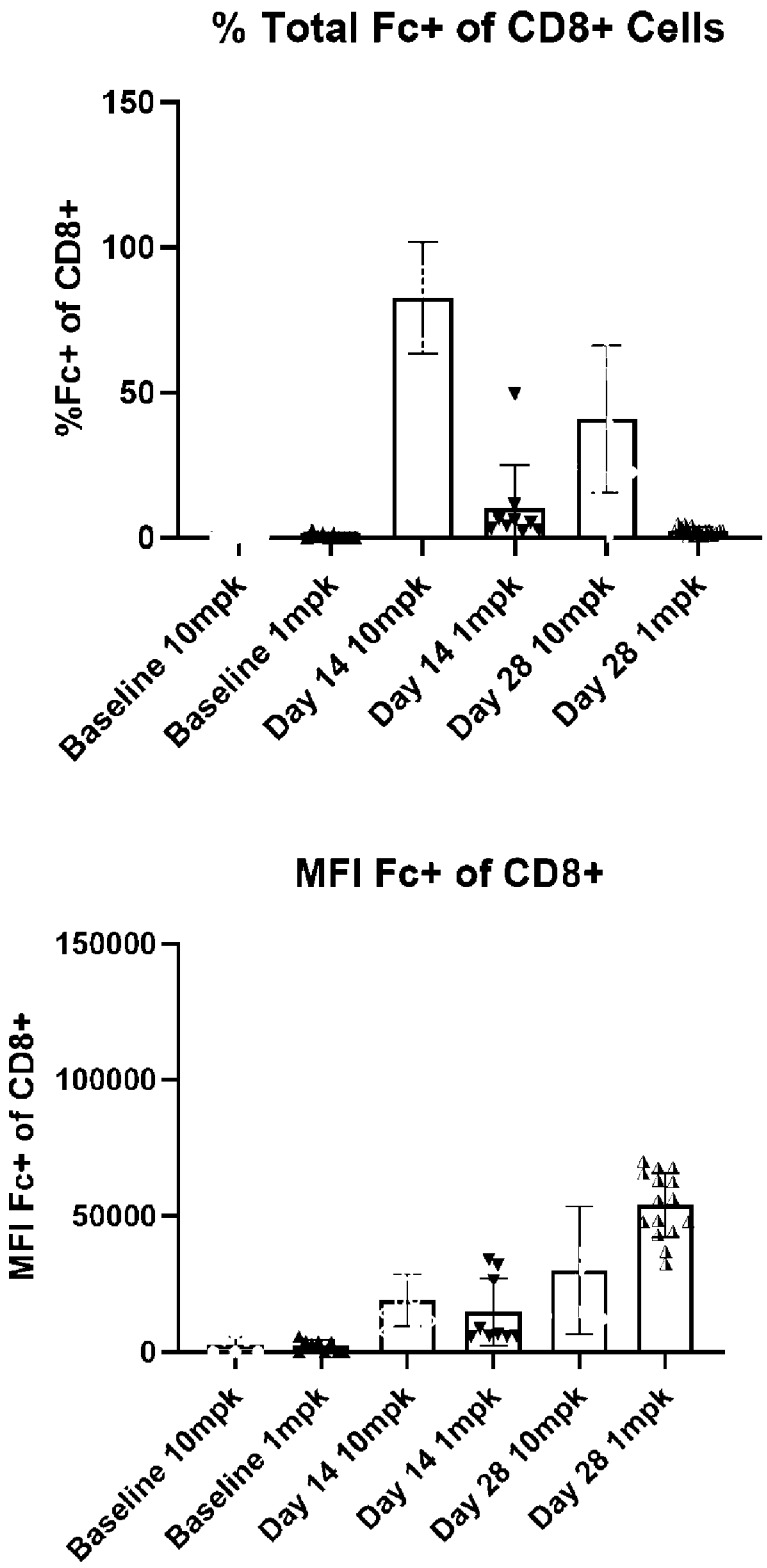

% Total Fc+ of CD8+ Cells
%Fc+ of CD8+
150
100
50
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk
MFI Fc+ of CD8+
MFI Fc+ of CD8+
150000
100000
50000
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 7A

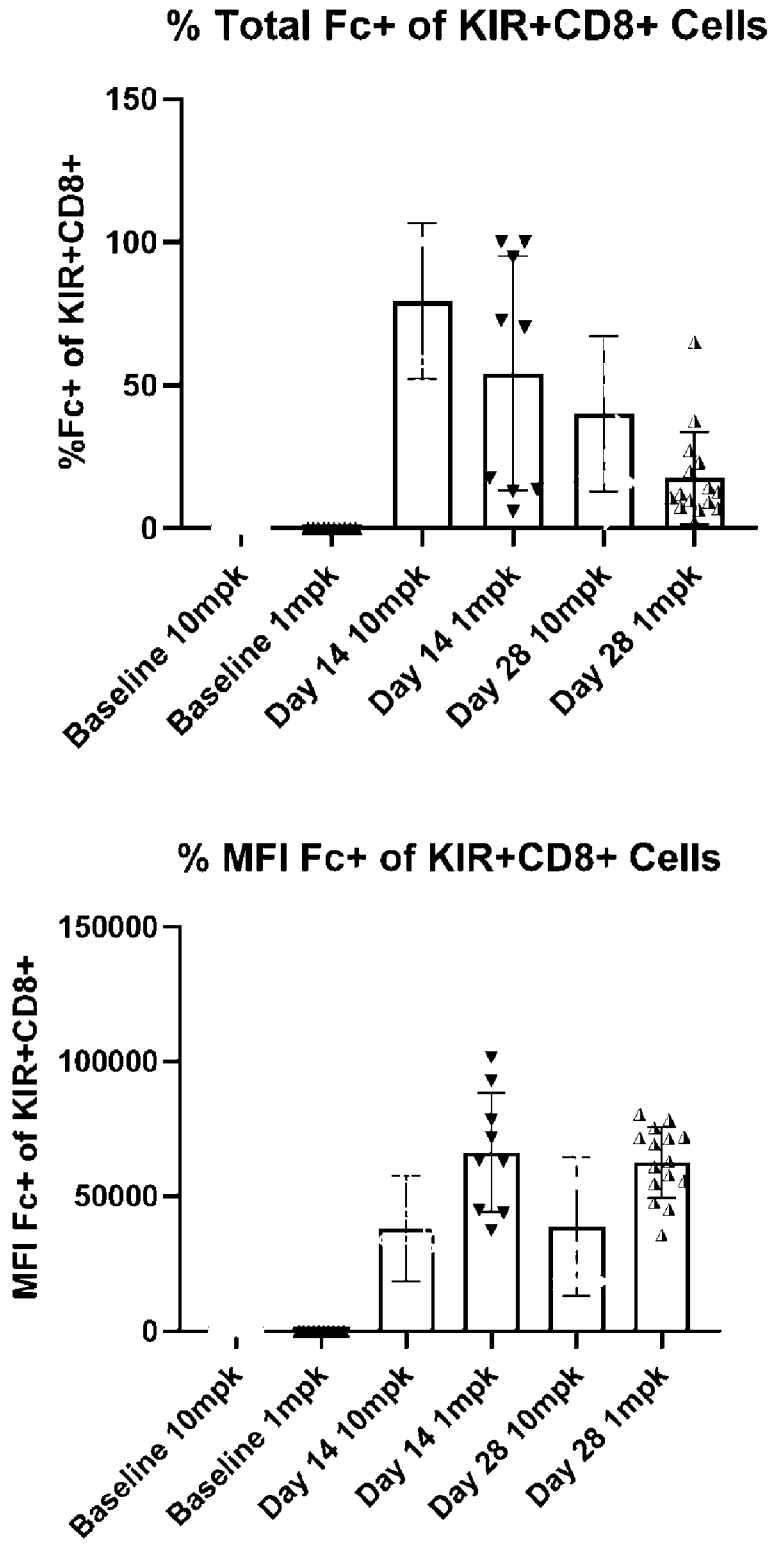

% Total Fc+ of KIR+CD8+ Cells
%Fc+ of KIR+CD8+
150
100
50
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk
% MFI Fc+ of KIR+CD8+ Cells
MFI Fc+ of KIR+CD8+
150000
100000
50000
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 7B

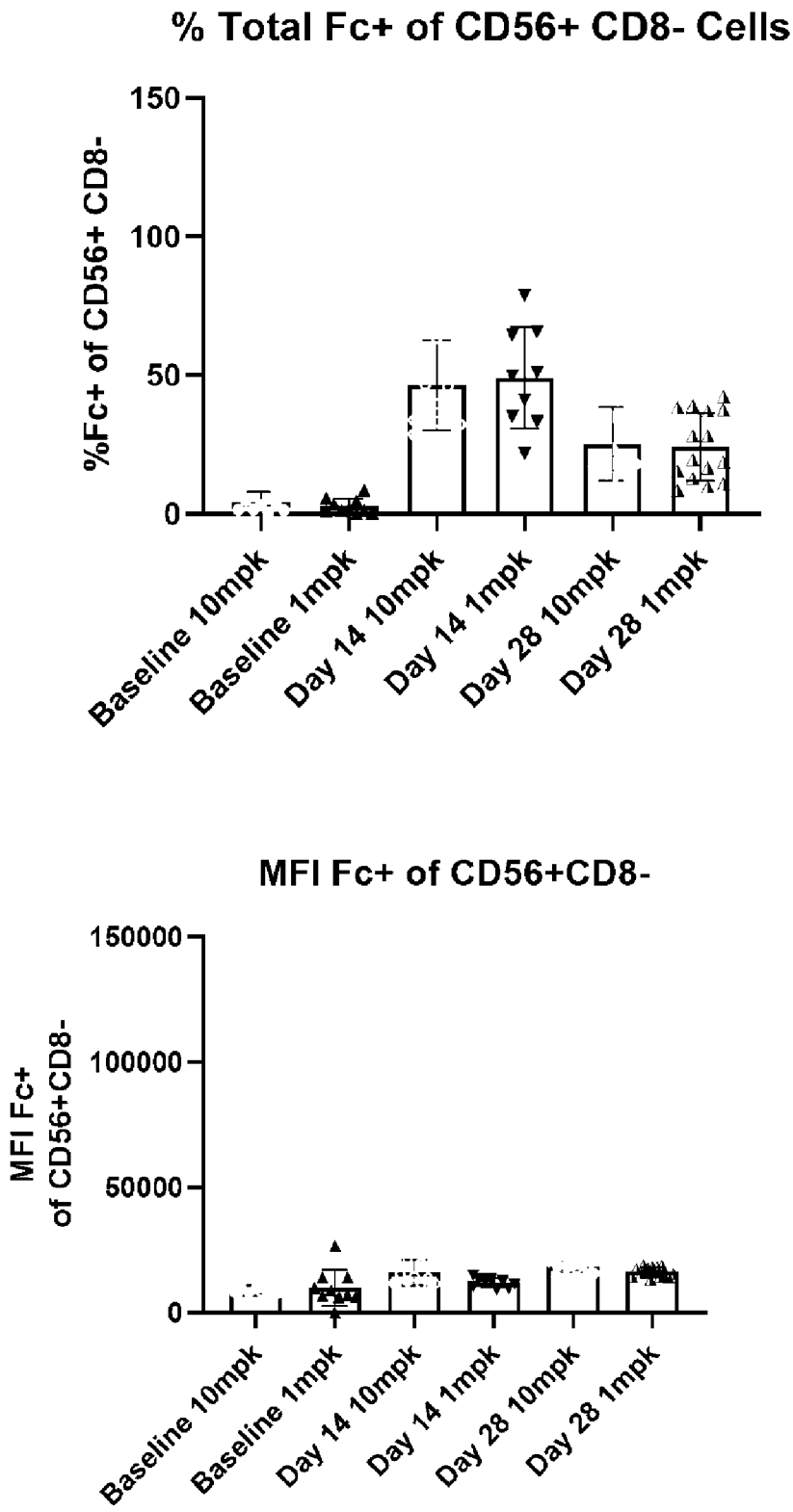

% Total Fc+ of CD56+ CD8- Cells
%Fc+ of CD56+ CD8-
150
100
50
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk
MFI Fc+ of CD56+CD8-
MFI Fc+ of CD56+CD8-
150000
100000
50000
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 7C

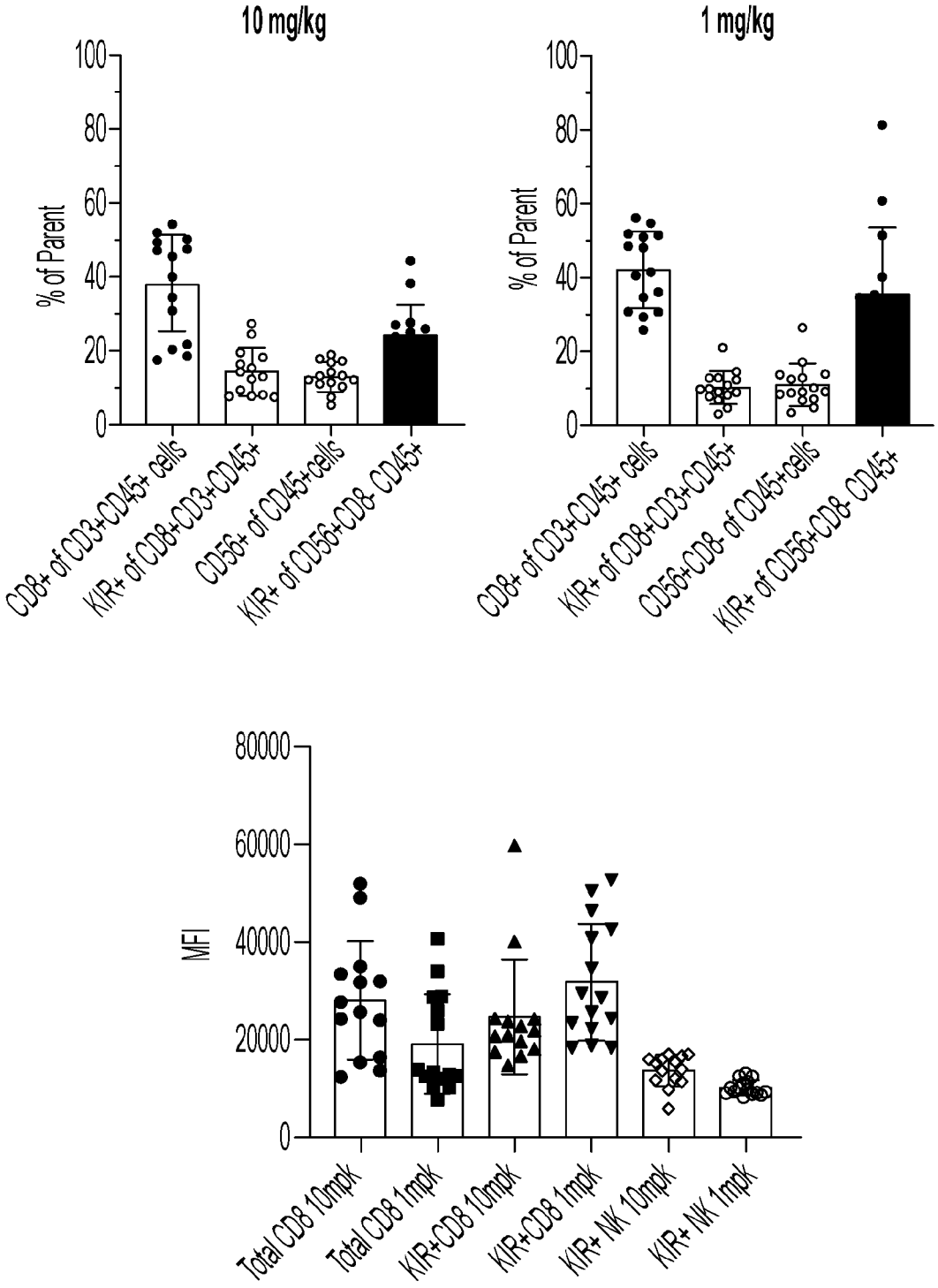

10 mg/kg
1 mg/kg
% of Parent
100
80
60
40
20
0
CD8+ of CD3+CD45+ cells
KIR+ of CD8+CD3+CD45+
CD56+ of CD45+cells
KIR+ of CD56+CD8- CD45+
MFI
80000
60000
40000
20000
0
Total CD8 10mpk
Total CD8 1mpk
KIR+CD8 10mpk
KIR+CD8 1mpk
KIR+ NK 10mpk
KIR+ NK 1mpk

FIG. 7D

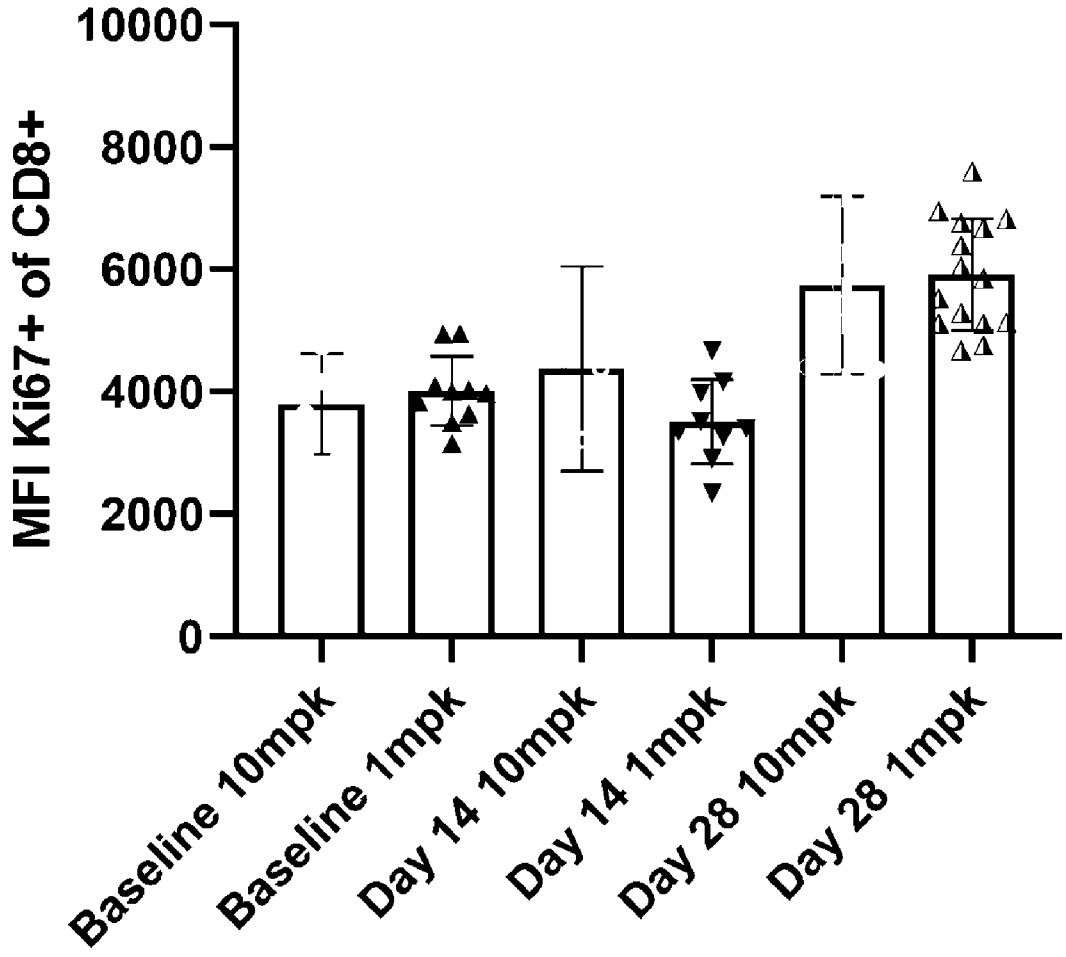

MFI Ki67+ of CD8+
10000
8000
6000
4000
2000
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 8A

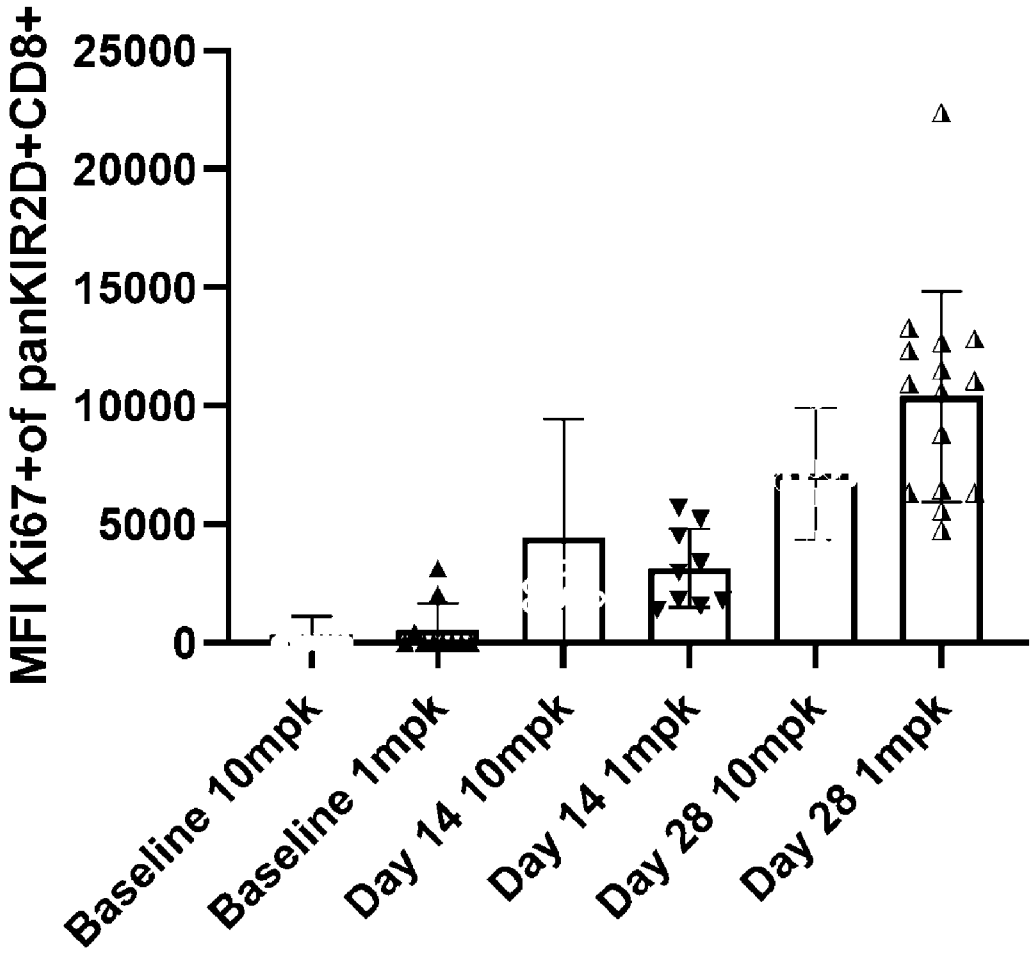

MFI Ki67+of panKIR2D+CD8+
25000
20000
15000
10000
5000
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 8B

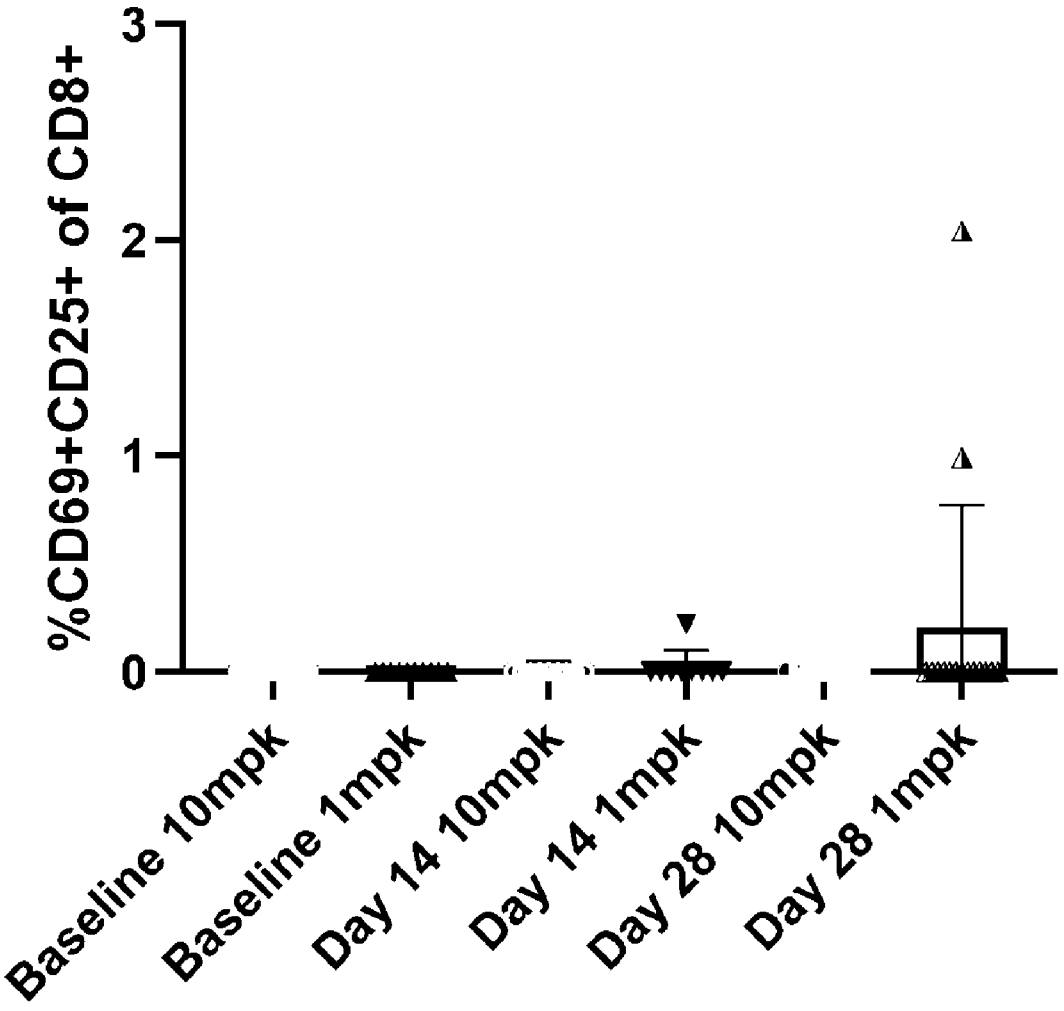

%CD69+CD25+ of CD8+
3
2
1
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 8C

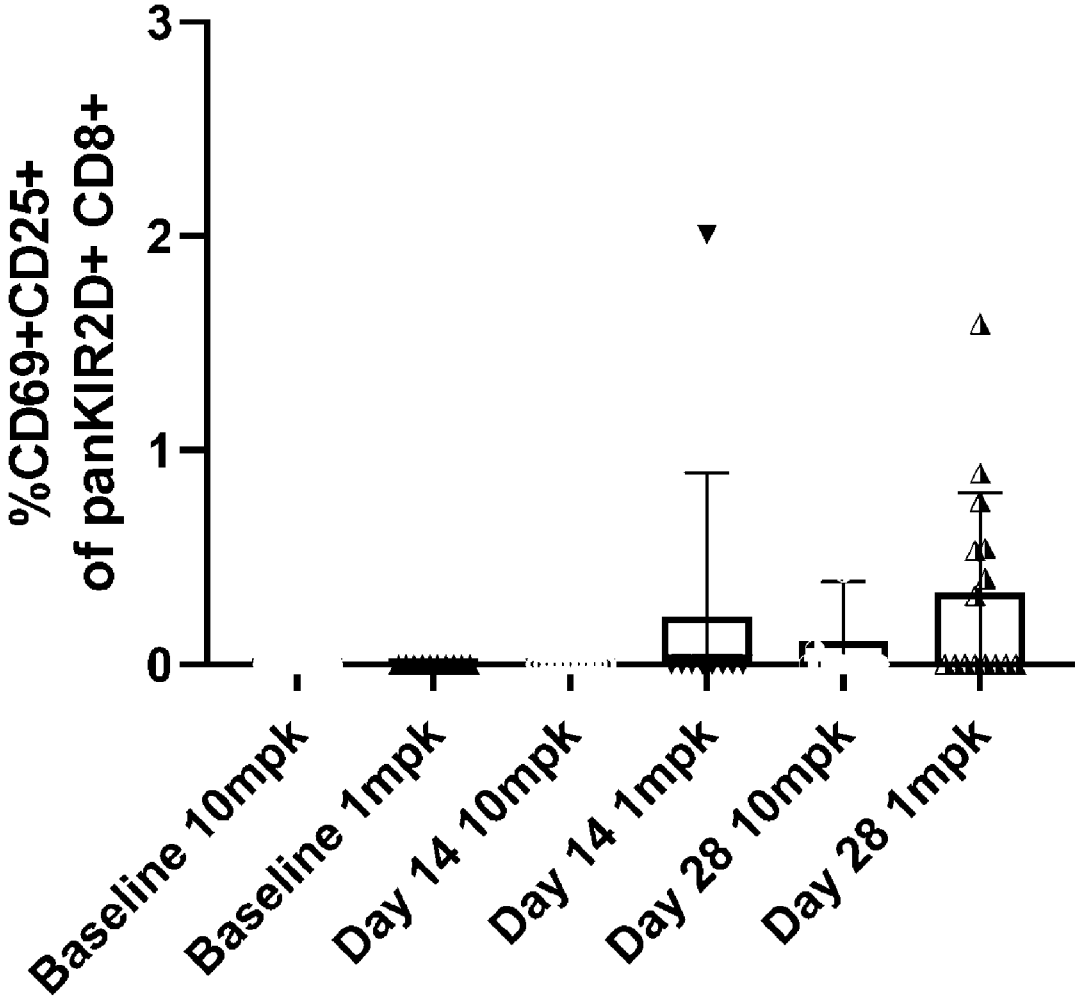

%CD69+CD25+ of panKIR2D+ CD8+
3
2
1
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 8D

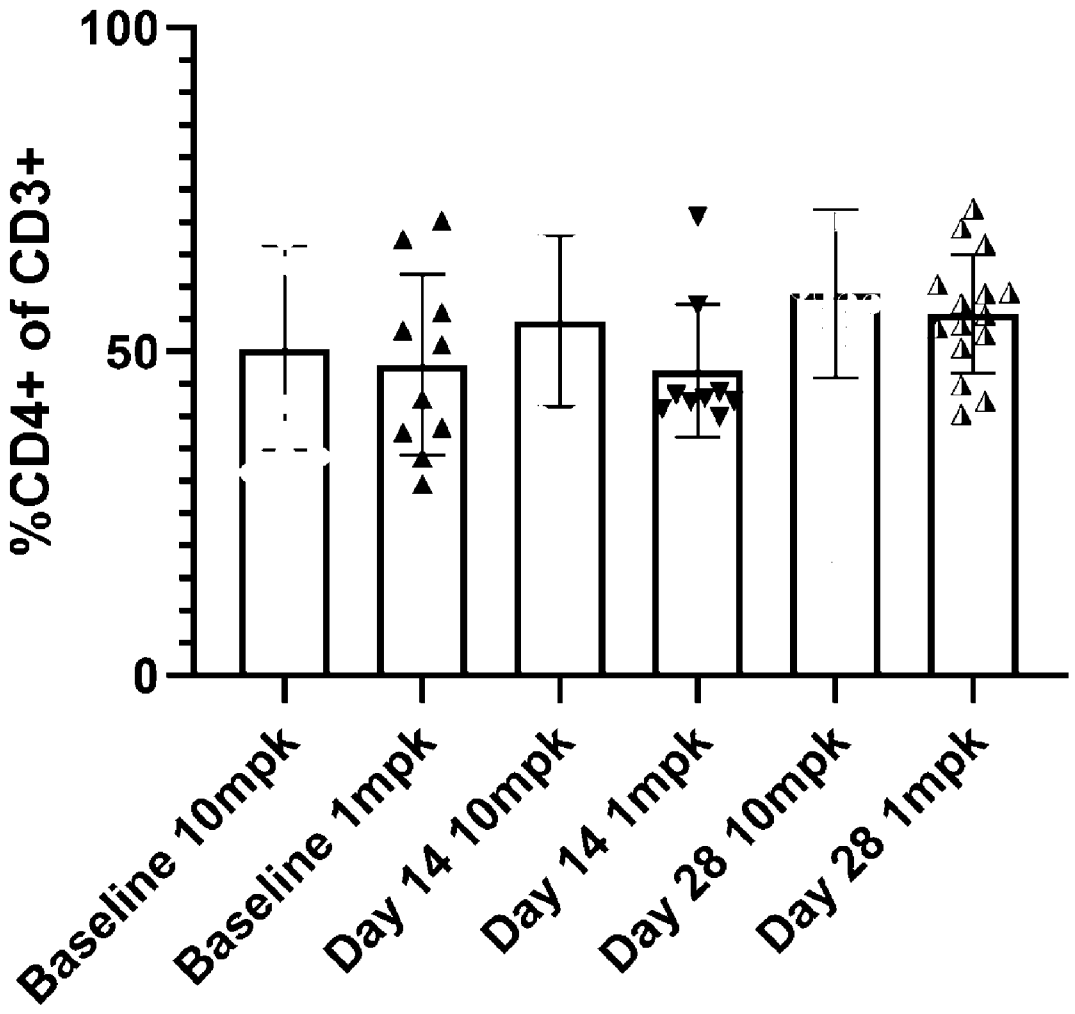

100
50
0
%CD4+ of CD3+
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 9A

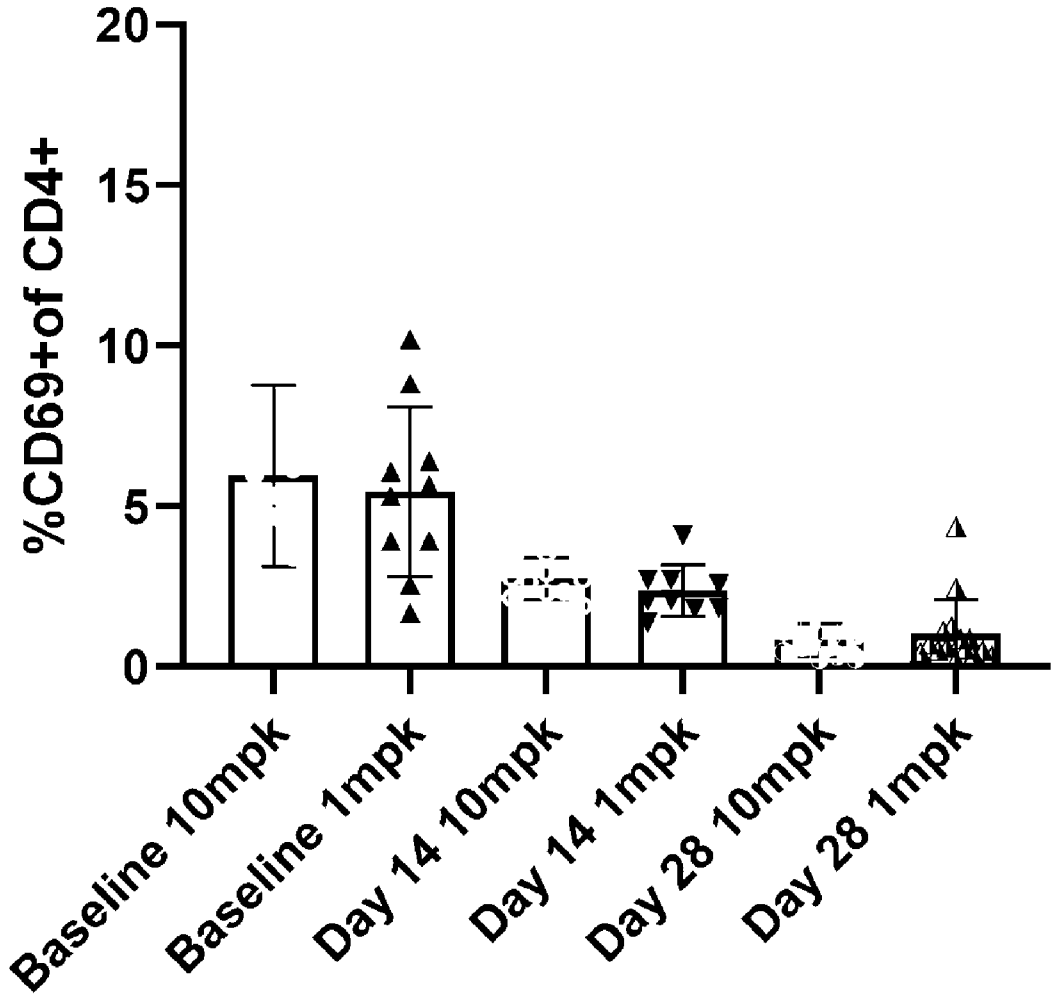

%CD69+of CD4+
20
15
10
5
0
Baseline 10mpk
Baseline 1mpk
Day 14 10mpk
Day 14 1mpk
Day 28 10mpk
Day 28 1mpk

FIG. 9B

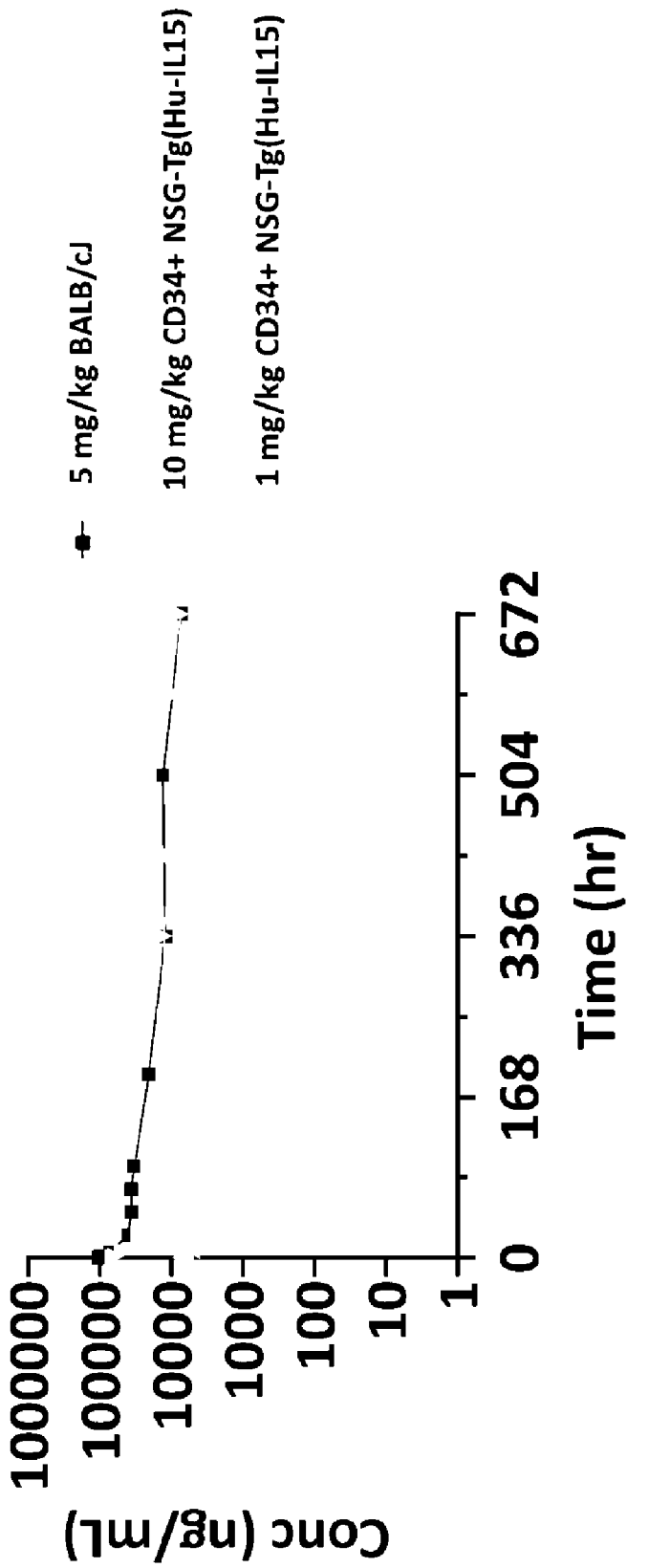

5 mg/kg BALB/cJ
10 mg/kg CD34+ NSG-Tg(Hu-IL15)
1 mg/kg CD34+ NSG-Tg(Hu-IL15)
Conc (ng/mL)
1000000
100000
10000
1000
100
10
1
0
168
336
504
672
Time (hr)

FIG. 10

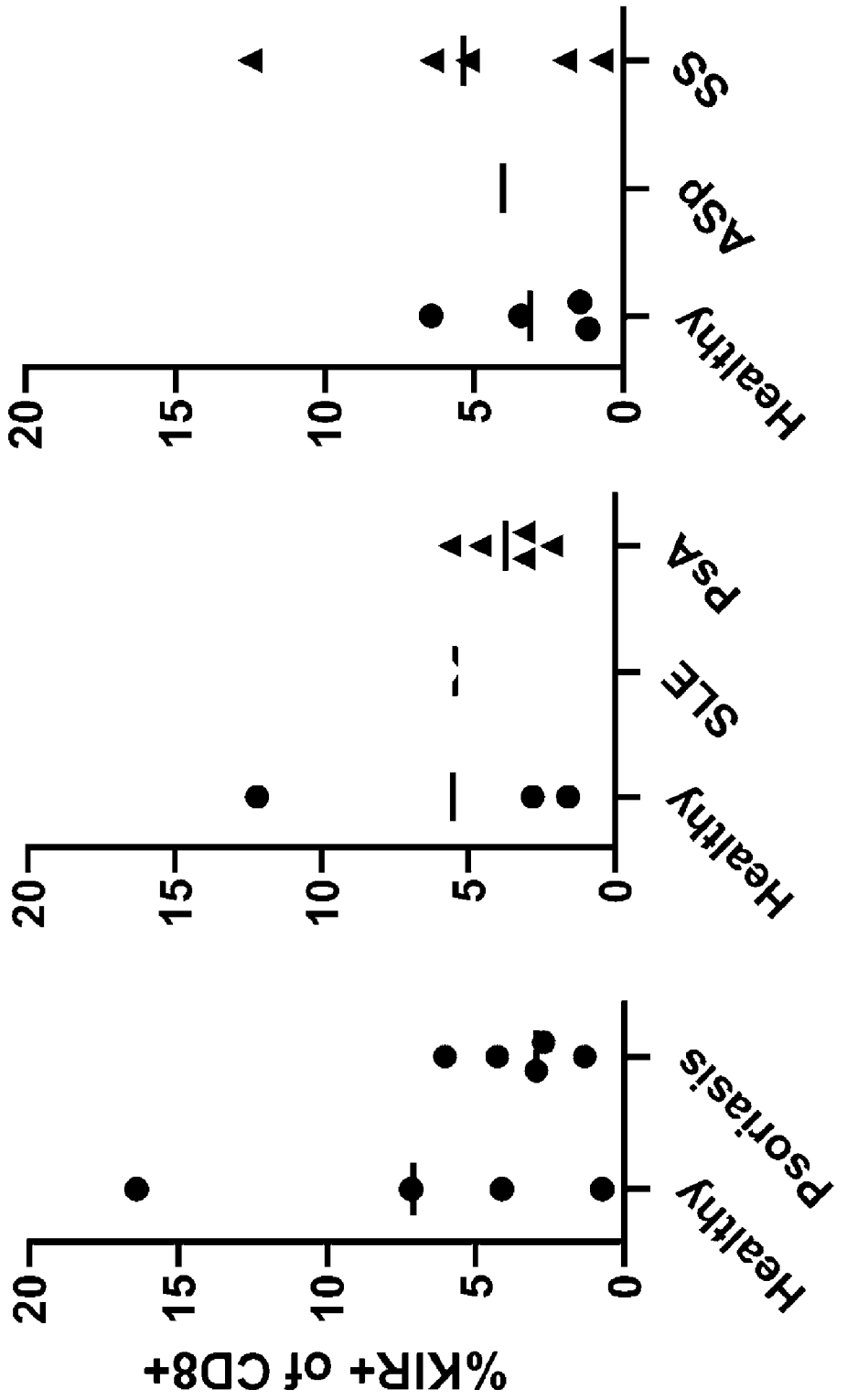

%KIR+ of CD8+
0
5
10
15
20
Healthy
Psoriasis
Healthy
SLE
PsA
Healthy
ASp
SS

FIG. 11

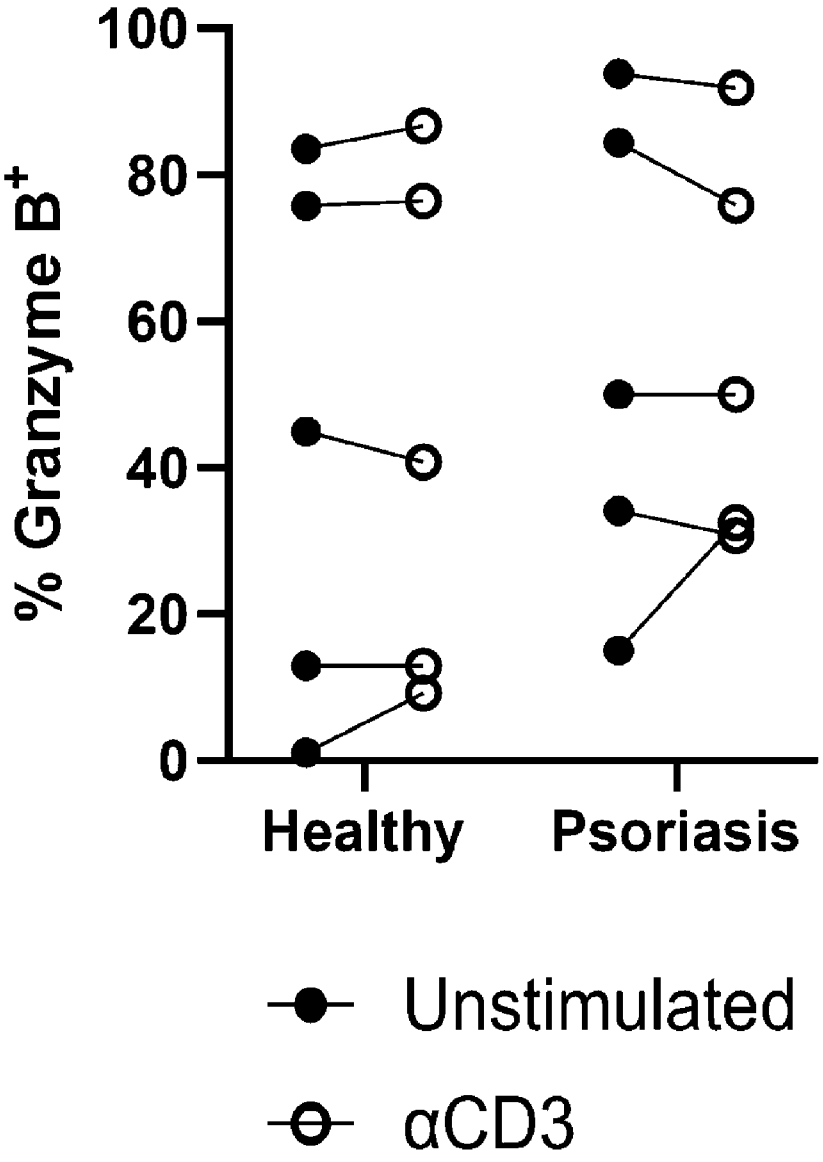

100
80
60
40
20
0
% Granzyme B+
Healthy
Psoriasis
Unstimulated
αCD3

FIG. 12A

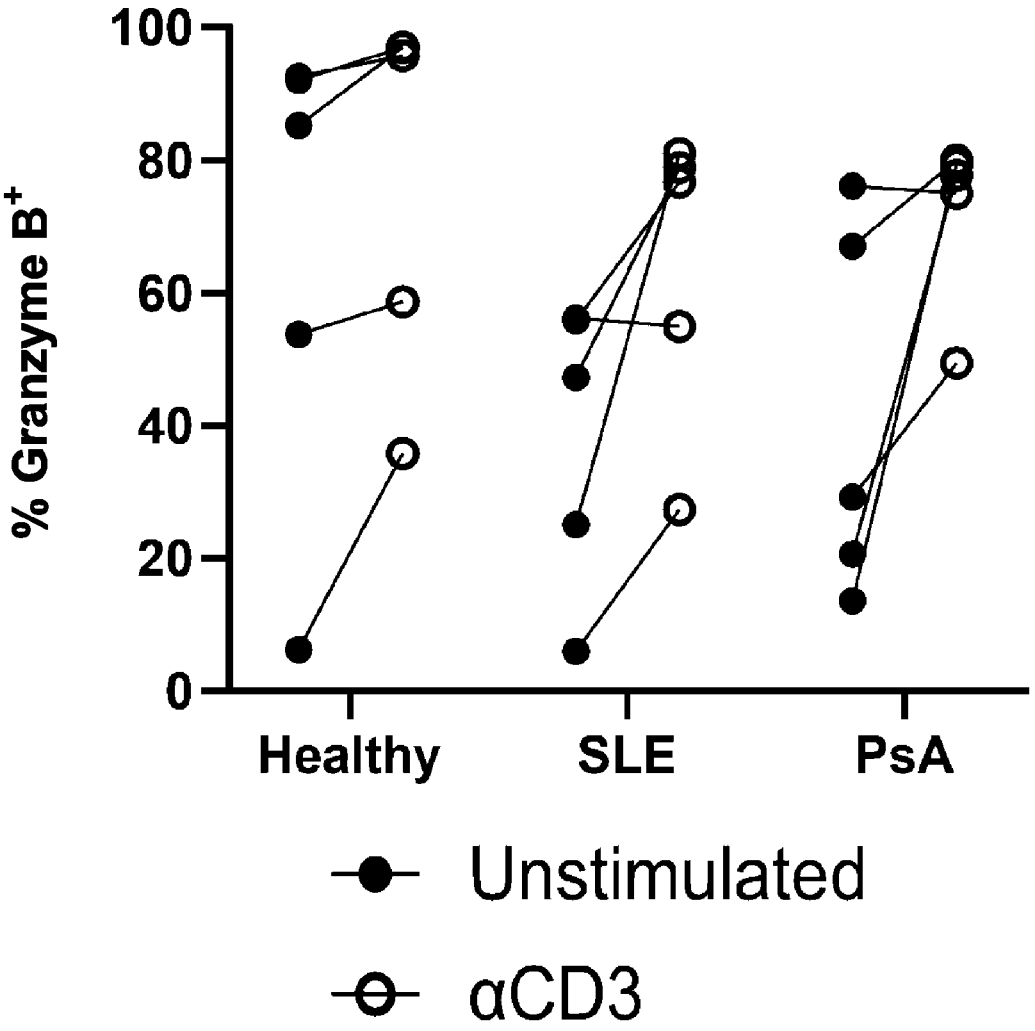

100
80
60
40
20
0
% Granzyme B+
Healthy
SLE
PsA
Unstimulated
αCD3

FIG. 12B

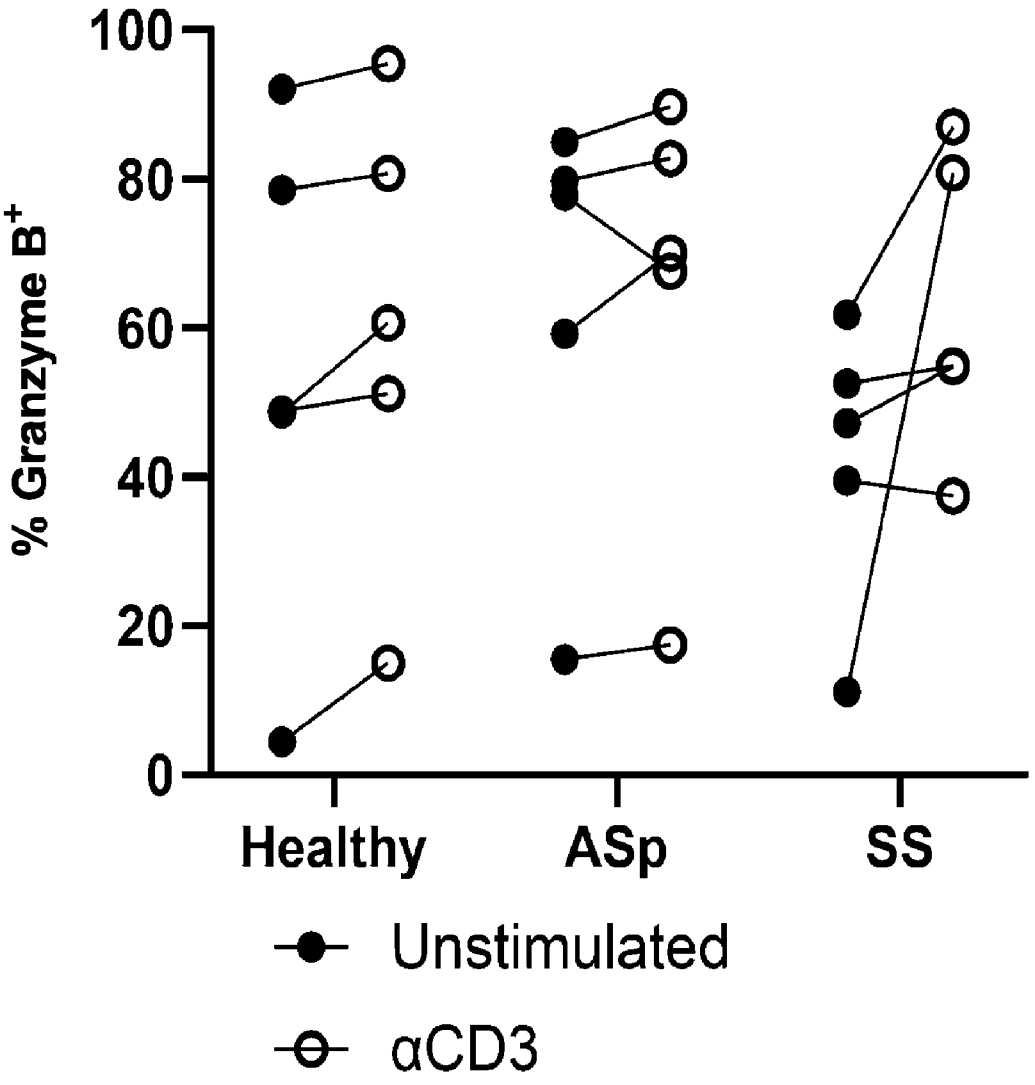
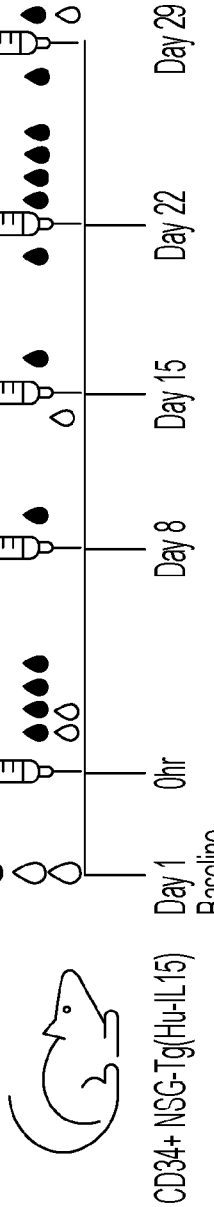


FIG. 12C



- PK time points (●): to evaluate exposure, microsamples were collected pre-dose and following dosing on Day 1 and 22 at 0.5, 2, 24, 96, 168 hours; time points were also collected post-dose on Day 8,15 and 29
- Flow cytometry time points (○): pre-dose on Day 1 and Day 15 and post-dose on Day 29
- Serum cytokine time points (○): pre-dose on Day 1 and at 8 and 24 hr post-dose

FIG. 13A

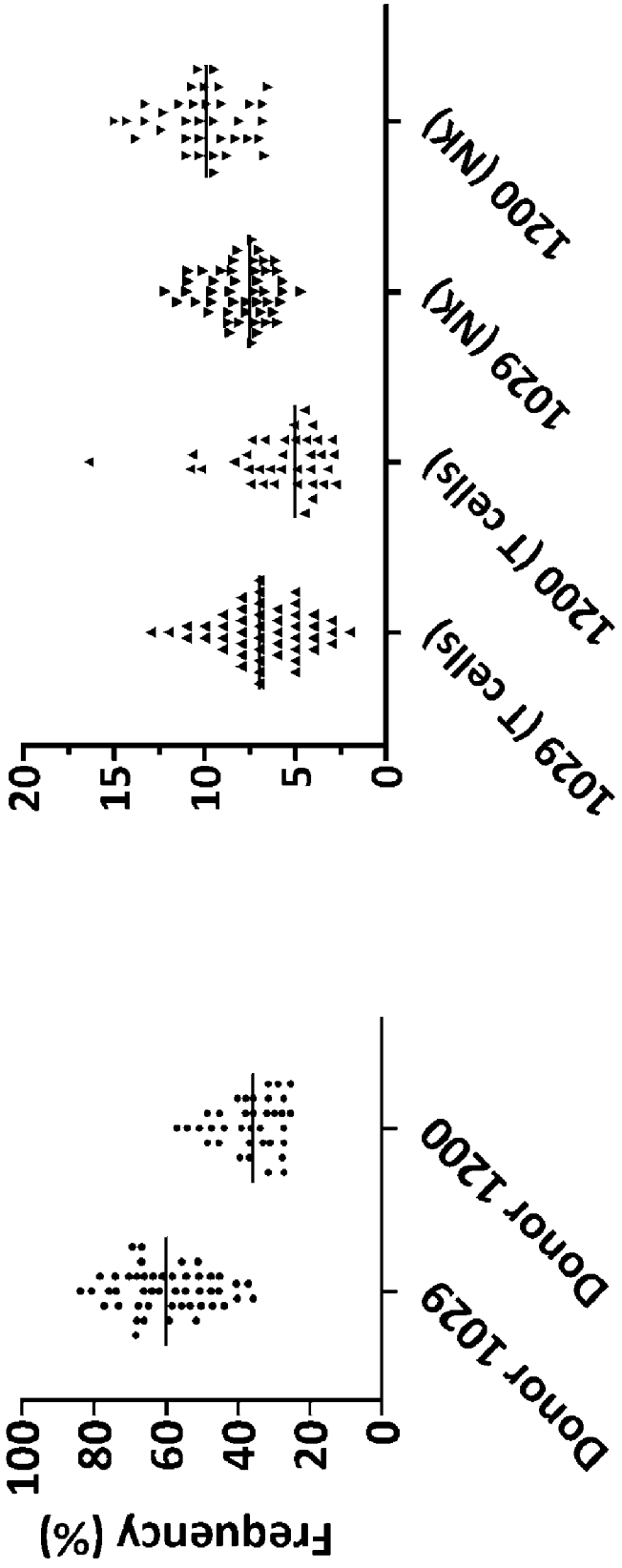

Frequency (%)
100
80
60
40
20
0
Donor 1029
Donor 1200
20
15
10
5
0
1029 (T cells)
1200 (T cells)
1029 (NK)
1200 (NK)

FIG. 13B

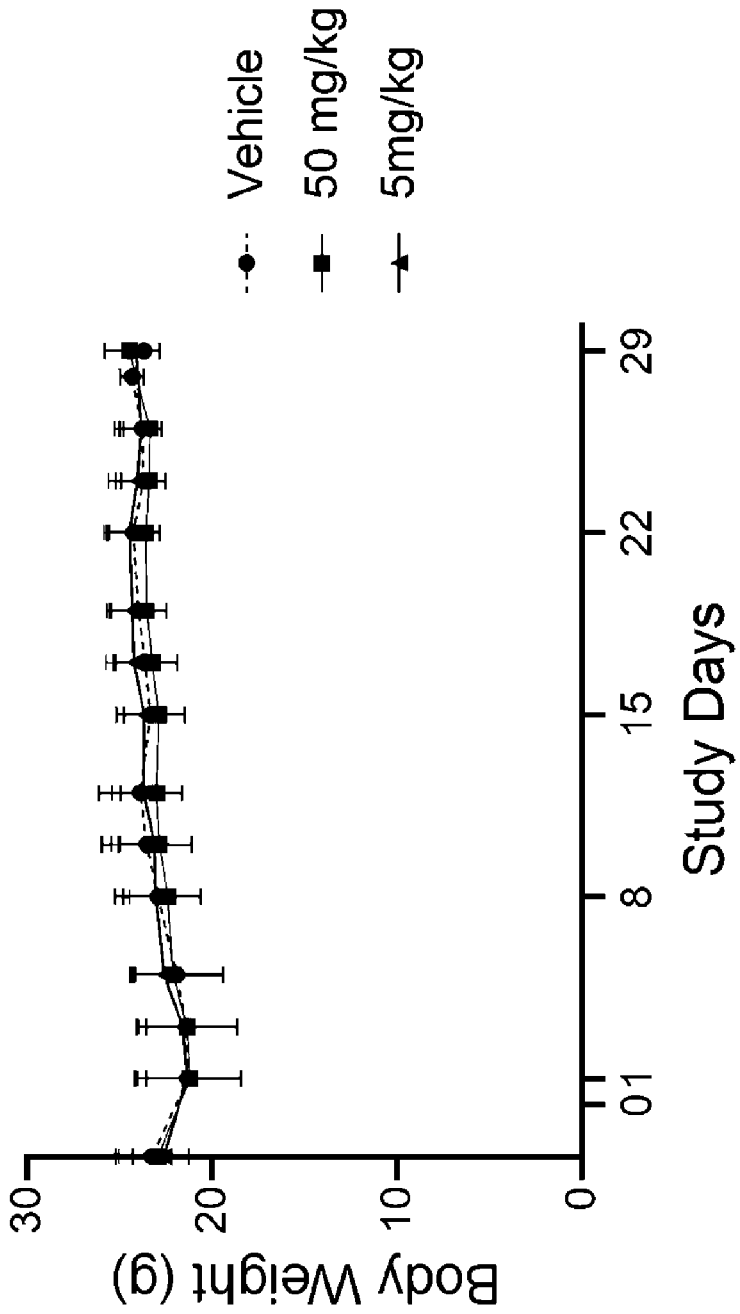

Vehicle
50 mg/kg
5mg/kg
Body Weight (g)
0
10
20
30
01
8
15
22
29
Study Days

FIG. 13C

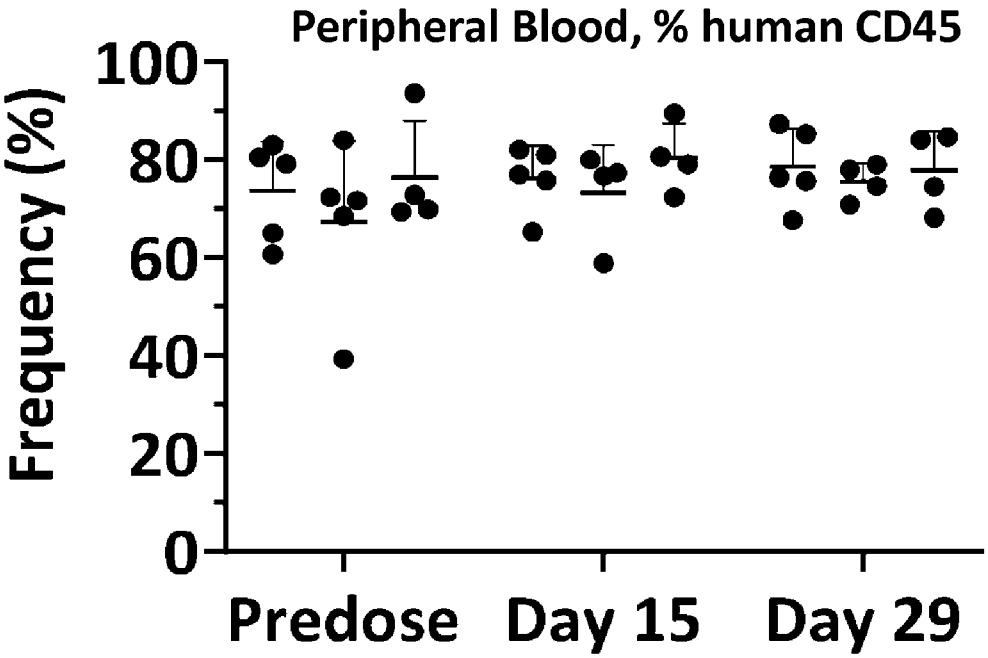


FIG. 13D

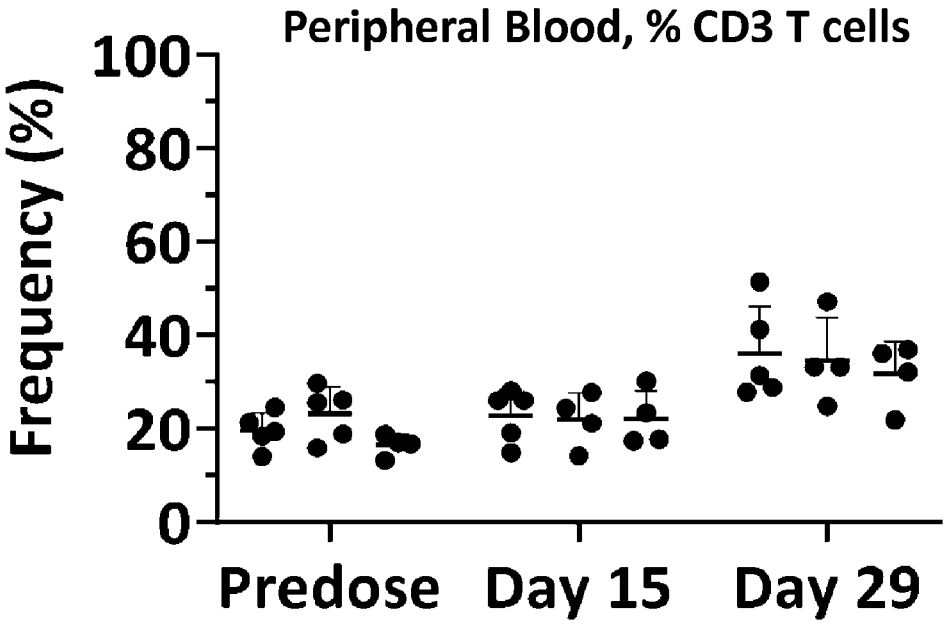


FIG. 13E

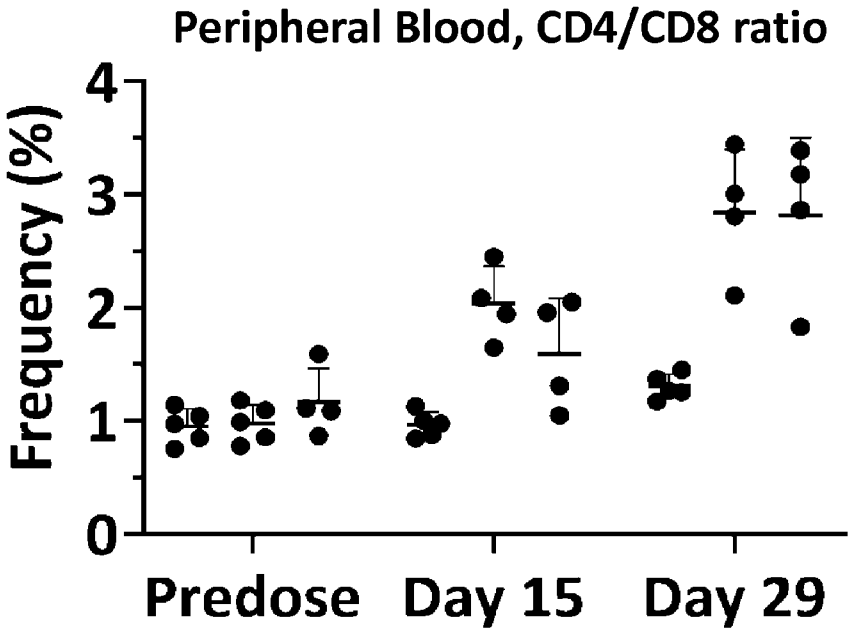


FIG. 13F

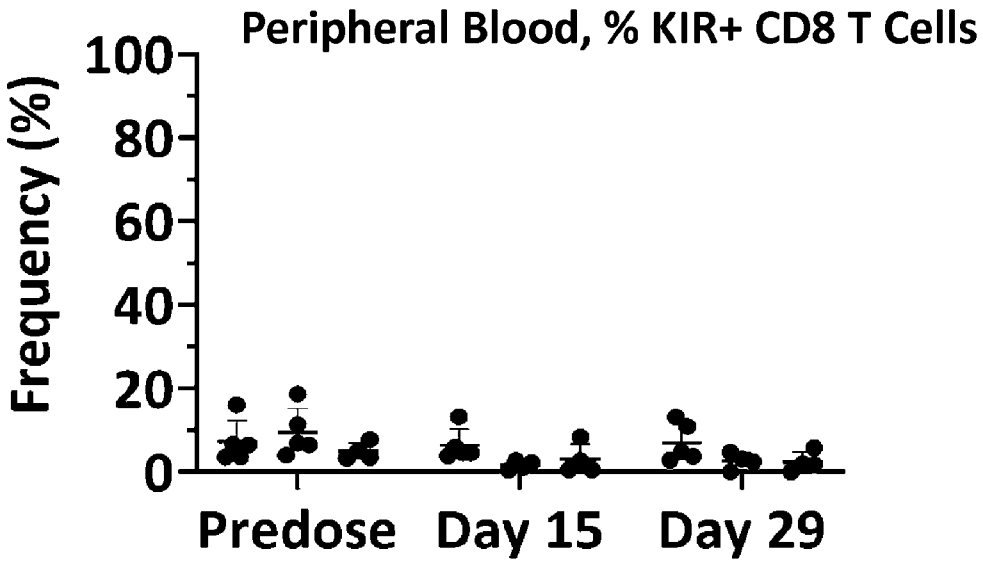


FIG. 13G

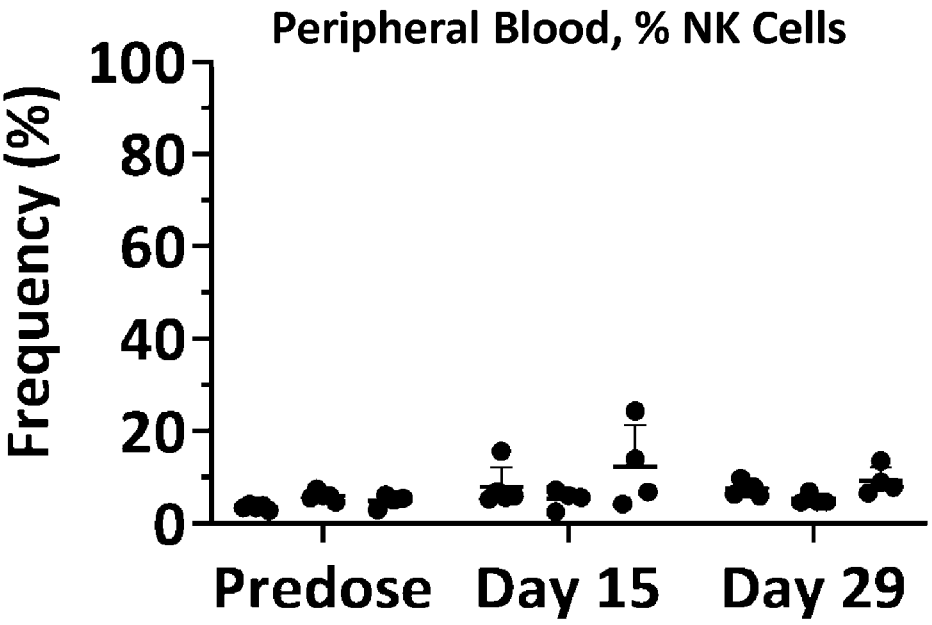


FIG. 13H

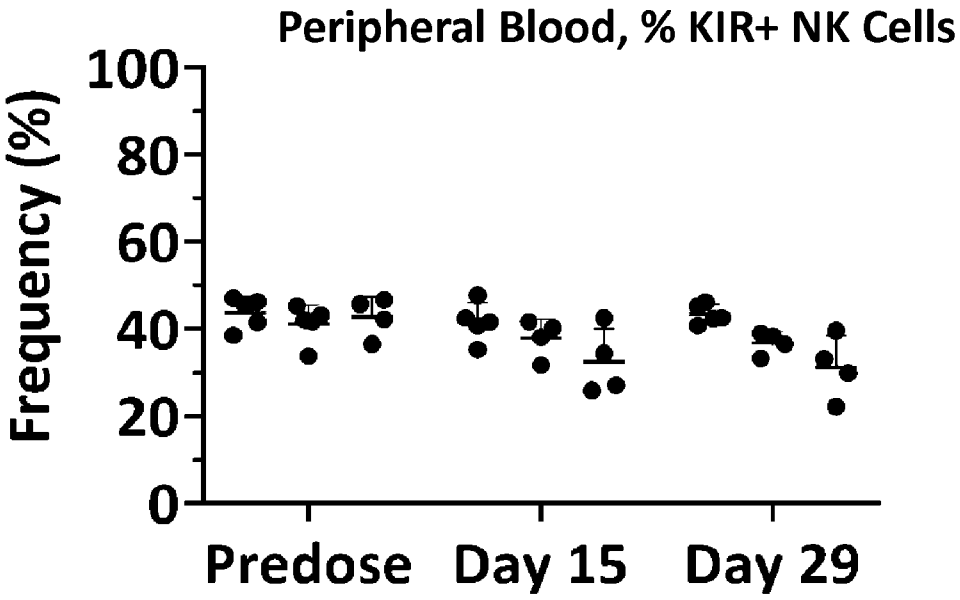


FIG. 13I

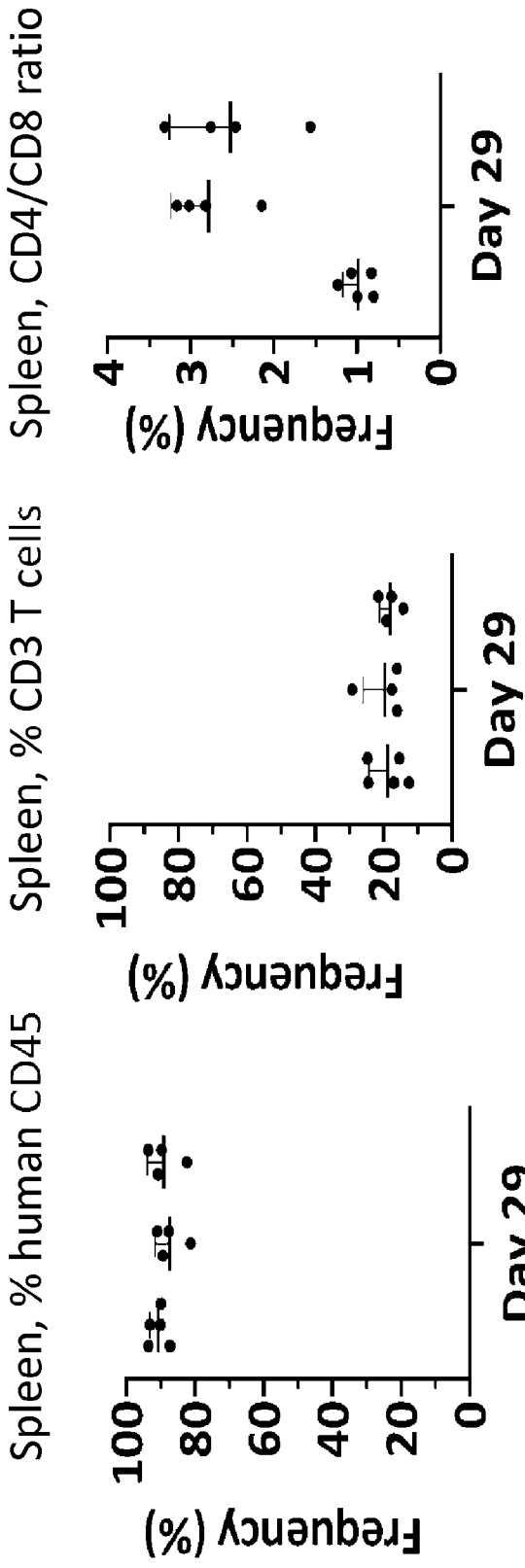


FIG. 13J

FIG. 13K

FIG. 13L

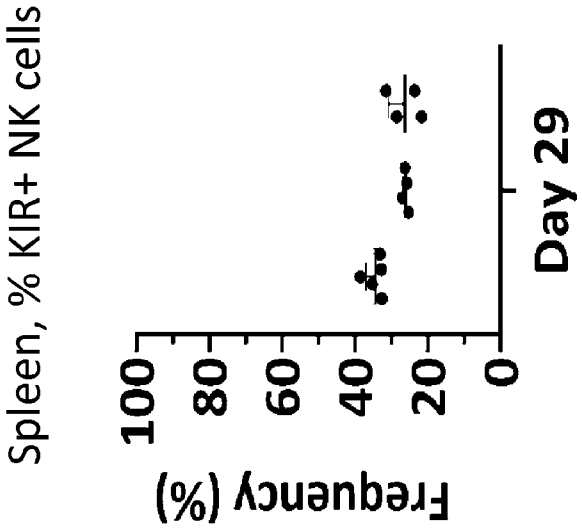


FIG. 13O

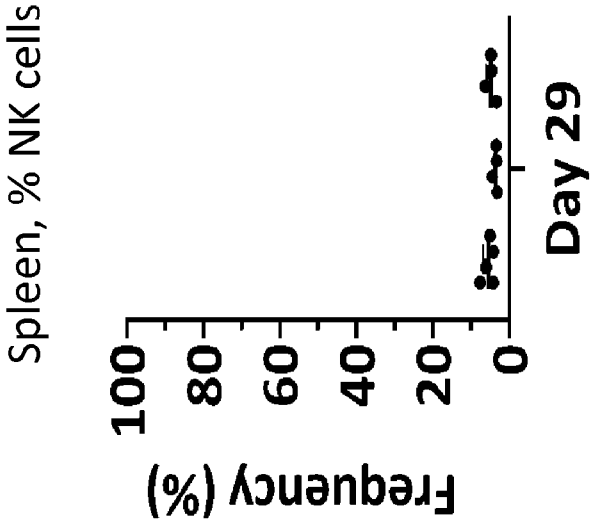


FIG. 13N

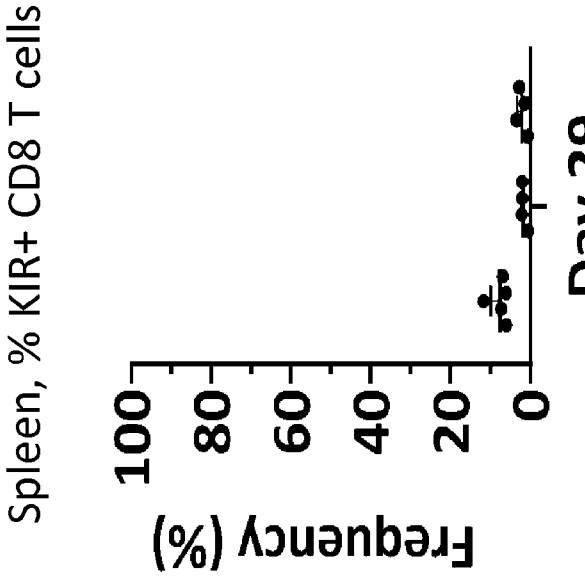


FIG. 13M

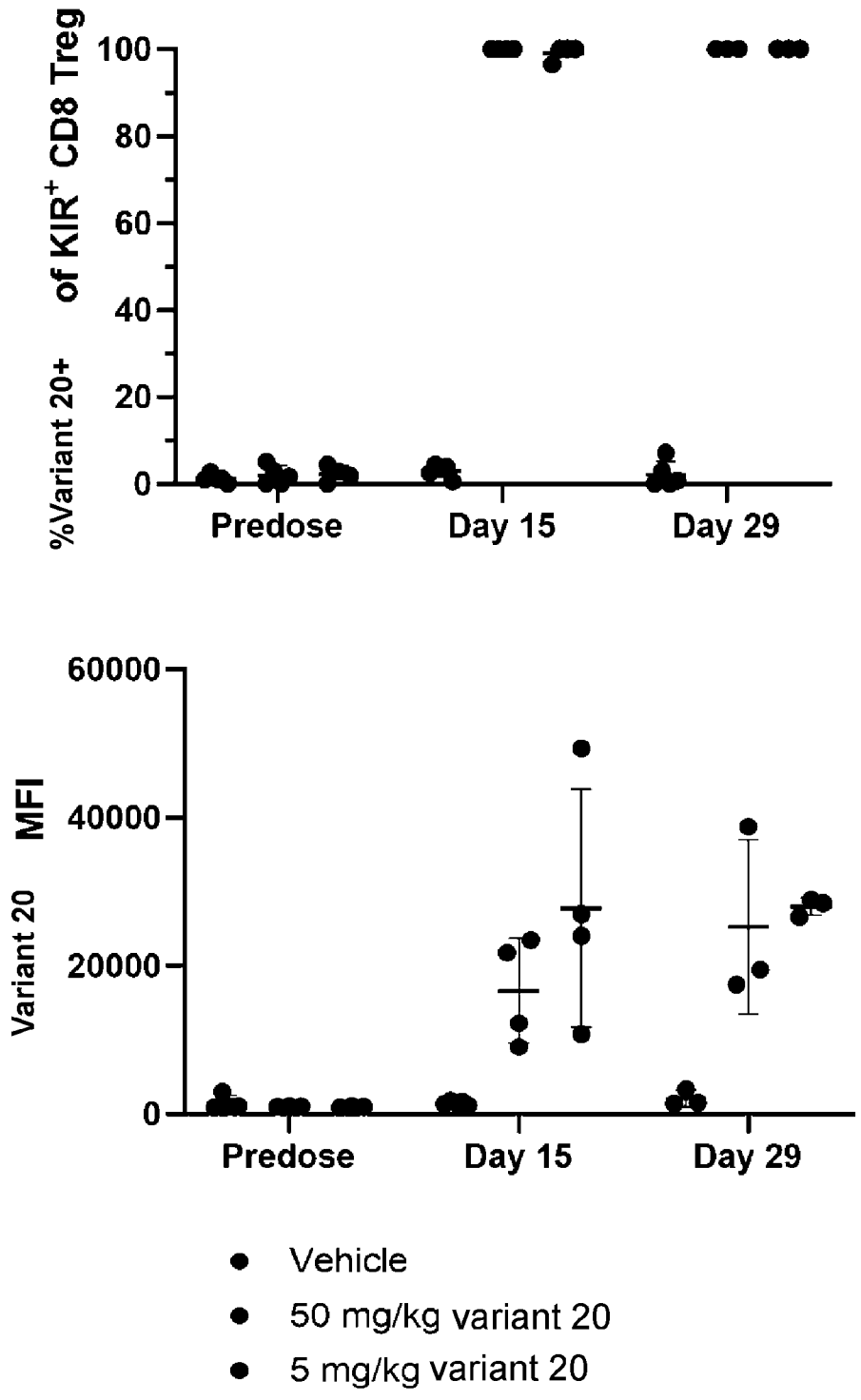

%Variant 20+ of KIR+ CD8 Treg
100
80
60
40
20
0
Predose
Day 15
Day 29
Variant 20 MFI
60000
40000
20000
0
Predose
Day 15
Day 29
Vehicle
50 mg/kg variant 20
5 mg/kg variant 20

FIG. 14A

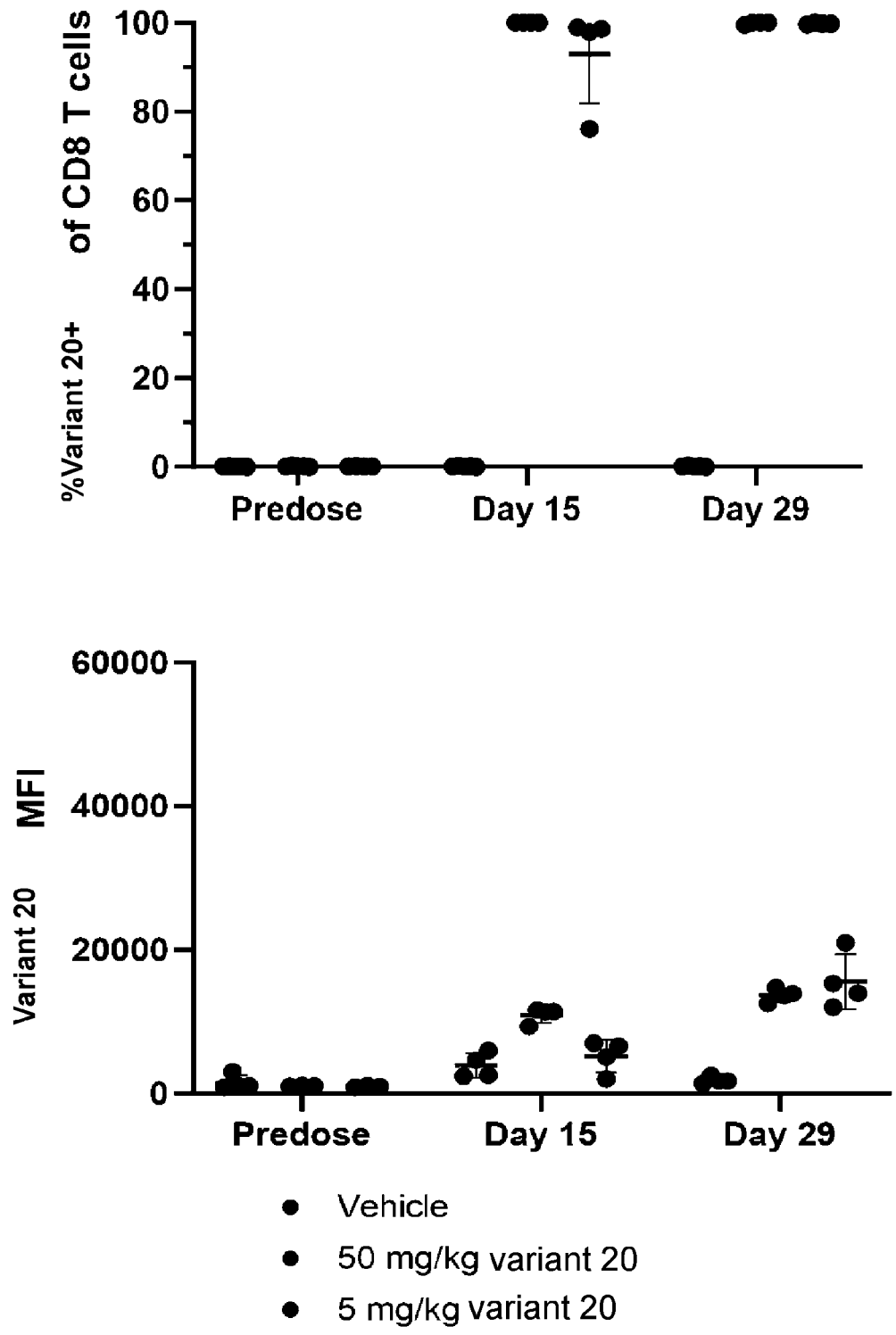

%Variant 20+ of CD8 T cells
100
80
60
40
20
0
Predose
Day 15
Day 29
Variant 20 MFI
60000
40000
20000
0
Predose
Day 15
Day 29
Vehicle
50 mg/kg variant 20
5 mg/kg variant 20

FIG. 14B

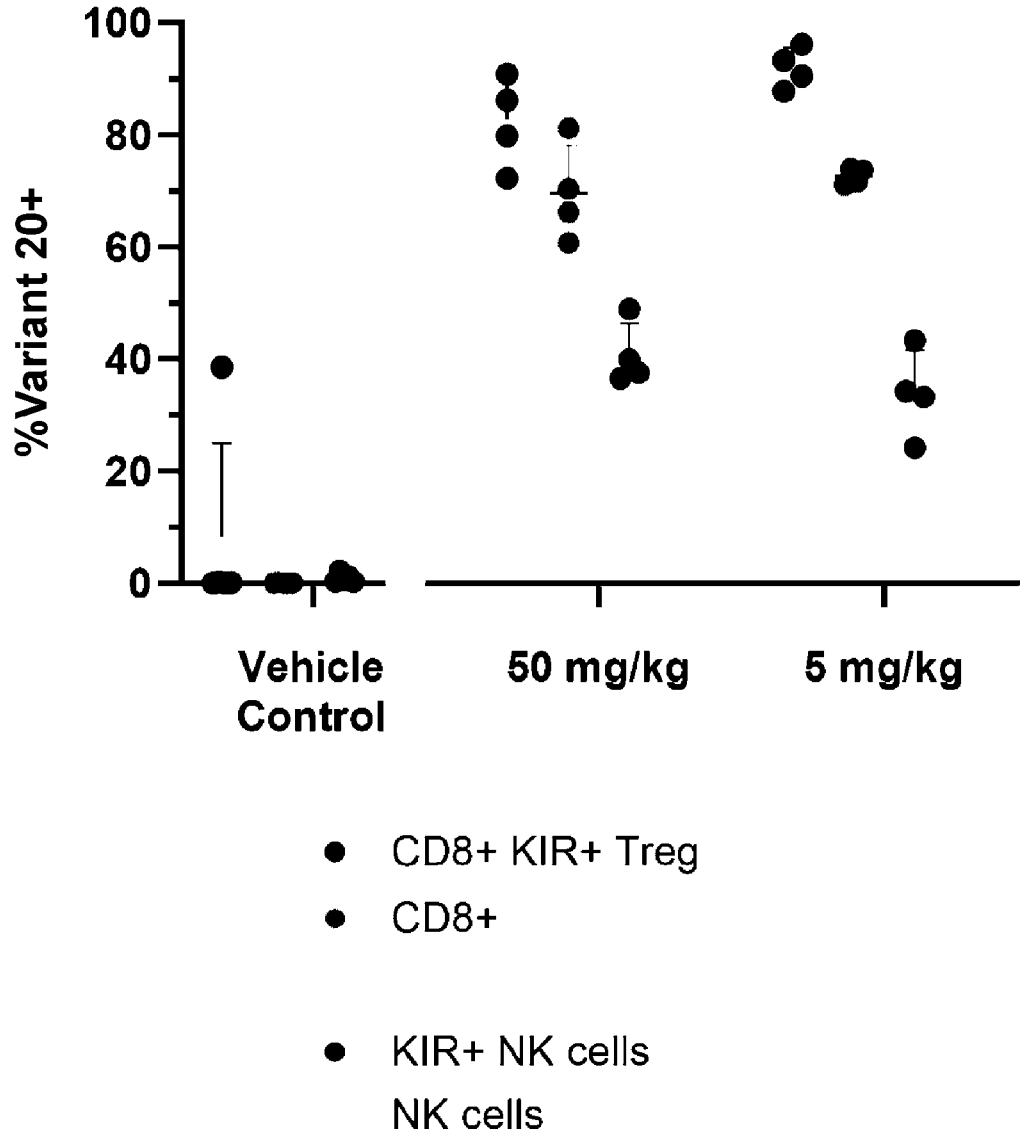

100
80
60
40
20
0
%Variant 20+
Vehicle Control
50 mg/kg
5 mg/kg
CD8+ KIR+ Treg
CD8+
KIR+ NK cells
NK cells

FIG. 14C

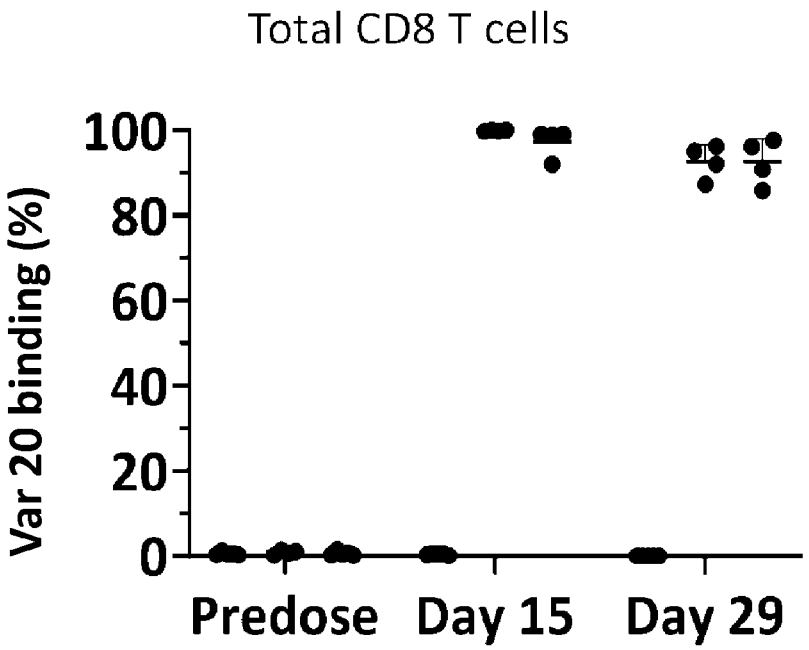


FIG. 14D

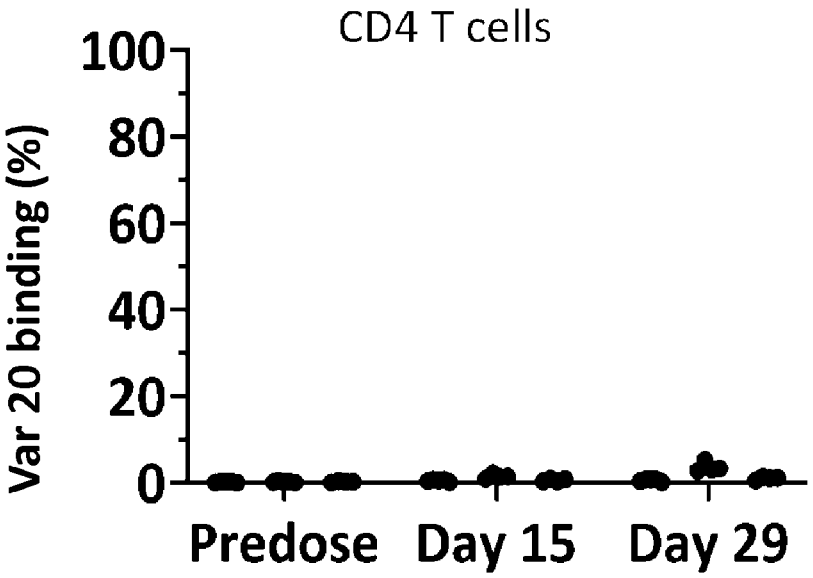


FIG. 14E

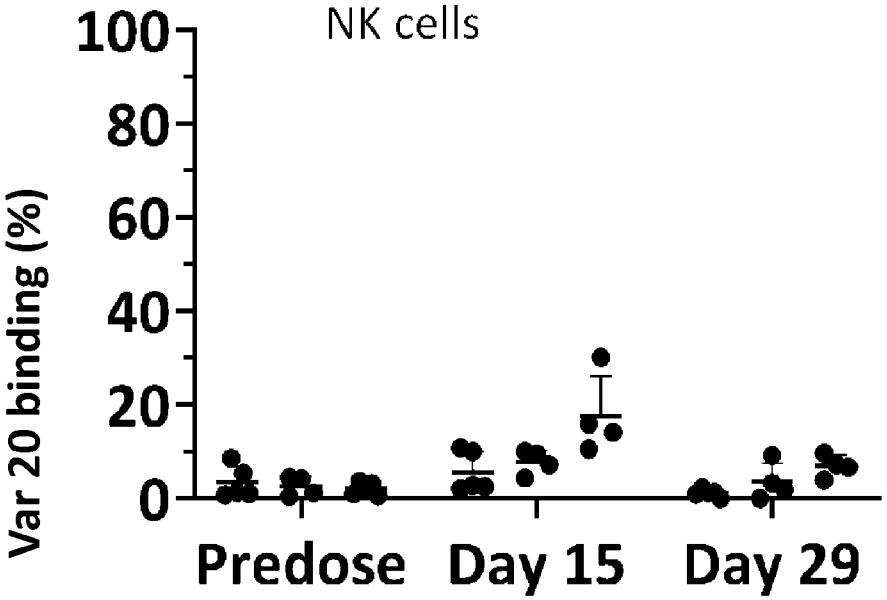


FIG. 14F

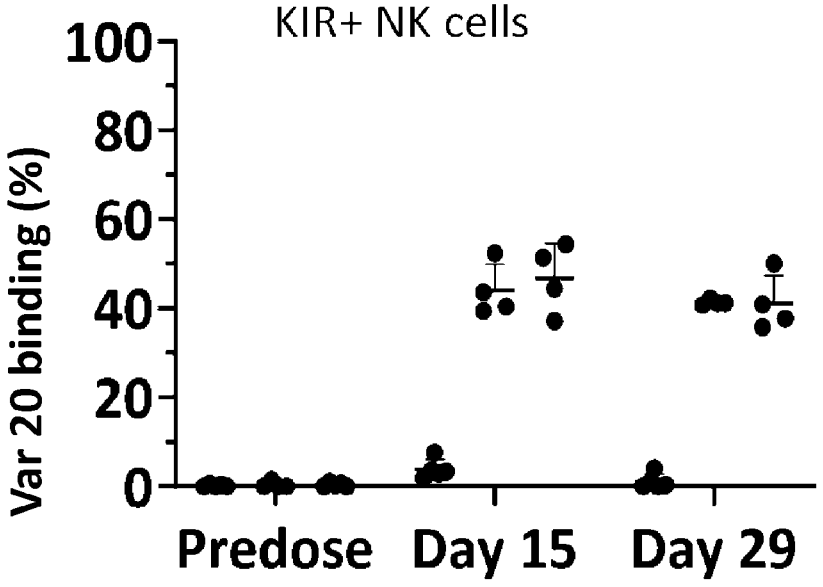


FIG. 14G

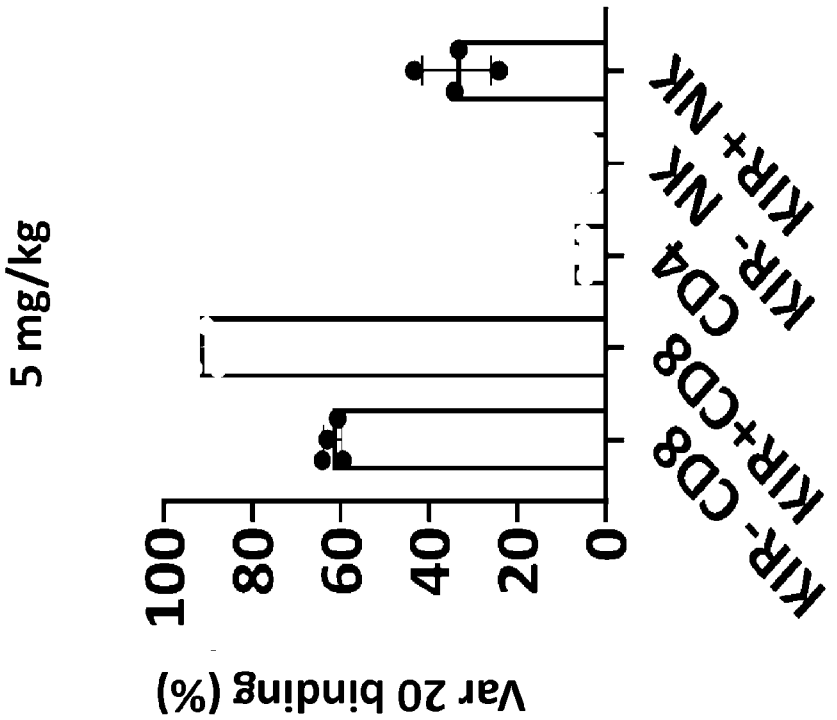


FIG. 14I

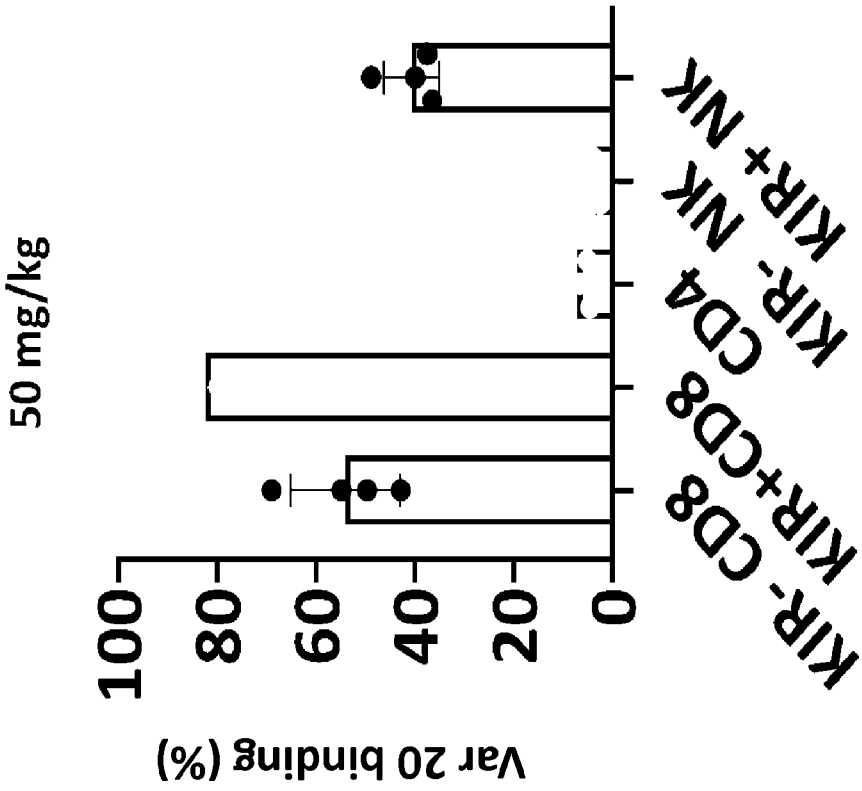


FIG. 14H

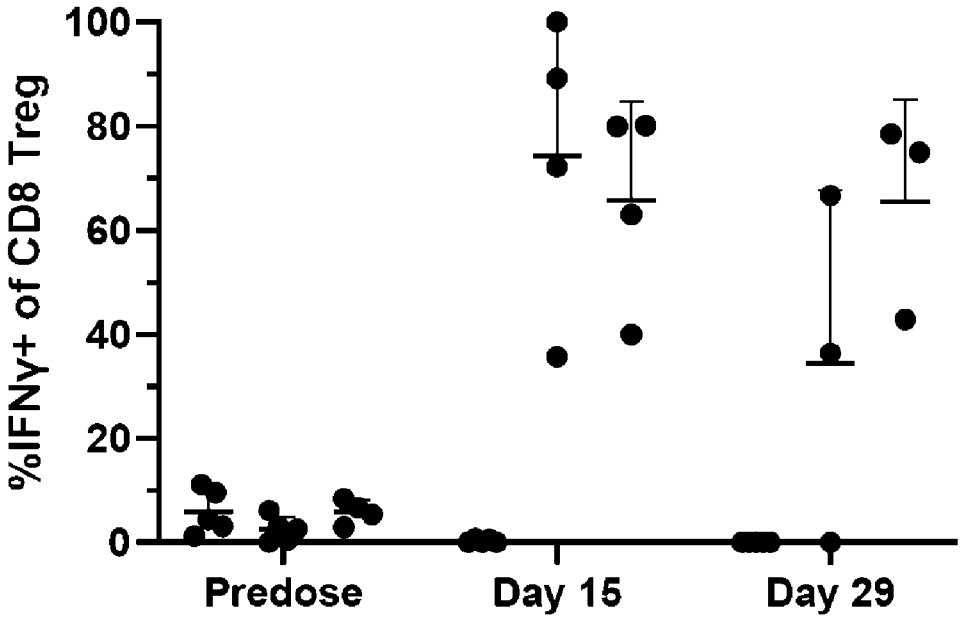


FIG. 15A

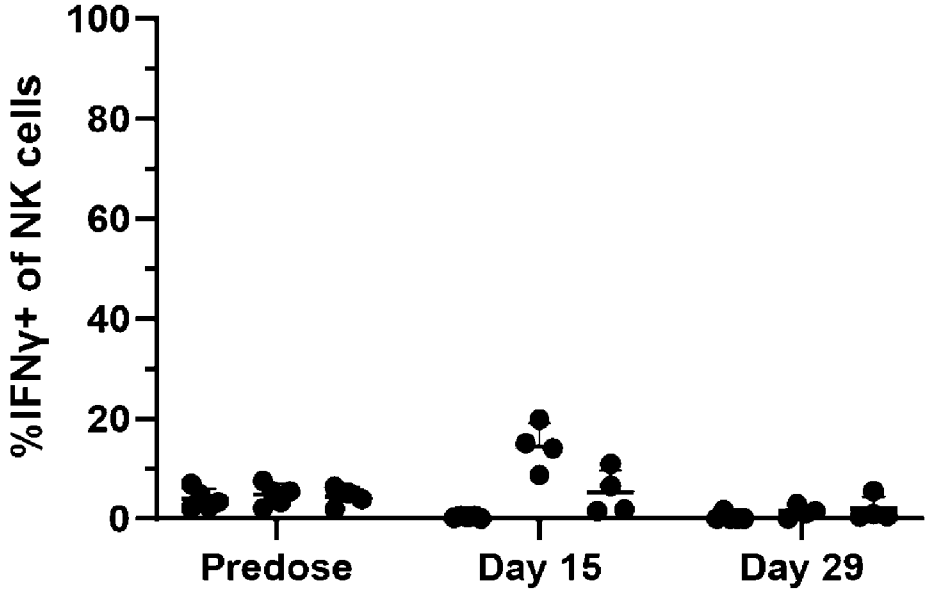


FIG. 15B

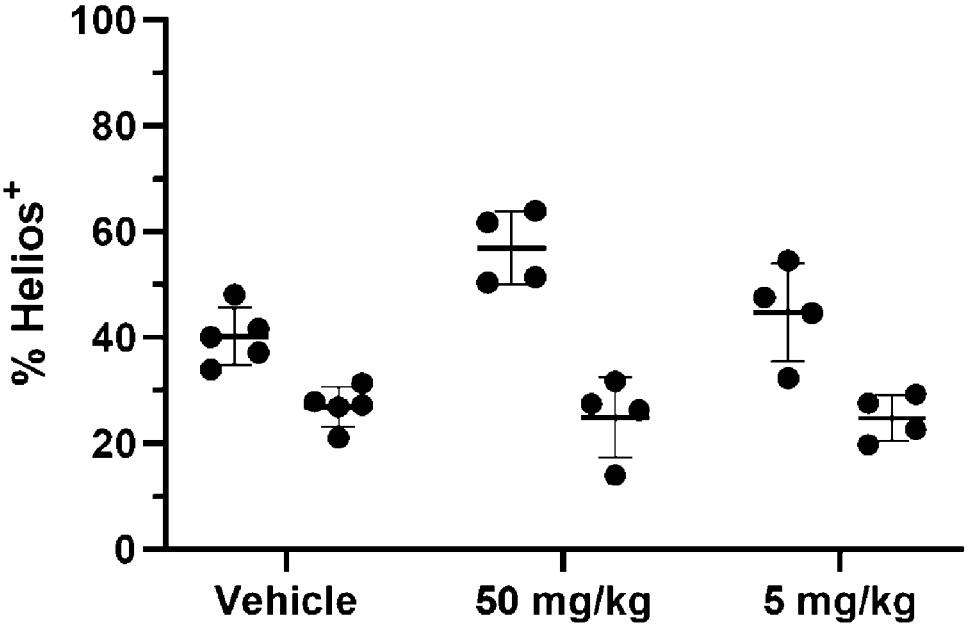


FIG. 15C

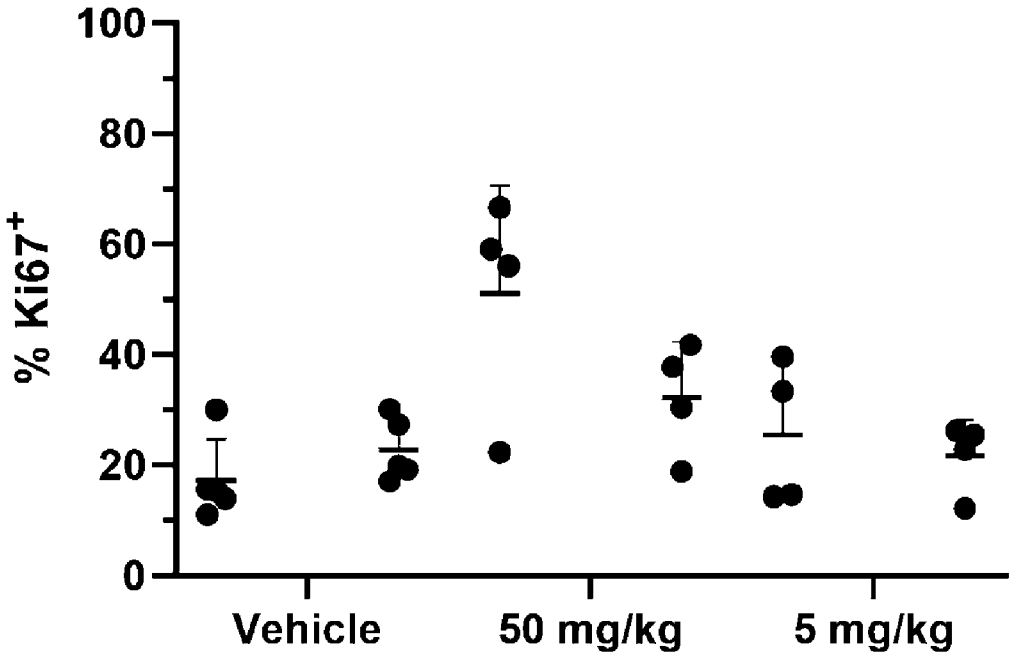


FIG. 15D

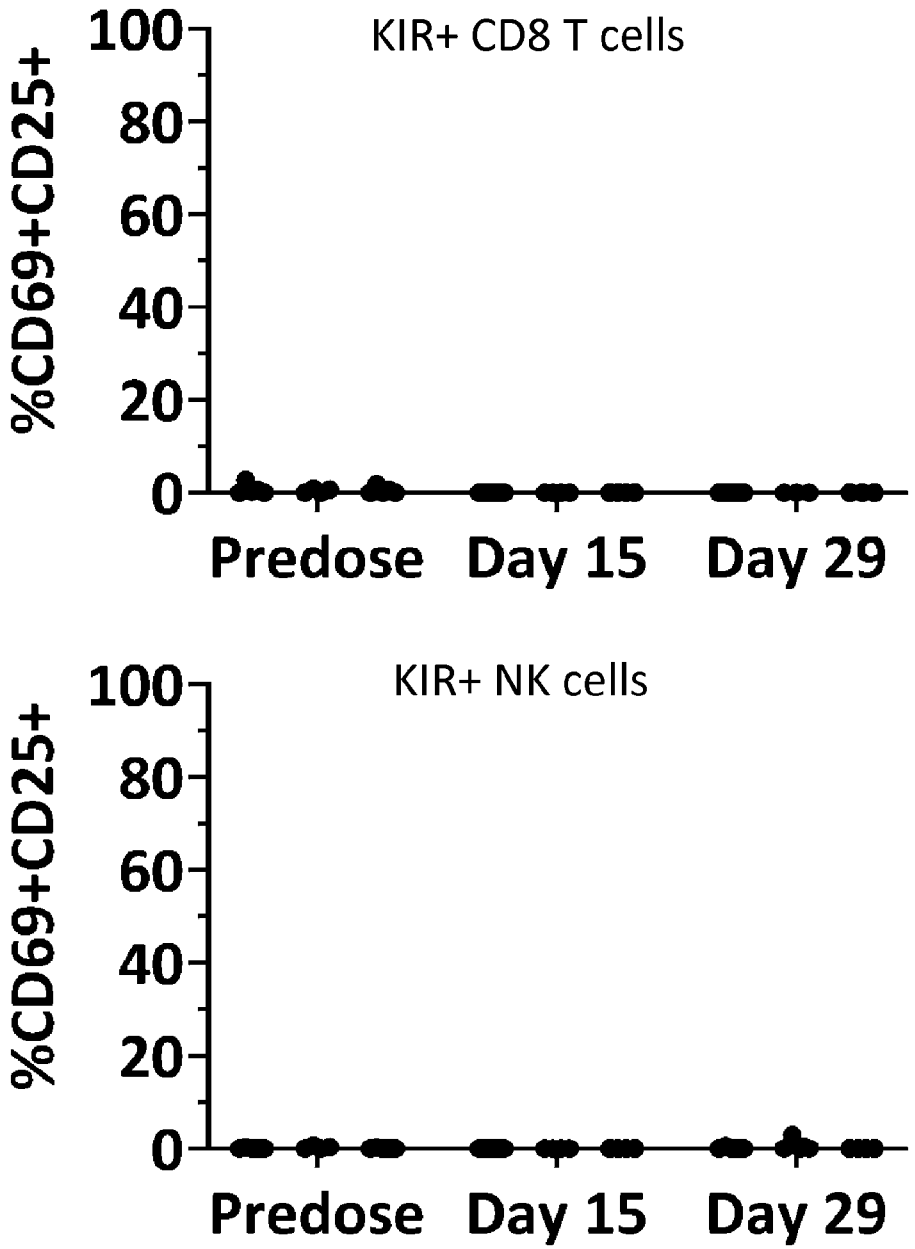

KIR+ CD8 T cells
%CD69+CD25+
100
80
60
40
20
0
Predose
Day 15
Day 29
KIR+ NK cells
%CD69+CD25+
100
80
60
40
20
0
Predose
Day 15
Day 29

FIG. 15E

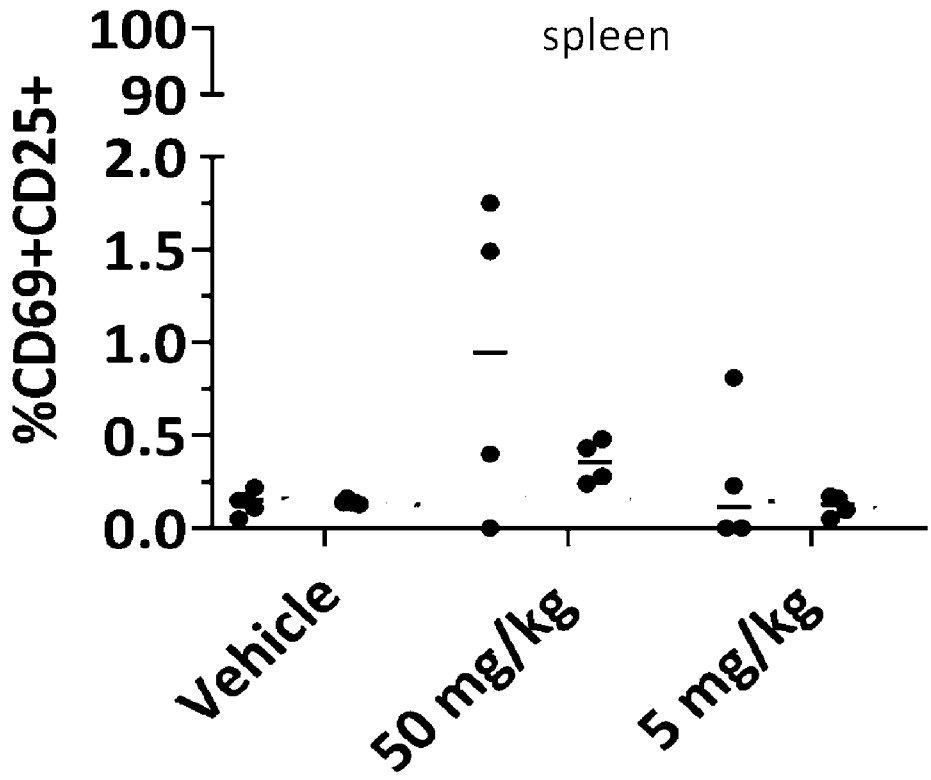


FIG. 15F

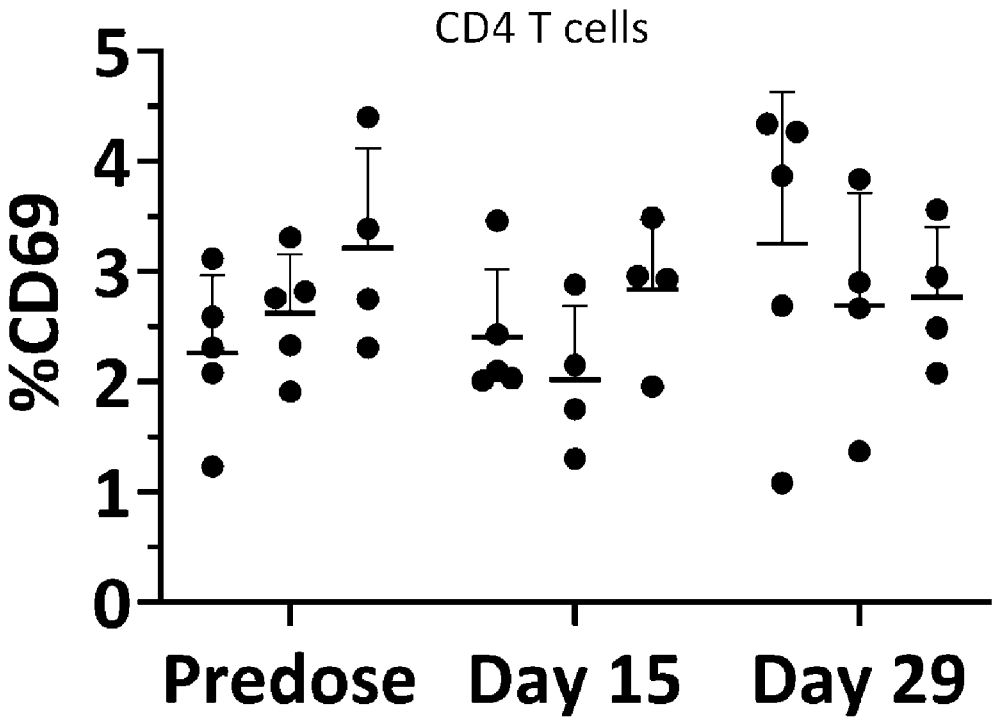


FIG. 15G

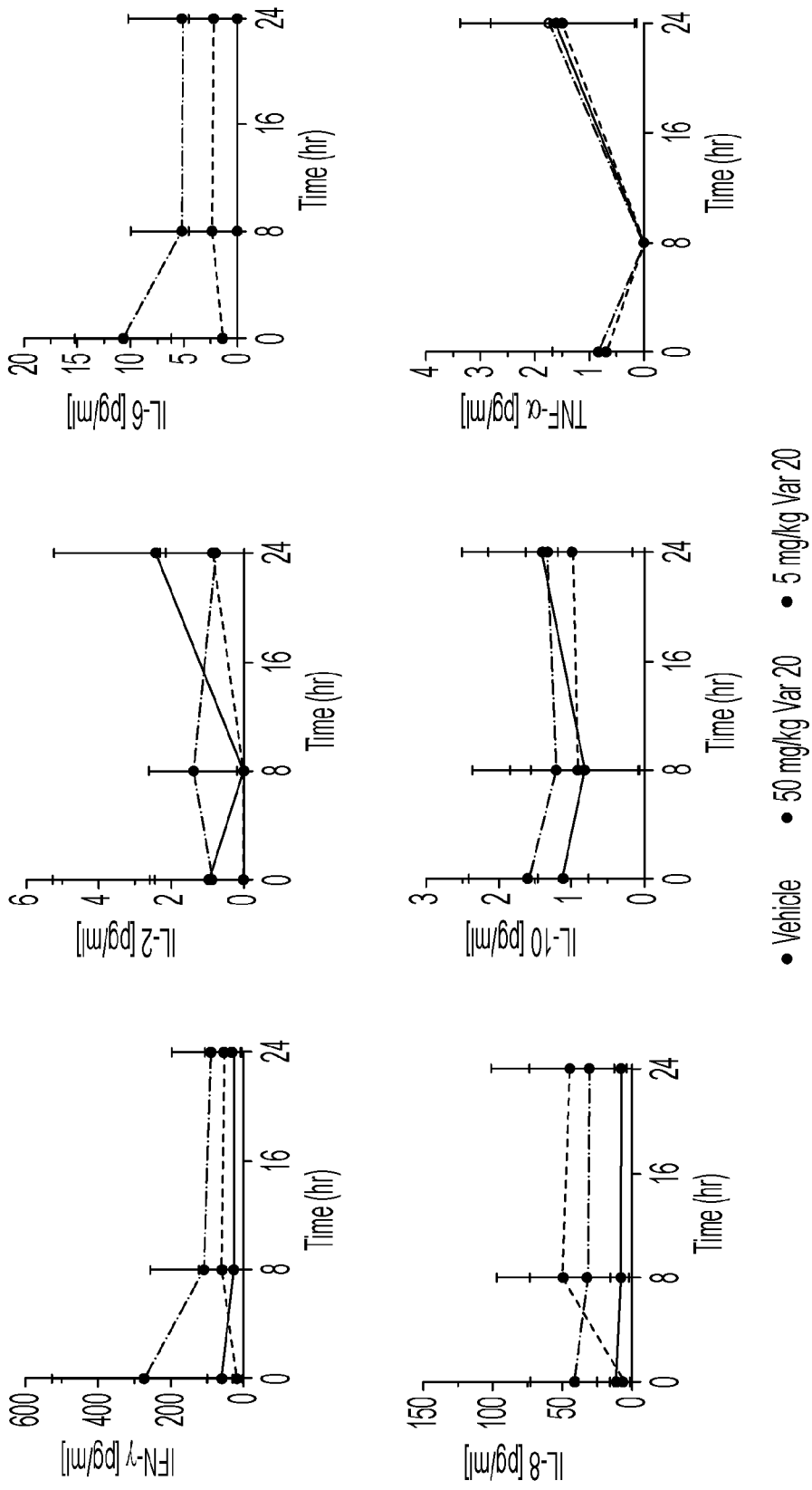

IL-6 [pg/ml]
TNF-α [pg/ml]
IL-2 [pg/ml]
IL-10 [pg/ml]
IFN-γ [pg/ml]
IL-8 [pg/ml]
Time (hr)
• Vehicle
• 50 mg/kg Var 20
• 5 mg/kg Var 20

FIG. 16

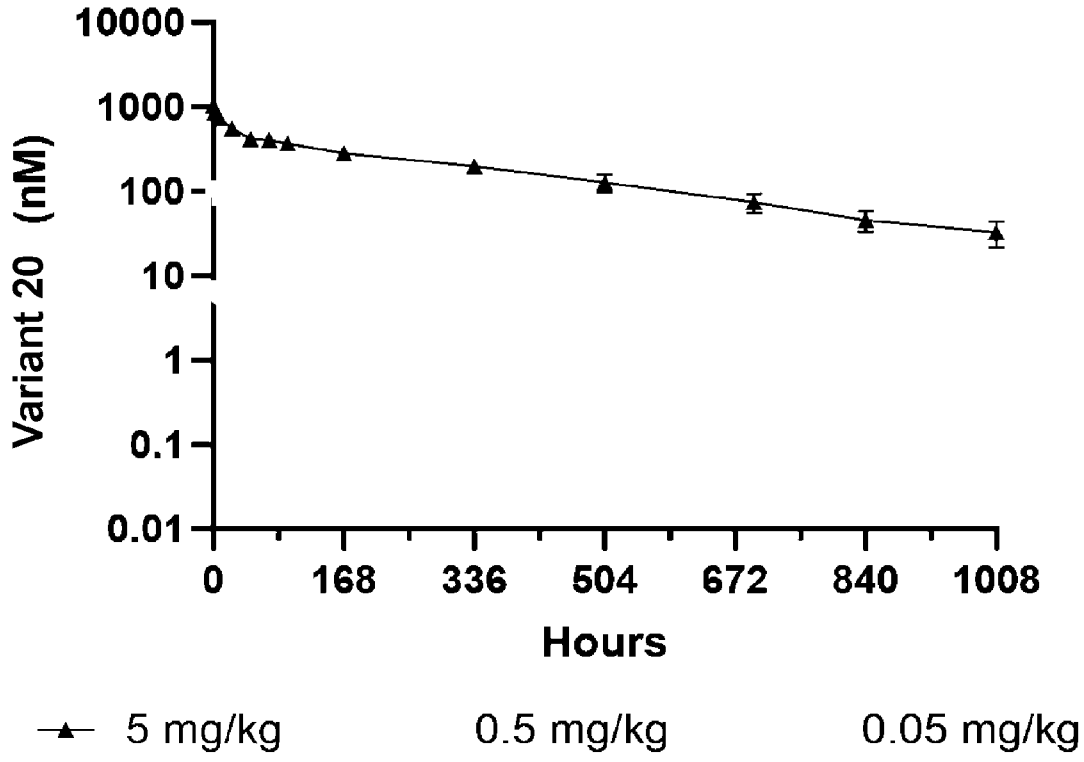

10000
1000
100
10
1
0.1
0.01
Variant 20 (nM)
0
168
336
504
672
840
1008
Hours
5 mg/kg
0.5 mg/kg
0.05 mg/kg

FIG. 17A

	Variant 20		
	0.05 mg/kg	**0.5 mg/kg**	**5 mg/kg**
C_{max} (µg/mL)	0.905	13.7	128
AUC_{0-t} (hr*µg/mL)	144	2318.4	21264
AUC_{0-inf} (hr*µg/mL)	153.84	2544	22704
$t_{1/2}$ (hr)	231.6	276	249.6
C_L (mL/hr/kg)	0.325	0.200	0.223
Vz (mL/kg)	108	77.6	80

FIG. 17B

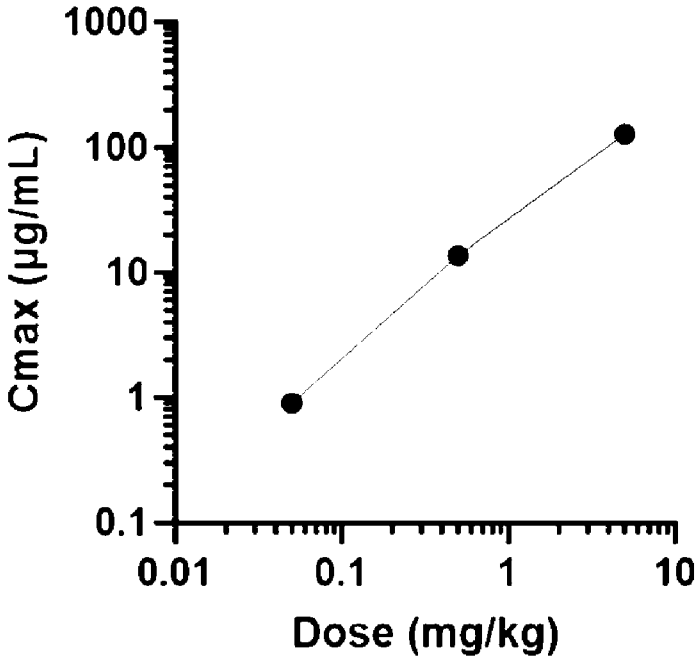


FIG. 17C

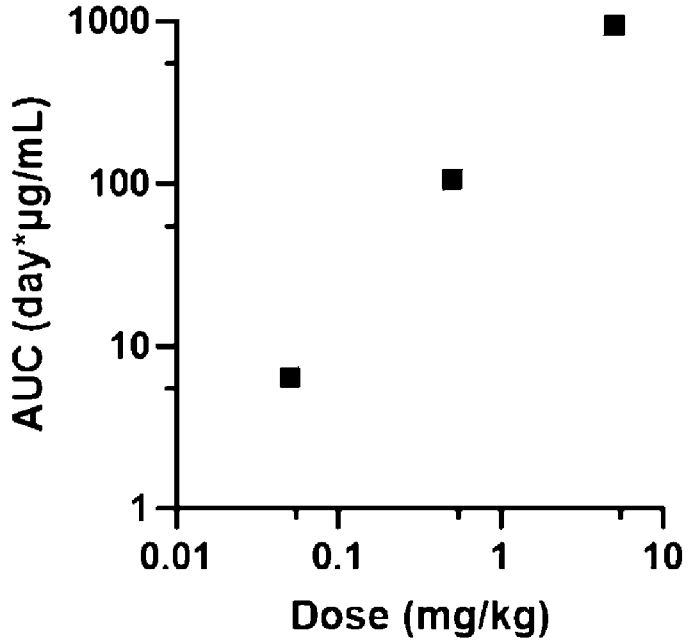


FIG. 17D

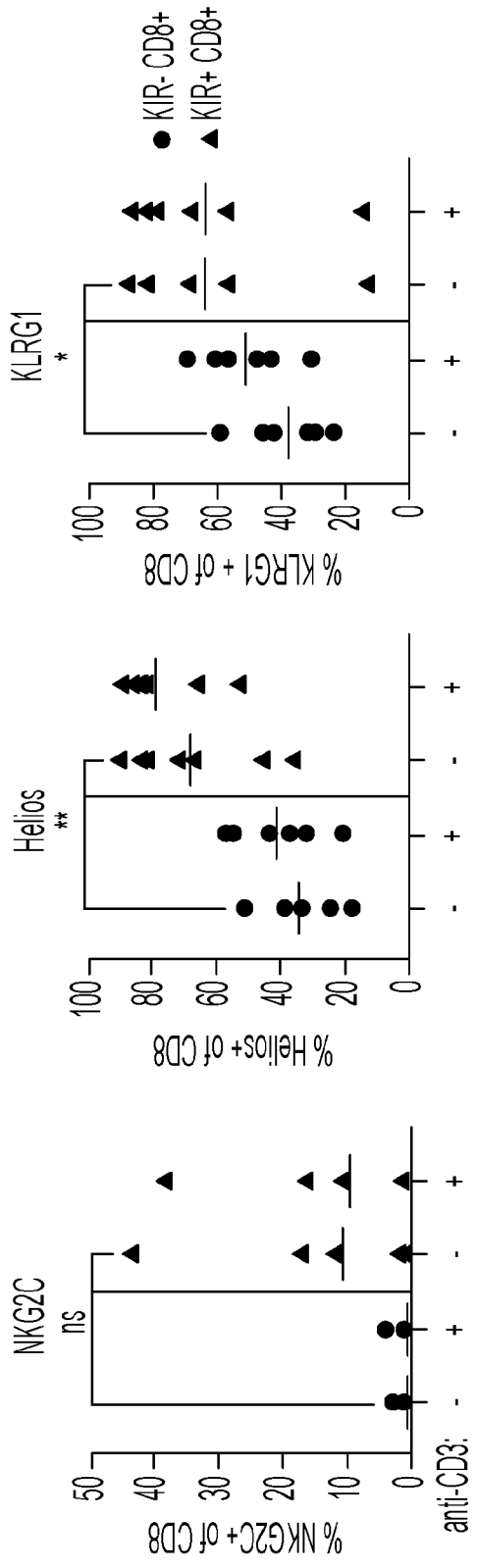

KIR- CD8+
KIR+ CD8+
KLRG1
*
% KLRG1 + of CD8
Helios
**
% Helios+ of CD8
NKG2C
ns
% NKG2C+ of CD8
anti-CD3:

FIG. 18A

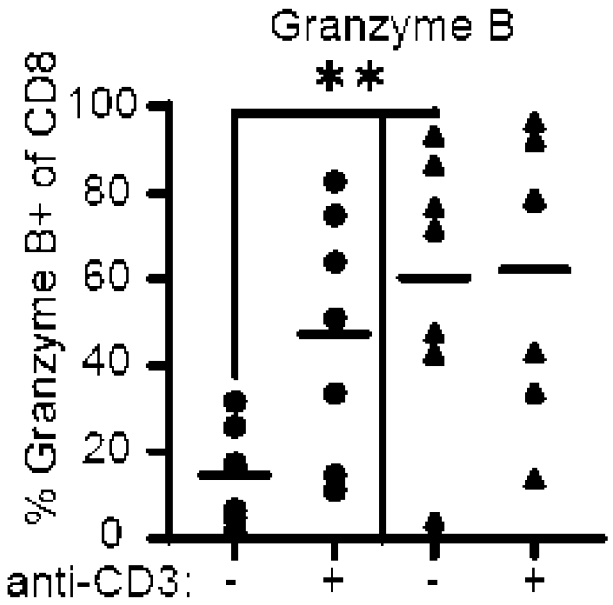


FIG. 18B

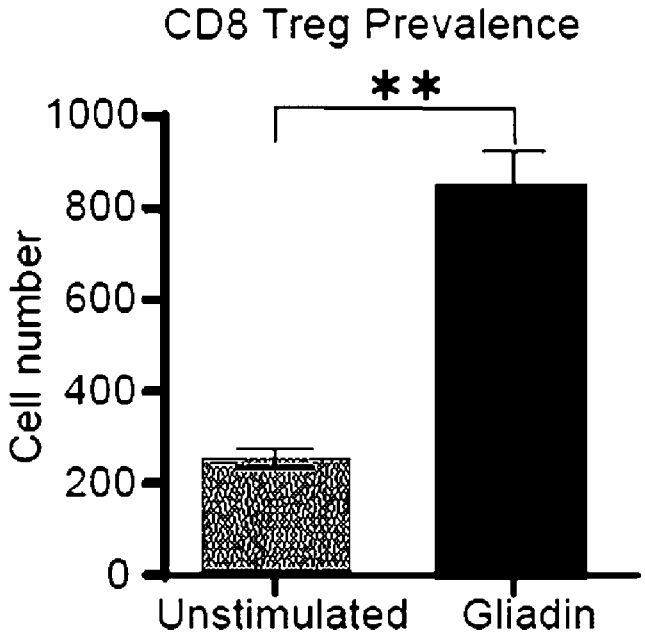


FIG. 18C

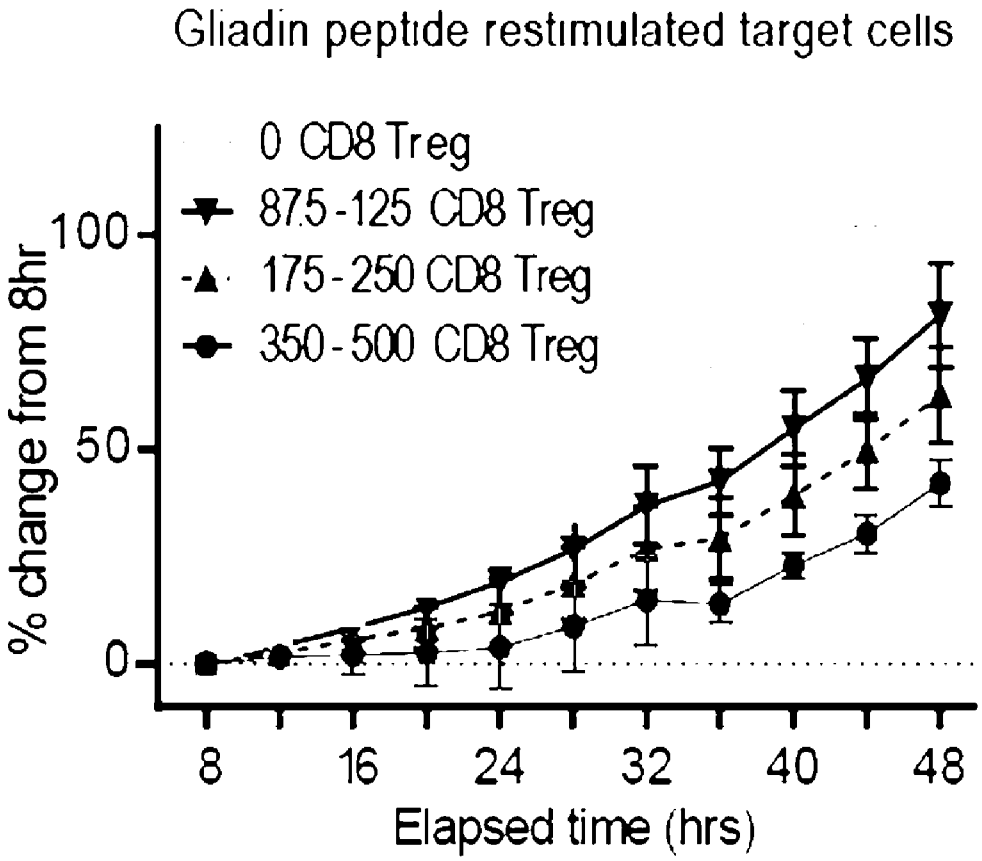

Gliadin peptide restimulated target cells
0 CD8 Treg
875-125 CD8 Treg
175-250 CD8 Treg
350-500 CD8 Treg
% change from 8hr
100
50
0
8
16
24
32
40
48
Elapsed time (hrs)

FIG. 18D

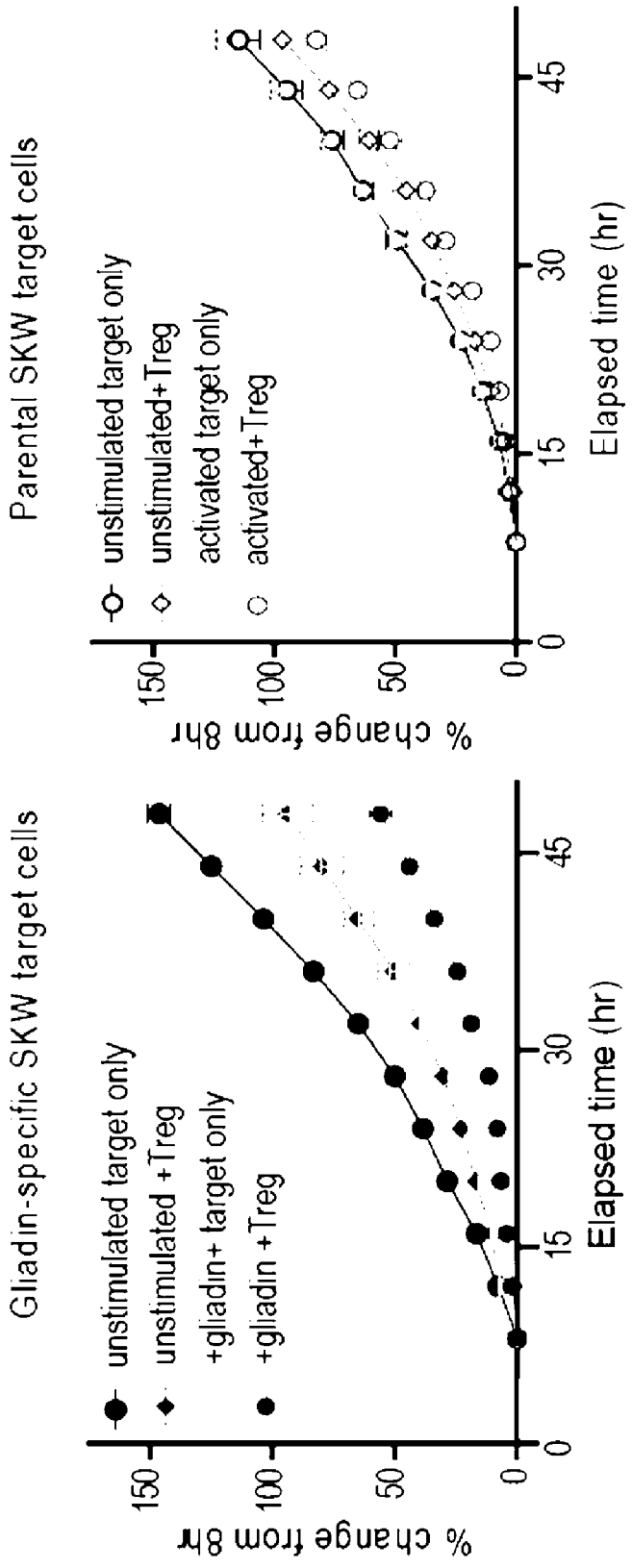

Gliadin-specific SKW target cells
unstimulated target only
unstimulated +Treg
+gliadin+ target only
+gliadin +Treg
% change from 8hr
Elapsed time (hr)
Parental SKW target cells
unstimulated target only
unstimulated+Treg
activated target only
activated+Treg
% change from 8hr
Elapsed time (hr)

FIG. 18E

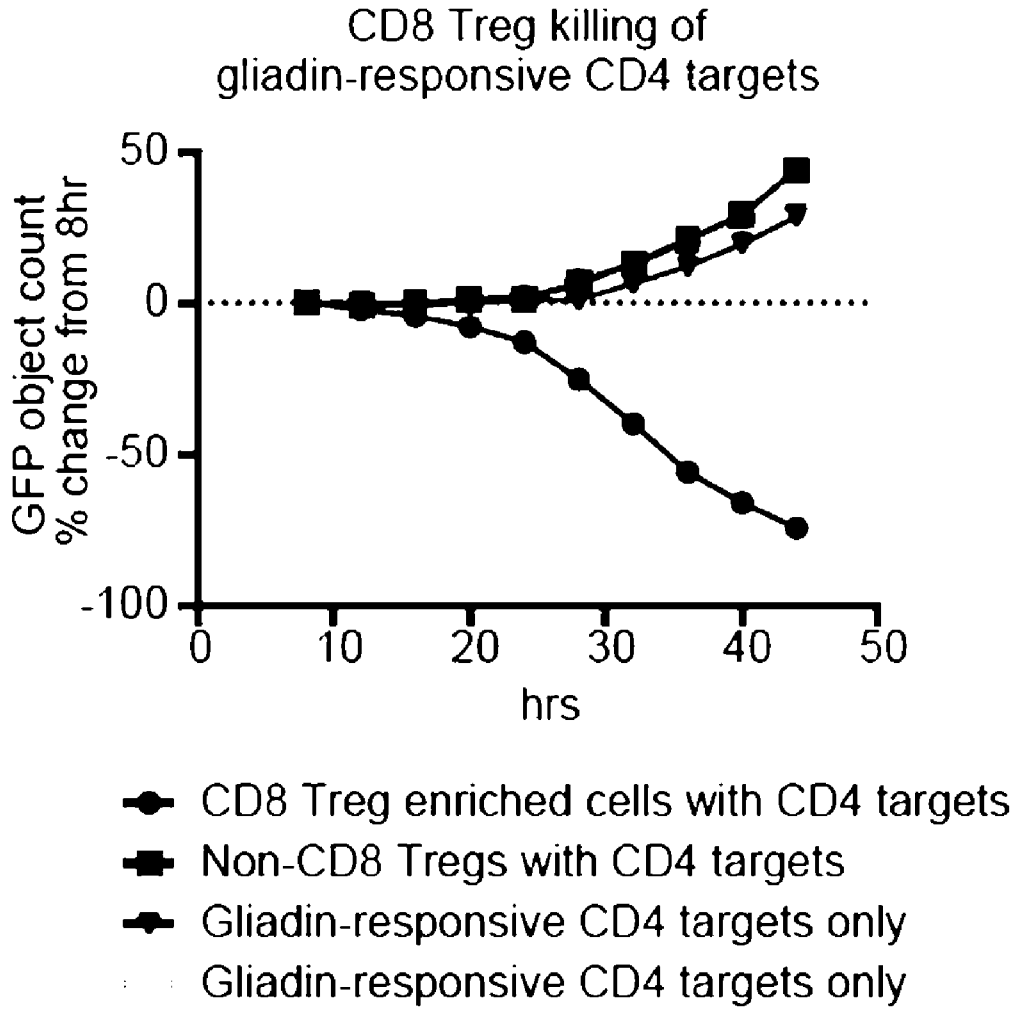

CD8 Treg killing of
gliadin-responsive CD4 targets
GFP object count
% change from 8hr
50
0
-50
-100
0
10
20
30
40
50
hrs
CD8 Treg enriched cells with CD4 targets
Non-CD8 Tregs with CD4 targets
Gliadin-responsive CD4 targets only
Gliadin-responsive CD4 targets only

FIG. 18F

FIG. 18G

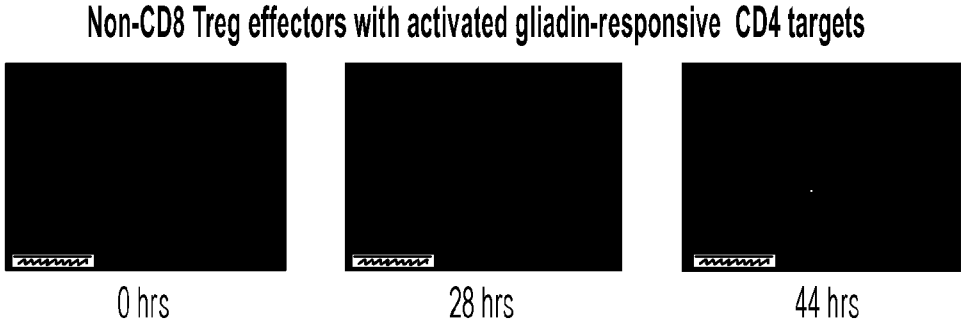


FIG. 18H

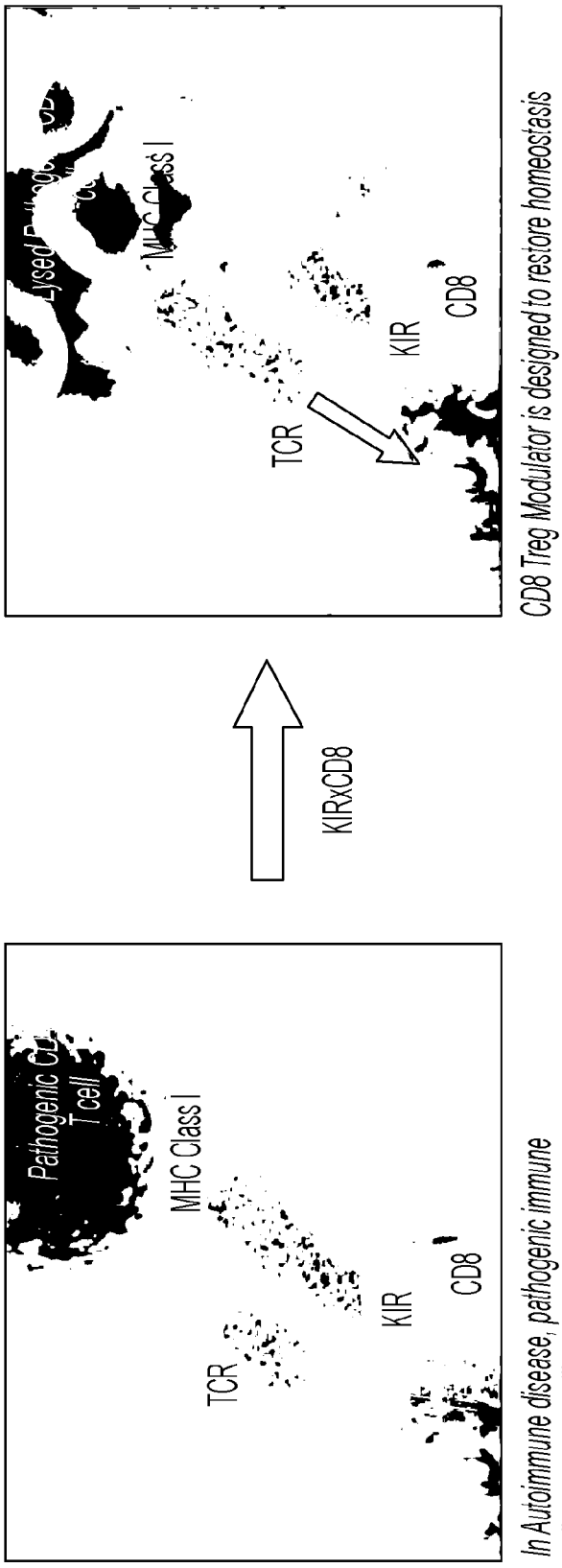

Pathogenic CD8 T cell
MHC Class I
TCR
KIR
CD8
In Autoimmune disease, pathogenic immune cells escape surveillance
KIRxCD8
Lysed Pathogenic CD8 T cell
MHC Class I
TCR
KIR
CD8
CD8 Treg Modulator is designed to restore homeostasis

FIG. 18I

Antigen	k_a(1/Ms) Association rate constant	k_d(1/s) Dissociation rate constant	K_D(M)
KIR2DL1-His	2.3E+05	2.5E-04	1.1E-09
KIR2DL2-His	4.9E+05	1.4E-04	2.8E-10
KIR2DL3-His	3.7E+05	2.6E-04	7.0E-10
CD8α-His	1.4E+05	1.6E-04	1.1E-09

FIG. 19A

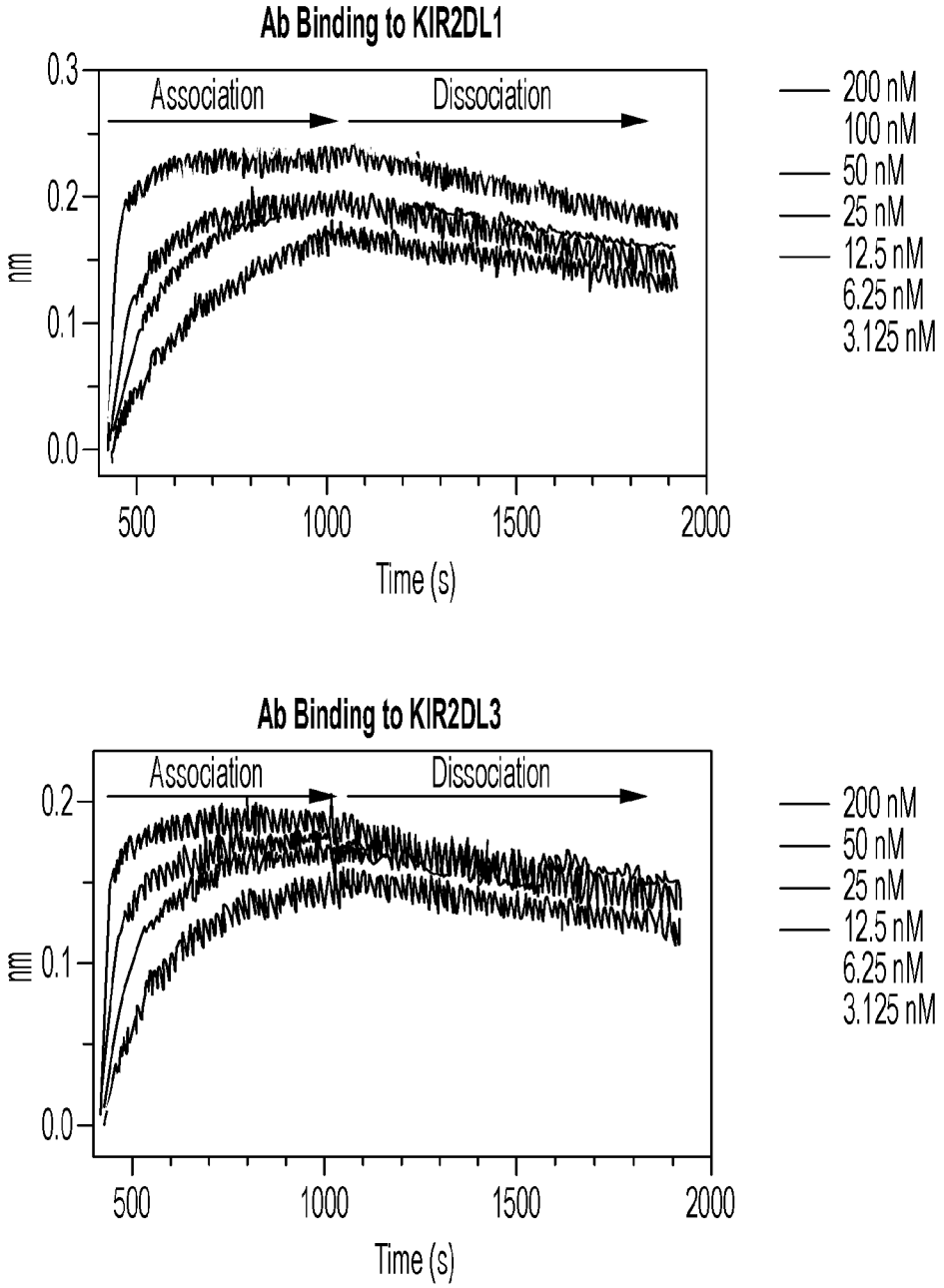

Ab Binding to KIR2DL1
Association
Dissociation
nm
0.3
0.2
0.1
0.0
500
1000
1500
2000
Time (s)
200 nM
100 nM
50 nM
25 nM
12.5 nM
6.25 nM
3.125 nM
Ab Binding to KIR2DL3
Association
Dissociation
nm
0.2
0.1
0.0
500
1000
1500
2000
Time (s)
200 nM
50 nM
25 nM
12.5 nM
6.25 nM
3.125 nM

FIG. 19B

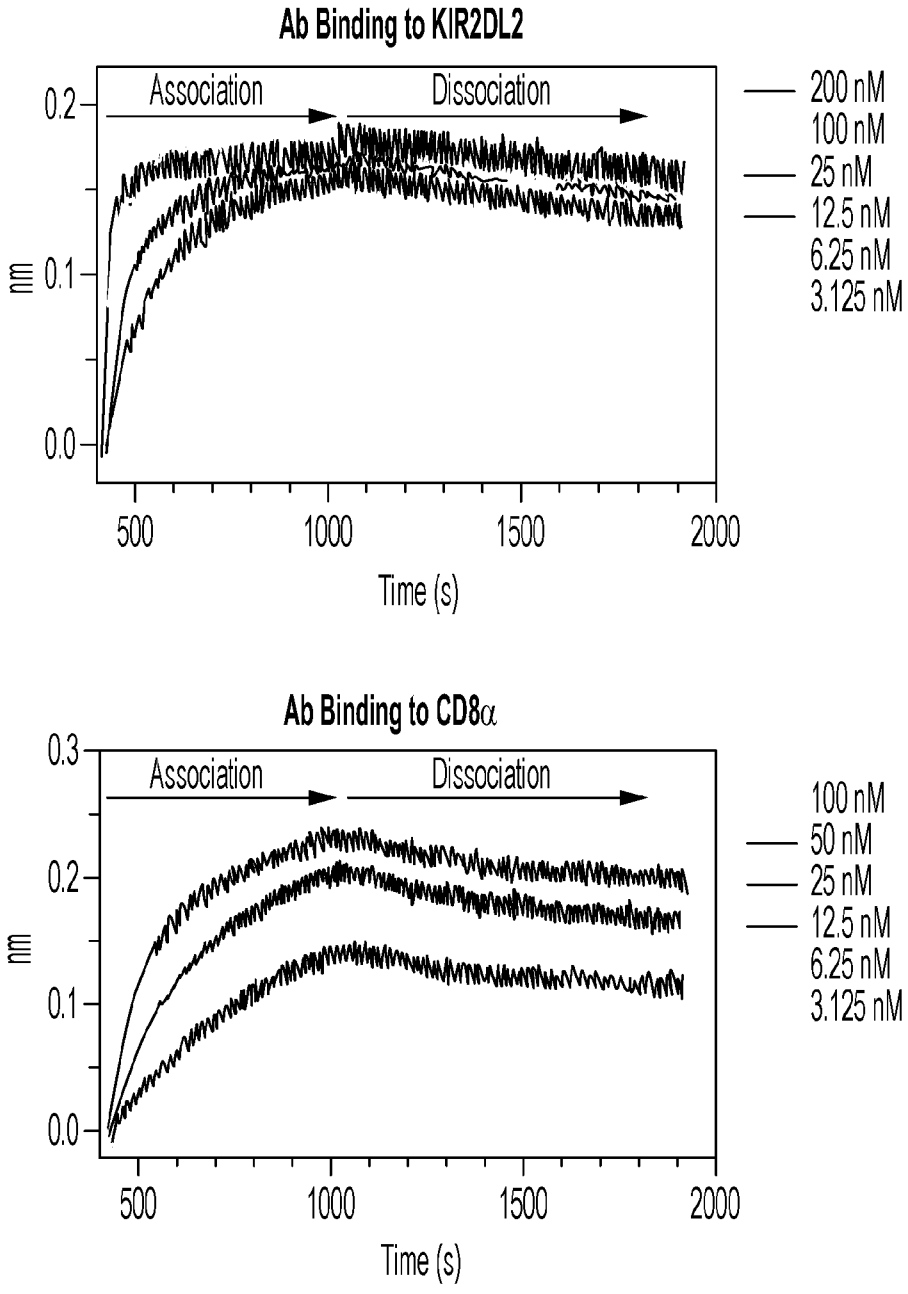


FIG. 19B
CONTINUED

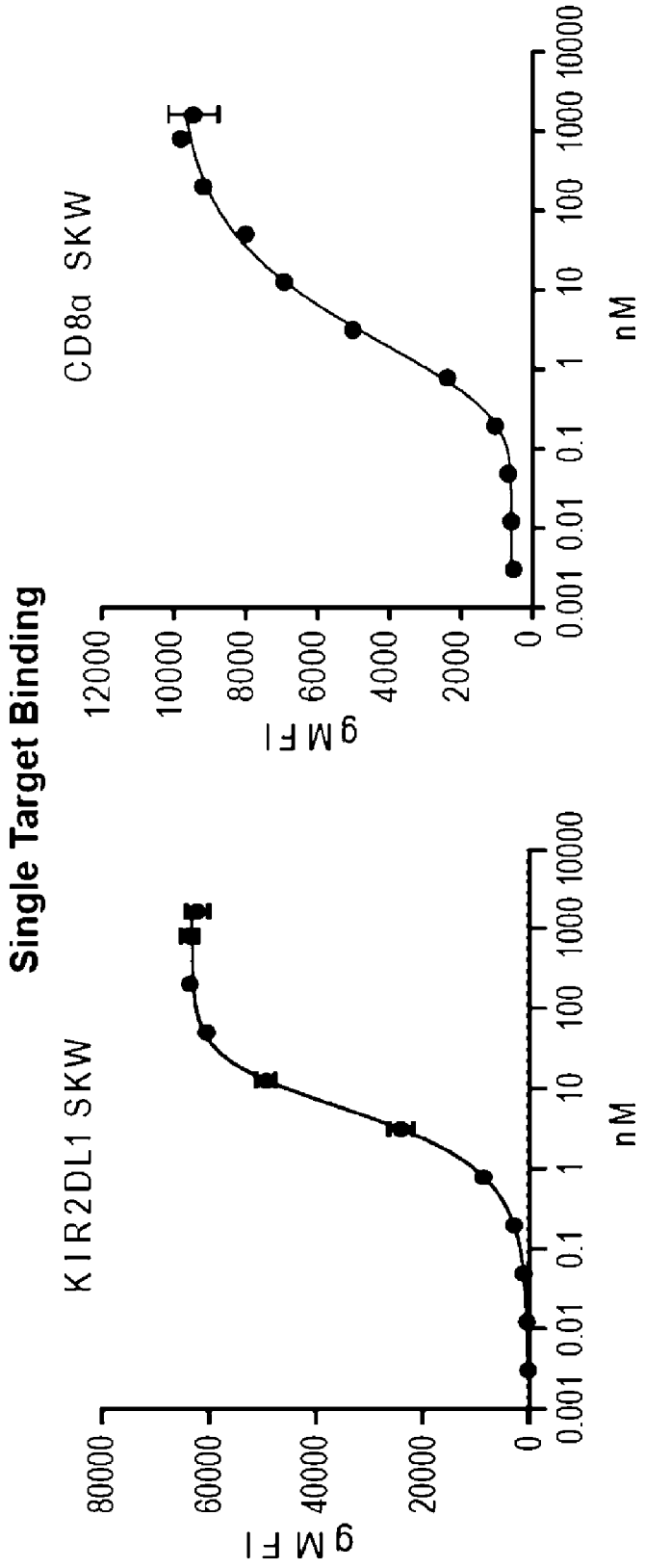

Single Target Binding
KIR2DL1 SKW
gMFI
80000
60000
40000
20000
0
0.001 0.01 0.1 1 10 100 1000 10000
nM
CD8α SKW
gMFI
12000
10000
8000
6000
4000
2000
0
0.001 0.01 0.1 1 10 100 1000 10000
nM

FIG. 19C

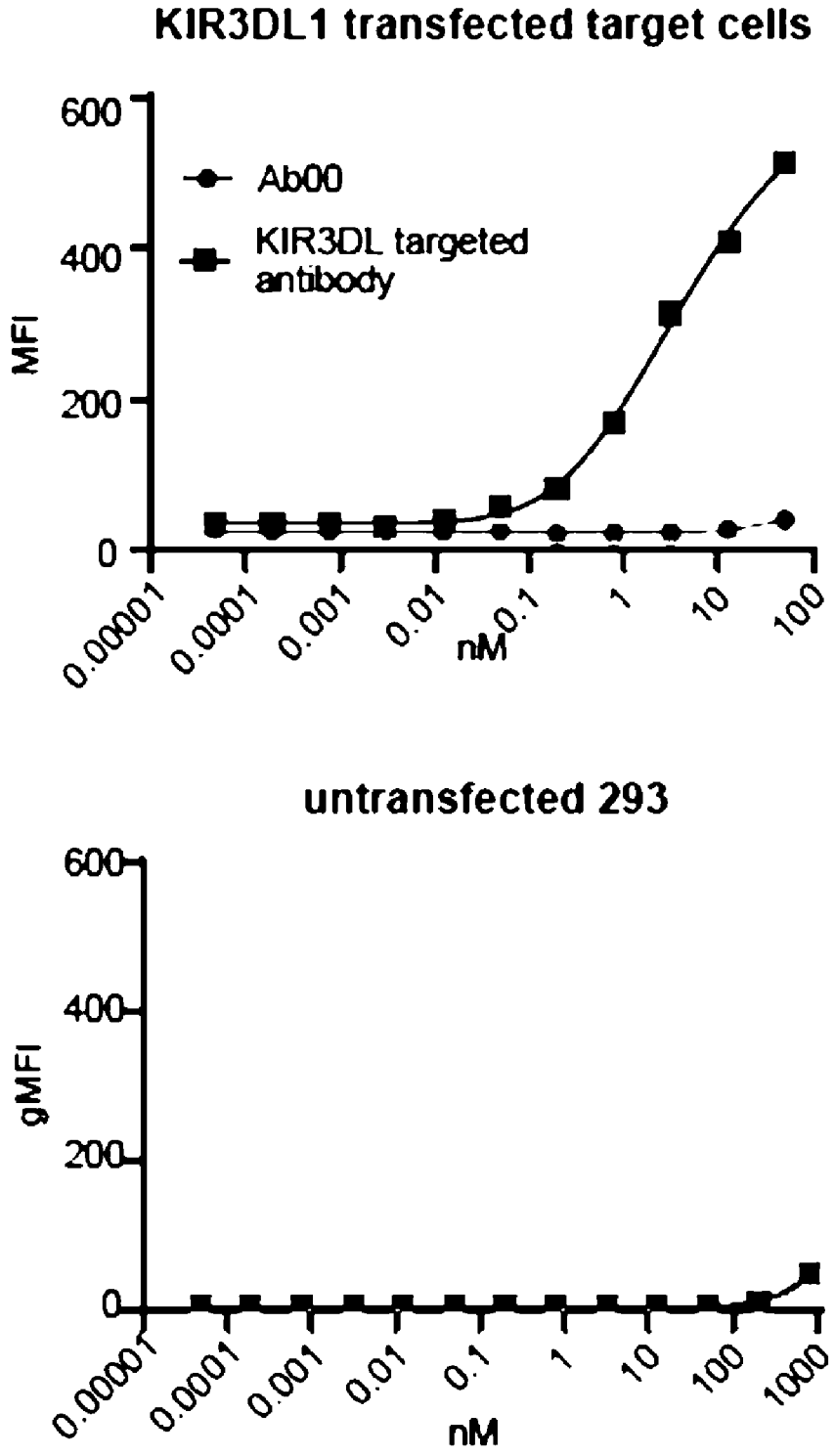

KIR3DL1 transfected target cells
600
400
200
0
MFI
Ab00
KIR3DL targeted antibody
0.00001
0.0001
0.001
0.01
0.1
1
10
100
nM
untransfected 293
600
400
200
0
gMFI
0.00001
0.0001
0.001
0.01
0.1
1
10
100
1000
nM

FIG. 19D

	KIR2DS2	KIR2DS3	KIR2DS1	KIR2DL2	KIR2DL3	KIR2DL1	KIR2DS2	KIR2DL1	CD8A	CD8A	FZD5
Sample ID (concentration)	246	1006	1058	927	2240	1942	3468	3402	4948	5802	1819
Confirmation Screen Results-FIXED											
Ab20 (20 µg/mL)	med/strong	weak	strong	strong	strong	strong	strong	strong	med/strong	med	v.weak
Ab20 (20 µg/mL)	med/strong	weak	strong	strong	strong	strong	strong	strong	med	med	v.weak
KIRFabFc (20 µg/mL)	med/strong	weak	strong	strong	strong	strong	strong	strong	neg	neg	v.weak
KIRFabFc (20 µg/mL)	med/strong	weak	strong	strong	strong	strong	strong	strong	neg	neg	v.weak
CD8 scFv (20 µg/mL)	neg	neg	neg	neg	neg	neg	neg	neg	med	weak	neg
CD8 scFv (20 µg/mL)	neg	neg	neg	neg	neg	neg	neg	neg	med	weak/med	neg
Confirmation Screen Results-UNFIXED											
Ab20 (20 µg/mL)	weak/med	v.weak	med	med	med	weak/med	med	strong	med/strong	v.weak	weak
KIRFabFc (20 µg/mL)	weak/med	v.weak	weak/med	med	weak/med	weak	med	strong	neg	neg	weak
CD8 scFv (20 µg/mL)	neg	neg	neg	neg	neg	neg	neg	neg	med/strong	v.weak	neg

FIG. 19E

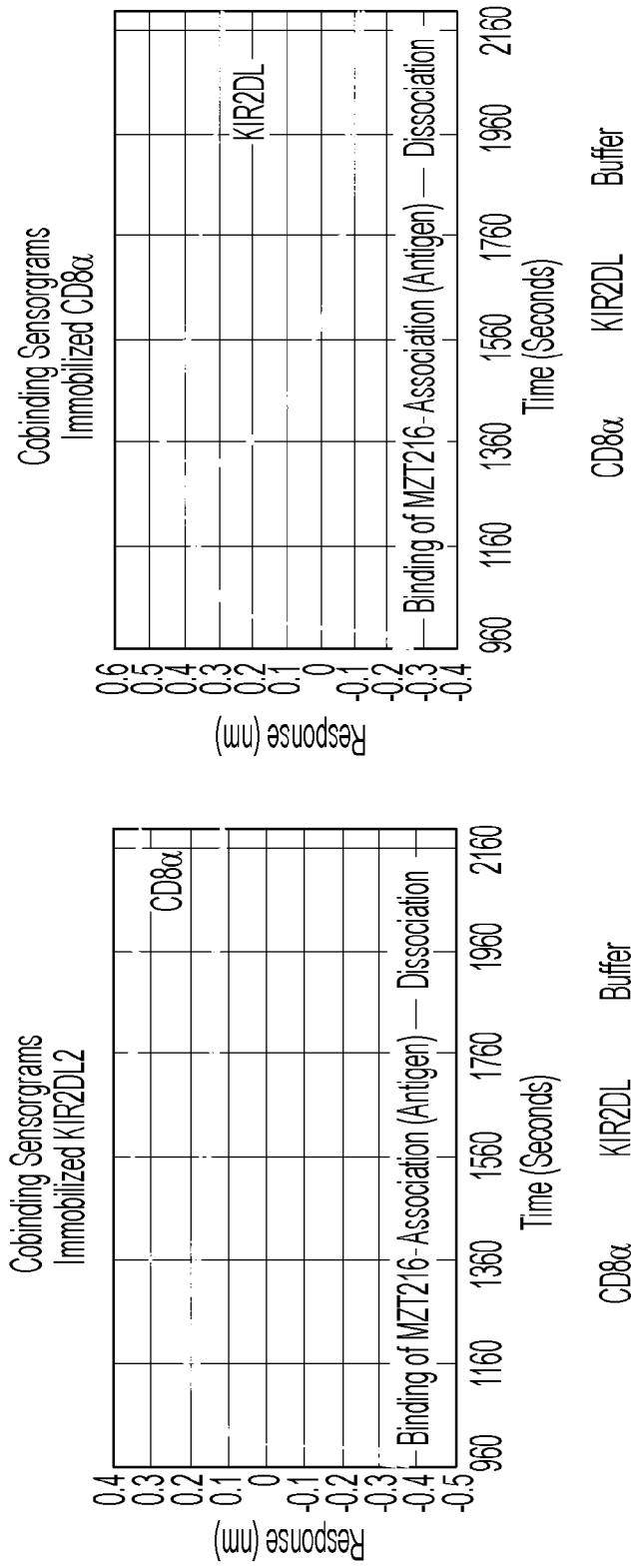

Cobinding Sensorgrams
Immobilized KIR2DL2
Response (nm)
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
960
1160
1360
1560
1760
1960
2160
Time (Seconds)
CD8α
Binding of MZT216-Association (Antigen)
Dissociation
CD8α
KIR2DL
Buffer
Cobinding Sensorgrams
Immobilized CD8α
Response (nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
960
1160
1360
1560
1760
1960
2160
Time (Seconds)
KIR2DL
Binding of MZT216-Association (Antigen)
Dissociation
CD8α
KIR2DL
Buffer

FIG. 20A

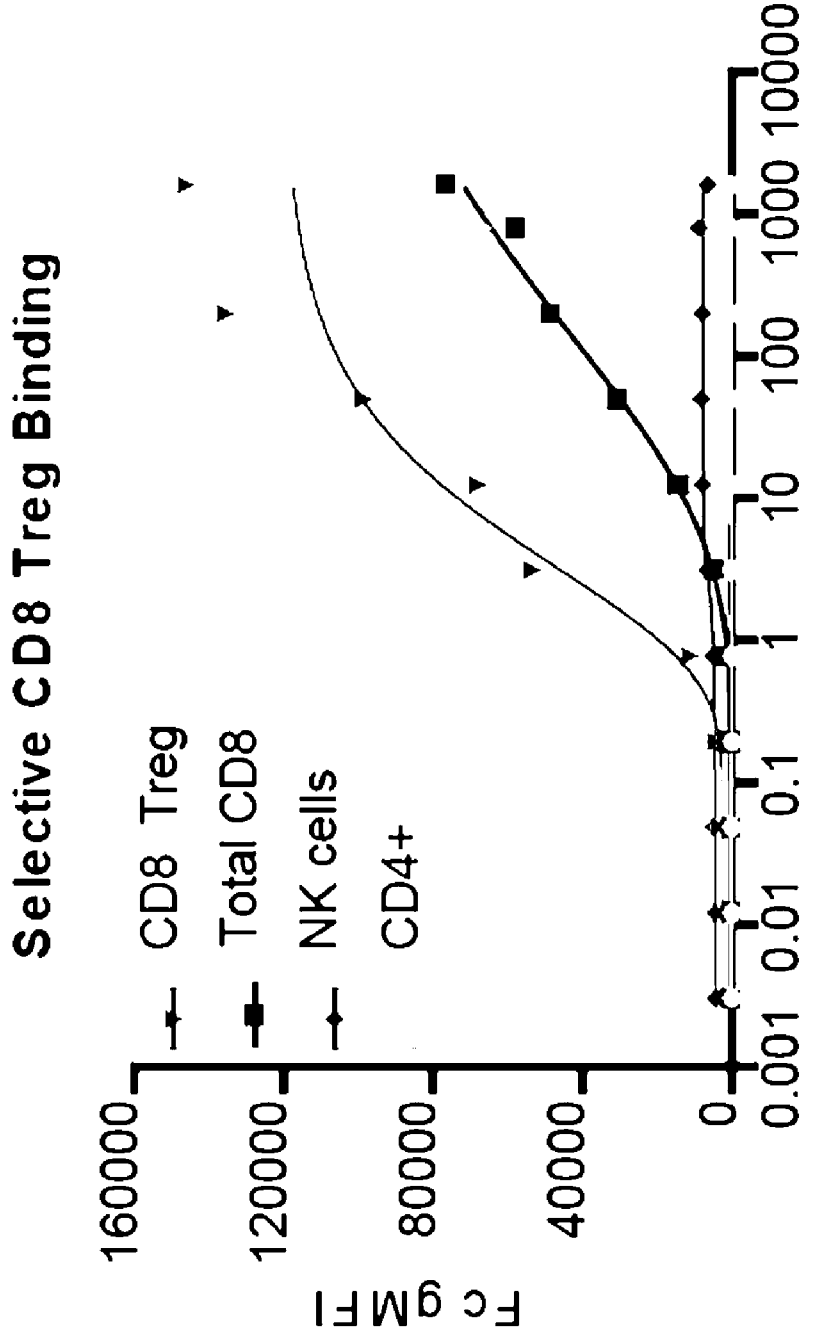

Selective CD8 Treg Binding
CD8 Treg
Total CD8
NK cells
CD4+
Fc gMFI
160000
120000
80000
40000
0
0.001
0.01
0.1
1
10
100
1000
10000
Ab Concentration (nM)

FIG. 20B

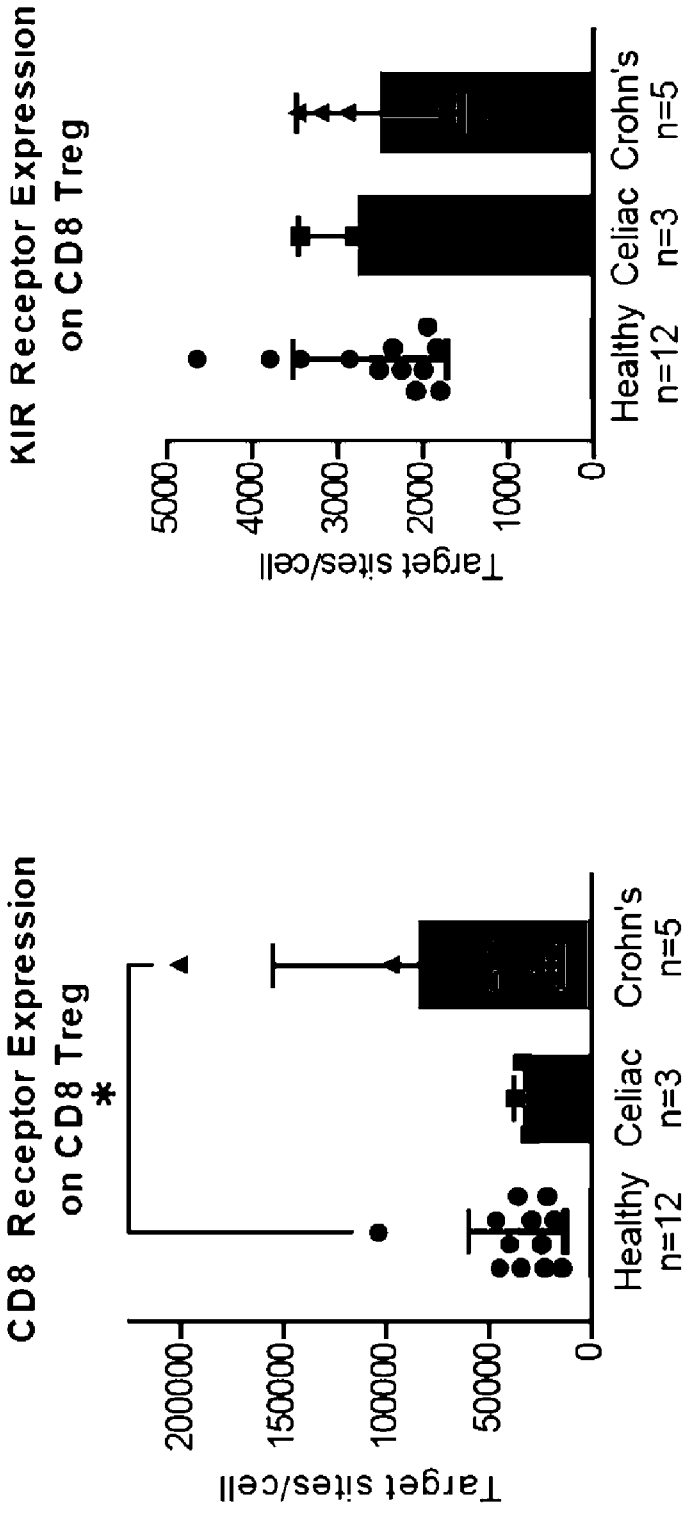

CD8 Receptor Expression on CD8 Treg
*
Target sites/cell
200000
150000
100000
50000
0
Healthy n=12
Celiac n=3
Crohn's n=5
KIR Receptor Expression on CD8 Treg
Target sites/cell
5000
4000
3000
2000
1000
0
Healthy n=12
Celiac n=3
Crohn's n=5

FIG. 20C

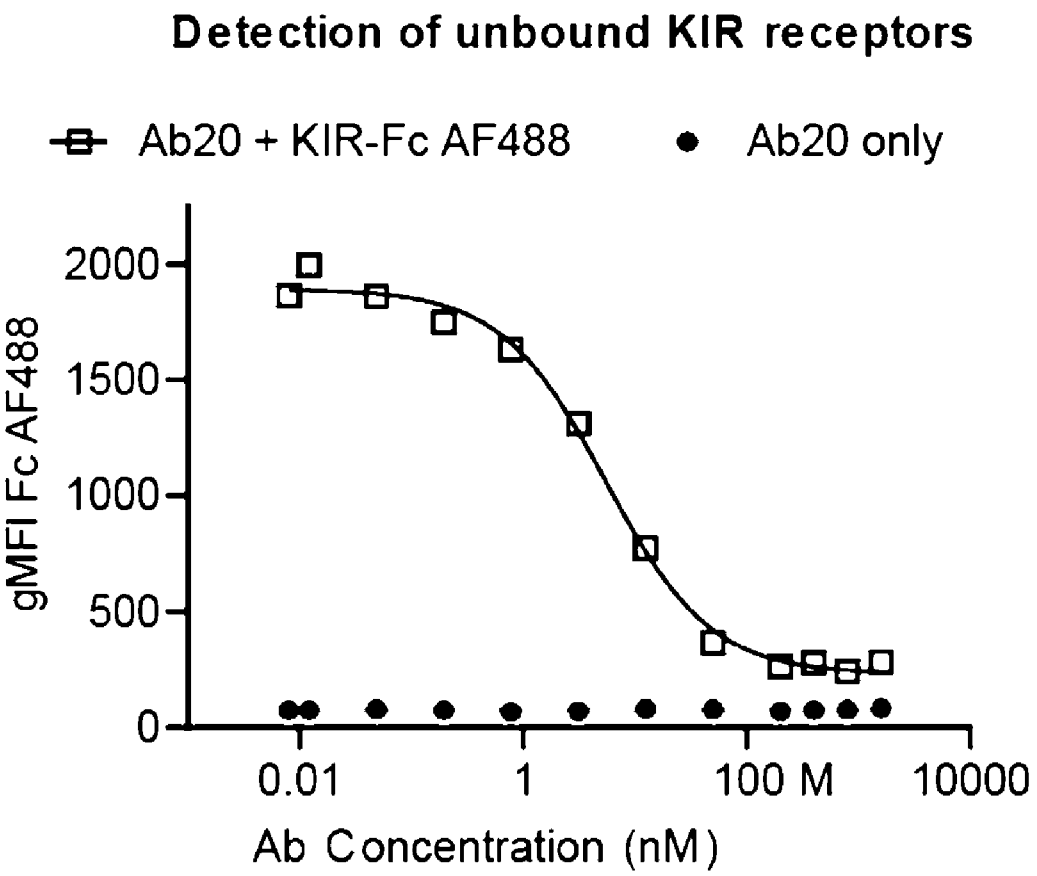

Detection of unbound KIR receptors
Ab20 + KIR-Fc AF488
Ab20 only
gMFI Fc AF488
2000
1500
1000
500
0
0.01
1
100 M
10000
Ab Concentration (nM)

FIG. 20D

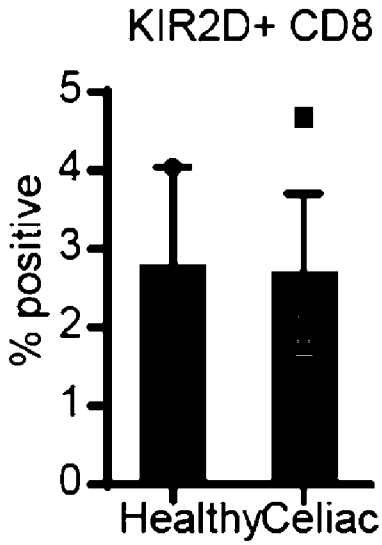


FIG. 21A

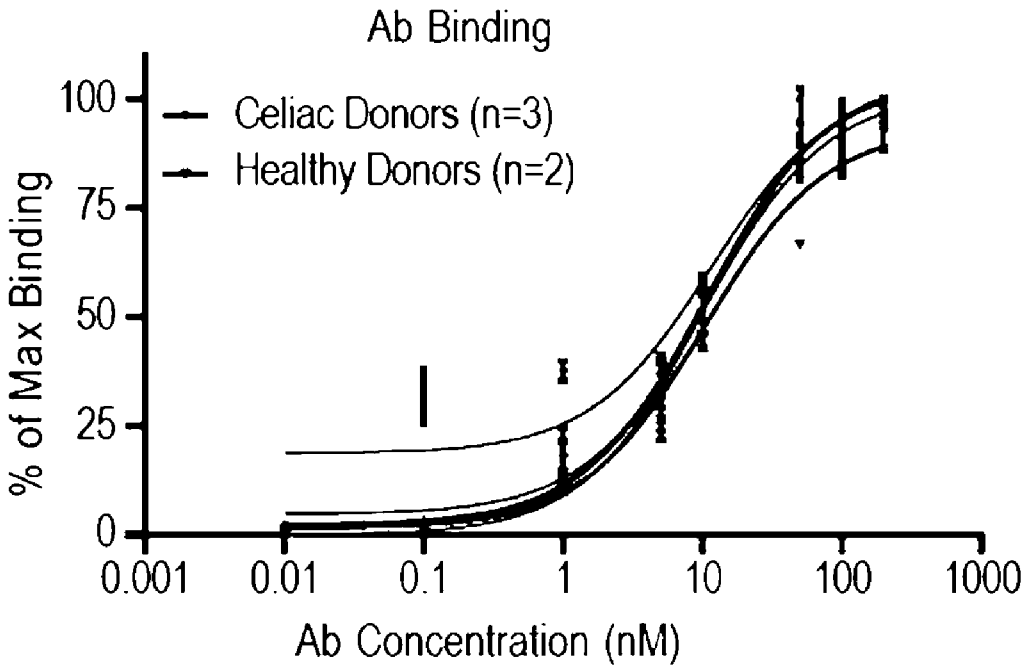


FIG. 21B

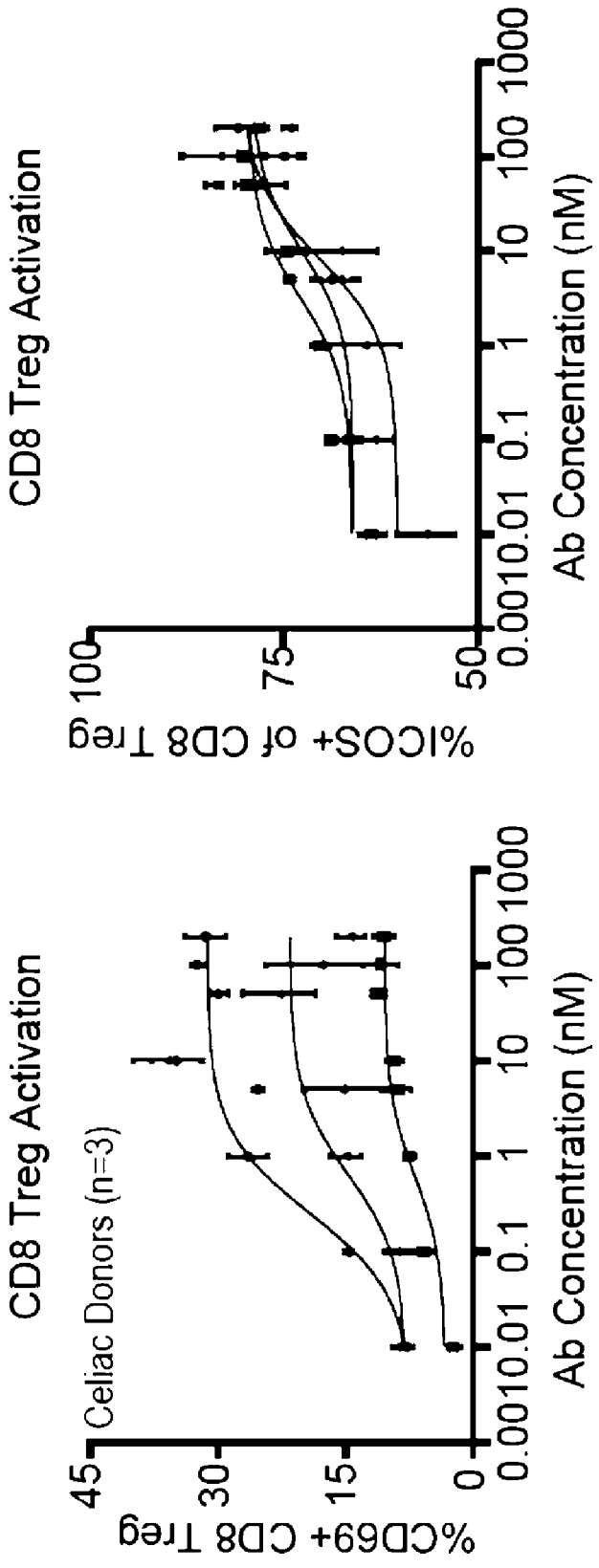

CD8 Treg Activation
%ICOS+ of CD8 Treg
100
75
50
0.0001
0.01
0.1
1
10
100
1000
Ab Concentration (nM)
CD8 Treg Activation
Celiac Donors (n=3)
%CD69+ CD8 Treg
45
30
15
0
0.0001
0.01
0.1
1
10
100
1000
Ab Concentration (nM)

FIG. 21C

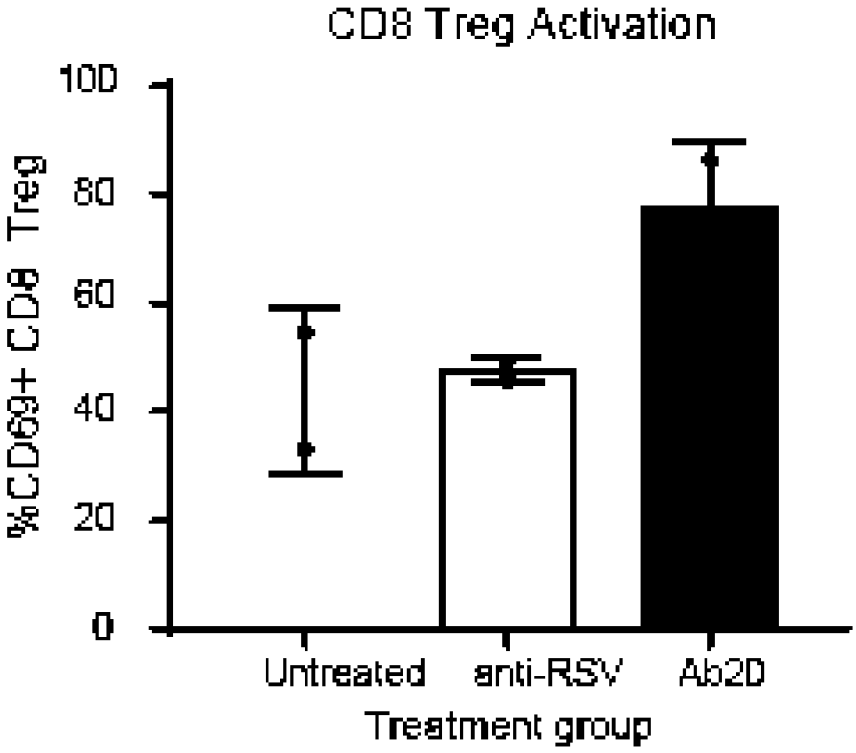

CD8 Treg Activation
100
80
60
40
20
0
%CD69+ CD8 Treg
Untreated
anti-RSV
Ab2D
Treatment group

FIG. 21D

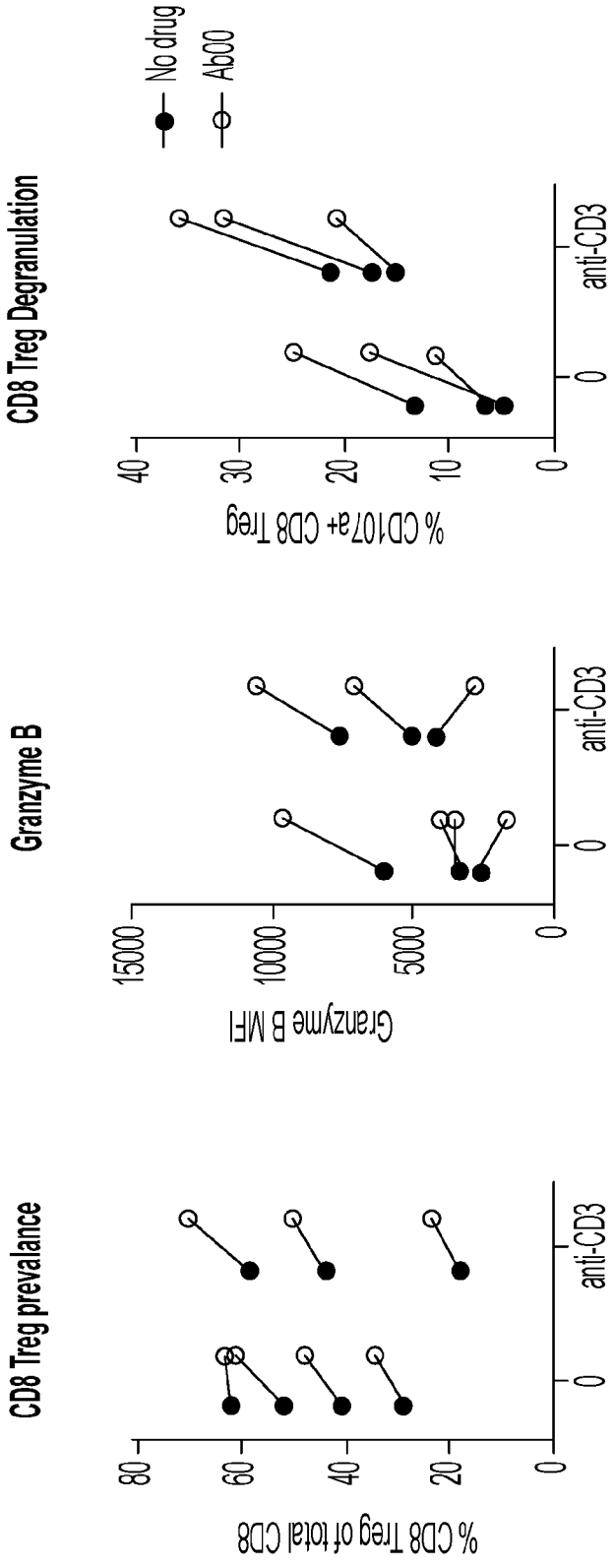

CD8 Treg prevalance
% CD8 Treg of total CD8
80
60
40
20
0
0
anti-CD3
Granzyme B
Granzyme B MFI
15000
10000
5000
0
0
anti-CD3
CD8 Treg Degranulation
% CD107a+ CD8 Treg
40
30
20
10
0
0
anti-CD3
No drug
Ab00

FIG. 22A

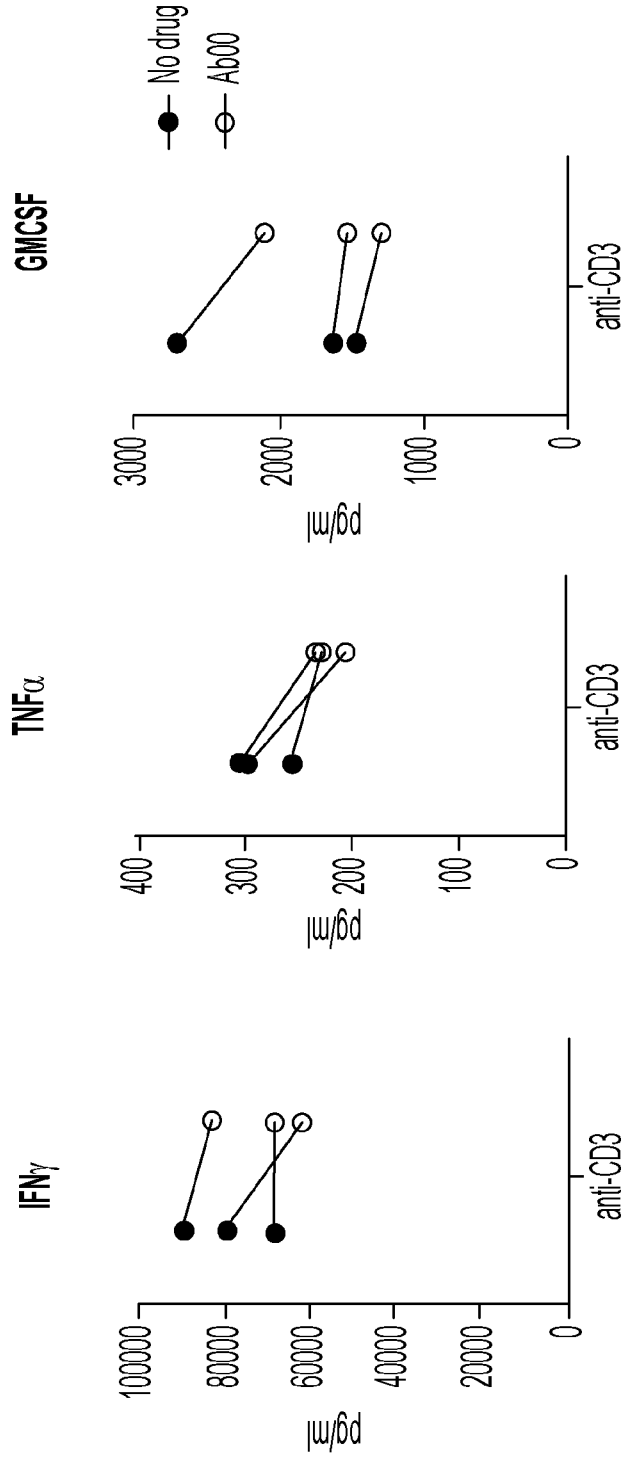

No drug
Ab00
GMCSF
TNFα
IFNγ
pg/ml
anti-CD3
3000
2000
1000
0
400
300
200
100
100000
80000
60000
40000
20000

FIG. 22B

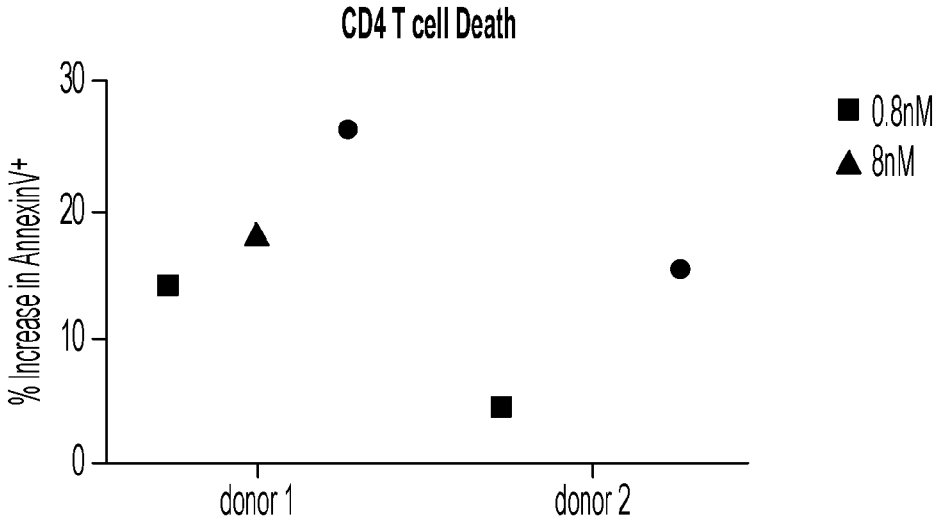

CD4 T cell Death
% Increase in AnnexinV+
30
20
10
0
donor 1
donor 2
0.8nM
8nM

FIG. 22C

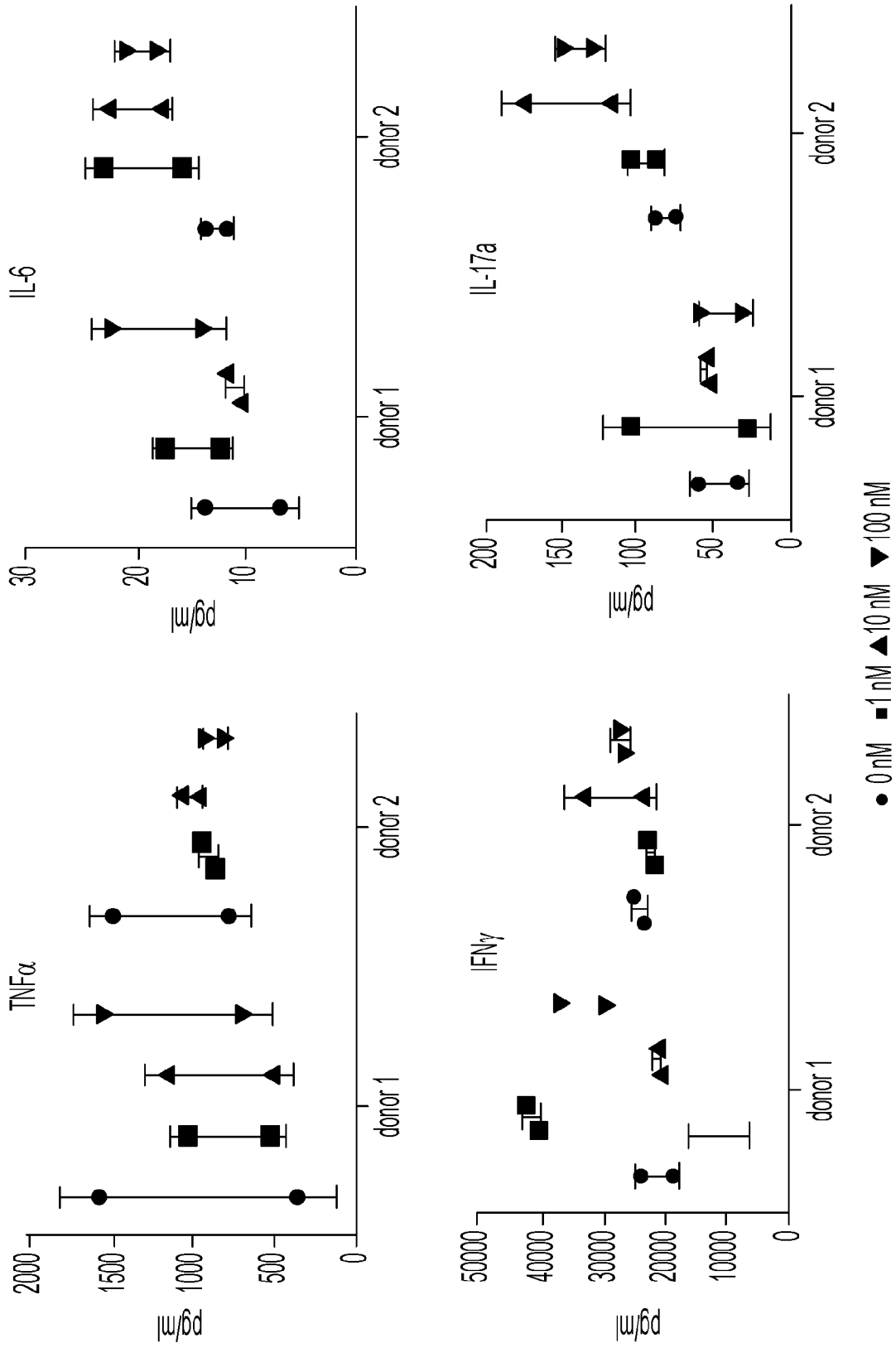

IL-6
pg/ml
30
20
10
0
donor 1
donor 2
IL-17a
pg/ml
200
150
100
50
0
donor 1
donor 2
TNFα
pg/ml
2000
1500
1000
500
0
donor 1
donor 2
IFNγ
pg/ml
50000
40000
30000
20000
10000
0
donor 1
donor 2
● 0 nM ■ 1 nM ▲ 10 nM ▼ 100 nM

FIG. 22D

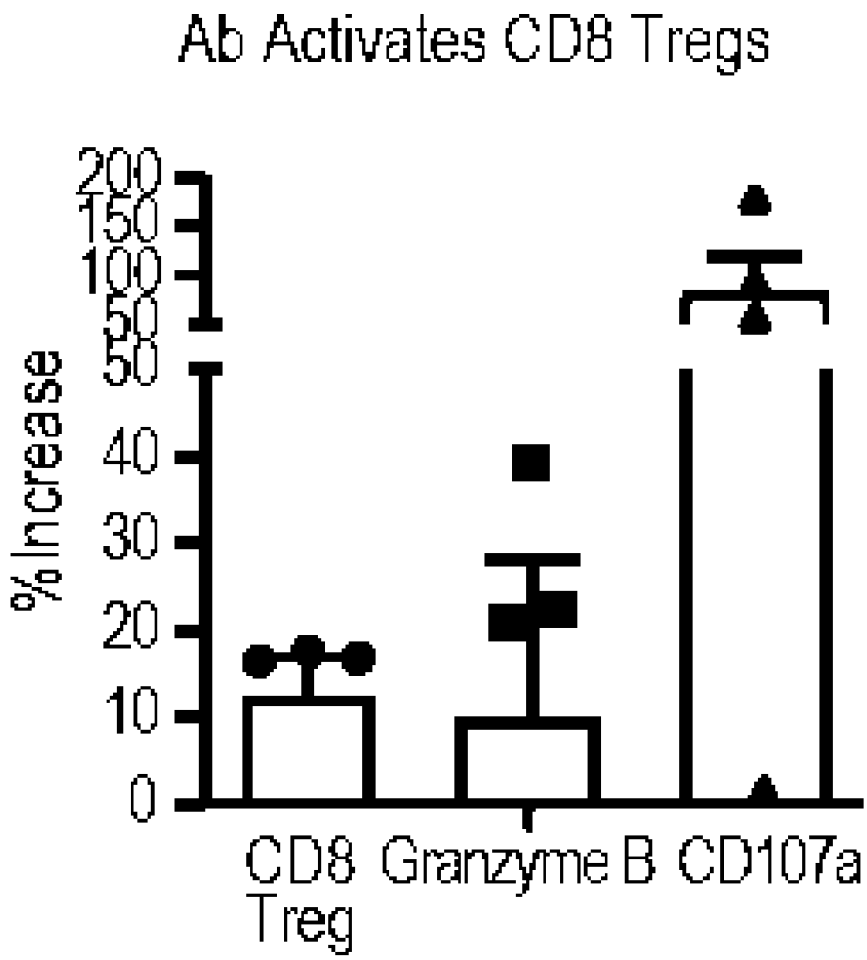

Ab Activates CD8 Tregs
% Increase
200
150
100
50
50
40
30
20
10
0
CD8 Treg
Granzyme B
CD107a

FIG. 22E

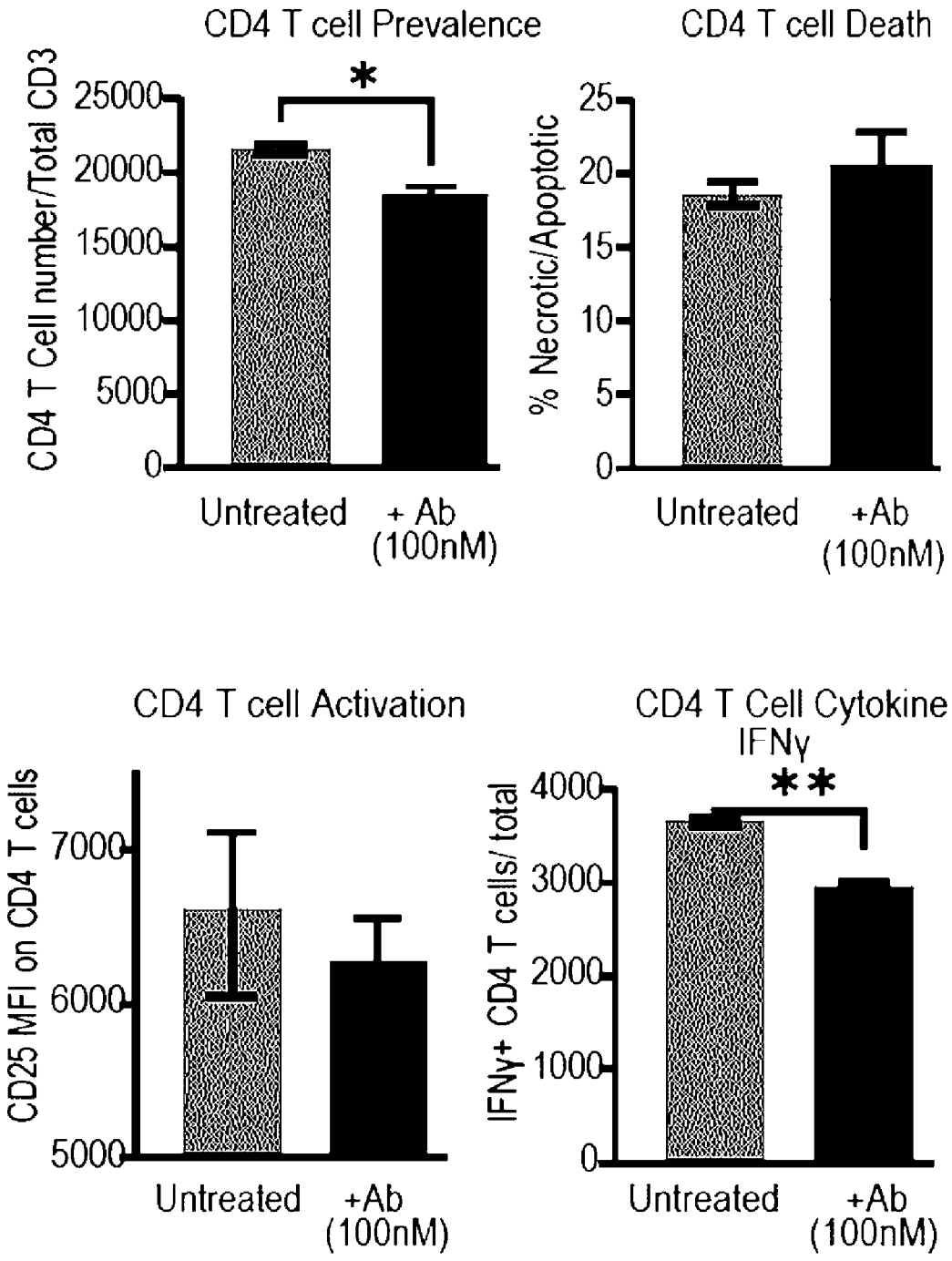

CD4 T cell Prevalence
CD4 T Cell number/Total CD3
25000
20000
15000
10000
5000
0
*
Untreated
+ Ab (100nM)
CD4 T cell Death
% Necrotic/Apoptotic
25
20
15
10
5
0
Untreated
+Ab (100nM)
CD4 T cell Activation
CD25 MFI on CD4 T cells
7000
6000
5000
Untreated
+Ab (100nM)
CD4 T Cell Cytokine IFNγ
IFNγ+ CD4 T cells/ total
4000
3000
2000
1000
0
**
Untreated
+Ab (100nM)

FIG. 22F

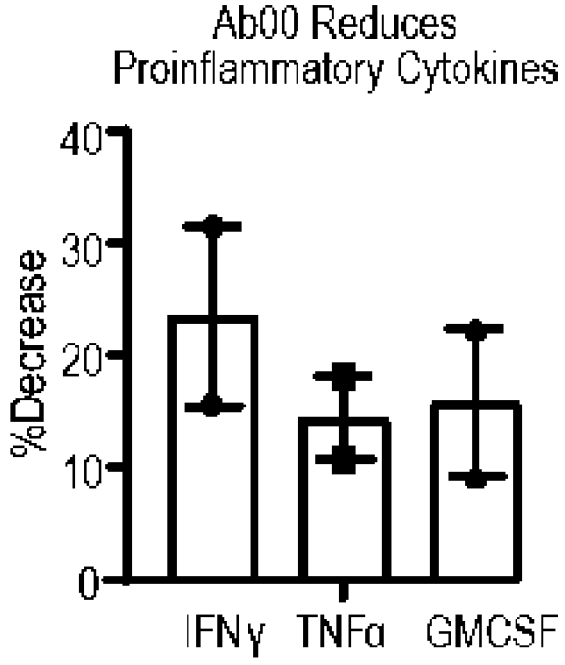


FIG. 22G

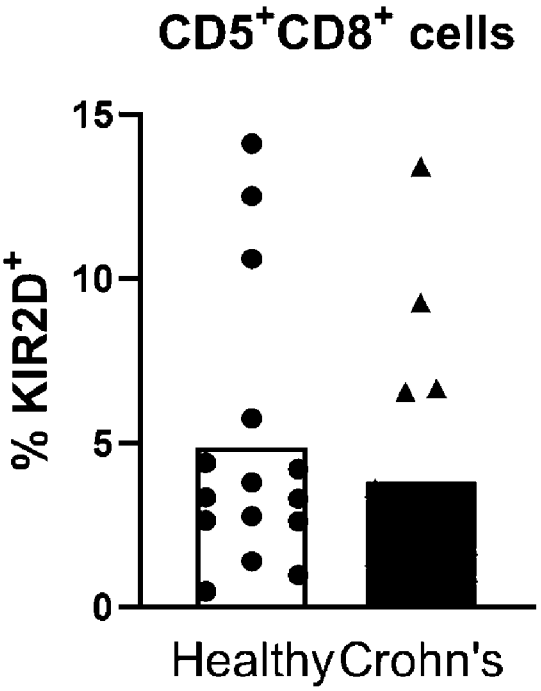


FIG. 23A

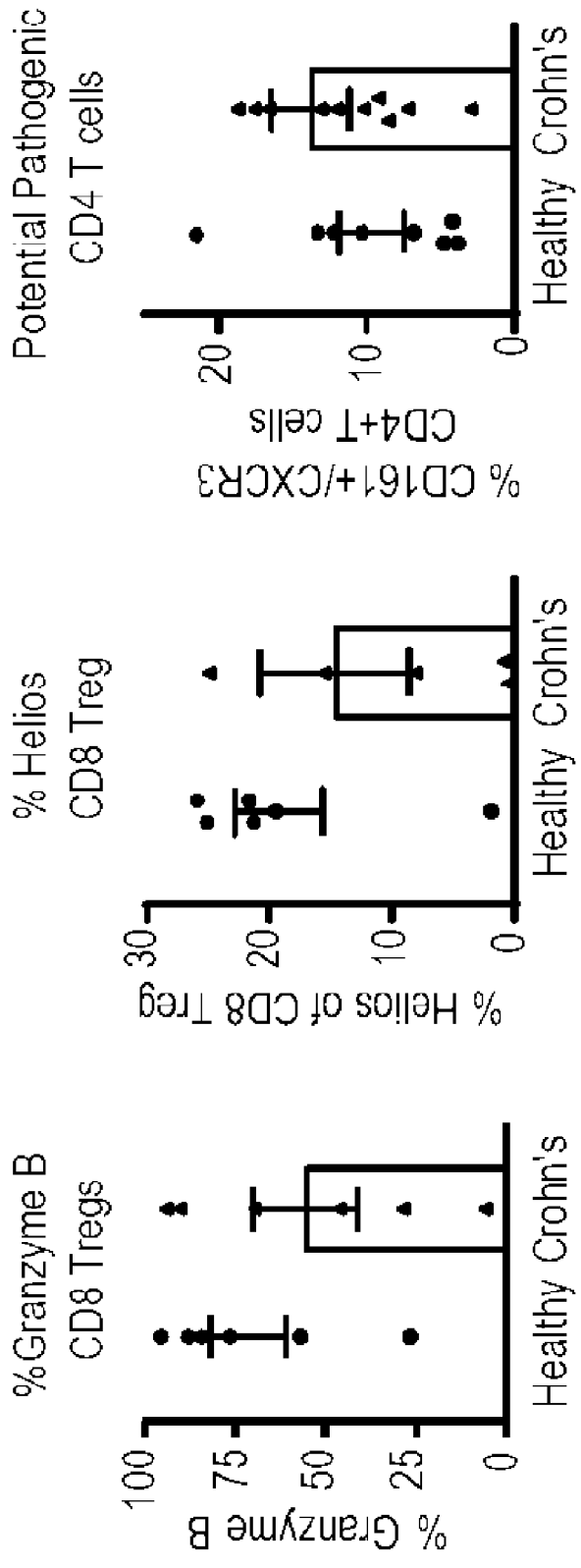

%Granzyme B
CD8 Tregs
% Granzyme B
100
75
50
25
0
Healthy
Crohn's
% Helios
CD8 Treg
% Helios of CD8 Treg
30
20
10
0
Healthy
Crohn's
Potential Pathogenic
CD4 T cells
% CD161+/CXCR3
CD4+T cells
20
10
0
Healthy
Crohn's

FIG. 23B

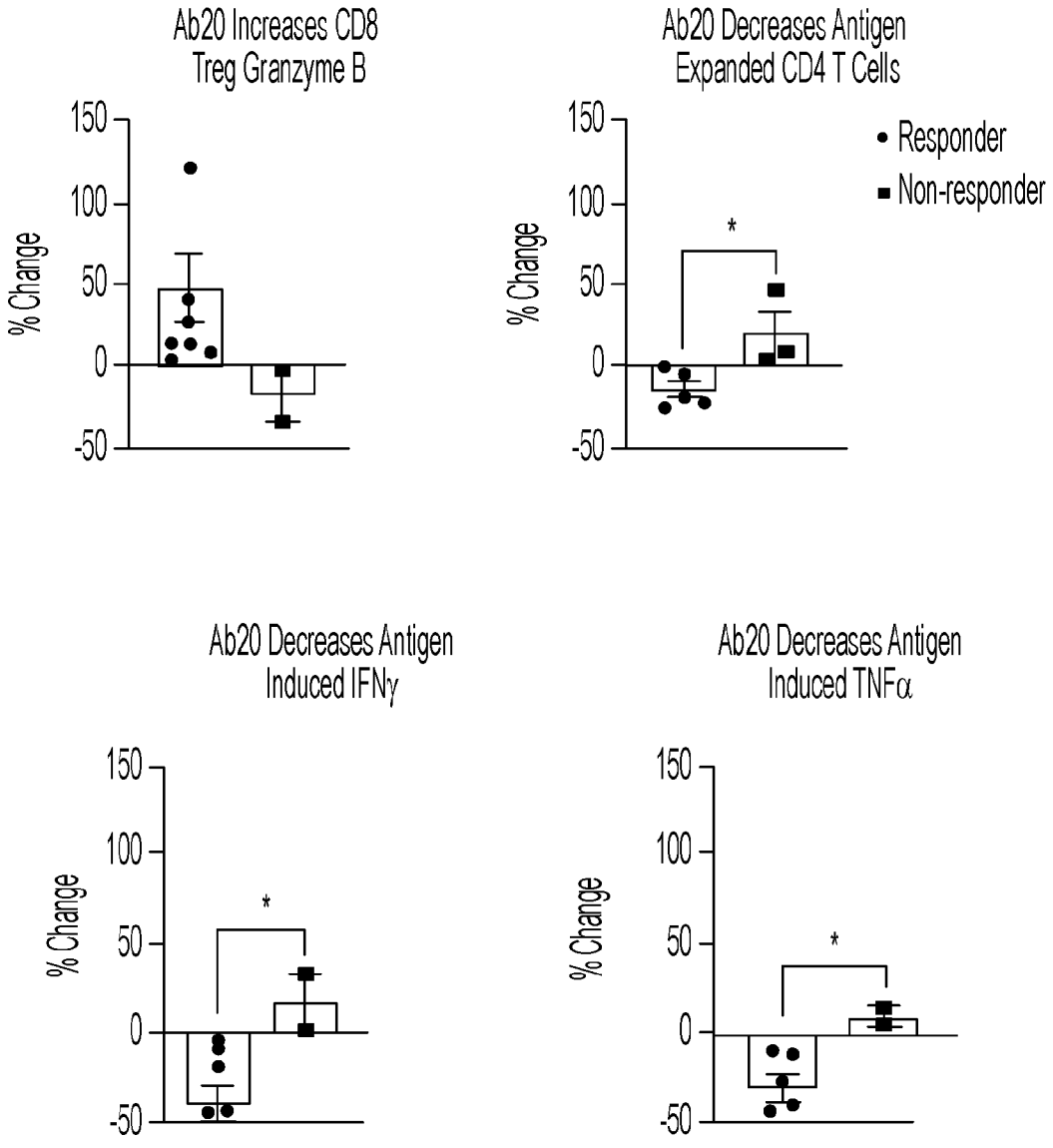

Ab20 Increases CD8
Treg Granzyme B
% Change
150
100
50
0
-50
Ab20 Decreases Antigen
Expanded CD4 T Cells
Responder
Non-responder
*
Ab20 Decreases Antigen
Induced IFNγ
Ab20 Decreases Antigen
Induced TNFα

FIG. 23C

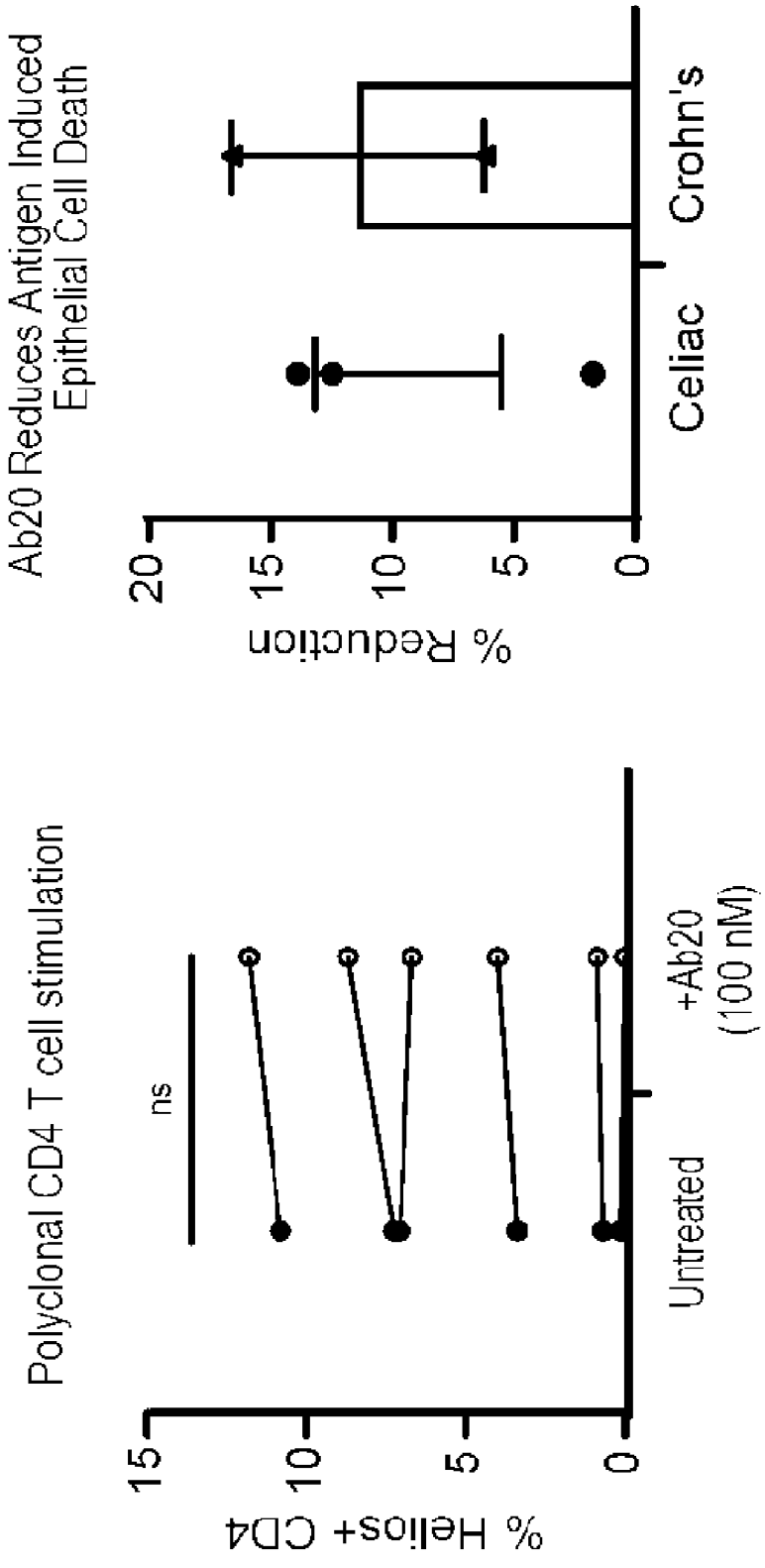

Ab20 Reduces Antigen Induced Epithelial Cell Death
% Reduction
20
15
10
5
0
Celiac
Crohn's
Polyclonal CD4 T cell stimulation
% Helios+ CD4
15
10
5
0
ns
Untreated
+Ab20 (100 nM)

FIG. 23D

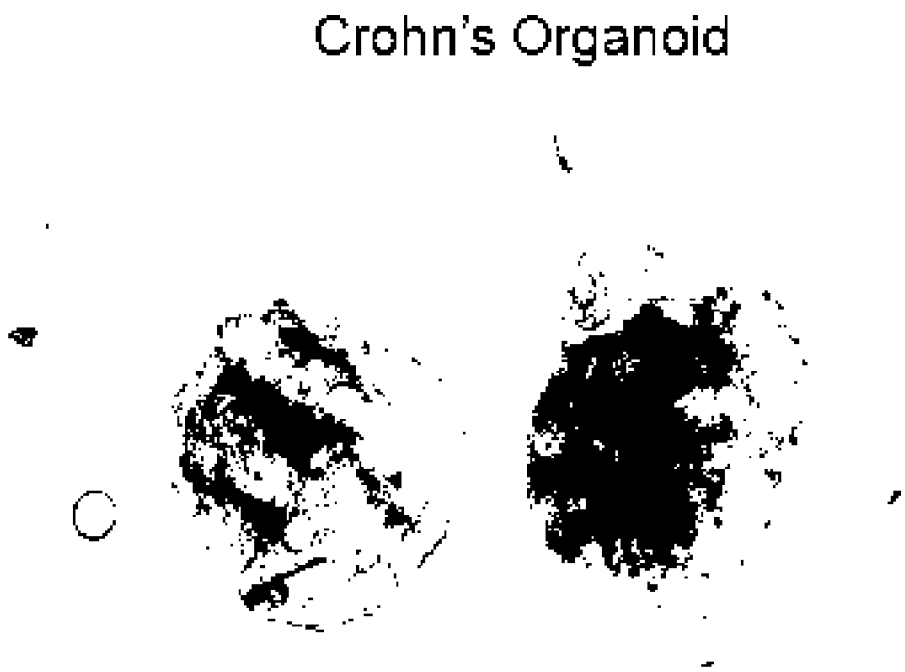


FIG. 24A

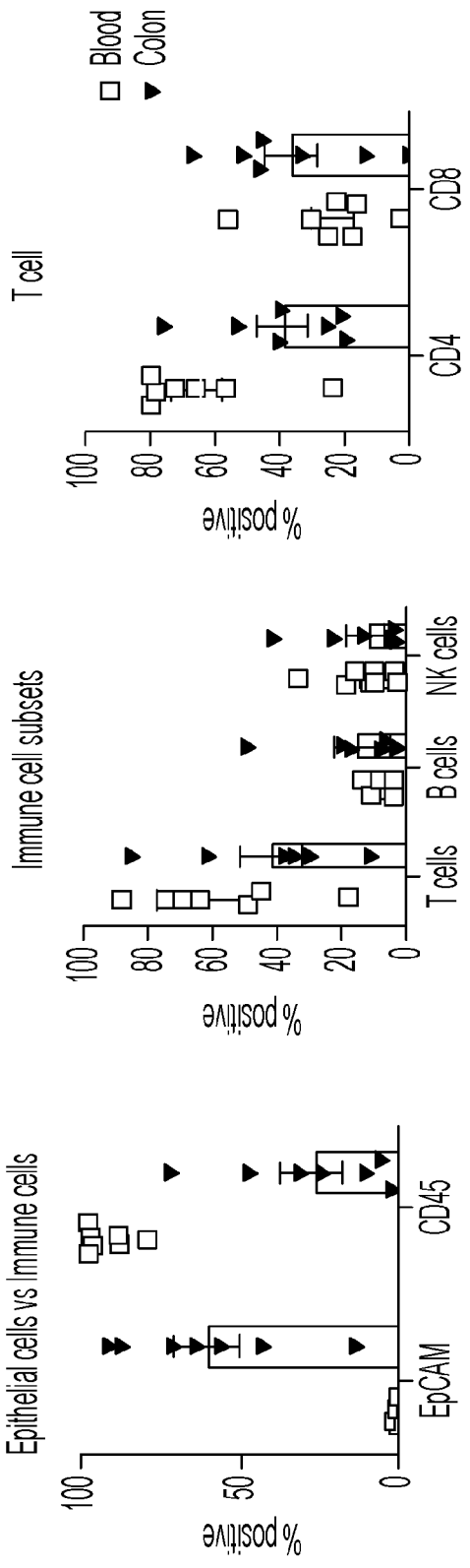

Epithelial cells vs Immune cells
% positive
100
50
0
EpCAM
CD45
Immune cell subsets
% positive
100
80
60
40
20
0
T cells
B cells
NK cells
T cell
% positive
100
80
60
40
20
0
CD4
CD8
Blood
Colon

FIG. 24B

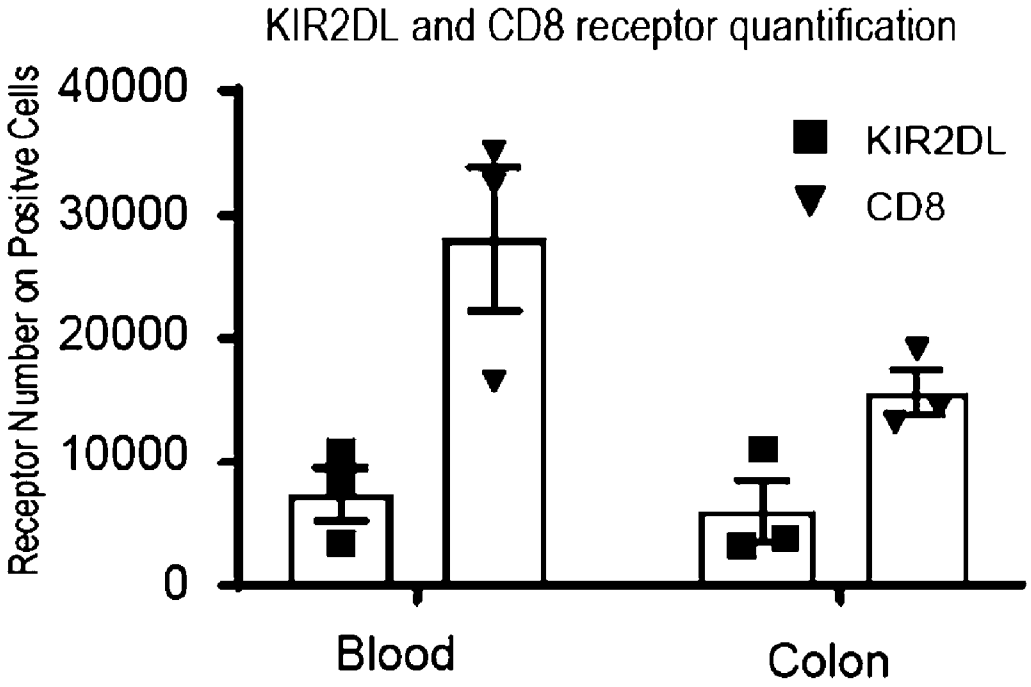

KIR2DL and CD8 receptor quantification
Receptor Number on Positive Cells
40000
30000
20000
10000
0
KIR2DL
CD8
Blood
Colon

FIG. 24C

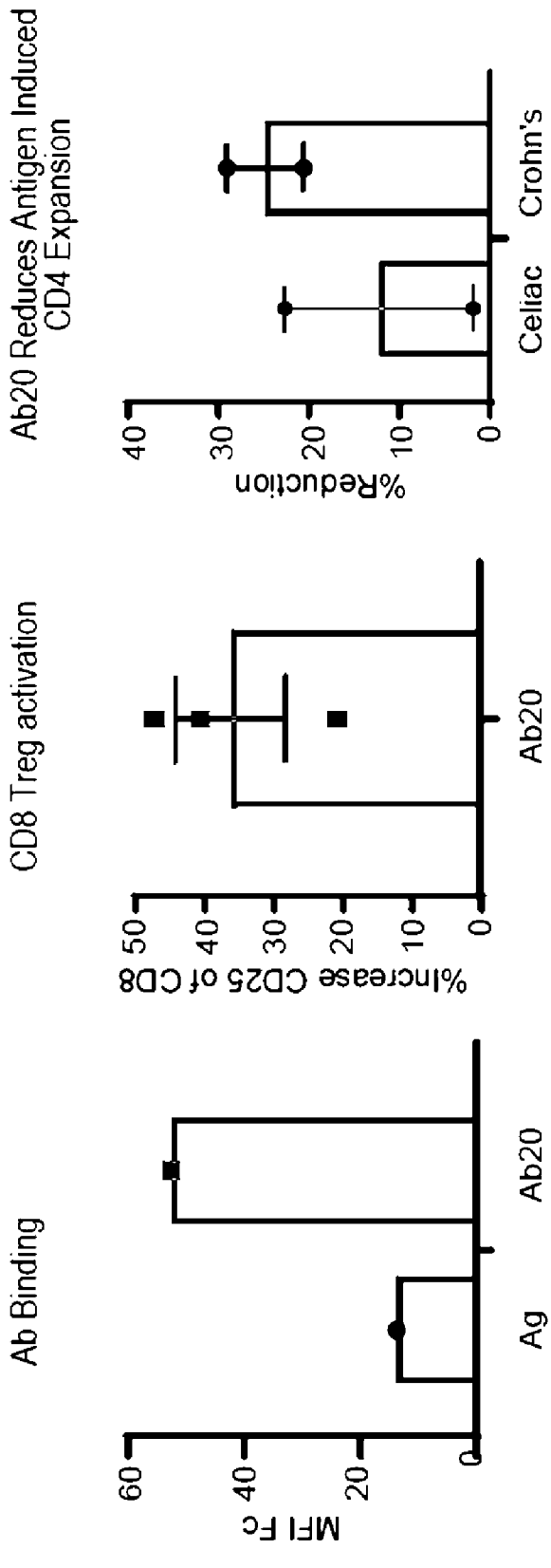

Ab Binding
MFI Fc
60
40
20
0
Ag
Ab20
CD8 Treg activation
%Increase CD25 of CD8
50
40
30
20
10
0
Ab20
Ab20 Reduces Antigen Induced CD4 Expansion
%Reduction
40
30
20
10
0
Celiac
Crohn's

FIG. 24D

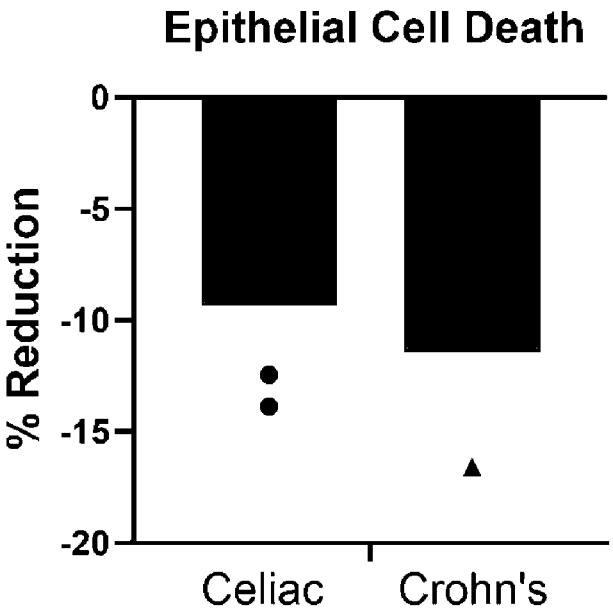


FIG. 24E

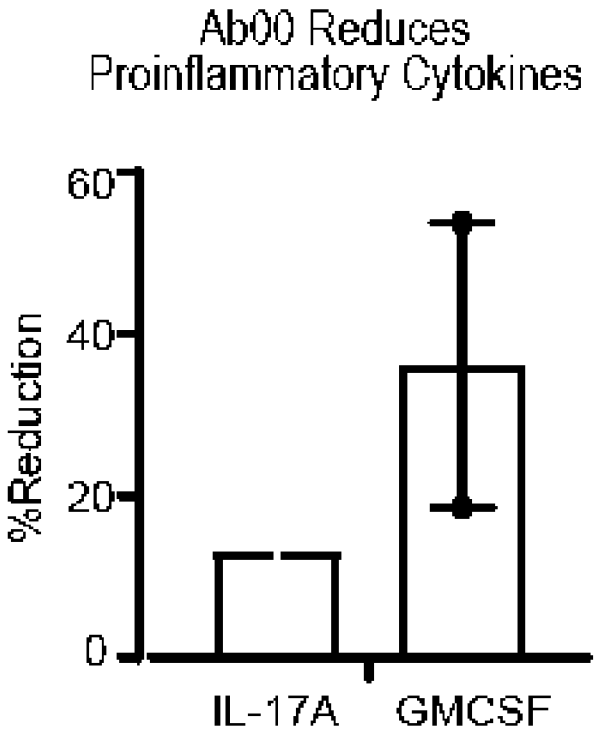


FIG. 24F

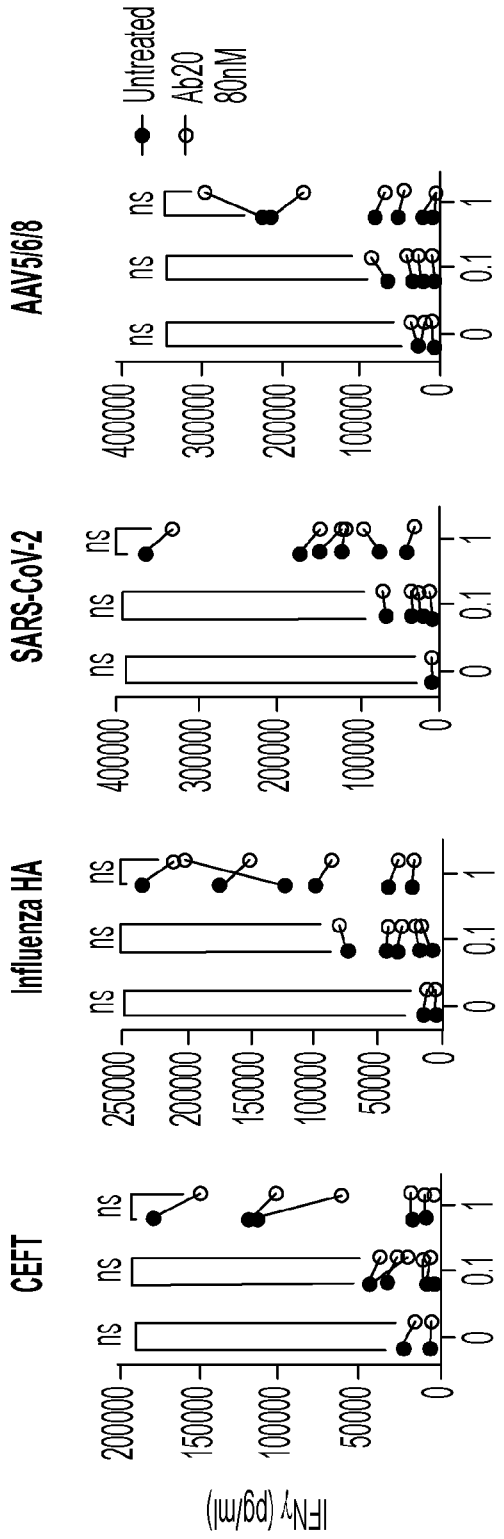

Untreated
Ab20
80nM
AAV5/6/8
SARS-CoV-2
Influenza HA
CEFT
IFNγ (pg/ml)
400000
300000
200000
100000
0
250000
150000
50000
ns
0
0.1
1

FIG. 25A

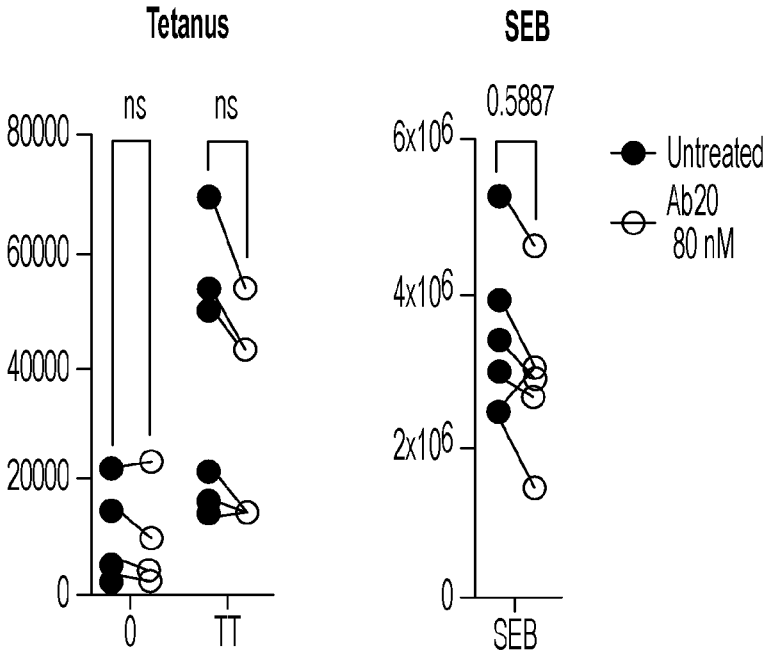

Tetanus
SEB
ns
ns
0.5887
80000
60000
40000
20000
0
6x10^6
4x10^6
2x10^6
0
0
TT
SEB
Untreated
Ab20
80 nM

FIG. 25B

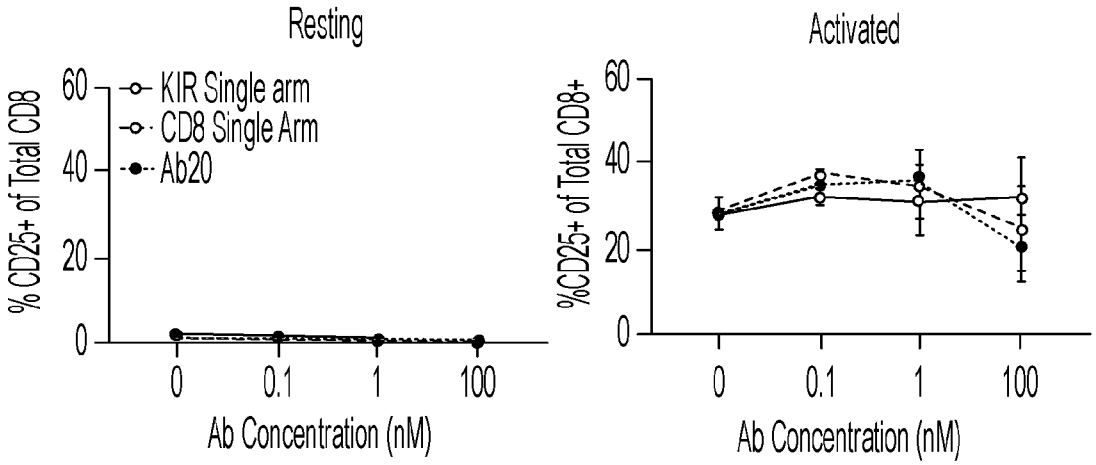


FIG. 26A

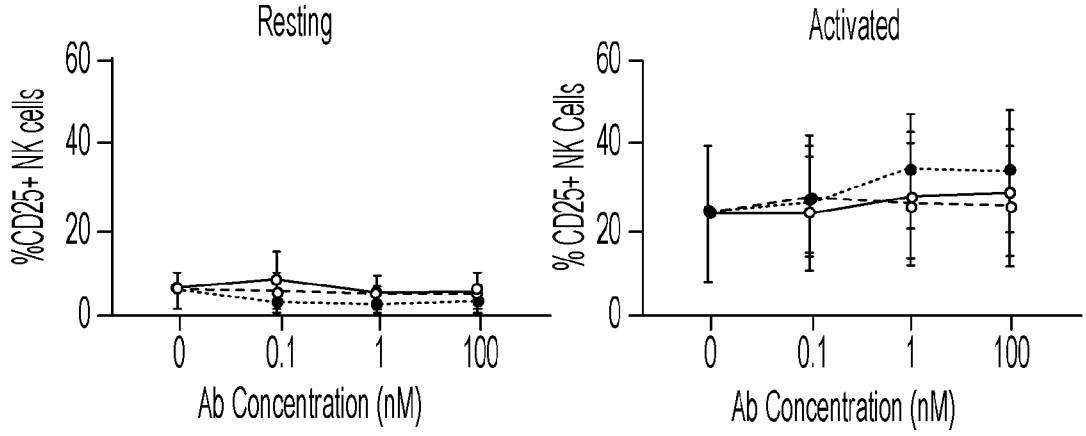


FIG. 26B

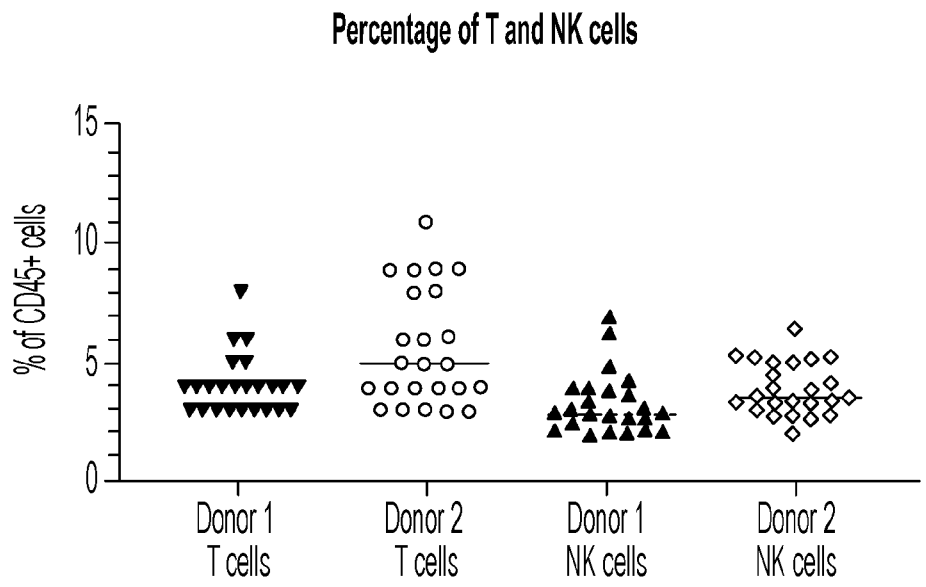

Percentage of T and NK cells
% of CD45+ cells
15
10
5
0
Donor 1 T cells
Donor 2 T cells
Donor 1 NK cells
Donor 2 NK cells

FIG. 26C

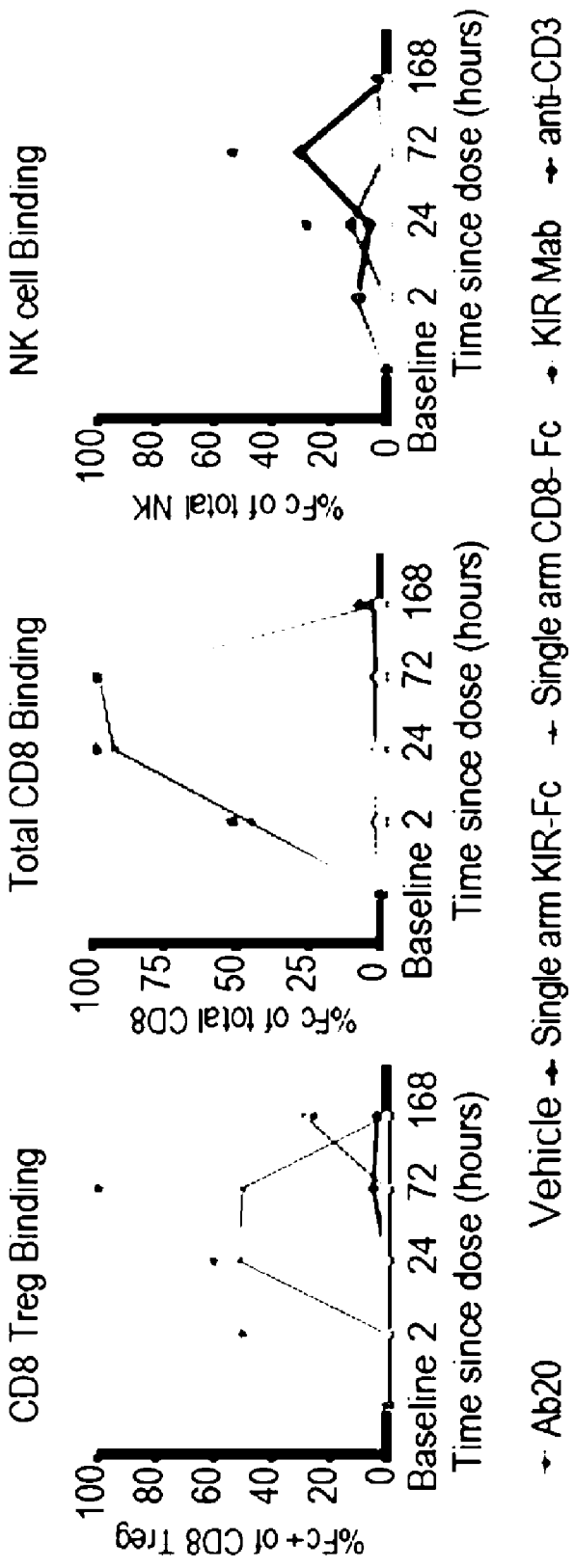

CD8 Treg Binding
%Fc+ of CD8 Treg
100
80
60
40
20
0
Baseline 2 24 72 168
Time since dose (hours)
Total CD8 Binding
%Fc of total CD8
100
75
50
25
0
Baseline 2 24 72 168
Time since dose (hours)
NK cell Binding
%Fc of total NK
100
80
60
40
20
0
Baseline 2 24 72 168
Time since dose (hours)
Ab20
Vehicle
Single arm KIR-Fc
Single arm CD8- Fc
KIR Mab
anti-CD3

FIG. 26D

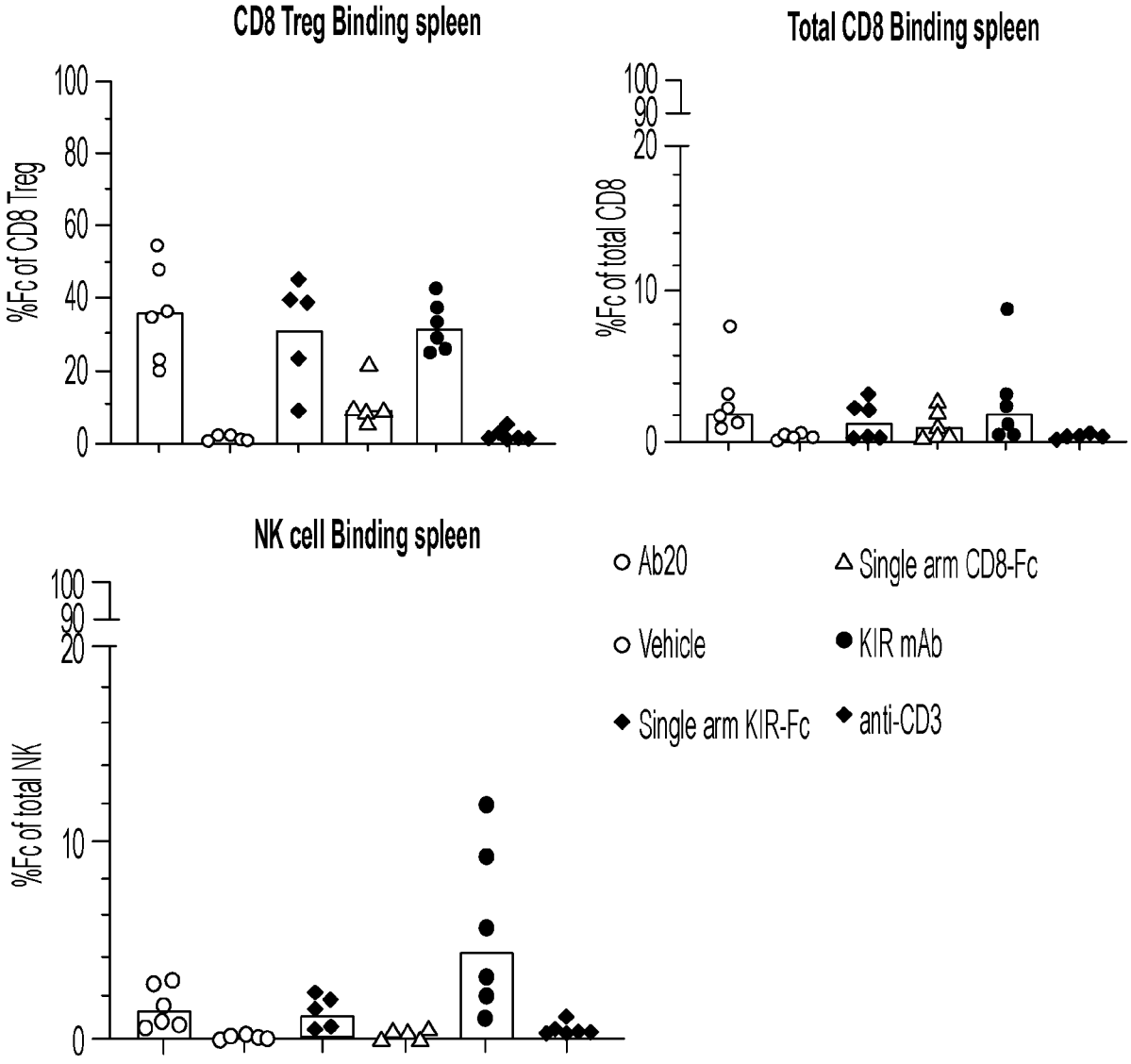

CD8 Treg Binding spleen
%Fc of CD8 Treg
100
80
60
40
20
0
Total CD8 Binding spleen
%Fc of total CD8
100
90
20
10
0
NK cell Binding spleen
%Fc of total NK
100
90
20
10
0
Ab20
Vehicle
Single arm KIR-Fc
Single arm CD8-Fc
KIR mAb
anti-CD3

FIG. 26E

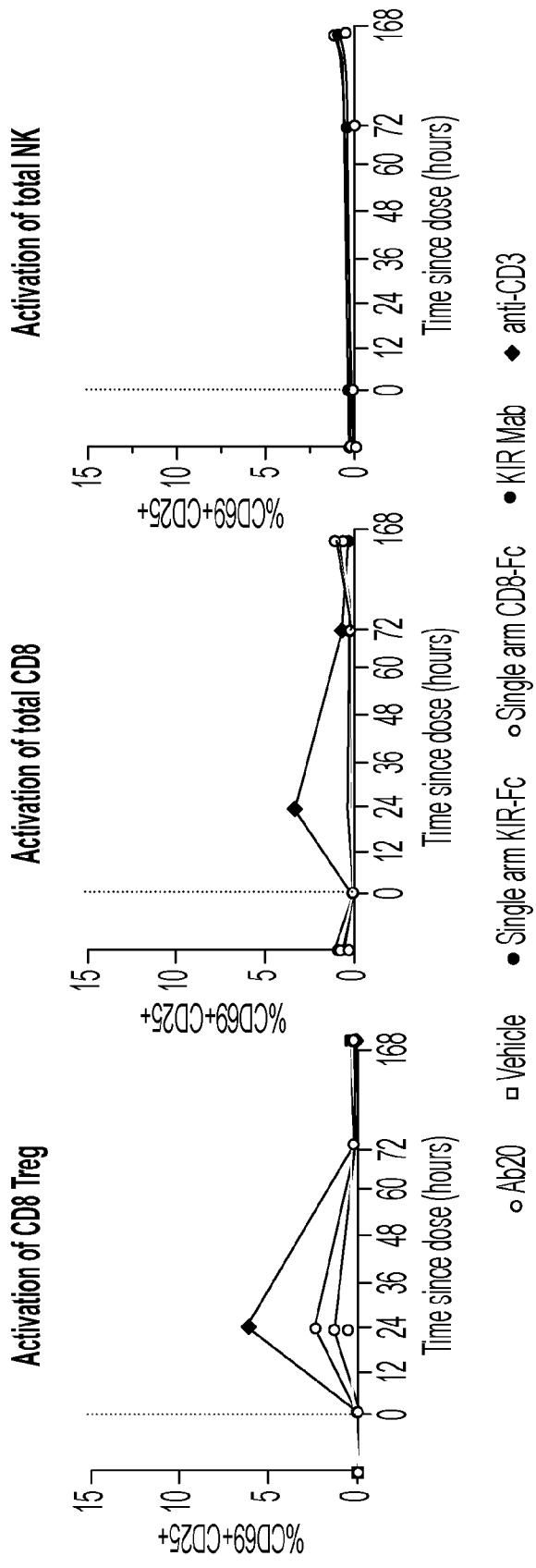

Activation of CD8 Treg
Activation of total CD8
Activation of total NK
%CD69+CD25+
0
5
10
15
0 12 24 36 48 60 72 168
Time since dose (hours)
Ab20
Vehicle
Single arm KIR-Fc
Single arm CD8-Fc
KIR Mab
anti-CD3

FIG. 26F

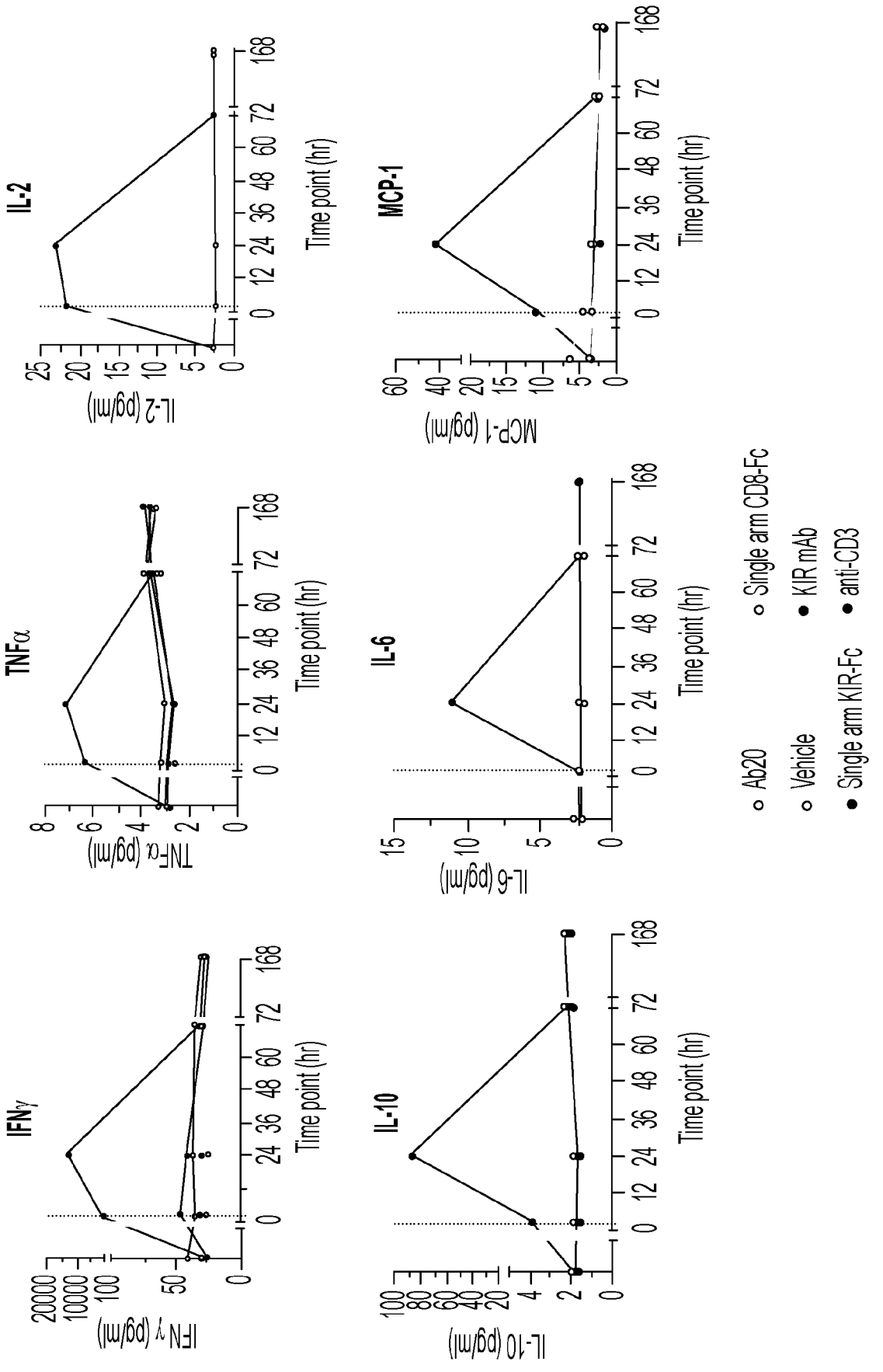

IL-2
IL-2 (pg/ml)
Time point (hr)
MCP-1
MCP-1 (pg/ml)
Time point (hr)
TNFα
TNFα (pg/ml)
Time point (hr)
IL-6
IL-6 (pg/ml)
Time point (hr)
IFNγ
IFN γ (pg/ml)
Time point (hr)
IL-10
IL-10 (pg/ml)
Time point (hr)
Ab20
Vehicle
Single arm KIR-Fc
Single arm CD8-Fc
KIR mAb
anti-CD3

FIG. 26G

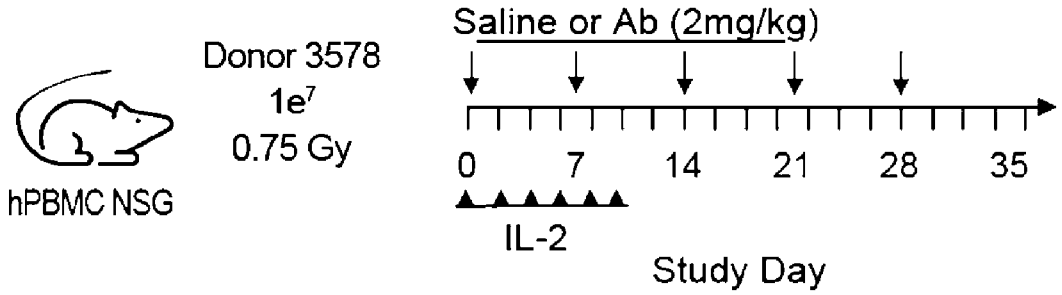


FIG. 27A

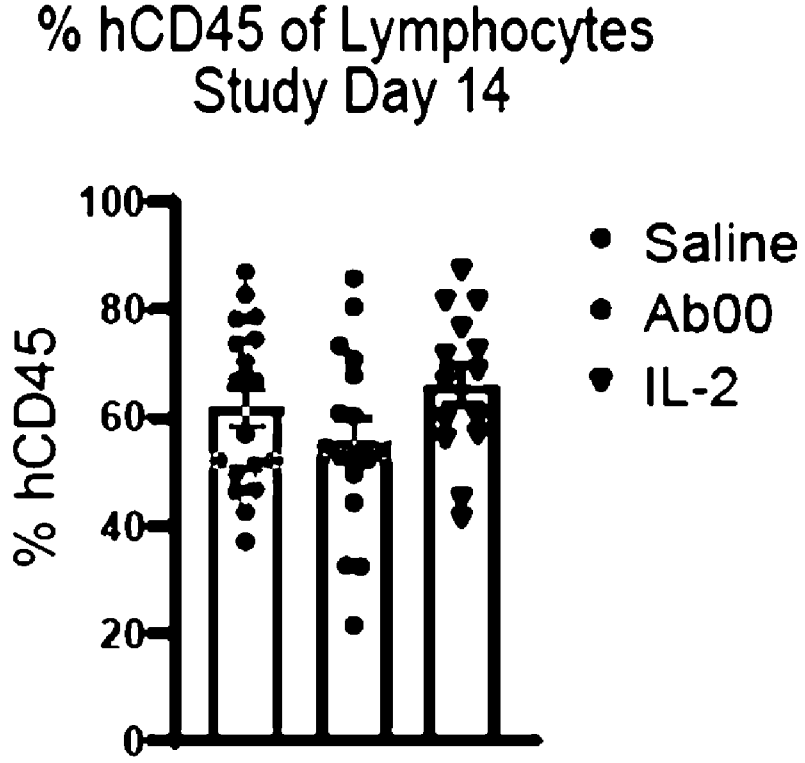


FIG. 27B

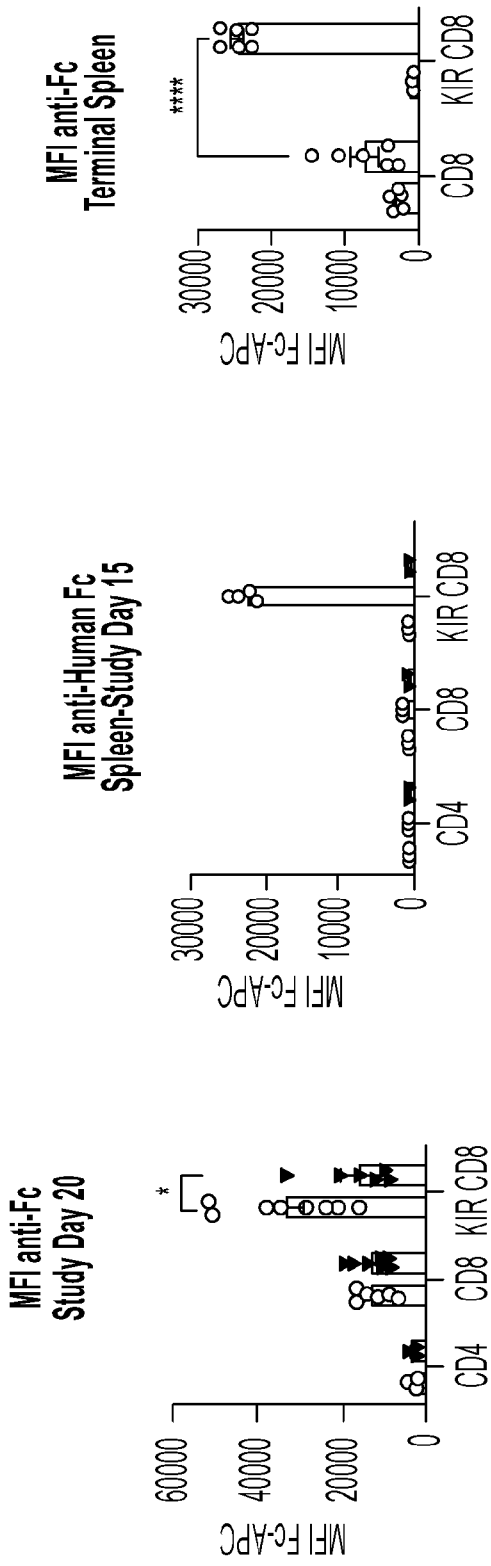

MFI anti-Fc
Study Day 20
MFI Fc-APC
60000
40000
20000
0
CD4
CD8
KIR CD8
*
MFI anti-Human Fc
Spleen-Study Day 15
MFI Fc-APC
30000
20000
10000
0
CD4
CD8
KIR CD8
Saline
Ab
IL-2
MFI anti-Fc
Terminal Spleen
MFI Fc-APC
30000
20000
10000
0
CD8
KIR CD8
****

FIG. 27C

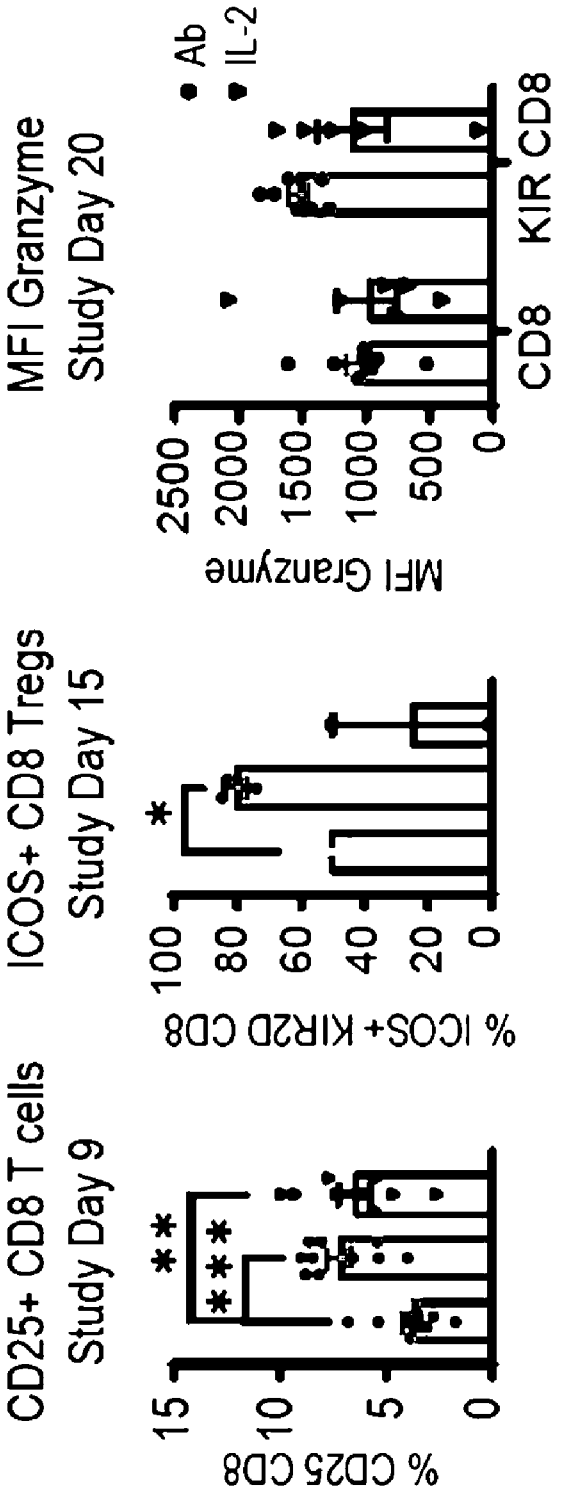

CD25+ CD8 T cells
Study Day 9
% CD25 CD8
15
10
5
0
**
***
ICOS+ CD8 Tregs
Study Day 15
% ICOS+ KIR2D CD8
100
80
60
40
20
0
*
MFI Granzyme
Study Day 20
MFI Granzyme
2500
2000
1500
1000
500
0
Ab
IL-2
CD8
KIR CD8

FIG. 27D

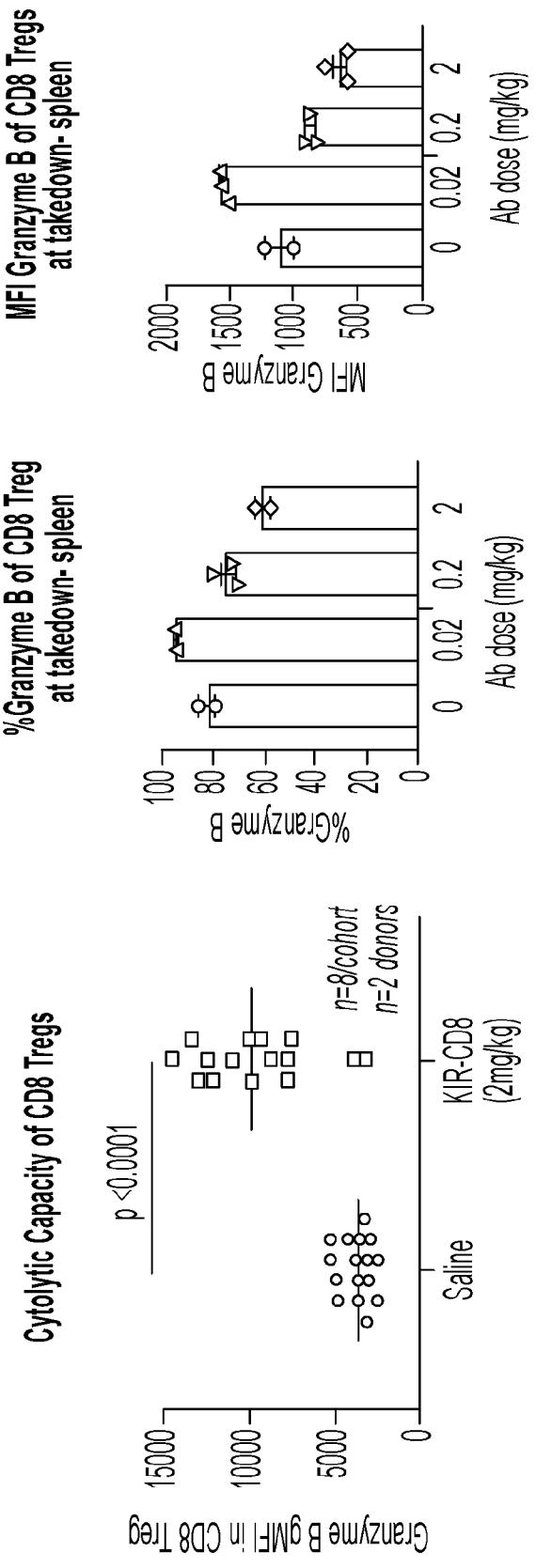

Cytolytic Capacity of CD8 Tregs
Granzyme B gMFI in CD8 Treg
15000
10000
5000
0
p <0.0001
n=8/cohort
n=2 donors
Saline
KIR-CD8
(2mg/kg)
%Granzyme B of CD8 Treg
at takedown- spleen
%Granzyme B
100
80
60
40
20
0
0
0.02
0.2
2
Ab dose (mg/kg)
MFI Granzyme B of CD8 Tregs
at takedown- spleen
MFI Granzyme B
2000
1500
1000
500
0
0
0.02
0.2
2
Ab dose (mg/kg)

FIG. 27E

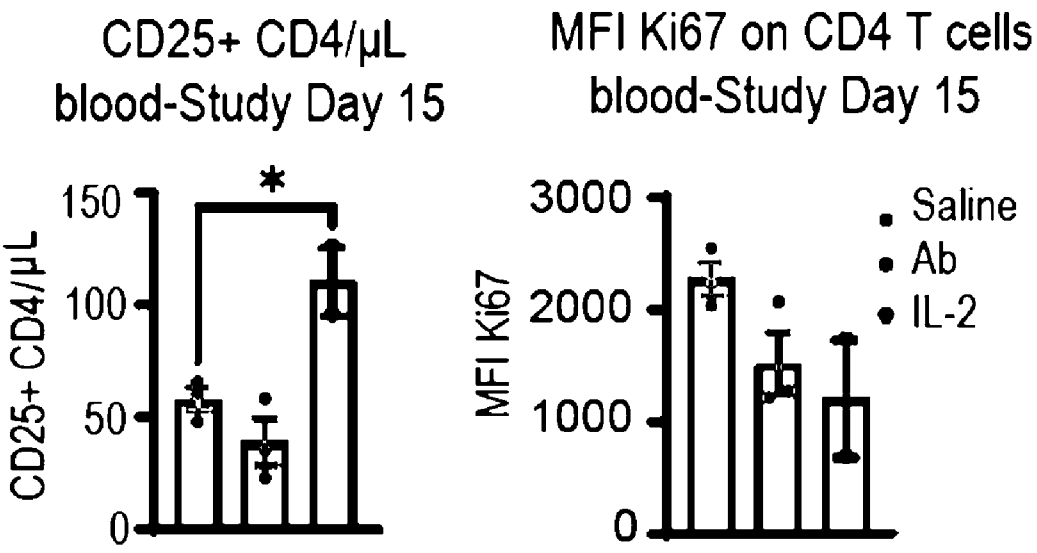


FIG. 27F

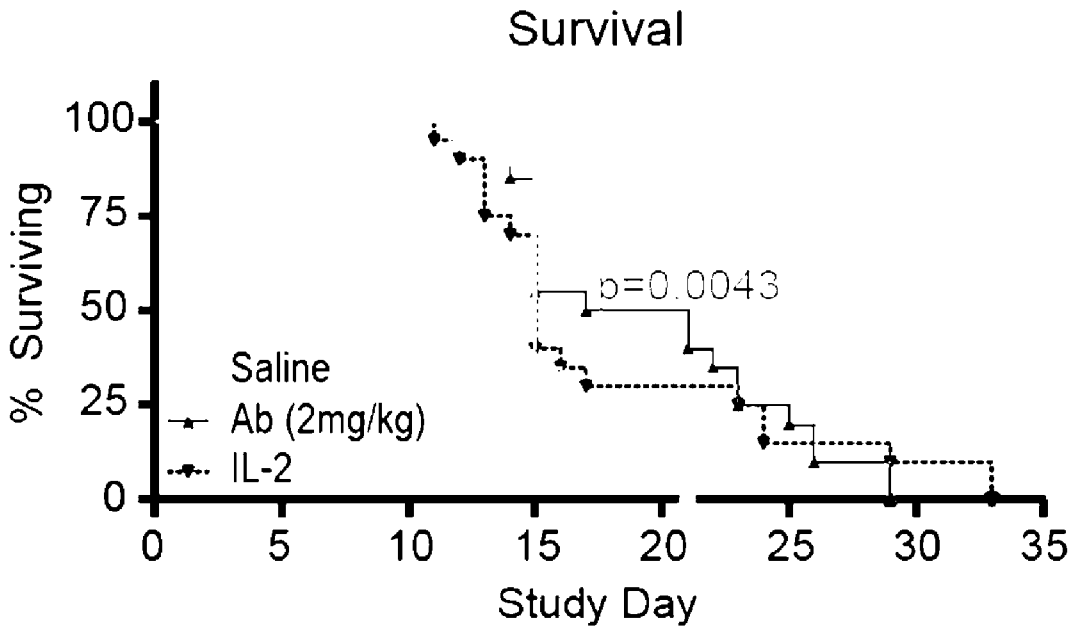


FIG. 27G

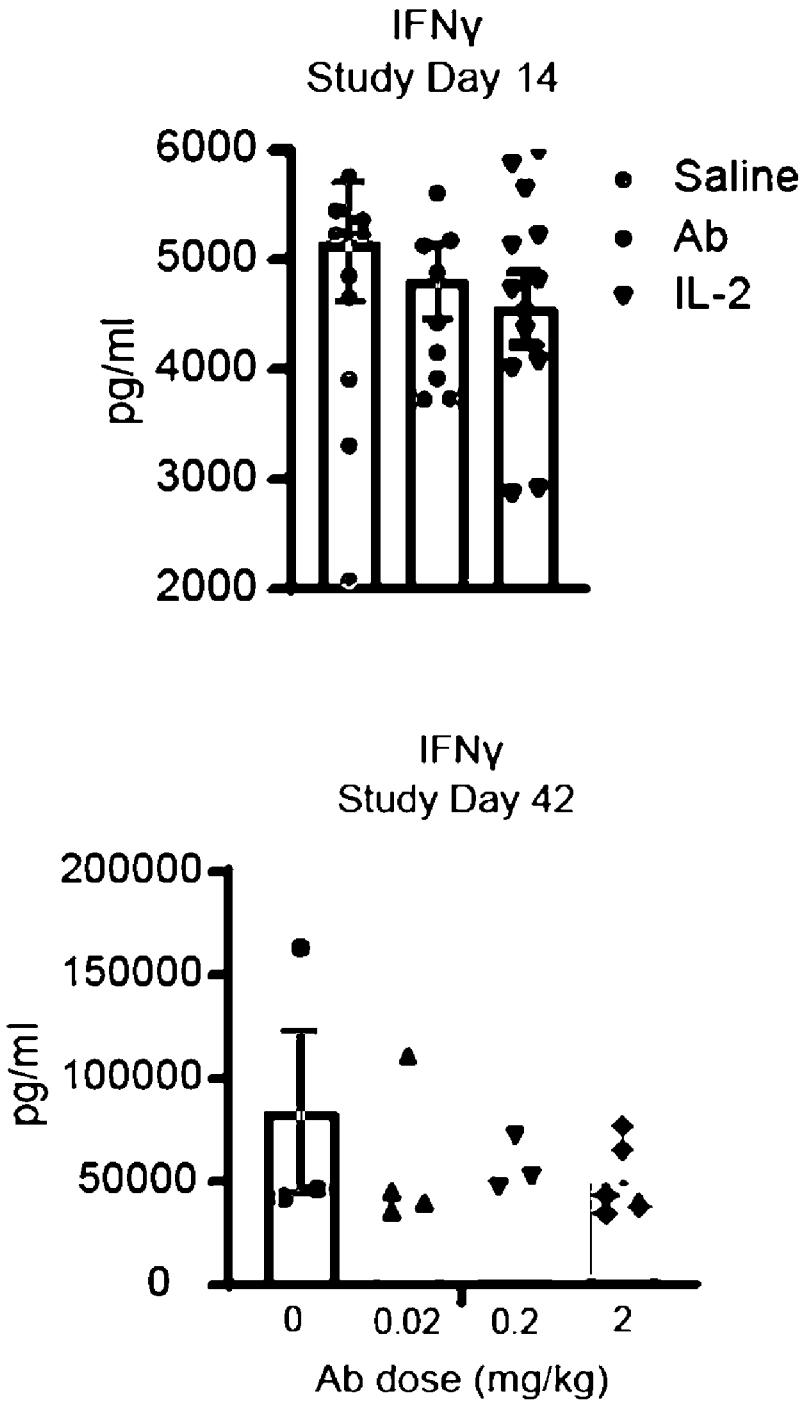

IFNγ
Study Day 14
6000
5000
4000
3000
2000
pg/ml
Saline
Ab
IL-2
IFNγ
Study Day 42
200000
150000
100000
50000
0
pg/ml
0
0.02
0.2
2
Ab dose (mg/kg)

FIG. 27H

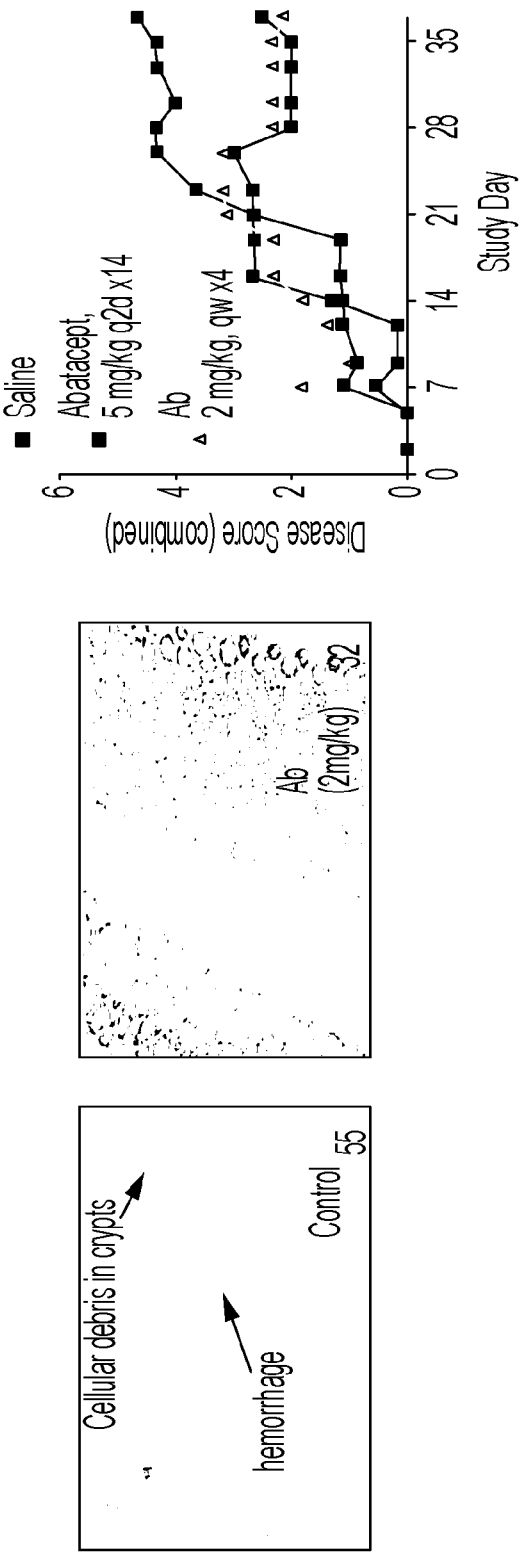

Saline
Abatacept, 5 mg/kg q2d x14
Ab 2 mg/kg, qw x4
Disease Score (combined)
0
2
4
6
0
7
14
21
28
35
Study Day
Ab (2mg/kg)
32
Cellular debris in crypts
hemorrhage
Control
55

FIG. 27I

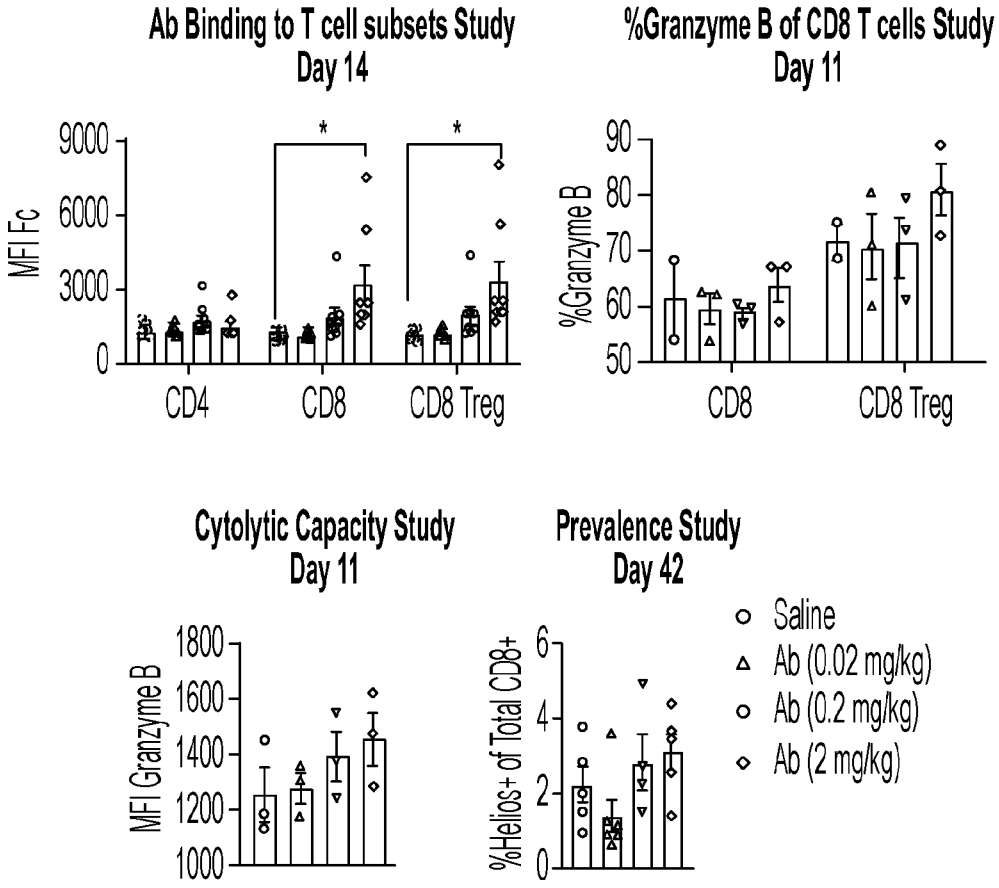

Ab Binding to T cell subsets Study
Day 14
MFI Fc
9000
6000
3000
0
*
*
CD4
CD8
CD8 Treg
%Granzyme B of CD8 T cells Study
Day 11
%Granzyme B
90
80
70
60
50
CD8
CD8 Treg
Cytolytic Capacity Study
Day 11
MFI Granzyme B
1800
1600
1400
1200
1000
Prevalence Study
Day 42
%Helios+ of Total CD8+
6
4
2
0
Saline
Ab (0.02 mg/kg)
Ab (0.2 mg/kg)
Ab (2 mg/kg)

FIG. 27J

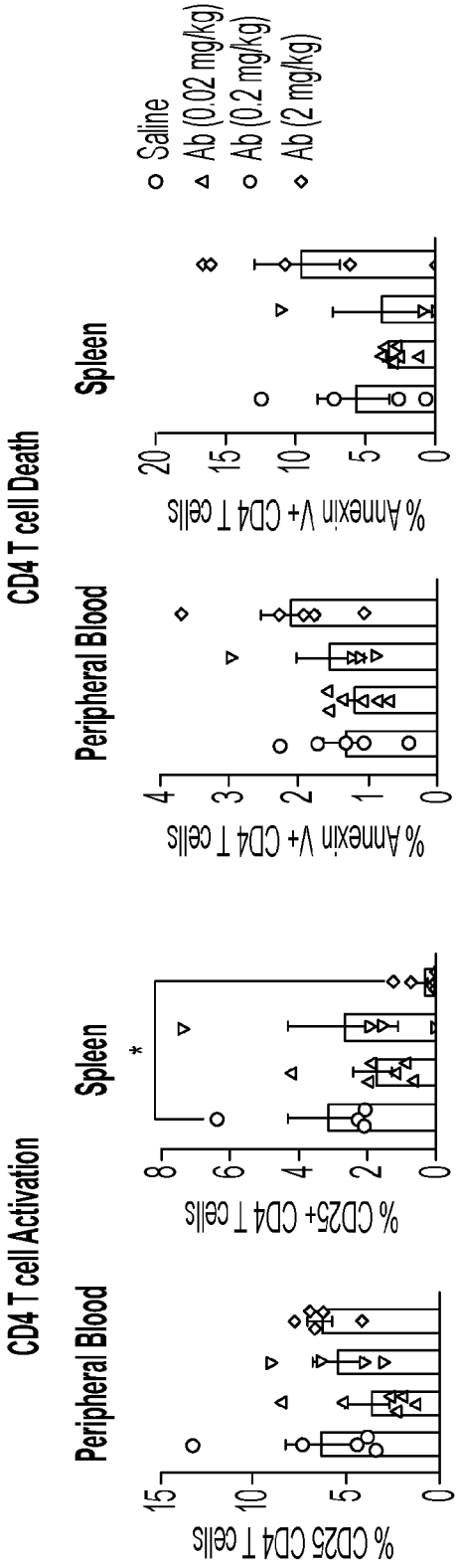

CD4 T cell Activation
Peripheral Blood
% CD25 CD4 T cells
Spleen
% CD25+ CD4 T cells
CD4 T cell Death
Peripheral Blood
% Annexin V+ CD4 T cells
Spleen
% Annexin V+ CD4 T cells
Saline
Ab (0.02 mg/kg)
Ab (0.2 mg/kg)
Ab (2 mg/kg)

FIG. 27K

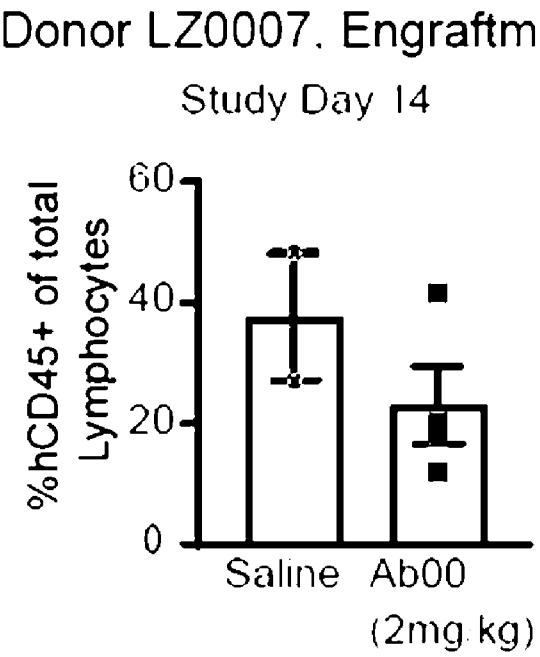


FIG. 28A

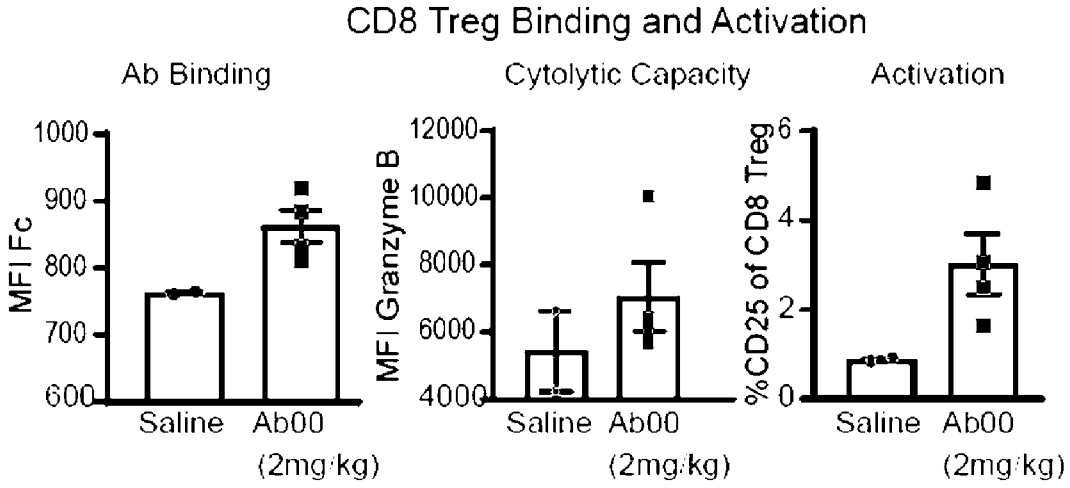


FIG. 28B

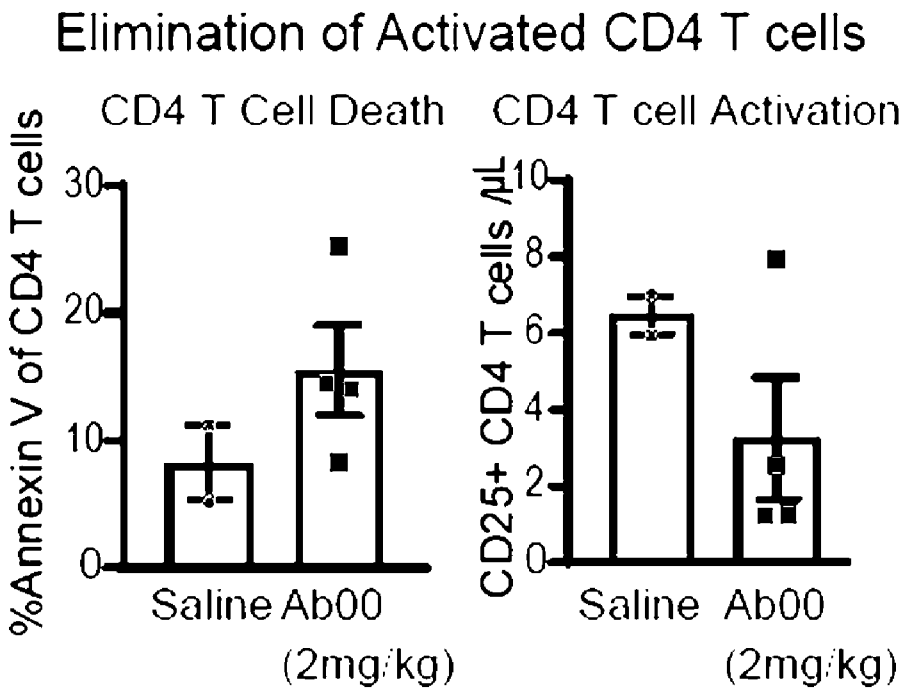


FIG. 28C

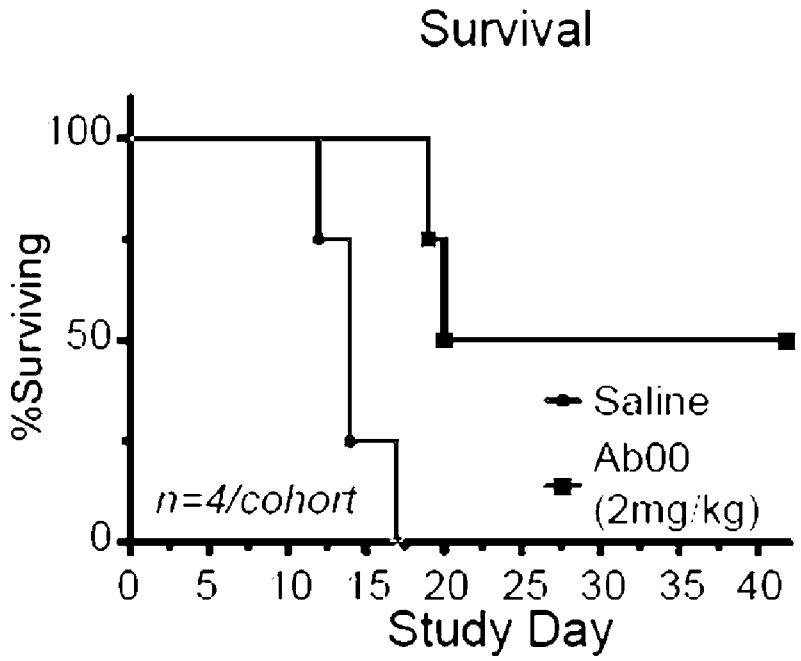


FIG. 28D

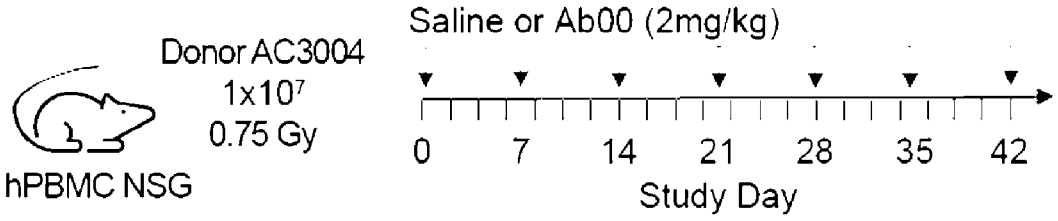


FIG. 28E

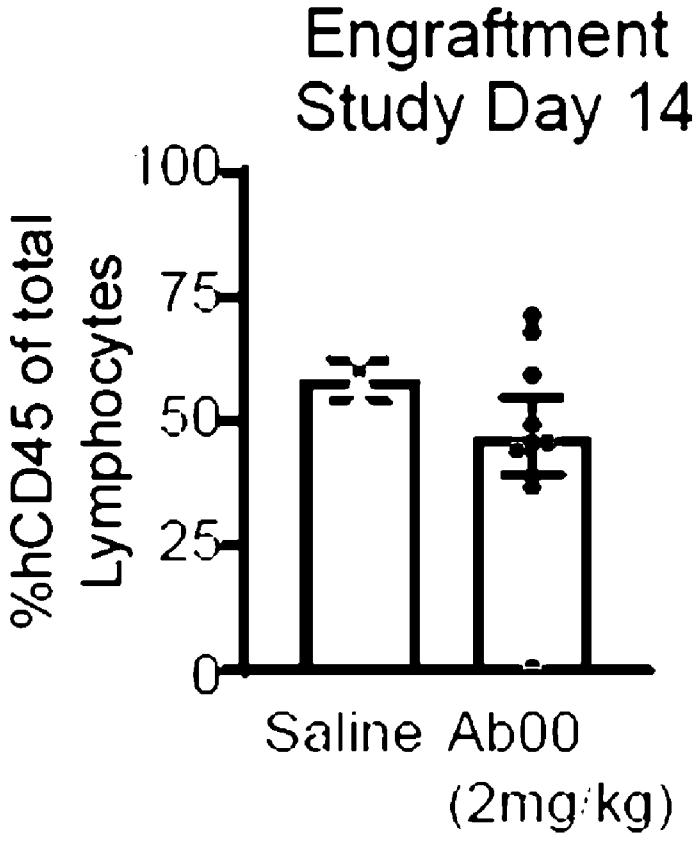


FIG. 28F

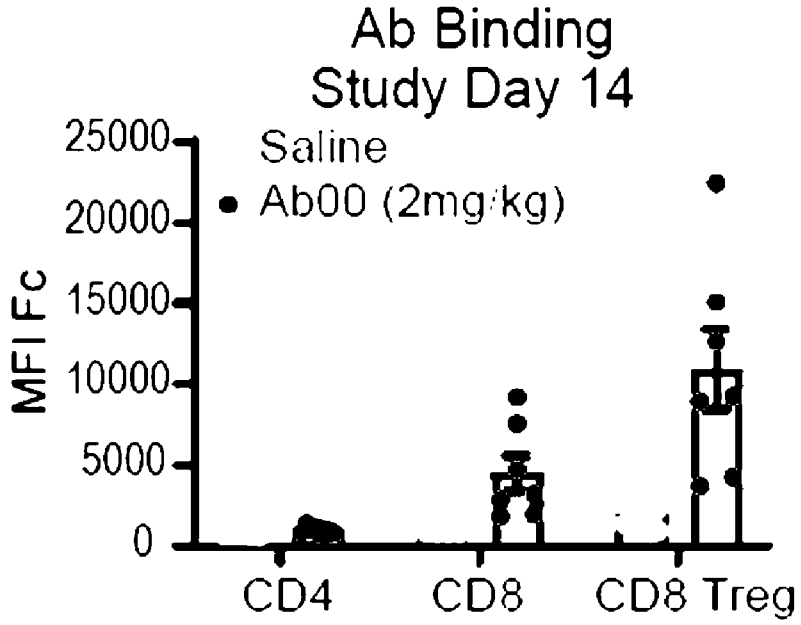


FIG. 28G

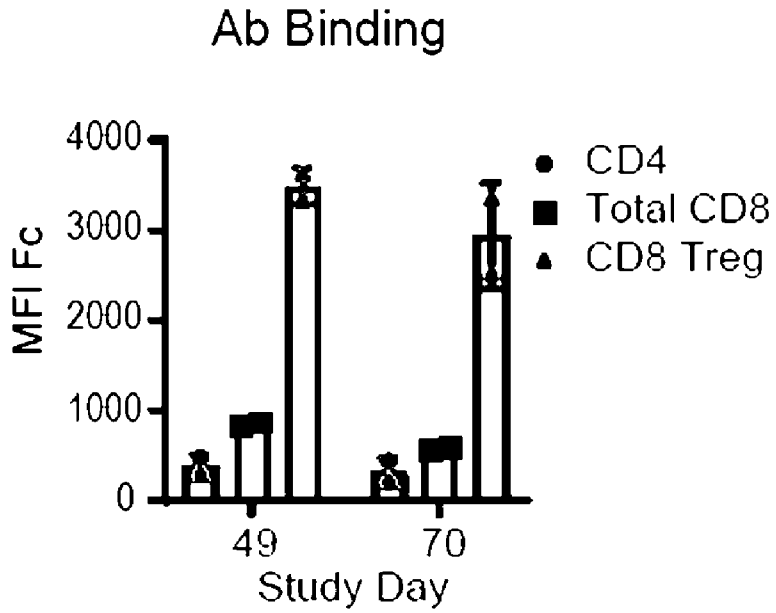


FIG. 28H

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2024/030804

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/221507 A1 (GUDAS JEAN MARIE [US] ET AL) 9 August 2018 (2018-08-09)	1-14, 36-50, 53-100
Y	See e.g. SEQ ID NOs: 161-163, Figures 8 and 9; Example 11	1-14, 36-50, 53-100

X	US 2017/029507 A1 (HO DAVID T [US] ET AL) 2 February 2017 (2017-02-02)	1-14, 36-50, 53-100
Y	See e.g. SEQ ID NOs: 3, 9, 12-15, 20, 22, 42, 46	1-14, 36-50, 53-100

X	WO 2021/001289 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 7 January 2021 (2021-01-07) See e.g. SEQ ID NO: 8	1-14, 36-50, 53-100

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 September 2024	08/11/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Valcárcel, Rafael

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	WO 2024/030956 A2 (MOZART THERAPEUTICS INC [US]) 8 February 2024 (2024-02-08) See e.g. SEQ ID NO: 54	48
X	WO 2022/169825 A1 (MOZART THERAPEUTICS INC [US]) 11 August 2022 (2022-08-11)	48
Y	See e.g. para 3 or Example 12	48

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/030804

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/030804

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-47, 49-51, 53-100(all partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1, 48(completely); 2-14, 36-47, 49, 50, 53-100(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2024/030804

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2018221507 A1	09-08-2018	US 2018221507 A1	09-08-2018
		WO 2018147960 A1	16-08-2018
US 2017029507 A1	02-02-2017	AU 2014249243 A1	08-10-2015
		AU 2019202676 A1	16-05-2019
		CA 2904969 A1	09-10-2014
		CN 105163765 A	16-12-2015
		CN 113045660 A	29-06-2021
		EP 2968622 A1	20-01-2016
		EP 3889182 A1	06-10-2021
		EP 4411378 A2	07-08-2024
		JP 6815196 B2	20-01-2021
		JP 6856611 B2	07-04-2021
		JP 2016520514 A	14-07-2016
		JP 2019054819 A	11-04-2019
		KR 20150131208 A	24-11-2015
		KR 20210016067 A	10-02-2021
		US 2014271462 A1	18-09-2014
		US 2016024209 A1	28-01-2016
		US 2017029507 A1	02-02-2017
		US 2019382487 A1	19-12-2019
		US 2019382488 A1	19-12-2019
		US 2020140550 A1	07-05-2020
		WO 2014164553 A1	09-10-2014
WO 2021001289 A1	07-01-2021	AR 119338 A1	09-12-2021
		CN 114051500 A	15-02-2022
		EP 3994169 A1	11-05-2022
		JP 2022538139 A	31-08-2022
		TW 202115115 A	16-04-2021
		WO 2021001289 A1	07-01-2021
WO 2024030956 A2	08-02-2024	NONE	
WO 2022169825 A1	11-08-2022	AU 2022218137 A1	24-08-2023
		CA 3209479 A1	11-08-2022
		EP 4288455 A1	13-12-2023
		IL 304736 A	01-09-2023
		JP 2024505600 A	06-02-2024
		KR 20230152032 A	02-11-2023
		US 2024101673 A1	28-03-2024
		WO 2022169825 A1	11-08-2022

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 48(completely); 2-14, 36-47, 49, 50, 53-100(partially)

A binding protein comprising the VL of SEQ ID NO: 55 and the VH of SEQ ID NO: 56. Even if claim 1 section (a) refers to a binding protein comprising at least 90% identity to SEQ ID NO: 55 and at least 90% identity to SEQ ID NO: 56, said definition is not clear, not supported and not sufficiently disclosed (see also the incomplete search reasoning). The minimal characterization of an antibody requires the 6 exact CDR sequences and the binding specificity or the exact VH and VL pair sequences and the binding specificity. A 10 % variation in a VH sequence and 10% variation in a VL sequence does not make technical sense in a "binding protein" since the binding specificity can be completely altered by these variations (even a single amino acid substitution in a CDR can alter the binding specificity). Thus, either the six exact CDRs must be fully defined (however the combination of the 6 CDRs of SEQ ID NOs 1-6 was known in anti CD8 antibodies) or the exact VH and VL pairs must be recited in the claim. Thus, invention 1 has been considered to refer to the first VL/VH pair defined in the claims (in independent claim 1) and this corresponds to variant 20 as designated by the Applicant.
Here it is noted that independent claim 47 refers to a binding protein according to Table 3. This definition is unclear since it is open to interpretation which sequences of said Table 3 must be combined to define an invention. However for the sake of procedural efficiency and taking independent claim 48 as guidance which clearly recites a binding molecule comprising SEQ ID NOs: 78, 80 and 120, the part of claim 47 referring to the combination recited in independent claim 48 has been considered as part of invention 1 since it is considered to correspond to "Variant 20 anti-CD8/anti-KIR bottle-opener molecule".
Also part of this subject is DNA sequences encoding said molecules, vectors host cells, methods of treatment and medical uses involving the above mentioned molecules.

2-22. claims: 2-47, 49, 50, 53-100(all partially)

As subject 1 but for each of the other 21 variants as designated by the Applicant, for example subject 2 would be variant 1, defined in independent claim 15 and comprising the exact VL of SEQ ID NO: 17 and the exact VH of SEq ID NO: 18 (NO VARIATIONS POSSIBLE, see the comments on subject 1).

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23. claims: 51(completely); 53, 54(partially)

A nucleic acid molecule comprising SEQ ID NOs: 139, 141, and

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

141 (sic). Vector and host cell comprising said molecule.
SEQ ID NO: 139 is the anti-KIR arm light chain DNA sequence without signal sequence.
SEQ ID NO: 141 is the anti-KIR arm heavy chain DNA sequence without signal sequence.
This means that the molecule of independent claim 51 has no CD8 binding arm.
Here it is noted that the claim is unclear since SEQ ID NO: 141 is repeated twice. Assuming there is a mistake it is not clear which would be the correction, i.e. which other sequence different from SEQ ID NO: 141 has to be included in the molecule. Although in general no additional seearch fees should be requested for subject-matter which could not be considered an invention, the subject-matter of independent claim 51 is listed here as a potental invention in case the Applicant could justify a correction of an obvious mistake even if prima facie it does not appear to be possible. Thus, the Applicant is not adviced to pay for this invention unless he is confident that the invention could be properly defined.

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24. claims: 52(completely); 53, 54(partially)

A nucleic acid molecule comprising SEQ ID NOs: 138, 140, and 142. Vector and host cell comprising said molecule.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-47, 49-51, 53-100(all partially)

The reasons for which the subject-matter of claims 1-51 and 53-100 can not be completely searched are specified in the annexed provisional opinion accompanying the partial search results (EPO Form 1707).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.3), should the problems which led to the Article 17(2) PCT declaration be overcome.