SINGLE- AND MULTI-CYTOKINE FUSION PROTEINS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application includes a claim of priority under 35 U.S.C. §119(e) to U.S. provisional patent applications No. 63/472,939 filed June 14, 2023, No. 63/472,972, filed June 14, 2023, No. 63/527,948, filed July 20, 2023, No. 63/530,784, filed August 4, 2023, and No. 63/641,265, filed May 1, 2024, the entirety of each is hereby incorporated by reference.

REFERENCE TO SEQUENCE LISTING

[0002] This application contains a Sequence Listing submitted as a computer readable form named "096034_000006WOPT_SequenceListing.xml", having a size in bytes of 1,254,546 bytes, and created on June 13, 2024. The information contained in this computer readable form is hereby incorporated by reference in its entirety.

FIELD OF INVENTION

[0003] This invention relates to fusion proteins and their uses in treating disease conditions such as cancer.

SUMMARY OF THE INVENTION

[0004] The following embodiments and aspects thereof are described and illustrated in conjunction with compositions and methods which are meant to be exemplary and illustrative, not limiting in scope.

[0005] Various embodiments provide for a fusion protein comprising a scaffold polypeptide and at least two of (a)-(c):

a. one or more of an interleukin 15 (IL-15) variant,
b. one or more of an interleukin 18 (IL-18), a fragment of the IL-18, an IL-18 variant, or a fragment of the IL-18 variant, and
c. one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA).

[0006] In various embodiments, the one or more polypeptides capable of binding the TAA can comprise a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH.

[0007] In various embodiments, the TAA can comprise EGFR, HER2, or DLL.

[0008] In various embodiments, the activation receptor can comprise cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, or wherein the costimulatory receptor can comprise cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40.

[0009] In various embodiments, the fusion protein can comprise one or more of an interleukin 15 (IL-15) variant and one or more of an interleukin 18 (IL-18), a fragment of the IL-18, an IL-18 variant. In various embodiments, the fusion protein can comprise one or more of IL-15 variants and one or more IL-18 variants.

[0010] In various embodiments, the fusion protein can further comprise a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, and wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40.

[0011] In various embodiments, wherein the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, can be each independently fused to the C-terminus of the scaffold polypeptide. In various embodiments, the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, can be each independently fused to the N-terminus of the scaffold polypeptide. In various embodiments, the scaffold polypeptide is an antibody, the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, can be each independently fused to any one of one of a C-terminus or an N-terminus of a heavy chain or a light chain, or fused to any one of a C_H2 domain, or hinge region of the antibody.

[0012] In various embodiments, the scaffold polypeptide can be an antibody or a fragment thereof. In various embodiments, the antibody can be an IgA, IgM, IgG, or IgE antibody. In various embodiments, the antibody can be an anti-PD-1 antibody or anti-PD-L1 antibody.

[0013] In various embodiments, the scaffold polypeptide can be a fragment crystallizable (Fc) region or a fragment thereof.In various embodiments, the fragment crystallizable (Fc) region can be an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG1. In various embodiments, the fragment crystallizable (Fc) region can be a knobs-in-hole (KiH) Fc.

[0014] In various embodiments, the scaffold polypeptide can be a polypeptide or protein capable of translocating into an endoplasmic reticulum (ER), or a fragment thereof.

[0015] In various embodiments, the scaffold polypeptide can be selected from fragment crystallizable (Fc) region, human serum albumin (HSA), beta2microglobulin, transferrin, fragment antigen-binding region (Fab region), VHH antibody, single-chain variable fragment (scFv), anticalin, designed ankyrin repeat protein (DARPin), a binding domain thereof, and a fragment thereof.

[0016] In various embodiments, IL-15 variant can comprise the sequence of Formula I: X_1WVX_4VISDLKKIEDLIQSMHIX_{22}ATLYTESX_{30}VHPSCKVTAMX_{41}CFLX_{45}ELQX_{49}ISLX_{53}SGDASIHDTVX_{64}N L$X_{67}X_{68}$LANNSLSSNGX_{79}VTESGCKECEELEX_{93}KNIKEFLQSX_{103}VHIVX_{108}MFIX_{112}TS, wherein

a. X_1 is any amino acid,

b. X_4 is any amino acid,
c. X_{22} is any amino acid,
d. X_{30} is any amino acid,
e. X_{41} is any amino acid,
f. X_{45} is any amino acid,
g. X_{49} is any amino acid except V,
h. X_{53} is any amino acid except E,
i. X_{64} is any amino acid,
j. X_{67} is any amino acid,
k. X_{68} is any amino acid,
l. X_{79} is any amino acid,
m. X_{93} is any amino acid,
n. X_{103} is any amino acid,
o. X_{108} is any amino acid, and
p. X_{112} is any amino acid; (SEQ ID NO:533).

[0017] In various embodiments, the IL-18 variant can comprise

a1. amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDS DVRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDN KMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251) with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of SEQ ID NO:251, optionally further comprising amino acid substitutions at one or more of C74, C104, C112, and C164, each independently substituted to valine, alanine or serine, OR

a3. an IL-18 variant selected from Table 6B; and

b1. optionally, wherein the one to five amino acid substitutions are one or more of: E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; or M149V or M149I; or

b2. optionally, wherein the one to five amino acid substitutions are E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; and M149V or M149I.

[0018] In various embodiments, the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant further can comprise its propeptide (PP) or a PP variant, and optionally, wherein the PP or the PP variant is on the N-terminus end relative to the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant.

[0019] In various embodiments, the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant can further comprise a short polypeptide or protein.

[0020] In various embodiments, the fusion protein can further comprise one or more cleavage sites and the fusion protein is cleaved at the one or more cleavage sites by one or more proteases. In various embodiments, the one or more cleavage sites can be between the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant and the scaffold polypeptide, or within the PP, between PP or the PP variant and the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, or within the PP, between the PP or the PP variant and the scaffold polypeptide, or within the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, or within the PP, or a combination thereof.

[0021] In various embodiments, the ROR1 VHH can comprise:

a polypeptide having SEQ ID NO:325 (complementarity-determining region (CDR) 1 of 2A11), a polypeptide having SEQ ID NO:326 (CDR2 of 2A11), a polypeptide having SEQ ID NO:327 (CDR3 of 2A11), or a combination thereof; OR

a variant of the polypeptide having SEQ ID NO:325 (CDR1 of 2A11), a variant of the polypeptide having SEQ ID NO:326 (CDR2 of 2A11), a variant of the polypeptide having SEQ ID NO:327 (CDR3 of 2A11), or a combination thereof, wherein the variant of the polypeptide having SEQ ID NO:325 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:325, wherein the variant of the polypeptide having SEQ ID NO:326 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:326, wherein the variant of the polypeptide having SEQ ID NO:327 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:327, and

wherein:

the variant of the polypeptide having SEQ ID NO:325 and the variant of the polypeptide having SEQ ID NO:327 do **not** replace cysteine residues in the polypeptide having SEQ ID NO:325 and the polypeptide having SEQ ID NO:327, or

the variant of the polypeptide having SEQ ID NO:325 and the variant of the polypeptide having SEQ ID NO:327 **replaces** one or both of the cysteine residues in the polypeptide having SEQ ID NO:325 and/or one or both of the cysteine residues in the polypeptide having SEQ ID NO:327 **with** an amino acid that contains a cross-linking functional group.

[0022] Various embodiments provide for a polynucleotide encoding any one of the fusion proteins of the invention described above. Various embodiments provide for a polynucleotide encoding any one of the fusion proteins of the invention described herein.

[0023] Various embodiments provide for an expression vector comprising any one of the polynucleotides of the invention described above. Various embodiments provide for an expression vector comprising any one of the polynucleotides of the invention described herein.

[0024] Various embodiments provide for a cell transfected with any one of the expression vectors of the invention described above. Various embodiments provide for a cell transfected with any one of the expression vectors of the invention described herein. In various embodiments, the cell can be a mammalian cell. In various embodiments, the mammalian cell can be a CHO cell or a HEK-293 cell. In various embodiments, the cell can be a bacterial cell or yeast cell.

[0025] Various embodiments provide for a method of producing any one of the fusion proteins described above, comprising: culturing any one of the cells described above, in cell culture medium to allow the fusion protein to be produced, and optionally secreted into the cell culture medium. Various embodiments provide for a method of producing any one of the fusion proteins described herein, comprising: culturing any one of the cells described herein, in cell culture medium to allow the fusion protein to be produced, and optionally secreted into the cell culture medium. In various embodiments, the method can further comprise isolating the fusion protein. In various embodiments, the method can further comprise purifying the fusion protein.

[0026] Various embodiments of the invention provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering any one of the fusion proteins described above to a subject in need thereof. Various embodiments of the invention provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering any one of the fusion proteins described herein to a subject in need thereof. In various embodiments, the subject has cancer.

[0027] Various embodiments provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering any one of the fusion proteins described above to a subject in need thereof. Various embodiments provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering any one of the fusion proteins described herein to a subject in need thereof. In various embodiments, the disease or condition can be cancer.

[0028] Various embodiments provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering at least two of the following to a subject in need thereof:

a. interleukin 15 (IL-15) variant or IL-15 fusion protein,
b. interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein,
c. a multispecific antibody construct comprising
    i. a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and

ii. a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell,

wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, and

wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40, and

d. one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA).

[0029] In various embodiments, the subject has cancer.

[0030] Various embodiments provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering at least two of the following to a subject in need thereof:

a. interleukin 15 (IL-15) variant or IL-15 fusion protein,

b. interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein,

c. a multispecific antibody construct comprising

iii. a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and

iv. a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell,

wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, and

wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40, and

d. one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA).

[0031] In various embodiments, the disease or condition is cancer.

[0032] Various embodiments provide for an interleukin 15 (IL-15) variant comprising the sequence of Formula I:

$X_1WVX_4VISDLKKIEDLIQSMHIX_{22}ATLYTESX_{30}VHPSCKVTAMX_{41}CFLX_{45}ELQX_{49}ISLX_{53}SGDASIHDTVX_{64}NLX_{67}X_{68}LANNSLSSNGX_{79}VTESGCKECEELEX_{93}KNIKEFLQSX_{103}VHIVX_{108}MFIX_{112}TS$, wherein

a. X_1 is any amino acid,

b. X_4 is any amino acid,

c. X_{22} is any amino acid,

d. X_{30} is any amino acid,

e. X_{41} is any amino acid,

f. X_{45} is any amino acid,
g. X_{49} is any amino acid except V,
h. X_{53} is any amino acid except E,
i. X_{64} is any amino acid,
j. X_{67} is any amino acid,
k. X_{68} is any amino acid,
l. X_{79} is any amino acid,
m. X_{93} is any amino acid,
n. X_{103} is any amino acid,
o. X_{108} is any amino acid, and
p. X_{112} is any amino acid; (SEQ ID NO:533).

[0033] In various embodiments of the IL-15 variant,

a. X_1 can be N (SEQ ID NO:534),
b. X_4 can be N (SEQ ID NO:535),
c. X_{22} can be D (SEQ ID NO:536),
d. X_{30} can be D (SEQ ID NO:537),
e. X_{41} can be K (SEQ ID NO:538),
f. X_{45} can be L (SEQ ID NO:539),
g. X_{64} can be E (SEQ ID NO:540),
h. X_{67} can be I (SEQ ID NO:541),
i. X_{68} can be I (SEQ ID NO:542),
j. X_{79} can be N (SEQ ID NO:543),
k. X_{93} can be E (SEQ ID NO:544),
l. X_{103} can be F (SEQ ID NO:545),
m. X_{108} can be Q (SEQ ID NO:546), or
n. X_{112} can be N (SEQ ID NO:547), or
o. any two or more of a-o.

[0034] In various embodiments of the IL-15 variant,

a. X_1 can be N (SEQ ID NO:534),
b. X_4 can be N (SEQ ID NO:535),
c. X_{22} can be D (SEQ ID NO:536),
d. X_{30} can be D (SEQ ID NO:537),
e. X_{41} can be K (SEQ ID NO:538),
f. X_{45} can be L (SEQ ID NO:539),
g. X_{49} can be R or K (SEQ ID NO:548),

h. X_{53} can be G, K, I or A (SEQ ID NO:549),
i. X_{64} can be E (SEQ ID NO:540),
j. X_{67} can be I (SEQ ID NO:541),
k. X_{68} can be I (SEQ ID NO:542),
l. X_{79} can be N (SEQ ID NO:543),
m. X_{93} can be E (SEQ ID NO:544),
n. X_{103} can be F (SEQ ID NO:545),
o. X_{108} can be Q (SEQ ID NO:546), or
p. X_{112} can be N (SEQ ID NO:547), or
q. any two or more of a-p.

[0035] In various embodiments of the IL-15 variant,

a. X_1 can be N or G (SEQ ID NO:550),
b. X_4 can be N, K or L (SEQ ID NO:551),
c. X_{22} can be D or A (SEQ ID NO:552),
d. X_{30} can be D or N (SEQ ID NO:553),
e. X_{41} can be K or Q (SEQ ID NO:554),
f. X_{45} can be L or S (SEQ ID NO:555),
g. X_{49} can be R or K (SEQ ID NO:548),
h. X_{53} can be G, K, I, or A (SEQ ID NO:549),
i. X_{64} can be E or Q (SEQ ID NO:556),
j. X_{67} can be I or T (SEQ ID NO:557),
k. X_{68} can be I or S (SEQ ID NO:558),
l. X_{79} can be N or Y (SEQ ID NO:559),
m. X_{93} can be E or A (SEQ ID NO:560),
n. X_{103} can be F or L (SEQ ID NO:561),
o. X_{108} can be Q or T (SEQ ID NO:562), or
p. X_{112} is N or R (SEQ ID NO:563), or
q. any two or more of a-p.

[0036] In various embodiments, X_{49} can be R or K, and X_{53} can be G, K, I or A; (SEQ ID NO:564). In various embodiments, X_{30} can be N (SEQ ID NO:565). In various embodiments, X_{64} can be Q (SEQ ID NO:566). In various embodiments, X_1 can be G (SEQ ID NO:567).

[0037] In various embodiments, the IL-15 variant can comprise an IL-15 variant listed in Table 1A or Table 3A.

[0038] Various embodiments provide for an IL-15 fusion protein, comprising any one of the IL-15 variants described above; and a scaffold polypeptide. Various embodiments provide for an IL-15 fusion protein, comprising any one of the IL-15 variants described herein; and a scaffold polypeptide.

[0039] In various embodiments, the IL-15 variant can be fused to the C-terminus of the scaffold polypeptide.

[0040] In various embodiments, the scaffold polypeptide can be an antibody or a fragment thereof. In various embodiments, the antibody can be an IgA, IgM, IgG, or IgE antibody. In various embodiments, the antibody can be an anti-PD-1 antibody or anti-PD-L1 antibody.

[0041] In various embodiments, the scaffold polypeptide can be a Fc region or a fragment thereof. In various embodiments, the scaffold polypeptide can be a Fc region or a fragment thereof and the scaffold polypeptide does not comprise an Fab. In various embodiments, the Fc region can be an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG1. In various embodiments, the Fc region can be a knobs-in-hole (KiH) Fc. In various embodiments, the IL-15 variant can be fused to the knob of the KiH Fc. In various embodiments, the IL-15 variant can be fused to the hole of the KiH Fc.

[0042] Various embodiments provide for a polynucleotide encoding any one of the IL-15 variants described above, or any one of the Il-15 fusion proteins described above. Various embodiments provide for a polynucleotide encoding any one of the IL-15 variants described herein, or any one of the Il-15 fusion proteins described herein.

[0043] Various embodiments provide for an expression vector comprising any one of the polynucleotides of encoding any one of the IL-15 variants described above, or any one of the Il-15 fusion proteins described above. Various embodiments provide for an expression vector comprising any one of the polynucleotides of encoding any one of the IL-15 variants described herein, or any one of the IL-15 fusion proteins described herein.

[0044] Various embodiments provide for a cell transfected with any one of the expression vector comprising any one of the polynucleotides of encoding any one of the IL-15 variants described above, or any one of the Il-15 fusion proteins described above. Various embodiments provide for a cell transfected with any one of the expression vector comprising any one of the polynucleotides of encoding any one of the IL-15 variants described herein, or any one of the Il-15 fusion proteins described herein. In various embodiments, the cell can be a mammalian cell. In various embodiments, the mammalian cell is a CHO cell or a HEK-293 cell. In various embodiments, the cell can be a bacterial cell or yeast cell.

[0045] Various embodiments provide for a method of producing any one of the IL-15 variants or any one of the IL-15 fusion proteins described above, comprising: culturing any one of the cells described above, in cell culture medium to allow the IL-15 variant or the fusion protein to be produced, and optionally secreted into the cell culture medium. Various embodiments provide for a method of producing any one of the IL-15 variants or any one of the IL-15 fusion proteins described herein, comprising: culturing any one of the cells described herein, in cell culture medium to allow the IL-15 variant or the fusion protein to be produced, and optionally secreted into the cell culture medium. In various embodiments, the method can further comprise isolating the IL-15 variant or the fusion protein, or purifying the IL-15 variant or the fusion protein.

[0046] Various embodiments provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering any of the IL-15 variants described above, or any one of the IL-15 fusion protein described above to a subject in need thereof. Various embodiments provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering any of the IL-15 variants described herein, or any one of the IL-15 fusion protein described herein to a subject in need thereof. In various embodiments, the subject has cancer.

[0047] Various embodiments provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering any of the IL-15 variants described above, or any one of the IL-15 fusion protein described above to a subject in need thereof. Various embodiments provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering any of the IL-15 variants described herein, or any one of the IL-15 fusion protein described herein to a subject in need thereof. In various embodiments, the subject has cancer.

[0048] Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various features of embodiments of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0049] Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[0050] Figures 1A-1D depict nonlimiting examples of the fusion proteins in accordance with various embodiments of the present invention.

[0051] Figures 2A-2D show the impact of an exemplary anti-PD1-pro-IL-18 antibody, one of three exemplary anti-PD1-mutant-IL-15 antibodies or the combination of the anti-PD1-pro-IL-18 antibody and of each of the three mutant IL-15 antibody fusions on IFNγ release mediated by purified T cells exposed to a suboptimal dose of anti-CD3 (Pelicluster CD3, which is capable of crosslinking CD3 and activating T cells independent of FcγR binding) for 72 hours. For anti-PD1-pro-IL-18, the exemplary test article was Fuse694, which incorporates one pro-IL-18 (granzyme B cleavable IL18mutAS) on the C-terminal knob or hole of an IgG1-LALA version of nivolumab (Fuse691). For anti-PD1-mutant-IL-15, the three test articles were Fuse765 (FIG. 2A, open triangle, dashed line), Fuse773 (FIG. 2B, open triangle, dashed line) and Fuse774 (FIG. 2C, open triangle, dashed line), which respectively incorporated one IL-15m4, IL15m9 or IL15m10 on the C-terminal knob or hole of Fuse691. FIGs 2A-2C are non-linear x-y plots of test article concentration versus IFNγ release. A summary table of EC50, Emax and AUC (area under the curve) values is shown in FIG. 2D.

[0052] Figure 3 shows the impact of an exemplary anti-PD1-pro-IL-18 antibody, an exemplary anti-PD1-mutant-IL-15 antibody and an anti-PD1 antibody incorporating both the aforementioned exemplary pro-IL-18 and mutant

IL-15, on IFNγ release mediated by purified T cells exposed to a suboptimal dose of anti-CD3 (PeliCluster CD3, which is capable of crosslinking CD3 and activating T cells independent of FcγR binding) for 72 hours. A summary table of EC50, Emax and AUC (area under the curve) values is shown below the x-y plot.

[0053] Figures 4A-4B show the capacity of an NKG2D x ROR1 bispecific antibody (bsAb) with or without the incorporation of an exemplary pro-IL18 and IL15 mutant to induce NK cell mediated tumor cell killing and IFNγ release. FIGs 4A and 4B are non-linear x-y plots of tumor cell killing versus test article concentration and IFNγ release versus test article concentration, respectively. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

[0054] Figures 5A-5B show the impact of targeting an exemplary IL-15 variant and an exemplary pro-IL18 to NKG2D on NK cell expansion in PBMC. FIG. 5A and FIG. 5B are x-y plots of NK cell percentages and NK cell numbers measured on day 12, respectively, illustrated as a function of a titration of each test article. Relative to the Fuse926, 100 nM of Fuse916 induced about a 6-fold expansion of NK cells.

[0055] Figures 6A-6B show the impact of targeting an exemplary IL-15 variant and an exemplary pro-IL18 to NKp46 on NK cell expansion in PBMC.

[0056] Figures 7A-7B show the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to γ9δ2 T cells via a Vγ9Vδ2 TCR x ROR1 bsAb cytokine fusion to induce expansion of γ9δ2 T cells in human PBMC. FIG. 7A and FIG. 7B are x-y plots of γ9δ2 T cell percentages and γ9δ2 T cell numbers measured on day 12, respectively, illustrated as a function of a titration of each test article. Relative to the Fuse966, 100 nM of Fuse923 induced about a 12-fold expansion of γ9δ2 T cells.

[0057] Figures 8A-8B show the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to γ9δ2 T cells via a Vγ9Vδ2 TCR x ROR1 bsAb cytokine fusion to induce γ9δ2 T cell mediated IFNγ release and tumor cell killing. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

[0058] Figures 9A-9C shows the impact of targeting an exemplary IL-15 variant and an exemplary IL18 variant to NKp30 plus or minus NKp46 on NK cell expansion from PBMC. Both NK cell frequency and numbers were measured after 21 days of incubating PBMC from a healthy human donor with 100 pM of the test articles described below.

[0059] Figures 10A-10B show the agonist activity of γ9δ2 TCR specific VHH fused to the Fc domain of human IgG1 to induce redirected lysis of P815 cells stably transduced with eGFP and firefly luciferase (P815) by expanded γ9δ2 T cells. Non linear x-y plots of killing percentage as a function of test article concentration is shown. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

[0060] Figures 11A-11B show the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to γ9δ2 T cells via a Vγ9Vδ2 TCR x ROR1 bsAb cytokine fusion to induce γ9δ2 T cell mediated IFNγ release and tumor cell killing. Shown are non-linear x-y plots of a titration of each test article as a function of tumor cell killing

(FIG. 11A) or IFNγ release (FIG. 11B). Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

[0061] Figures 12A-12C show the agonist activity of human NKp46 (FIGs 12A-12B) and human NKp30 (FIG. 12C) specific VHH fused to the Fc domain of human IgG1 to induce redirected lysis of P815 cells stably transduced with eGFP and firefly luciferase (P815) by expanded γ9δ2 T cells. Non linear x-y plots of killing percentage as a function of test article concentration is shown . Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

[0062] Figures 13A-13B show the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to human NK cells via a NKp46 x ROR1 bsAb cytokine fusion to induce NK cell mediated IFNγ release and tumor cell killing. Shown are non-linear x-y plots of a titration of each test article as a function of tumor cell killing (FIG. 13A) or IFNγ release (FIG. 13B). Relative to Fuse1125, the incorporation of cytokine(s) into the bsAb enhanced both tumor cell killing and IFNγ release in the rank order of Fuse1124>Fuse1127>Fusee1126 indicating that the combination of IL-18 and IL-15 was the most potent. The differential readout between test articles was more apparent for IFNγ release compared to tumor cell killing. This is likely due to the high E:T ratio of 3:1 at which maximum tumor cell killing may be reached at a lower NK cell signaling threshold than IFNγ release. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

[0063] Figures 14-15 show the capacity of an exemplary IL-15 variant and an exemplary pro-IL18 targeted to human NK cells via a (1) human NKp30 x human ROR1 bsAb cytokine fusion (Fuse1147 and Fuse1145) or (2) NKp30 x NKp46 ROR1 bsAb cytokine fusion (Fusc1148 and Fusc1146), to induce NK cell mediated IFNγ release and tumor cell killing. Shown are non-linear x-y plots of a titration of each test article as a function of tumor cell killing (FIG. 14) or IFNγ release (FIG. 15). Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

[0064] Figure 16 depicts an exemplary fusion protein construct having an IL-15 variant in accordance with various embodiments of the invention.

[0065] Figure 17A-17C depict the functional activity of IL-15 variants as it relates to the induction and release of secreted embryonic alkaline phosphatase (SEAP) by HEK-Blue IL-2 reporter cells.

[0066] Figure 18A-18B depict the functional activity of IL-15 variants as it relates to T cell mediated IFNγ release induced by PD1 targeted IL-15 variants in the presence of a low "suboptimal" concentration of anti-CD3.

[0067] Figure 19 depicts the functional activity of IL-15 variants as it relates to T cell mediated IFNγ release induced by PD1 targeted IL-15 variants in the presence of allogeneic dendritic cells (DC).

[0068] Figure 20A-20M depict illustrations of formats for pro-IL-18 fusion proteins. All proteins incorporate a human IgG scaffold that is either wild type homodimeric IgG1 or comprising a knob-in-hole (KIH) heterodimeric IgG1 Fc. Pro-IL-18, when included in the fusion protein is fused as (a) a singular cassette to either the C-terminal end of the knob (or hole) chain or (b) as singular cassettes to both the C-terminal ends of the knob chain and the hole chain.

Cleavage sites, when present in the pro-IL-18 cassette, are indicated as a star shape. A tumor associated antigen binding domain (TAA), when present, is fused as a singular cassette to the N-terminal end of a knob (or hole) chain, or both chains of the KIH heterodimer IgG Fc. The TAA binding domain may be a VHH (e.g. EGFR specific clone 9G8) or a fab (e.g. EGFR specific clone C225 from cetuximab).

[0069] Figure 21A-21B depict the functional activity as it relates to Antibody Dependent Cellular Cytotoxicity (FIG. 21A) and IFNγ release (FIG. 21B) induced by FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), and FUSE686 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18).

[0070] Figure 22A-22B depict the functional activity as it relates to Antibody Dependent Cellular Cytotoxicity (FIG. 22A) and IFNγ release (FIG. 22B) induced by of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1; targets both EGFR and FcγR), or the combination of FUSE556 and FUSE422 (IgG1-pro-IL-18; only targets FcγR).

[0071] Figure 23 depicts the functional activity as it relates IFNγ release induced by of FUSE691 (nivolumab), FUSE645 (EGFR targeted IgG1-pro-IL-18; non-targeted in this system), FUSE694 (nivolumab-pro-IL-18) and human recombinant IL-18 on T cell/allogeneic DC co-culture.

[0072] Figure 24A-24B depict the functional activity as it relates to Antibody Dependent Cellular Cytotoxicity of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), and FUSE516 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18). Graphs illustrating killing of EGFR+ MDA-MB-231 tumor cells by PBMC from each of four normal human donors are shown in FIGs 24A-24B.

[0073] Figure 25A-25B depicts the functional activity as it relates to pro-IL-18 fusion protein induced Antibody Dependent Cellular Cytotoxicity (FIG. 25A), IFNγ release (FIG. 25A), Granzyme B release (FIG. 25B), and IL-18 release/consumption (FIG. 25B) after 48 hours of co-culture of PBMC and EGFR+ MDA-MB-231 tumor cells. The pro-IL-18 fusion proteins tested were FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), and FUSE516 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18).

[0074] Figure 26A-26B depicts the functional activity as it relates to Antibody Dependent Cellular Cytotoxicity induced by FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), variants of pro-IL-18 fused to the C-terminus of FUSE566 with different protease cleavage sites between the Fc domain and pro-IL-18 (FIG. 26A), and a variant that contains the PGLALA mutation in its Fc domain to abolish targeting to FcγRs (FUSE627; FIG. 26B). The protease sites tested were Granzyme A (FUSE658), Granzyme B (FUSE516), MMP2/9 (FUSE659), and the combination of Granzyme A, Granzyme B and MMP2/9 (FUSE660).

[0075] Figure 27A-27B depicts the functional activity as it relates to pro-IL-18 fusion protein induced Antibody Dependent Cellular Cytotoxicity (FIG 27A) and IFNγ release (FIG 27B) after 48 hours of co-culture of expanded NK cells and EGFR+ MDA-MB-231 tumor cells at E:T ratios of 1:1 and 1:5. The pro-IL-18 fusion proteins tested were FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), and FUSE516 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18).

[0076] Figure 28A-28B depicts the functional activity as it relates to pro-IL-18 fusion protein induced Antibody Dependent Cellular Cytotoxicity (FIG 28A) and IFNγ release (FIG 28B) after 48 hours of co-culture of expanded NK cells and EGFR+ MDA-MB-231 tumor cells at E:T ratios of 2:1. The pro-IL-18 fusion protein tested was FUSE555 (dual armed anti-EGFR Fab C225-IgG1-pro-IL-18), which was compared to cetuximab.

[0077] Figure 29A-29B depicts the functional activity as it relates to pro-IL-18 fusion protein induced Antibody Dependent Cellular Cytotoxicity (FIG 29A) and IFNγ release (29B) after 48 hours of co-culture of expanded NK cells and HER2+ T47D tumor cells at E:T ratios of 5:1. The pro-IL-18 fusion proteins tested were FUSE624 (dual armed anti-HER2 Fab IgG1-pro-IL-18) and trastuzumab (Herceptin).

[0078] Figure 30 shows the assessment of the capacity of an anti-PDL1 antibody incorporating a pro-IL-18amut2 variant, designed so that it cannot be cleaved by Granzyme B, to induce T cell mediated IFNγ release. A summary table of EC50, Emax and AUC (area under the curve) is shown beneath of x-y plot.

[0079] Figure 31A-31B shows the assessment of the capacity of an anti-PD1 antibody, incorporating one of two pro-IL-18 variants (pro-IL18amut2 or pro-IL18amut9), designed so that it cannot be cleaved by Granzyme B, to induce T cell mediated tumor cell king (FIG. 27A) and IFNγ release (FIG. 27B). A summary table of EC50, Emax and AUC (area under the curve) is shown beneath each x-y plot.

[0080] Figure 32 shows the capacity of several IL-18 variants to induce non-targeted trans based release of SEAP from HEK-Blue-IL-18.

[0081] Figure 33A-33B shows the capacity of several IL-18 variants targeted to human PD-1 to induce cis based release of IFNγ from human PBMC derived from a healthy human donor (FIG. 29A) or human T cells (FIG. 29B).

[0082] Figure 34A-34B shows the capacity of an exemplary IL-18 variant targeted to mouse PD-1 to mediate tumor growth inhibition of an aggressive syngeneic melanoma tumor in a fully immunocompetent mouse tumor model.

[0083] Figure 34C show the number of different lymphocyte subsets per gram of tumor was assessed from tumor bearing mice treated with PBS (light grey), anti-PD1 (black), and Fuse1113 (horizontal lines).

[0084] Figure 34D depicts the frequency of total myeloid cells (CD11b+ cells), M2 macrophages (CD11b+ F4-80+ CD11c-CD206+ cells) and the ratios of CD8+ T cells to total Myeloid Derived Suppressor Cells (MDSC; CD11b+ Ly6C+ Ly6G- [M-MDSC] and CD11b+ Ly6C-mid Ly6G+ [G-MDSC]) , CD8+ T cells to M2 macrophages, anti-PD-1 responsive CD8+ T cells to MDSC, and CD8+ T cells to CD4+ T cells.

[0085] Figure 35A-35B shows the capacity of an exemplary IL-18 variant targeted to mouse PD-1 to mediate tumor growth inhibition in a syngeneic colorectal cancer tumor in a fully immunocompetent mouse tumor model.

[0086] Figure 36A-36B shows he capacity of an exemplary IL-18 variant targeted to mouse PD-1 to mediate tumor growth inhibition in a syngeneic rapidly growing colorectal cancer tumor in a fully immunocompetent mouse tumor model.

[0087] Figure 37A shows C57BL/6 mice injected subcutaneously with MC38i. Serum was collected at 0, 24, 72 and 144 hours after the Day 0 treatment and mouse IFNγ measured.

[0088] Figure 37B shows C57BL/6 mice that harbored MC38i tumors and experienced a CR following treatment with αPD1-HT18cis or αPD-1-IL-18 were re-challenged with MC38i after 90 days.

[0089] Figure 38 shows BALB/c mice that harbored CT26.C tumors and experienced a CR following treatment with αPD1-HT18cis were rechallenged with CT26.C after 65 days on the left flank and parental CT26 after 70 days on the right flank. As a means of comparison, a group of 5 naïve mice were subjected to the equivalent tumor inoculation.

[0090] Figure 39 shows C57BL/6 mice were injected subcutaneously with B16-F10R. When mean tumor volume ranged between 75-100 mm3, mice were randomized into groups of 5 and treated with PBS, αPD-1, αPD1-HT18cis on Days 0, 3 and 6 at 15mg/kg. Mice that experienced a CR following treatment with αPD1-HT18cis were rechallenged with B16-F10 after 60 days.

[0091] Figure 40 shows C57BL/6 mice harboring B16-F10R tumors were treated on Days 0 and 3. Mice were euthanized on Day 5, tumors collected and dispersed into singe cell suspensions for flow cytometric assessment of TIL (CD45+ cells). TEM and TCM were defined CD44+/CD62L- and CD44+/CD62L-, respectively within the CD8+ pool. αPD-1 responsive CD8+ T cells were defined as PD1+/TCF1+ cells within the CD8+ pool.

[0092] Figure 41 shows C57BL/6 mice harboring B16-F10R tumors were treated on Days 0 and 3. Mice were euthanized on Day 5, tumors collected and dispersed into singe cell suspensions for flow cytometric assessment of TIL (CD45+ cells). All myeloid subsets were gated on CD11b. Within that pool, MDSC were defined as Ly6G+/Ly6Cmid+Ly6G-/Ly6Chigh and M2 macrophages as F480+/CD206+.

[0093] Figure 42A and 42B depicts the impact of exemplary anti-PD1-pro-IL-18 antibodies containing different IL-18 variants on IFNγ release mediated by PBMC activated with PeliCluster CD3 for 48 hours, washed and the re-exposed to a 10 fold lower concentration of PeliCluster CD3 for an additional 48 hours.

[0094] Figure 43 (panels A-E) depicts exemplary fusion proteins in which the N-terminus of pro-IL-18 is fused to the C-terminus of the knob of a knob-into-hole heterodimeric IgG1 protein; which has a structure from N- to C-terminus comprising knobs-in-hole (KiH) Fc – propeptide (PP) – enterokinase-cleavable site (EK) – IL-18 wild type or its variants. This depicts exemplary fusion proteins such as IDs: FUSE-480, FUSE-481, and FUSE-442 in Table 6. Further modification was made to pro-IL-18 to reduce aggregation of the molecule, wherein each cysteine residue in both the pro-peptide and mature IL-18 was replaced with serine (as in FUSE-480, denoted as "IL-18AS"), with alanine (as in FUSE-481, denoted as "IL-18AA"), or with valine (as in FUSE-442, denoted as "IL-18AV"). Alternatively, the N-terminus of pro-IL-18 can be fused to the C-terminus of the hole chain of a KiH heterodimeric IgG1 proteins. The biological activity defined as the EC50-SEAP for each compound is shown in panel E.

[0095] Figure 44 (panels A-E) depicts exemplary fusion proteins in which the N-terminal of pro IL-18 was fused to the C-terminal of an IgG1 CH3 domain (which is also a knob chain of a knob-into-hole heterodimeric IgG1 protein as in Figure 43), and the pro IL-18 incorporated four amino acid substitutions hypothesized to reduce binding to IL-18BP while maintaining wild type binding to the IL-18 receptor complex, denoted as "pro-IL-18mut2". These fusion

proteins have a structure from N- to C-terminus comprising knobs-in-hole (KiH) Fc – propeptide (PP) – enterokinase-cleavable site (EK) – IL-18mut2. Further modification was made to the pro-IL-18mut2 to reduce aggregation of the molecule, wherein each cysteine residue in both the pro-peptide and mature IL-18mut2 was substituted with serine (denoted as "IL-18mut2AS", as in FUSE-422; panel B), with alanine (denoted as "IL-18mut2AA", as in FUSE-423; panel C), or with valine (denoted as "IL-18mut2AV", as in FUSE-424; panel D). The biological activity defined as the EC50-SEAP for each compound is shown in panel E.

[0096] Figure 45 (panels A-D) depicts exemplary fusion proteins with (panel A) or without (panel B) the propeptide to examine the impact on masking of "IL-18AV" biological activity (panel C), wherein Fc fusion variants were generated incorporating "IL-18AV" with the propeptide (panel A; FUSE-442) or without (panel B; FUSE-505) the propeptide. The biological activity defined as the EC50-SEAP for each compound is shown in panel D.

[0097] Figure 46 (panels A-D) depicts exemplary fusion proteins with (panel A) or without (panel B) the propeptide to examine the impact of propeptide on masking of "IL-18mut2AV" biological activity, wherein Fc fusion variants were generated incorporating "IL-18mut2AV" without the propeptide (hence, a mature IL-18 with mutation, denoted as "matIL-18mut2-AV", see panel B; FUSE-441) or with the propeptide (panel A; FUSE-424). For a fusion protein devoid of the propeptide, the EK cleavage site that replaced the Caspase 1 site was moved to a position directly in between the CH3 domain of the knob and the mature IL-18AV without the addition of a flexible linker. Panel C depicts the activation readout using the HEK-Blue IL-18AV reporter cell assay following exposure to a titration of FUSE-441 (Fc-EK-IL-18AV) or FUSE-424 (Fc-EKpp-IL-18AV) with or without treatment with EK. The biological activity defined as the EC50-SEAP for each compound is shown in panel D.

[0098] Figure 47 (panels A-D) depicts exemplary fusion proteins in which the N-terminus of pro-IL-18 is fused to the C-terminus of IgG1 Fc protein or IgG4 Fc; which has a structure from N- to C-terminus comprising IgG1 Fc-propeptide (PP) - IL-18AV (FUSE-507; panel A) and IgG4 Fc- propeptide (PP) - IL-18AV (FUSE-509; panel B). Panel C depicts the activation readout using the HEK-Blue IL-18AV reporter cell assay of exposing the cells to a titration of FUSE-507 or FUSE-509 with or without treatment with EK. The biological activity defined as the EC50-SEAP for each compound is shown in panel D.

[0099] Figure 48 (panels A-E) depicts exemplary fusion proteins in which the N-terminus of pro-IL-18 is fused to the C-terminus of HSA with or without the propeptide (PP); which has a structure from N- to C-terminus comprising HSA- propeptide (PP) - IL-18AV (FUSE-501; panel A) and HSA- IL-18AV (FUSE-503; panel B). Panels C and D depict the activation readout. The biological activity defined as the EC50-SEAP for each compound is shown in panel E.

[0100] Figure 49 (panels A-E) depicts exemplary fusion proteins in which the N-terminus of pro-IL-18mut2 is fused to the C-terminus of HSA with or without the propeptide (PP); which has a structure from N- to C-terminus comprising HSA- propeptide (PP) - IL-18mut2AV (FUSE-502; panel A) and HSA- IL-18mut2AV (FUSE-504; panel B). Panels C and D depict the activation readout. The biological activity defined as the EC50-SEAP for each compound is shown in panel E.

[0101] Figure 50 (panels A-D) depicts exemplary fusion proteins in which the C-terminus of pro-IL-18 is fused to the N-terminus of the knob of a knob-into-hole heterodimeric IgG1 protein with or without the propeptide (PP); which has a structure from N- to C-terminus comprising propeptide (PP) - IL-18AV - knobs-in-hole (KiH) Fc (FUSE-499; panel A) and IL-18AV - knobs-in-hole (KiH) Fc (FUSE-500; panel B). Panel C depicts the activation readout. The biological activity defined as the EC50-SEAP with or without exposure to Caspase 1 for each compound is shown in panel D.

[0102] Figure 51A and 51D depicts exemplary fusion proteins with a structure from N- to C- terminus: Fc-ppMMP2/9-cleavage sites-IL-18-AV (FUSE-486), Fc-ppMMP9/2-cleavage sites-IL-18-AV (FUSE-487), in which the cleavage sites are specific for the metalloproteases, MMP2 and MMP9, with preferred enzyme to the left of the forward-slash, or FUSE-485 (Fc-GzmBpp-IL-18AV) and FUSE-462 (Fc-GzmBpp-IL-18mut2AV). Figure 51B depicts the activation readout relating to FUSE-486 and FUSE-487, with or without MMP2 treatment. The biological activity defined as the EC50-SEAP for each of FUSE-486 and FUSE-487, with or without MMP2 treatment, is shown in figure 51B. FIG. 51C shows that FUSE587 was about 3,000-fold attenuated relative to recombinant human IL-18. Interestingly, we observed that cleavage of FUSE587 with MMP2 released and IL-18AV variant that was still about 100-fold attenuated relative to recombinant IL-18. In contrast, cleavage with Granzyme B released an IL-18AV variant with activity similar activity as recombinant IL-18. Cleavage with Granzyme B results in release of mature IL-18AV without any N-terminal residues constituting an overhang, whereas 11 and 15 amino acid N-terminal polypeptide overhangs remain after cleavage of FUSE486 and FUSE587, respectively, with MMP2. We speculated that these overhangs might be attenuating IL-18AV activity, albeit to a lesser degree than the full size variant propeptide. This phenomenon was further investigated in FIG. 53 and FIG. 55. Figures 51D and 51G also depicts exemplary fusion proteins with a structure from N- to C- terminus: Fc-ppGb-cleavage sites-IL-18-AV (FUSE-485; B-19D) or Fc-ppGb-cleavage sites-IL-18mut2-AV (FUSE-462; 51D) or Fab-Cetuximab-Fc-ppGb-cleavage sites-IL-18-AV (FUSE-517; 51G), in which the cleavage site is specific for granzyme B (Gb). Figure 51E depicts the activation readout relating to FUSE-462 and FUSE-485, with or without granzyme B treatment. Figure 51F shows the biological activity defined as the EC50-SEAP for each of FUSE-462 and FUSE-485, with or without granzyme B treatment. Figure 51H depicts the activation readout relating to FUSE-517, with or without enzyme treatment. Figure 51I shows the biological activity defined as the EC50-SEAP for FUSE-517, with or without granzyme B treatment.

[0103] Figure 52 (panels A-G) depicts the impact of IL-18BP on the biological activity of recombinant human IL-18 (rhIL-18) and the EK cleavage products of the exemplary fusion proteins, Fc-ppEK-IL-18-AV (FUSE-442) and Fc-ppEK-IL-18mut2AV (FUSE-424). The biological activity defined as the EC50-SEAP for each compound with and without the addition of IL-18BP (competition assay) is shown in panels B, D and F, with corresponding biological activity defined as the EC50-SEAP shown in panels C, E, and G, respectively.

[0104] Figure 53 (panel A) depicts diagrams of exemplary fusion proteins in which the C-terminus of pro-IL-18 is fused to the N-terminus of the knob of a knob-into-hole heterodimeric IgG1 protein with different size polypeptides

fused to the N-terminus of mature IL-18. Figure 53 (panel B) depicts the biological activity of each fusion protein using the IL-18 reporter cell line, HEK-Blue IL-18.

[0105] Figure 54A, 54B(i), 54B(ii), 54C, 54D(i), 54D(ii) and 54E depict human IL-18 engineered mutant fusion proteins in accordance with various embodiments of the invention.

[0106] Figure 55 shows the impact of the size of polypeptides fused to the N-terminus of mature IL-18 on the biological activity of a single IL-18AV fused to the N-terminus of IgG1 Fc.

[0107] Figure 56A-56H shows the impact of the substituting the cysteine residue in the pro-peptide and cysteine residues in the mature IL18, which were fused together to form the pro-IL-18 variant cassette, on the biological activity of each variant using the HEK Blue IL18 assay system.

[0108] Figure 57A-57B shows the impact of targeting pro-IL18 to within close proximity of its receptor complex (i.e., "cis activity).

[0109] Figure 58A-58E depict schematics of multiple formats of anti-ROR1/CD3 bispecific antibodies, also termed T cell engagers (TCEs), illustrating protein designs of TCE fusion proteins comprising a knob-in-hole (KIH) heterodimeric IgG1 Fc scaffold with (1) a CD3-specific fragment antigen-binding region (Fab) on the N-terminal end of a knob chain (shown in panels 58A, 58B, 58D and 58E) or a hole chain (shown in 58C) of the KIH, and (2) one or more VHH or single-chain variable fragment (scFV) specific for a tumor-associated antigen (TAA) (e.g., ROR1) in one or more positions. The examples shown illustrate the TAA binder linked to (a) the N-terminal end of the hole chain in tandem (58A), (b) the N-terminal end of the hole chain and on the C-terminal end of the CD3-specific (anti-CD3) Fab light chain (58B), (c) the N-terminal end of the knob chain and on the C-terminal end of the CD3-specific (anti-CD3) Fab light chain (58C), (d) the N and C-terminal end of the hole chain (58D) and (e) the N-terminal end of the hole chain only (58E).

[0110] Figure 59A-59B show the production yield (59A) and the melting temperature (Tm) (2B) of exemplary fusion proteins (denoted as a FUSE ID, "FUSE-XXX").

[0111] Figure 60A-60D shows the apparent binding affinity of exemplary TCEs for cell membrane expressed CD3 in human T cells and in non-human primate (NHP), Cynomolgus T cells.

[0112] Figure 61A-61E depict the apparent binding affinity of exemplary TCEs for cell membrane expressed ROR1 using a series of engineered MDA-MB-231 variant cell lines expressing different cell surface densities of the ROR1. Figure 61F-61K depict the capacity of exemplary TCEs specific for ROR1 to bind the membrane distal ROR1 Ig domain.

[0113] Figure 62A-62D depict the binding affinity (KD) for ROR1, determined via Bio-layer Interferometry (BLI), of TCEs: FUSE-211 (in FIG. 62A), FUSE-393 (in FIG. 62B), and FUSE-394 (in FIG. 62C), with their calculated binding affinity shown in FIG. 62D.

[0114] Figure 62E-62G depict the binding affinity for ROR1 of a 2A11 VHH – Fc fusion protein (denoted as FUSE-112, in FIG. 62E) and of a 2A11 VHH *mutant* – Fc fusion protein (denoted as FUSE-453, in FIG. 62F), with their

calculated binding affinity shown in FIG. 62G, which demonstrated the impact of replacing the cysteine residues in CDR1 and CDR3 of the ROR1-specific VHH.

[0115] Figure 62H-62J depict the binding affinity for ROR1 of a 5A1 VHH – Fc fusion protein (denoted as FUSE-179, in FIG. 62H) and of a 5A1 VHH *mutant* – Fc fusion protein (denoted as FUSE-454, in FIG. 64), with their calculated binding affinity shown in FIG. 62J, which demonstrated the impact of replacing the cysteine residues in CDR1 and CDR3 of the ROR1-specific VHH.

[0116] Figure 63A-63D illustrate TCEs induction of human peripheral blood mononuclear cells (PBMC)-mediated, ROR1-dependent cytotoxicity (characterized as percentage killed) of MDA-MB-231 tumor cells, wherein the tested TCEs include FUSE-211, FUSE-393, and FUSE-394 and the human PBMC were from donor 1 (FIG. 63A), donor 2 (FIG. 63B), or donor 3 (FIG. 63C). FIG. 63D summarizes the calculated EC_{50} of each TCE for inducing a killing percentage of 50% of the tumor cells.

[0117] Figure 63E-6H illustrate TCEs induction of IFN-γ release from human PBMC (characterized as concentration of IFN-γ in cell culture supernatant) in the presence of MDA-MB-231 tumor cells. The tested TCEs include FUSE-211, FUSE-393, and FUSE-394, and the human PBMC were from donor 1 (FIG. 63E), donor 2 (FIG. 63F), or donor 3 (FIG. 63G). FIG. 63H summarizes the calculated EC_{50} of each TCE for inducing 50% of a maximum IFN-γ release.

[0118] Figure 63I-63O illustrate the capacity of FUSE-211, FUSE-393, and FUSE-394 to mediate reduced ROR1-dependent IFN-γ release from human PBMC (average of three donors) or Pan-T cells while maintaining cytotoxic efficacy (maximum killing of MDA-MB-231), or "decoupling of cytotoxicity from cytokine release" compared to a FUSE-277, a prototypical highly potent TCE that serves as the "non-decoupling" control TCE.

[0119] Figure 63P-63V depict ROR1 dependent killing, induction of cytokine release and calculated decoupling ratios following 24, 48 and 72 hour co-cultures of PBMC effectors with ROR1+ MDA-MB-231 tumor targets.

[0120] Figure 64A-64C illustrates TCEs induction of human PBMC-mediated, ROR1-independent cytotoxicity of T47-D tumor cells (which do not express ROR1), characterized as IFN-γ concentration in the co-culture supernatant. Panels A-C show the results wherein the human PBMC were from donor 1 (panel A), donor 2 (panel B), and donor 3 (panel C), respectively.

[0121] Figure 65A-65E and 65G-65K depict TCEs induction of human PBMC-mediated, ROR1-dependent cytotoxicity and cytokine release, respectively, as a function of the cell surface density of ROR1. Data was generated using a series of cell lines of different origins expressing different numbers of ROR1 molecules on their cell surfaces. The cell lines are shown in a descending order of cell surface ROR1 density, from high to low. That is, NCCIT (FIG. 65A,G), MDA-MB-231 (FIG. 65B,H), NCI-H1975 (FIG. 65C,I), DU-145 (FIG. 65D,J) and ROR1-negative T-47D (FIG. 65E,K). FIG. 65F and 65L summarize the potencies for cytotoxicity and cytokine release induced by each of the TCEs

towards a series of cell lines with different cell-surface ROR1 densities. FIG. 65M illustrates the calculated decoupling ratio for each TCE across the cell line series.

[0122] Figure 66A-66E and 66G-66K depict TCEs induction of human PBMC-mediated, ROR1-dependent cytotoxicity and cytokine release, respectively, as a function of the cell surface density of ROR1 using a series of engineered MDA-MB-231 variant cell lines expressing different cell surface densities of the ROR1. The surface densities of ROR1 on the engineered MDA-MB-231 were as follows: ~2,000,000 molecules per cell (MDA++; FIG. 66A, 66G), ~500,000 molecules per cell (MDA+; FIG. 66B, 66H), ~35,000 molecules per cell (MDA; FIG. 66C, 66I), ~ 17,000 molecules per cell (MDA-low; FIG. 66D, 66J), and 0 molecule per cell (MDA-KO; FIG. 66E, 66K). FIG. 66F and 66L summarize the potencies for cytotoxicity and cytokine release induced by each of the TCEs towards MDA-MB-231 cells with different cell-surface ROR1 densities. FIG. 66M represents the calculated decoupling ratios (cytotoxicity from cytokine release) for each TCE and FIG. 66N is a regression analysis between the magnitude of IFNγ versus ROR1 cell surface density of a non-decoupling TCE control versus an exemplary decoupling TCE.

[0123] FIGs. 67A and 67B depict TCE-induced expression of activation markers, CD69 and CD25, respectively, on T cells following co-culture of PBMCs with ROR1-expressing tumor cells. Fig. 67C summarizes the potency by which each TCE induces CD69 or CD25, defined as the EC_{50}-binding. FIG 67D and FIG. 67F are bar charts illustrating the decoupling of cytotoxicity from the induction of the cell surface biomarkers of T cell activation, CD69 and CD25. FIG. 67E and FIG. 67G are bar charts illustrating the decoupling of the induction CD69 and CD25 from cytokine release.

[0124] Figure 68A-68C depict the pharmacokinetics (PK) of exemplary TCEs in a single dose of either 5 mg/kg (FIG. 68A) or 0.5 mg/kg (FIG. 68B) administered to wild type C57BL/6 mice over a 10-day period. FIG. 68C depicts the PK parameters for each test article.

[0125] Figure 69A-169E depict a schematics of two humanized mouse xenograft tumor models (FIGs 69A and 69C). In both cases, the highly aggressive human $ROR1^+$ teratoma, NCCIT was injected into immune incompetent mice. Tumor growth over time for the first model is shown in FIG. 69B and second model in FIGs 69D and 69E. In both cases, the test articles consisted of FUSE-394 and the non-decoupled control TCE, FUSE-399.

[0126] Figure 70A-70E depict the capacity of an exemplary TCE to induce T cell mediated killing of ROR1-positive tumor cells (MDA-MB-231; FIG. 70A), ROR1 negative tumor cells (T-47D; FIG. 70B) and a mixture of ROR1-positive tumor cells and ROR1 negative tumor cells (FIG. 70C and FIG. 70D). For the latter, killing of the ROR1 positive tumor is shown in FIG. 70C. Killing of the ROR1 negative tumor is termed "bystander killing", the comparison of which to a non-decoupling TCE is shown in FIG. 70D. FIG. 70E depicts IFNγ production by the same T cells used to assess "bystander killing" as a function of exemplary TCE concentration.

[0127] Figure 71A-71B depict the (a) percent killing of ROR1-positive tumor cells (MDA-MB-231) as a function of T cell numbers (FIG. 71A) in the context of exemplary TCEs and (b) TCE mediated "serial killing" calculated as the average number of TCE induced ROR1-positive tumor cells killed per T cell in a 24-hour period (FIG. 71B).

[0128] Figure 72 depicts the apparent binding affinity (EC50-binding) of the ROR1 specific VHH-Fc incorporating either the non humanized VHH clone 5A1 (FUSE179; black circle), the humanized clone 5A1-FA9-HK1 (FUSE559; black square) or the humanized clone 5A1-HK1 (FUSE524; black triangle) was calculated.

[0129] Figure 73A-73B depict the affinity (KD) of the ROR1 specific VHH-Fc incorporating either the non humanized VHH clone 5A1 (FUSE179; FIG. 73A) or the humanized clone 5A1-FA9-HK1 (FUSE559; FIG. 73B) was calculated. FIGs. C-74A-74B depict the apparent binding affinity (EC50-binding) of the two bispecific antibodies (bsAbs).

[0130] Figure 75A-75B depict capacity of several ROR1 x CD3 specific bsAbs to induce (1; FIG. 75A) T cell mediated killing of ROR1+ MDA-MB-231 tumor cells and (2; FIG. 75B) T cell mediated release of IFNγ when co-mixed with ROR1+ MDA-MB-231 tumor cells.

[0131] Figure 76A-76I depict capacity of several ROR1 x CD3 specific bsAbs to induce (1; FIGs 76A-76D) PBMC mediated killing of four different tumor cell lines and (2; FIGs 76E-76H), PBMC mediated release of IFNγ when co-mixed with the aforementioned tumor cells.

[0132] Figure 77A-77F depict capacity of several ROR1 x CD3 specific bsAbs to induce (1; FIG. 77A) PBMC mediated killing of ROR1+ MDA-MB-231 tumor cells and (2; FIG. 77B), PBMC mediated release of IFNγ when co-mixed with ROR1+ MDA-MB-231 tumor cells, (3. FIG. 77C) upregulation of cell surface CD69 expression on T cells within PBMC when co-mixed with ROR1+ MDA-MB-231 tumor cells, (4. FIG. 77D) upregulation of cell surface PD-1 expression on T cells within PBMC when co-mixed with ROR1+ MDA-MB-231 tumor cells, and (5. FIG. 77E) upregulation of cell surface TIGIT expression on T cells within PBMC when co-mixed with ROR1+ MDA-MB-231 tumor cells.

[0133] Figure 78A-78C depict capacity of FUSE399 (black reverse triangle) and FUSE608 (black circle) to induce CD8+ T cell mediated killing of a constant number (5000 cells) of ROR1+ MDA-MB-231 cells across a range of CD8+ T cell numbers from 313 to 40,000.

[0134] Figure 79A-22D depict whether FUSE608 induced bystander killing of ROR1 negative tumors co-mixed with ROR1 positive tumors.

[0135] Figure 80A-80B depict the capacity of FUSE608 and the non-decoupled benchmark ROR1 x CD3 bsAb, FUSE399, to mediate tumor growth inhibition (TGI) in a mouse tumor xenograft model.

DESCRIPTION OF THE INVENTION

[0136] All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology 3rd ed., Revised*, J. Wiley & Sons (New York, NY 2006); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure 7th ed.*, J. Wiley & Sons (New York, NY 2013); and Sambrook and Russel,

Molecular Cloning: A Laboratory Manual 4th ed., Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2012), provide one skilled in the art with a general guide to many of the terms used in the present application. For references on how to prepare antibodies, see D. Lane, *Antibodies: A Laboratory Manual 2nd ed.* (Cold Spring Harbor Press, Cold Spring Harbor NY, 2013); Kohler and Milstein, (1976) Eur. J. Immunol. 6: 511; Queen et al. U. S. Patent No. 5,585,089; and Riechmann et al., Nature 332: 323 (1988); 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 334:544-54 (1989); Tomlinson I. and Holliger P. (2000) Methods Enzymol, 326, 461-479; Holliger P. (2005) Nat. Biotechnol. Sep;23(9):1126-36).

[0137] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

[0138] As used herein the term "about" or "approximately" when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 5% of that referenced numeric indication, unless otherwise specifically provided for herein. For example, the language "about 50%" covers the range of 45% to 55%. In various embodiments, the term "about" when used in connection with a referenced numeric indication can mean the referenced numeric indication plus or minus up to 4%, 3%, 2%, 1%, 0.5%, or 0.25% of that referenced numeric indication, *if* specifically provided for in the claims.

[0139] Percent (%) sequence identity with respect to a reference polypeptide sequence is the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are known for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Appropriate parameters for aligning sequences are able to be determined, including algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0140] As used herein the term "immunoglobulin heavy chain constant region" is used interchangeably with the term "Fc region" and is understood to mean the carboxyl-terminal portion of an immunoglobulin heavy chain constant region, or an analog or portion thereof capable of binding an Fc receptor. Each immunoglobulin heavy chain constant region comprises four or five domains. The domains are named sequentially as follows: CH1-hinge-CH2-CH3(-CH4). CH4 is present in IgM, which has no hinge region. The immunoglobulin heavy chain constant region suitable for the invention preferably comprises an immunoglobulin hinge region, and preferably also includes a CH3 domain. The immunoglobulin heavy chain constant region most preferably comprises an immunoglobulin hinge region, a CH2 domain and a CH3 domain.

[0141] As used herein, the term immunoglobulin "hinge region" is understood to mean an entire immunoglobulin hinge region or at least a portion of the immunoglobulin hinge region sufficient to form one or more disulfide bonds with a second immunoglobulin hinge region.

[0142] As used herein, the term "vector" is understood to mean any nucleic acid comprising a nucleotide sequence competent to be incorporated into a host cell and to be recombined with and integrated into the host cell genome, or to replicate autonomously as an episome. Such vectors include linear nucleic acids, plasmids, phagemids, cosmids, RNA vectors, viral vectors and the like. Non-limiting examples of a viral vector include a retrovirus, an adenovirus and an adeno-associated virus.

[0143] As used herein, the term "gene expression" or "expression" of a protein or polypeptide, is understood to mean the transcription of a DNA sequence, translation of the mRNA transcript, and secretion of a protein or polypeptide product. In some embodiments, the expression process also includes or is followed by purification; for example, protein A affinity chromatography or other means such as size exclusion chromatography can be used for purification.

[0144] The term "linker" with respect to amino acid linker in a polypeptide can be a short peptide, such as a dimer of two amino acids, a tri-mer of three amino acids, a 4-mer of four amino acids, a 5-mer of five amino acids or a peptide selected from the group consisting of T, PT, MPT, S, GS, GGS, GGGS (SEQ ID NO:235), and (GGGGX_λ (SEQ ID NO:236))n wherein X_λ is Q, A, E or S and n=1-5 or an integer larger than 5. In some embodiments, the amino acid linker has the amino acid sequence of (GGGGS (SEQ ID NO:237))n where n is an integer between 1 and 5, thereby an amino acid linker of 25 amino acids or shorter in length. Additional examples include (X_λGGGG (SEQ ID NO:317))$_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX_λGGG (SEQ ID NO:318))$_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGX_λGG (SEQ ID NO:319))$_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGGX_λG (SEQ ID NO:320))$_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5. Still additional examples include (X_λGGG)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX_λGG)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGX_λG)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGGX_λ)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5. Still additional examples include (X_λGG)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX_λG)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGX_λ)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments. Still additional examples include (X_λG)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX_λ)n wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5. As further examples, the short polypeptide is 1, 2, 3, or 4 amino acids, or flexible linkers or amino acid chain of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, or 35 amino acids. In some embodiments, the amino acid linker is an IL-18 propeptide or IL-18 propeptide variant. In some embodiments, the amino acid linker is a fragment of an IL-18 propeptide or IL-18

propeptide variant; for example, about 30-36 amino acids in length, about 5-10, 11-20, 21-30, or 31-40 amino acids in length.

[0145] As used herein, the term "cis" in the context of interactions refers to the interaction between two molecules on the same cell, such as proteins expressed on the same cell. A nonlimiting example is the interaction between B7-1 and PD-L1. "Cis" interaction as used herein also includes multispecific proteins that bind two proteins on the same cell simultaneously.

[0146] As used herein, the term "trans" in the context of interactions refers to the interaction between two molecules on different cells, such as proteins on different cells. A nonlimiting example is the interaction between the prototypical interaction of the T cell Receptor (TCR) and its ligand, an MHC-I/peptide complex. "Trans" interaction as used herein also include multispecific proteins that bind one protein on one cell and a second protein on a separate cell; hence a "bridge" would be considered as occurring in trans. Also included as a "trans" interaction is an interaction between a soluble protein and a cell surface protein, although not forming a cellular bridge.

[0147] As used herein, "IL-18 fusion protein" refers to a fusion protein that includes wild-type IL-18 or IL-18 variants, unless specifically noted as only including the wild-type IL-18, or only including the IL-18 variant. Thus, in particular embodiments, the "IL-18 fusion protein" only includes any one of the IL-18 variants as described herein.

[0148] "Antibody" refers to all isotypes of immunoglobulins (IgG, IgA, IgE, IgM, IgD, and IgY) including various monomeric, polymeric and chimeric forms, unless otherwise specified. Specifically encompassed by the term "antibody" are VHH antibodies or nanobody antibodies that consist of antigen binding fragment(s) of heavy chain only antibodies fused to an Fc, where the VHH are preferably derived from camelids, polyclonal antibodies, monoclonal antibodies (mAbs), and antibody-like polypeptides, such as chimeric antibodies and humanized antibodies.

[0149] "Antigen-binding fragments" or "bispecific antigen-binding fragments" are any proteinaceous structure that may exhibit binding affinity for a particular antigen. Antigen-binding fragments include those provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. Some antigen-binding fragments are composed of portions of intact antibodies that retain antigen-binding specificity of the parent antibody molecule. For example, antigen-binding fragments may comprise at least one variable region (either a heavy chain or light chain variable region) or one or more CDRs of an antibody known to bind a particular antigen. Examples of suitable antigen-binding fragments include, without limitation, diabodies, Fab, F(ab')2, Fc, Fabc, and Fv molecules, single chain (Sc) antibodies, individual antibody light chains, individual antibody heavy chains, chimeric fusions between antibody chains or CDRs and other proteins, protein scaffolds, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, a monovalent fragment consisting of the VL, VH, CL and CH1 domains, or a monovalent antibody, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region, a Fd fragment consisting essentially of the V_H and C_{H1} domains; a Fv fragment consisting essentially of the V_L and V_H domains of a single arm of an antibody, a dAb fragment (e.g., Ward et al., Nature 341, 544-546 (1989)), which consists essentially of a V_H domain and also called domain antibodies (e.g., Holt et al; Trends Biotechnol. 2003

November; 21(11):484-90); camelid VHH or nanobodies (e.g., Revets et al; Expert Opin Biol Ther. 2005 January; 5(1):111-24); an isolated complementarity determining region (CDR), and the like. All antibody isotypes may be used to produce antigen-binding fragments. Additionally, antigen-binding fragments may include non-antibody proteinaceous frameworks that may successfully incorporate polypeptide segments in an orientation that confers affinity for a given antigen of interest, such as protein scaffolds (e.g., a receptor-ligand pair; for example, a PD1-Fc fusion would target PDL1 and PDL2). Antigen-binding fragments may be recombinantly produced or produced by enzymatic or chemical cleavage of intact antibodies. The phrase "an antibody or antigen-binding fragment thereof" may be used to denote that a given antigen-binding fragment incorporates one or more amino acid segments of the antibody referred to in the phrase.

[0150] "VHH" refers to the single variable domain on a heavy chain. It may also be termed as a nanobody. The VHH (or nanobody) includes three CDR domains that make up the majority of the paratope or antigen binding fragment of heavy chain only antibodies (HcAb). Generally, HcAb is naturally produced by camelids and sharks.

[0151] "Single-chain variable fragment" or "scFv" refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins, connected with a short flexible linker peptide (generally about ten to 25 amino acids long).

[0152] In pharmacokinetics study, half-life (t1/2) generally refers to the time that the drug concentration needs to decrease by 50%. "T1/2 alpha" refers to the half-life of distribution phase, whereas "T1/2 beta" refers to the half-life of elimination phases. By some descriptions, the alpha half-life is the rate of decline in plasma concentrations due to the process of drug redistribution from the central to the peripheral compartment, and the beta half-life is the rate of decline due to the process of drug elimination due to metabolism. During the distribution phase, changes in the concentration of drug in plasma reflect primarily movement of drug from the circulation to internal compartments, rather than loss from, the body. However, once the drug in the plasma and tissues has reached equilibrium, the decline of plasma concentration is driven by elimination of the drug from the body, called the elimination phase (late phase).

[0153] "ROR1" (Receptor Tyrosine Kinase-Like Orphan Receptor 1) refers to the 106-kDa member of the receptor tyrosine kinase family having a UniProt Accession Number Q01973 (human) and Q9Z139 (mouse).

[0154] The term "CD3" refers to the human CD3 protein multi-subunit complex. The CD3 protein multi-subunit complex is composed to 6 distinctive polypeptide chains. These include a CD3γ chain (e.g., SwissProt P09693), a CD3δ chain (e.g., SwissProt P04234), two CD3ε chains (SwissProt P07766), and one CD3ζ chain homodimer (e.g., SwissProt 20963), and which is associated with the T cell receptor α and β chain. CD3 is further clustered with the T cell receptor (TCR) or TCR/CD3 complex, which represents the key activation receptor expressed on T cells. Thus, crosslinking of CD3 or the TCR result in similar signaling pathways and activation of T cells. The term "CD3" includes any CD3 variant, isoform and species homolog which is naturally expressed by cells (including T cells) or can be expressed on cells transfected with genes or cDNA encoding those polypeptides, unless noted.

[0155] "Immunospecifically" when used in the context of antibodies, or antibody fragments, represents binding via domains encoded by immunoglobulin genes or fragments of immunoglobulin genes to one or more epitopes

of a protein of interest, without preferentially binding other molecules in a sample containing a mixed population of molecules. Phrases such as "anti-[antigen] antibody" (e.g., anti-ROR1 antibody) or "[antigen]-specific antibody" (e.g., ROR1-specific antibody" are meant to convey that the recited antibody specifically binds the recited antigen.

[0156] "Isolated" means a biological component (such as an antibody) has been substantially separated, produced apart from, or purified away from other biological components of the organism in which the component naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA, and proteins. Antibodies that have been "isolated" thus include antibodies purified by standard purification methods. "Isolated antibodies" can be part of a composition and still be isolated if such composition is not part of the native environment of the antibody. The term also embraces antibodies prepared by recombinant expression in a host cell as well as chemically synthesized antibodies.

Fusion proteins

[0157] Various embodiments provide for a fusion protein comprising a scaffold polypeptide and at least two of: (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more of a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH. In various embodiments, (b) is one or more IL-18 variants.

[0158] Various embodiments provide for a fusion protein comprising a scaffold polypeptide and at least two of: (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA). In various embodiments, (b) is one or more IL-18 variants. Polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a TAA include those described herein, and those known in the art.

[0159] Various embodiments provide for a fusion protein comprising a scaffold polypeptide and at least two of: (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more immune checkpoint-targeting fragment. In various embodiments, (b) is one or more IL-18 variants.

[0160] In various embodiments, the one or more polypeptides capable of binding the TAA comprise a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH. In various embodiments, the TAA comprises EGFR, HER2, or DLL. Polypeptides capable of binding the TAA include those described herein and those known in the art.

[0161] In various embodiments, the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, or the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40. Accordingly, Polypeptides capable of binding these activation receptors or costimulatory receptors include those described herein and those known in the art.

[0162] In various embodiments, the immune checkpoints include but are not limited to PD-1, PD-L1, CTLA-4, LAG-3. In various embodiments, one or more immune checkpoint-targeting fragment comprises immune checkpoint-targeting fragments of known antibodies. Examples of anti-PD1 fragments include fragments (E.g. Fab, Fv) from pembrolizumab, nivolumab, pidilizumab, AMP-224, AMP-514, spartalizumab, cemiplimab, penpulimab (AK105), prolgolimab (BCD-100), ezabenlimab (BI 754091), toripalimab (JS001), lipustobart (LZM009), retifanlimab (MGA012), Sym021, dostarlimab (TSR-042), tebotelimab (MGD013), cadonilimab (AK104), vudalimab (XmAb20717), tislelizumab, or PF-06801591. Additional examples of anti-PD1 fragments also include fragments (e.g., Fab, Fv) from vopratelimab, camrelizumab, sintilimab, AMP-224, AMP-514, and Acrixolimab. Examples of anti-PD-L1 antibodies include but are not limited to garivulimab (BGB-A333), cosibelimab (CK-301), FAZ053, envafolimab (KN035), MDX-1105, betifisolimab (MSB2311), adebrelimab (SHR-1316), atezolizumab, avelumab, durvalumab, BMS-936559, CK-301, and M7824. Thus, for example, the fusion protein comprises an anti-PD1 fragment from an anti-PD1 antibody (e.g., Fab, Fv), an IL-18 variant, and an IL-15 variant, and optionally, a linker.

[0163] In various embodiments, the fusion protein comprises the scaffold polypeptide and (a) one or more of an interleukin 15 (IL-15) variant, and (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant. In various embodiments, (b) is one or more IL-18 variants.

[0164] In various embodiments, the fusion protein comprises the scaffold polypeptide and all three of (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more of a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH. In various embodiments, (b) is one or more IL-18 variants.

[0165] In various embodiments, the fusion protein comprises the scaffold polypeptide and all three of (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA). In various embodiments, (b) is one or more IL-18 variants.

[0166] In various embodiments, the fusion protein comprises the scaffold polypeptide and all three of (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more immune checkpoint-targeting fragment. In various embodiments, (b) is one or more IL-18 variants.

[0167] In various embodiments, the one or more of the IL-15 variant is 2, 3, 4, 5, or 6, or up to 8 IL-15 variants. In various embodiments, the one or more of the one or more of the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant is 2, 3, 4, 5, or 6, or up to 8. In various embodiments, the one or more of the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH is 2, 3, 4, 5, or 6, or up to 8.

[0168] In various embodiments, the fusion protein further comprises a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, wherein optionally the

activation receptor comprises cluster of differentiation (CD) 3, CD16, NKp46, or NKG2D, and wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp30, CD2, ICOS, OX40, CD40L and CD40.

[0169] In various embodiments, the fusion protein further comprises a linker between the scaffold polypeptide and the (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, or (c) one or more of a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH.

[0170] In various embodiments, the fusion protein further comprises a linker between the scaffold polypeptide and the (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA). In various embodiments, (b) is one or more IL-18 variants.

[0171] In various embodiments, the fusion protein further comprises a linker between the scaffold polypeptide and the (a) one or more of an interleukin 15 (IL-15) variant, (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, and (c) one or more immune checkpoint-targeting fragment. In various embodiments, (b) is one or more IL-18 variants.

[0172] In various embodiments, the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, are each independently fused to the C-terminus of the scaffold polypeptide.

[0173] In various embodiments, the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, are each independently fused to the N-terminus of the scaffold polypeptide.

[0174] In various embodiments, IL-15 variant, the IL-18, the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, and the receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or the one or more polypeptides capable of binding a tumor associated antigen (TAA) are each independently fused to the C-terminus of the scaffold polypeptide. In various embodiments, (b) is one or more IL-18 variants.

[0175] In various embodiments, IL-15 variant, the IL-18, the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, and the receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or the one or more polypeptides capable of binding a tumor associated antigen (TAA) are each independently fused to the N-terminus of the scaffold polypeptide. In various embodiments, (b) is one or more IL-18 variants.

[0176] In various embodiments, IL-15 variant, the IL-18, the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, and the immune checkpoint-targeting fragment are each independently fused to the C-terminus of the scaffold polypeptide. In various embodiments, (b) is one or more IL-18 variants.

[0177] In various embodiments, IL-15 variant, the IL-18, the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, and the immune checkpoint-targeting fragment are each independently fused to the N-terminus of the scaffold polypeptide. In various embodiments, (b) is one or more IL-18 variants.

[0178] In various embodiments, the scaffold polypeptide is an antibody, and IL-15 variant, the IL-18, the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, and the receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or the one or more polypeptides capable of binding a tumor associated antigen (TAA), are each independently fused to any one of one of a C-terminus or an N-terminus of a heavy chain or a light chain, or fused to any one of a C_H2 domain, or hinge region of the antibody.

[0179] In various embodiments, the scaffold polypeptide is an antibody, and the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, are each independently fused to any one of one of a C-terminus or an N-terminus of a heavy chain or a light chain, or fused to any one of a C_H2 domain, or hinge region of the antibody.

[0180] In various embodiments, the scaffold polypeptide is an antibody, and the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the immune checkpoint-targeting fragment, are each independently fused to any one of one of a C-terminus or an N-terminus of a heavy chain or a light chain, or fused to any one of a C_H2 domain, or hinge region of the antibody.

[0181] In various embodiments, the scaffold polypeptide is an Fc region, and the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or the one or more polypeptides capable of binding a tumor associated antigen (TAA), are each independently fused to any one of one of a C-terminus or an N-terminus of the Fc region.

[0182] In various embodiments, the scaffold polypeptide is an Fc region, and the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, are each independently fused to any one of one of a C-terminus or an N-terminus of the Fc region.

[0183] In various embodiments, the scaffold polypeptide is an Fc region, and the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the immune checkpoint-targeting fragment, are each independently fused to any one of one of a C-terminus or an N-terminus of the Fc region.

[0184] In various embodiments, the scaffold polypeptide is an antibody or a fragment thereof. In various embodiments, the antibody is an IgA, IgM, IgG, or IgE antibody. Additional examples of antibodies include but are not

limited to monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, and single chain (ScFv). The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies). Preferably, the antibodies are human or humanized antibodies.

[0185] In various embodiments, the antibody is an anti-PD-1 antibody or anti-PD-L1 antibody. Examples of anti-PD-1 antibodies include but are not limited to pembrolizumab, nivolumab, pidilizumab, AMP-224, AMP-514, spartalizumab, cemiplimab, penpulimab (AK105), prolgolimab (BCD-100), ezabenlimab (BI 754091), toripalimab (JS001), lipustobart (LZM009), retifanlimab (MGA012), Sym021, dostarlimab (TSR-042), tebotelimab (MGD013), cadonilimab (AK104), vudalimab (XmAb20717), tislelizumab, PF-06801591, and anti-PD1 antibody expressing pluripotent killer T lymphocytes (PIK-PD-1). Examples of anti-PD-L1 antibodies include but are not limited to garivulimab (BGB-A333), cosibelimab (CK-301), FAZ053, envafolimab (KN035), MDX-1105, betifisolimab (MSB2311), adebrelimab (SHR-1316), atezolizumab, avelumab, durvalumab, BMS-936559, CK-301, and M7824.

[0186] In various embodiments, two arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can be heterodimerized by creating "knobs-in-holes" (KiH) mutations in the CH3 domain. This structural feature in the polypeptide arms allows for assembly of two half antibodies (e.g., Fc heterodimer; and VH–CH and VL–CL domains). For example, a heteromultimer (including a heterodimer) may comprise two polypeptides, each comprising a CH3 domain, wherein the polypeptides meet at an engineered interface within the CH3 domain, and the one polypeptide contains an engineered protuberance ("knob") in the interface with at least one contact residue replaced with an import residue having a larger side chain volume than the original residue, and another polypeptide contains an engineered cavity ("hole") in the interface with at least one contact residue replaced with an import residue having a smaller side chain volume than the original residue. In some embodiments, the engineered interface of a heteromultimer includes at least two protuberance-into-cavity mutant pairs. Volumes and accessible surface areas of each amino acid are described in A. A. Zamyatnin, *Prog. Biophys. Mol. Biol.* 24: 107-123, 1972 and C. Chothia, *J. Mol. Biol.* 105: 1-14, 1975. For example, import residues for the formation of a protuberance can be arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W); and preferably the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine. As another example, import residues for the formation of a cavity can be alanine (A), serine (S), threonine (T) and valine (V); and preferably the original residue for the formation of the cavity has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. For example, a T366W mutation in CH3 domain for the "knob"/protuberance chain, and a T366S/L368A/Y407V mutation in CH3 domain for the "hole"/cavity chain. Additionally, the KiH configuration may be coupled further mutations to permit S-S disulfide linkage between the two chains. In various aspects wherein the protein/polypeptide is a heterodimer of a the KiH configuration, the IL-18 (or its fragment, variant, or a fragment of its variant) is linked to one, and only one, of the two (or more) immunoglobulin heavy chain constant regions/chains (i.e. knob or hole).

[0187] In various embodiments, the scaffold polypeptide is a fragment crystallizable (Fc) region or a fragment thereof. In various embodiments, the fragment crystallizable (Fc) region is an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG1. In various embodiments, the fragment crystallizable (Fc) region is a knobs-in-hole (KiH) Fc.

[0188] In various embodiments, the Fc region is an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG1. In various embodiments, Fc region can be a human IgG1, IgG2, or IgG4.

[0189] In some embodiments, the two or more arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can contain another symmetric-to-asymmetric steric complementarity design (e.g., HA-TF, ZW1), a charge-to-charge swap interaction (DD-KK), a charge-to-steric complementarity swap plus additional long-range electrostatic interaction (e.g., EW-RVT), or an isotype strand swap design (e.g., strand-exchange engineered domain (SEED)), or Xmab, 7.8.60, Electrostatic Steering, A107, or Duobody, so as to form heterodimeris/heteromultimers. Further description of these configurations and exemplary mutations/residues are seen in *Front Immunol.* 2016; 7: 394.

[0190] In various embodiments, the scaffold polypeptide is selected from fragment crystallizable (Fc) region, human serum albumin (HSA), beta2microglobulin, transferrin, fragment antigen-binding region (Fab region), VHH antibody, single-chain variable fragment (scFv), anticalin, designed ankyrin repeat protein (DARPin), a binding domain thereof, and a fragment thereof.

[0191] In various embodiments, the scaffold polypeptide is a polypeptide or protein capable of translocating into an endoplasmic reticulum (ER), or a fragment thereof.

[0192] Additional details and embodiments of the scaffold polypeptide are described herein and intended to be included as embodiments for these multi-cytokine fusion proteins.

[0193] In various embodiments, IL-15 variant comprises the sequence of Formula I: X_1WVX_4VISDLKKIEDLIQSMHIX_{22}ATLYTESX_{30}VHPSCKVTAMX_{41}CFLX_{45}ELQX_{49}ISLX_{53}SGDASIHDTVX_{64}N L$X_{67}X_{68}$LANNSLSSNGX_{79}VTESGCKECEELEX_{93}KNIKEFLQSX_{103}VHIVX_{108}MFIX_{112}TS, wherein

X_1 is any amino acid,

X_4 is any amino acid,

X_{22} is any amino acid,

X_{30} is any amino acid,

X_{41} is any amino acid,

X_{45} is any amino acid,

X_{49} is any amino acid except V,

X_{53} is any amino acid except E,

X_{64} is any amino acid,

X_{67} is any amino acid,

X_{68} is any amino acid,

X_{79} is any amino acid,

X_{93} is any amino acid,

X_{103} is any amino acid,

X_{108} is any amino acid, and

X_{112} is any amino acid; (SEQ ID NO:533).

[0194] Additional details and embodiments of the IL-15 variant component of the fusion protein are described herein and intended to be included as embodiments for these multi-cytokine fusion proteins.

[0195] In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDS DVRDNAPRTIFIISMYKDSQPRGMAVTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDN KMQFESSSYEGYFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:250) with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of SEQ ID NO:250. In various embodiments, the one to five amino acid substitutions is one amino acid substitution. In other embodiments, the one to five amino acid substitutions are two amino acid substitutions. In other embodiments, the one to five amino acid substitutions are three amino acid substitutions. In other embodiments, the one to five amino acid substitutions are four amino acid substitutions. In other embodiments, the one to five amino acid substitutions are five amino acid substitutions. In various embodiments, the IL-18 variant comprises no more than five amino acid substitutions, with the exception of substituting cysteines. In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDS DCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDN KMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251) with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of SEQ ID NO:251.

[0196] In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDS DCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDN KMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251) with one or more amino acid substitutions at positions C74, C104, C112, and C164, and one to five amino acid substitutions at positions E42, M87, K89, M96, and M149.

[0197] In various embodiments, the amino acid substitutions at one or more of C74, C104, C112, and C164 are each independently substituted to valine, alanine or serine. In various embodiments, the one to five amino acid substitutions are one or more of: E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; or M149V or M149I. In various embodiments, the one to five amino acid substitutions are E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; and M149V or M149I.

[0198] In various embodiments, the fusion protein comprises an IL-18 variant selected from Table 6B. In various embodiments, the fusion protein comprising the IL-18 variant selected from Table 6B, further comprises a propeptide having an amino acid sequence selected from Propeptide column in Table 6B, and optionally from the same row as the IL-18 variant. In various embodiments, the fusion protein comprising the IL-18 variant selected from Table 6B and propeptide selected from Table 6B, further comprises a cleavage peptide selected from Table 6B, and optionally from the same row as the IL-18 variant and propeptide. As a particular example, IEQD (SEQ ID NO:88) can be used.

[0199] In various embodiments, the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant further comprises its propeptide (PP) or a PP variant. In various embodiments, the PP or the PP variant is on the N-terminus end relative to the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant.

[0200] In various embodiments, the fusion protein further comprises one or more cleavage sites and the fusion protein is cleaved at the one or more cleavage sites by one or more proteases.

[0201] In various embodiments, the one or more cleavage sites is between the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant and the scaffold polypeptide, or within the PP, between PP or the PP variant and the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, or within the PP, between the PP or the PP variant and the scaffold polypeptide, or within the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, or within the PP, or a combination thereof.

[0202] Additional details and embodiments of the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant component of the fusion protein are described herein and intended to be included as embodiments for these multi-cytokine fusion proteins.

[0203] In various embodiments, the ROR1 VHH comprises: a polypeptide having SEQ ID NO:325 (complementarity-determining region (CDR) 1 of 2A11), a polypeptide having SEQ ID NO:326 (CDR2 of 2A11), a polypeptide having SEQ ID NO:327 (CDR3 of 2A11), or a combination thereof; **OR** a variant of the polypeptide having SEQ ID NO:325 (CDR1 of 2A11), a variant of the polypeptide having SEQ ID NO:326 (CDR2 of 2A11), a variant of the polypeptide having SEQ ID NO:327 (CDR3 of 2A11), or a combination thereof, wherein the variant of the polypeptide having SEQ ID NO:325 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:325, wherein the variant of the polypeptide having SEQ ID NO:326 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:326, wherein the variant of the polypeptide having SEQ ID NO:327 comprises one or more deletions, additions or a

substitutions of an amino acid residue in the polypeptide having SEQ ID NO:327, and wherein: the variant of the polypeptide having SEQ ID NO:325 and the variant of the polypeptide having SEQ ID NO:327 do **not** replace cysteine residues in the polypeptide having SEQ ID NO:325 and the polypeptide having SEQ ID NO:327, or the variant of the polypeptide having SEQ ID NO:325 and the variant of the polypeptide having SEQ ID NO:327 **replaces** one or both of the cysteine residues in the polypeptide having SEQ ID NO:325 and/or one or both of the cysteine residues in the polypeptide having SEQ ID NO:327 **with** an amino acid that contains a cross-linking functional group.

[0204] Additional details and embodiments of the ROR1 VHH component of the fusion protein are described herein and intended to be included as embodiments for these multi-cytokine fusion proteins.

[0205] In embodiments wherein the fusion protein comprises the scaffold polypeptide and (a) one or more of an interleukin 15 (IL-15) variant, and (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, the (a) one or more of an interleukin 15 (IL-15) variant, and (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant can be as described herein and intended to be included as embodiments for these multi-cytokine fusion proteins.

[0206] In embodiments wherein the fusion protein comprises the scaffold polypeptide and (a) one or more of an interleukin 15 (IL-15) variant, and (b) one or more of an IL-18 variant, the (a) one or more of an interleukin 15 (IL-15) variant, and (b) one or more of an IL-18 variant can be as described herein and intended to be included as embodiments for these multi-cytokine fusion proteins.

[0207] In various embodiments, the fusion protein, comprising the scaffold polypeptide and (a) one or more of an interleukin 15 (IL-15) variant, and (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, is selected from Table 3A. For example, the fusion protein is FUSE-697, FUSE-916, FUSE-923, FUSE-943, FUSE-944, FUSE-1145, FUSE-1146, FUSE-1124 or FUSE 1136.

[0208] In various embodiments, the fusion protein, comprising the scaffold polypeptide and (a) one or more of an interleukin 15 (IL-15) variant, and (b) one or more of an interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, or a fragment of the IL-18 variant, is encoded by a polynucleotide selected from Table 3B. For example, the fusion protein is FUSE-697, FUSE-916, FUSE-923, FUSE-943, FUSE-944, FUSE-1145, FUSE-1146, FUSE-1124 or FUSE 1136.

Polynucleotides, expression vectors, cells and methods of producing fusion proteins.

[0209] Various embodiments provide for a polynucleotide encoding any one of the fusion protein of the present invention as described herein.

[0210] Various embodiments provide for an expression vector comprising any one of the polynucleotide of the present invention as described herein.

[0211] Various embodiments provide for a cell transfected with any one of the expression vector of the present invention as described herein. In various embodiments, the cell is a mammalian cell. Examples of mammalian cells include Chinese hamster ovary (CHO) cells, NS0 cells (a mouse myeloma cell line), PER.C6® cells, and human embryonic kidney cells (HEK cells). In various embodiments, the cell is a bacterial cell or yeast cell.

[0212] Various embodiments provide for a method of producing any one of the fusion proteins of the present invention described herein, comprising: culturing any one of the cells of the present invention described herein, in cell culture medium to allow the fusion protein to be produced, and optionally secreted into the cell culture medium. In various embodiments, the method further comprises isolating the fusion protein. In various embodiments, the method further comprises purifying the fusion protein.

[0213] In some embodiments, using a Chinese hamster ovary (CHO) expression system, the fusion protein is produced via a process including the steps of: (1) cell recovery, which may be to recover frozen CHO cells via water bath at 37°C; (2) cell subculturing, which may be to sub-culture the cells and adjust the cell density to 6×10^6/ml for transfection; (3) transfection and expression, using a solution 1 (in which a plasmid is diluted with a diluting agent), a solution 2 (in which a transection reagent is diluted with a/the diluting agent), and then mixing the solution 1, the solution 2 and the CHO cells, followed by incubating the mixture at a shaker for expression for 12-14 days at 32°C to collect the supernatant of the culture after centrifuge.

[0214] In some embodiments, a purification process is performed after the expression of the fusion protein. In some embodiments, a purification process includes the steps of: (1) washing a column with a binding buffer (10 times volume) at a flow rate of 1 mL/min; (2) loading a fusion-protein-containing sample into the column at a flow rate of 1 mL/min; (3) washing the column with 10x volumes of PBS buffer with a flow rate of 1 mL/min; (4) eluting the protein from the column with 40 mM sodium citrate (pH3.4); optionally the elution sample may be collected into tubes (1ml/min) and measured for optical density (OD) using NanoDrop at 280 nm; and (5) performing dialysis, e.g., against PBS buffer in a dialysis bag overnight.

Methods of use

[0215] Various embodiments provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering any one of the fusion protein of the present invention described herein, to a subject in need thereof. Expansion of T cells, B cells or natural killer (NK) cells, does not require binding to the tumor associated antigen, such as ROR1. In various embodiments, the subject has cancer.

[0216] Various embodiments provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering any one of the fusion protein of the present invention described herein, to a subject in need thereof. In various embodiments, the disease or condition is cancer.

[0217] Various embodiments provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering at least two of the following to a

subject in need thereof: (a) interleukin 15 (IL-15) variant or IL-15 fusion protein, (b) interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein, and (c) a multispecific antibody construct comprising a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, NKp46, or NKG2D, and wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp30, CD2, ICOS, OX40, CD40L and CD40. In various embodiments, the subject has cancer. Expansion of T cells, B cells or natural killer (NK) cells, does not require binding to the tumor associated antigen, such as ROR1.

[0218] Various embodiments provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering at least two of the following to a subject in need thereof: (a) interleukin 15 (IL-15) variant or IL-15 fusion protein, (b) interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein, (c) a multispecific antibody construct comprising a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, NKp46, or NKG2D, and wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp30, CD2, ICOS, OX40, CD40L and CD40, and (d) one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA). In various embodiments, the subject has cancer. Expansion of T cells, B cells or natural killer (NK) cells, does not require binding to the tumor associated antigen, such as ROR1.

[0219] A method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, administering at least two of the following to a subject in need thereof: (a) interleukin 15 (IL-15) variant or IL-15 fusion protein, (b) interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein, and (c) a multispecific antibody construct comprising a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, NKp46, or NKG2D, and wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp30, CD2, ICOS, OX40, CD40L and CD40. In various embodiments, the disease or condition is cancer.

[0220] A method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, administering at least two of the following to a subject in need thereof: (a) interleukin 15 (IL-15) variant or IL-15 fusion protein, (b) interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein, (c) a multispecific antibody construct comprising a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor

expressed on an immune cell, wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, NKp46, or NKG2D, and wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp30, CD2, ICOS, OX40, CD40L and CD40, and (d) one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA). In various embodiments, the disease or condition is cancer.

[0221] In some embodiments, the cancer is a ROR1-expressing cancer, such as lung cancer, hematological cancer, breast cancer, prostate cancer, pancreatic cancer, colon cancer, ovarian cancer, renal cancer, uterine cancer, bladder cancer, kidney cancer, melanoma, thyroid cancer, myeloid leukemia, mantle cell lymphoma, or multiple myeloma. The ROR1-expressing cancer can be a lung cancer, such as non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC). The ROR1-expressing cancer can be a hematological cancer, such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS, low or high risk), acute lymphocytic leukemia (ALL, including all subtypes), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML), or blastic plasmacytoid dendritic cell neoplasm (DPDCN). The ROR1-expressing cancer can be breast cancer. The ROR1-expressing cancer can be prostate cancer. The ROR1-expressing cancer can be pancreatic cancer. The ROR1-expressing cancer can be colon cancer. The ROR1-expressing cancer can be ovarian cancer. The ROR1-expressing cancer can be renal cancer. The ROR1-expressing cancer can be uterine cancer. The ROR1-expressing cancer can be melanoma.

IL-15 variant details and embodiments

[0222] Described herein are IL-15 variants that can be used in the multi-cytokine fusion proteins of the invention. Various embodiments provide for an interleukin 15 (IL-15) variant comprising the sequence of Formula I:
X_1WVX_4VISDLKKIEDLIQSMHIX_{22}ATLYTESX_{30}VHPSCKVTAMX_{41}CFLX_{45}ELQX_{49}ISLX_{53}SGDASIHDTVX_{64}N L$X_{67}X_{68}$LANNSLSSNGX_{79}VTESGCKECEELEX_{93}KNIKEFLQSX_{103}VHIVX_{108}MFIX_{112}TS, wherein

X_1 is any amino acid,

X_4 is any amino acid,

X_{22} is any amino acid,

X_{30} is any amino acid,

X_{41} is any amino acid,

X_{45} is any amino acid,

X_{49} is any amino acid except V,

X_{53} is any amino acid except E,

X_{64} is any amino acid,

X_{67} is any amino acid,

X_{68} is any amino acid,

X_{79} is any amino acid,

X_{93} is any amino acid,

X_{103} is any amino acid,

X_{108} is any amino acid, and

X_{112} is any amino acid; (SEQ ID NO:533).

[0100] In various embodiments of the IL-15 variant, X_1 is N (SEQ ID NO:534), X_4 is N (SEQ ID NO:535), X_{22} is D (SEQ ID NO:536), X_{30} is D (SEQ ID NO:537), X_{41} is K (SEQ ID NO:538), X_{45} is L (SEQ ID NO:539), X_{64} is E (SEQ ID NO:540), X_{67} is I (SEQ ID NO:541), X_{68} is I (SEQ ID NO:542), X_{79} is N (SEQ ID NO:543), X_{93} is E (SEQ ID NO:544), X_{103} is F (SEQ ID NO:545), X_{108} is Q (SEQ ID NO:546), or X_{112} is N (SEQ ID NO:547), or any combination thereof. In various embodiments, any combination thereof is any two combinations thereof. In various embodiments, any combination thereof is any 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 combinations thereof.

[0101] In various embodiments of the IL-15 variant, X_1 is N (SEQ ID NO:534), X_4 is N (SEQ ID NO:535), X_{22} is D (SEQ ID NO:536), X_{30} is D (SEQ ID NO:537), X_{41} is K (SEQ ID NO:538), X_{45} is L (SEQ ID NO:539), X_{49} is R or K (SEQ ID NO:548), X_{53} is G, K, I or A (SEQ ID NO:549), X_{64} is E (SEQ ID NO:540), X_{67} is I (SEQ ID NO:541), X_{68} is I (SEQ ID NO:542), X_{79} is N (SEQ ID NO:543), X_{93} is E (SEQ ID NO:544), X_{103} is F (SEQ ID NO:545), X_{108} is Q (SEQ ID NO:546), or X_{112} is N (SEQ ID NO:547), or any combination thereof. In various embodiments, any combination thereof is any two combinations thereof. In various embodiments, any combination thereof is any 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 combinations thereof.

[0102] In various embodiments of the IL-15 variant, X_1 is N or G (SEQ ID NO:550), X_4 is N, K or L (SEQ ID NO:551), X_{22} is D or A (SEQ ID NO:552), X_{30} is D or N (SEQ ID NO:553), X_{41} is K or Q (SEQ ID NO:554), X_{45} is L or S (SEQ ID NO:555), X_{49} is R or K (SEQ ID NO:548), X_{53} is G, K, I, or A (SEQ ID NO:549), X_{64} is E or Q (SEQ ID NO:556), X_{67} is I or T (SEQ ID NO:557), X_{68} is I or S (SEQ ID NO:558), X_{79} is N or Y (SEQ ID NO:559), X_{93} is E or A (SEQ ID NO:560), X_{103} is F or L (SEQ ID NO:561), X_{108} is Q or T (SEQ ID NO:562), or X_{112} is N or R (SEQ ID NO:563), or any combination thereof. In various embodiments, any combination thereof is any two combinations thereof. In various embodiments, any combination thereof is any 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 combinations thereof.

[0223] In various embodiments of the IL-15 variant, X_{49} is R or K, and X_{53} is G, K, I or A; (SEQ ID NO:564). In various embodiments of the IL-15 variant, X_{30} is N (SEQ ID NO:565). In various embodiments of the IL-15 variant, X_{64} is Q (SEQ ID NO:566). In various embodiments of the IL-15 variant, X_1 is G (SEQ ID NO:567).

[0224] In various embodiments, the IL-15 variant is a variant selected from Table 1A. In various embodiments, the IL-15 is a variant selected from Table 3A.

[0225] In various embodiments, the IL-15 variants are not IL-15 variants described in U.S. Patent Application Publ. No. US 2019/0263877, which is herein incorporated by reference.

[0226] Also described herein are fusion proteins comprising IL-15 variants and a scaffold polypeptide that can be used in various methods relating to administering separate fusions proteins described herein. Various embodiments of the invention provide for a fusion protein, comprising an IL-15 variant as described herein; and a scaffold polypeptide. These IL-15 fusion proteins can be used in accordance with the methods of the present invention as described herein.

[0227] In various embodiments, the fusion protein comprises two or more IL-15 variants as described herein; and a scaffold polypeptide. In various embodiments, the two or more IL-15 variants can be 3, 4, 5 or 6 IL-15 variants. In various embodiments, the two or more IL-15 variants can be up to 8 IL-15 variants.

[0228] In various embodiments, the IL-15 variant is fused to the C-terminus of the scaffold polypeptide. In other embodiments, the IL-15 variant is fused to the N-terminus of the scaffold polypeptide.

[0229] In still other embodiments, wherein the scaffold polypeptide is an antibody, the IL-15-variant is fused to any one of the C-terminus or N-terminus of the heavy chain or light chain, or fused to any one of the C_H2 domain, or hinge region (e.g., between the C_H2 and C_H1 domains).

[0230] In various embodiments, the scaffold polypeptide is an antibody or a fragment thereof. In various embodiments, the antibody is an IgA, IgM, IgG, or IgE antibody.

[0231] In various embodiments, the antibody is an anti-PD-1 antibody or anti-PD-L1 antibody. Examples of anti-PD-1 antibodies include but are not limited to pembrolizumab, nivolumab, pidilizumab, AMP-224, AMP-514, spartalizumab, cemiplimab, penpulimab (AK105), prolgolimab (BCD-100), ezabenlimab (BI 754091), toripalimab (JS001), lipustobart (LZM009), retifanlimab (MGA012), Sym021, dostarlimab (TSR-042), tebotelimab (MGD013), cadonilimab (AK104), vudalimab (XmAb20717), tislelizumab, PF-06801591, and anti-PD1 antibody expressing pluripotent killer T lymphocytes (PIK-PD-1). Examples of anti-PD-L1 antibodies include but are not limited to garivulimab (BGB-A333), cosibelimab (CK-301), FAZ053, envafolimab (KN035), MDX-1105, betifisolimab (MSB2311), adebrelimab (SHR-1316), atezolizumab, avelumab, durvalumab, BMS-936559, CK-301, and M7824.

[0232] In various embodiments, the scaffold polypeptide is a Fc region or a fragment thereof. In various embodiments, the scaffold polypeptide is a Fc region or a fragment thereof and the scaffold polypeptide does not comprise a Fab.

[0233] In various embodiments, two arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can be heterodimerized by creating "knobs-in-holes" (KiH) mutations in the CH3 domain. This structural feature in the polypeptide arms allows for assembly of two half antibodies (e.g., Fc heterodimer; and VH–CH and VL–CL domains). For example, a heteromultimer (including a heterodimer) may comprise two polypeptides, each comprising a CH3 domain, wherein the polypeptides meet at an engineered interface within the CH3 domain, and the one polypeptide contains an engineered protuberance ("knob") in the interface with at least one contact residue replaced with an import residue having a larger side chain volume than the original residue, and another polypeptide contains an engineered cavity ("hole") in the interface with at least one contact residue replaced with an import residue having a smaller side chain volume than the original residue. In some embodiments, the engineered interface of a heteromultimer

includes at least two protuberance-into-cavity mutant pairs. Volumes and accessible surface areas of each amino acid are described in A. A. Zamyatnin, *Prog. Biophys. Mol. Biol.* 24: 107-123, 1972 and C. Chothia, *J. Mol. Biol.* 105: 1-14, 1975. For example, import residues for the formation of a protuberance can be arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W); and preferably the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine. As another example, import residues for the formation of a cavity can be alanine (A), serine (S), threonine (T) and valine (V); and preferably the original residue for the formation of the cavity has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. For example, a T366W mutation in CH3 domain for the "knob"/protuberance chain, and a T366S/L368A/Y407V mutation in CH3 domain for the "hole"/cavity chain. Additionally, the KiH configuration may be coupled further mutations to permit S-S disulfide linkage between the two chains. In various aspects wherein the protein/polypeptide is a heterodimer of a the KiH configuration, the IL-18 (or its fragment, variant, or a fragment of its variant) is linked to one, and only one, of the two (or more) immunoglobulin heavy chain constant regions/chains (i.e. knob or hole).

[0234] As such, in some embodiments, the Fc region is a knobs-in-hole (KiH) Fc.

[0235] In various embodiments, the IL-15 variant is fused to the knob of the KiH Fc. In various embodiments, the IL-15 variant is fused to the hole of the KiH Fc.

[0236] In various embodiments, the Fc region is an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG In various embodiments, Fc region can be a human IgG1, IgG2, or IgG4.

[0237] In some embodiments, the two or more arms (or chains) of immunoglobulin heavy chain constar regions (e.g., Fc polypeptides) can contain another symmetric-to-asymmetric steric complementarity design (e.g., HA TF, ZW1), a charge-to-charge swap interaction (DD-KK), a charge-to-steric complementarity swap plus additional long range electrostatic interaction (e.g., EW-RVT), or an isotype strand swap design (e.g., strand-exchange engineere domain (SEED)), or Xmab, 7.8.60, Electrostatic Steering, A107, or Duobody, so as to forn heterodimeris/heteromultimers. Further description of these configurations and exemplary mutations/residues are seen i *Front Immunol.* 2016; 7: 394.

[0238] In various embodiments, the scaffold polypeptide of the fusion protein comprises a globular protei human serum albumin (HSA), beta2microglobulin, transferrin, fragment antigen-binding region (Fab region), VH antibody, single-chain variable fragment (scFv), anticalin, designed ankyrin repeat protein (DARPin), a binding domai thereof, or a fragment thereof.

[0239] In various embodiments, one or more polypeptides can be inserted between the IL-15 variant and th antibody of the IL-15 fusion proteins. In various embodiments, the polypeptide can be inserted or conjugated at the N terminus, at the C-terminus, or both the N-terminus and C- terminus of the antibody. In various embodiments, th polypeptide comprises a polypeptide linker conjugating the IL-15 variant and the antibody.

[0240] In various embodiments, one or more polypeptides can be inserted between the IL-15 variant and the F region of the IL-15 fusion proteins. In various embodiments, the polypeptide can be inserted or conjugated at the N

terminus, at the C-terminus, or both the N-terminus and C- terminus of the Fc region. In various embodiments, the polypeptide comprises a polypeptide linker conjugating the IL- 5 variant and the Fc region. In these embodiments, the fusion protein does not include Fab region of an antibody.

[0241] The antibodies that can be fused to IL-15 can include monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, and single chain (ScFv). The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies). Preferably, the antibodies are human or humanized antibodies.

[0242] In various embodiments, the IL-15 fusion proteins are IL-15 fusion proteins listed in Table 1A. That is, an IL-15 fusion protein comprising polypeptide 1, polypeptide 2, and polypeptide 3.

[0243] In various embodiments, the IL-15 fusion protein comprises polypeptide 1, polypeptide 2, and polypeptide 3 listed in Table 1A, except the linker in polypeptide 2 is a different peptide linker. For example, the linker can be a flexible linker that is generally about 10 to 25 amino acids in length. Additional examples of linkers include but are not limited to a dimer of two amino acids, a tri-mer of three amino acids, or a peptide selected from the group consisting of T, PT, MPT, S, GS, GGS, GGGS (SEQ ID NO:235), (GGGGX_5 (SEQ ID NO:236))n, and (X_5GGGG (SEQ ID NO:317))n wherein X_5 is Q, A, E or S and n=1-5 or an integer larger than 5. In some embodiments, the amino acid linker has the amino acid sequence of (GGGGS (SEQ ID NO:237))n where n is an integer between 1 and 5, theret an amino acid linker of 25 amino acids or shorter in length.

[0244] In various embodiments, the IL-15 fusion protein comprises an IL-15 variant listed in Table 1A (Table 3B.

Table 1A – Polypeptide sequences (IL-15 embodiments)

Note: polypeptide 1 is the "hole" heavy chain; Polypeptide 2 is the "knob" heavy chain, a linker (underlined) and an IL-15 variant; Polypeptide 3 is the light chain. IL-15 variant is also listed separately, but is included in Polypeptide 2.

	Polypeptide 1	Polypeptide 2	Polypeptide 3	IL-15 Variant
FUSE-696	QVQLVESGGGVVQPGRSLRLDCK ASGITFSNSGMHWVRQAPGKGLE WVAVIWYDGSKRYYADSVKGRFT ISRDNSKNTLFLQMNSLRAEDTA VYYCATNDDYWGQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNRFTQKSL SLSPGK (SEQ ID NO:574)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGG GSGGGGSGWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLGSGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:577)	EIVLTQSPATLSLSPGERATL SCRASQSVSSYLAWYQQKPGQ APRLLIYDASNRATGIPARFS GSGSGTDFTLTISSLEPEDFA VYYCQQSSNWPRTFGQGTKVE IKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO:75)	GWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLGS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:596)
FUSE-764	QVQLVESGGGVVQPGRSLRLDCK ASGITFSNSGMHWVRQAPGKGLE WVAVIWYDGSKRYYADSVKGRFT ISRDNSKNTLFLQMSSLRAEDTA VYYCATNDDYWGQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPEA AGGPSVFLFPPKPKDTLMISRTP	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEaaGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGG GSGGGGSGWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT	(SEQ ID NO:75)	GWVNVISDL KKIEDLIQS MHIDATLYT ESnVHPSCK VTAMKCFLL ELQrISLkS GDASIHDTV qNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF

EVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNRFTQKSL SLSPGK (SEQ ID NO:575)	AMKCFLLELQRISLKSGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:578)		LQSFVHIVQ MFINTS (SEQ ID NO:597)
FUSE-7665 (SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSGWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLISGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:579)	(SEQ ID NO:75)	GWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLIS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:598)
FUSE-7665 (SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSGWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQKISLASGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:580)	(SEQ ID NO:75)	GWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQKISLAS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID

FUSE-7676-7	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSGWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQKISLKSGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:581)	(SEQ ID NO:75)	GWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQKISLKS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:600)
FUSE-7676-8	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSGWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQKISLISGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:582)	(SEQ ID NO:75)	GWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQKISLIS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:601)
FUSE-7677-7	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY	(SEQ ID NO:75)	NWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLGS GDASIHDTV

2			KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLGSGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:583)	QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:602)
FUSE-7763	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVKVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLGSGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:584)	(SEQ ID NO:75)	NWVKVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLGS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:603)
FUSE-7774	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLGSGDASIHDTVQNLISLANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:585)	(SEQ ID NO:75)	NWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLGS GDASIHDTV QNLISLANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:604)

F	QVQLVESGGGVVQPGRSLKLDCKASGITFSNSGMHWVRQAPGKGL	(SEQ ID NO:75)	GWVNVISDL
U	ASGITFSNSGMHWVRQAPGKGLE	EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED	KKIEDLIQS
S	WVAVIWYDGSKRYYADSVKGRFT	TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT	MHIAATLYT
E	ISRDNSKNTLFLQMSSLRAEDTA	AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS	ESNVHPSCK
-	VYYCATNDDYWGQGTLVTVSSAS	VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC	VTAMKCFLL
8	TKGPSVFPLAPSSKSTSGGTAAL	PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK	ELQRISLGS
0	GCLVKDYFPEPVTVSWNSGALTS	FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY	GDASIHDTV
2	GVHTFPAVLQSSGLYSLSSVVTV	KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS	QNLIILANN
	PSSSLGTQTYICNVNHKPSNTKV	LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK	SLSSNGNVT
	DKKVEPKSCDKTHTCPPCPAPEA	LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGG	ESGCKECEE
	AGGPSVFLFPPKPKDTLMISRTP	GGSGGGGGWVNVISDLKKIEDLIQSMHIAATLYTESNVHPSCKVT	LEAKNIKEF
	EVTCVVVDVSHEDPEVKFNWYVD	AMKCFLLELQRISLGSGDASIHDTVQNLIILANNSLSSNGNVTES	LQSFVHIVQ
	GVEVHNAKTKPREEQYNSTYRVV	GCKECEELEAKNIKEFLQSFVHIVQMFINTS (SEQ ID	MFINTS
	SVLTVLHQDWLNGKEYKCKVSNK	NO:586)	(SEQ ID
	ALPAPIEKTISKAKGQPREPQVY		NO:605)
	TLPPSREEMTKNQVSLSCAVKGF		
	YPSDIAVEWESNGQPENNYKTTP		
	PVLDSDGSFFLVSKLTVDKSRWQ		
	QGNVFSCSVMHEALHNRFTQKSL		
	SLSPGK (SEQ ID NO:576)		
F	(SEQ ID NO:576)	QVQLVESGGGVVQPGRSLKLDCKASGITFSNSGMHWVRQAPGKGL	(SEQ ID NO:75)
U		EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED	
S		TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT	
E		AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS	
-		VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC	
8		PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK	
0		FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY	
3		KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS	
		LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK	
		LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGG	
		AMKCFLLELQRISLGSGDASIHDTVQNLIILANNSLSSNGNVTES	
		GCKECEELEAKNIKEFLQSLVHIVQMFINTS (SEQ ID	
		NO:587)	
F	(SEQ ID NO:576)	QVQLVESGGGVVQPGRSLKLDCKASGITFSNSGMHWVRQAPGKGL	(SEQ ID NO:75)
U		EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED	
S		TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT	

GWVNVISDL	
KKIEDLIQS	
MHIAATLYT	
ESNVHPSCK	
VTAMKCFLL	
ELQRISLGS	
GDASIHDTV	
QNLIILANN	
SLSSNGNVT	
ESGCKECEE	
LEAKNIKEF	
LQSFVHIVQ	
MFINTS (SEQ ID NO:606)	
GWVNVISDL	
KKIEDLIQS	
MHIDATLYT	

E-8044		AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGG GGSGGGGGWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMQCFLSELQRISLGSGDASIHDTVQNLTILANNSLSSNGYVTES GCKECEELEAKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:588)		ESNVHPSCK VTAMQCFLS ELQRISLGS GDASIHDTV QNLTILANN SLSSNGYVT ESGCKECEE LEAKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:607)
FU S E-8046	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEaaGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGG GSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLISGDASIHDTVQNLISLANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:589)	(SEQ ID NO:75)	NWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLIS GDASIHDTV QNLISLANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:608)
FU S E-8047	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEaaGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGGGGSGGG GSGGGGSNWVKVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT	(SEQ ID NO:75)	NWVKVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLIS GDASIHDTV QNLISLANN SLSSNGNVT ESGCKECEE LEEKNIKEF

	AMKCFLLELQRISLISGDASIHDTVQNLISLANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:590)		LQSFVHIVQ MFINTS (SEQ ID NO:609)
FUSE-8478	(SEQ ID NO:575)	(SEQ ID NO:75)	NWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLIS GDASIHDTV QNLISLANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVT MFINTS (SEQ ID NO:610)
	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEaaGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLISGDASIHDTVQNLISLANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVTMFINTS (SEQ ID NO:591)		
FUSE-8479	(SEQ ID NO:575)	(SEQ ID NO:75)	NWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLIS GDASIHDTV QNLISLANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFIRTS (SEQ ID NO:611)
	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEaaGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLISGDASIHDTVQNLISLANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFIRTS (SEQ ID NO:592)		
FUSE-8479	(SEQ ID NO:575)	(SEQ ID NO:75)	NWVNVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK
	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS		

- 8 5 0		VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEaaGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLISGDASIHDTVQNLISLANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVTMFTRTS (SEQ ID NO:593)		VTAMKCFLL ELQRISLIS GDASIHDTV QNLISLANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVT MFIRTS (SEQ ID NO:612)
F U S E - 8 5 3	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVKVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLISGDASIHDTVQNLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:594)		NWVKVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLIS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ MFINTS (SEQ ID NO:613)
F U S E - 8 5 4	(SEQ ID NO:575)	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGL EWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLFLQMSSLRAED TAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGG GSGGGGSNWVLVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVT AMKCFLLELQRISLISGDASIHDTVQNLIILANNSLSSNGNVTES		NWVLVISDL KKIEDLIQS MHIDATLYT ESNVHPSCK VTAMKCFLL ELQRISLIS GDASIHDTV QNLIILANN SLSSNGNVT ESGCKECEE LEEKNIKEF LQSFVHIVQ

		GCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:595)		MFINTS (SEQ ID NO:614)

Table 1B -Polypeptide sequences (IL-15 embodiments)

Note: Polypeptide 1 is the heavy chain and polypeptide 2 while left blank is identical to Polypeptide 1. Polypeptide 3 is the light chain.

	Polypeptide 1	Poly-peptide 2	Poly-peptide 3
FUSE-691	QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKNTLF LQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:16)		(SEQ ID NO:75)

Table 3A – Polypeptide sequences relating to IL15/IL18 fusion proteins

Note: polypeptide 1 is the "hole" heavy chain; Polypeptide 2 is the "knob" heavy chain; Polypeptide 3 is the light chain. Mature IL18-variant and IL-15 variant are also listed separately, but is included in Polypeptide 1, 2 or 3.

	Polypeptide 1	Polypeptide 2	Polypeptide 3	Propeptide	Cleavage	Mature IL18 Variants	IL15 Variants
FUSE-32	EVQLVESGGGLVQAGGSLRLS CAASGRTFSSYAMGWFRQAPG KEREFVVAINWSSGSTYYADS VKGRFTISRDNAKNTMYLQMN SLKPEDTAVYYCAAGYQINSG NYNFKDYEYDYWGQGTQVTVS SEPKSSDKTHTCPPCPAPEAA	EVQVQQSGPELVKPGTSVKISCKTSG YTFTEYTMHWVKQSHGESLEWIGGIV PNNGATNYNQKFRGKATLTVDKSSST AYMELRSLTSEDSAVYYCARRGLLWH FAYWGQGTLVTVSAAKTTPPSVYPLA PGSAAQTNSMVTLGCLVKGYFPEPVT VTWNSGSLSSGVHTFPAVLQSDLYTL	DILMTQSPSAMSVSLGDTVSIT CHASQGISSYIGWLQQKPGKSF KGLIYHGTNLEDGVPSRFSGSG SGADYSLTISSLESEDFADYYC IQYAQFPFTFGSGTKLEIKRAD AAPTVSIFPPSSEQLTSGGASV VCFLNNFYPKDINVKWKIDGSE				

O	GGPSVFLFPPKPKDTLMISRT	SSSVTVPSSTWPSETVTCNVAHPASS	RQNGVLNSWTDQDSKDSTYSMS			
	PEVTCVVVDVSHEDPEVKFNW	TKVDKKIVPRDCDKTHTCPPCPAPEA	STLTLTKDEYERHNSYTCEATH			
	YVDGVEVHNAKTKPREEQYNS	AGGPSVFLFPPKPKDTLMISRTPEVTI	KTSTSPIVKSFNRNEC (SEQ			
	TYRVVSVLTVLHQDWLNGKEY	CVVVDVSHEDPEVKFNWYVDGVEVHN	ID NO:647)			
	KCKVSNKALPAPIEKTISKAK	AKTKPREEQYNSTYRVVSVLTVLHQD				
	GQPREPQVYTLPPSREEMTKN	WLNGKEYKCKVSNKALPAPIEKTISK				
	QVSLSCAVKGFYPSDIAVEWE	AKGQPREPQVYTLPPSREEMTKNQVS				
	SNGQPENNYKTTPPVLDSDGS	LWCLVKGFYPSDIAVEWESNGQPENN				
	FFLVSKLTVDKSRWQQGNVFS	YKTTPPVLDSDGSFFLYSKLTVDKSR				
	CSVMHEALHNRFTQKSLSLSP	WQQGNVFSCSVMHEALHNHYTQKSLS				
	GK (SEQ ID NO:615)	LSPGK (SEQ ID NO:639)				
F	(SEQ ID NO:574)	SEQ ID NO:577)	(SEQ ID NO:75)			(SEQ ID NO:596)
U						
S						
E						
-						
6						
9						
6						
F	QVQLVESGGGVVQPGRSLRLD	(SEQ ID NO:577)	(SEQ ID NO:75)	(S	I (SEQ ID	(SEQ ID
U	CKASGITFSNSGMHWVRQAPG			EQ	E NO:97)	NO:596)
S	KGLEWVAVIWYDGSKRYYADS			ID	Q	
E	VKGRFTISRDNSKNTLFLQMN			NO	D	
-	SLRAEDTAVYYCATNDDYWGQ			:7	(	
6	GTLVTVSSASTKGPSVFPLAP			6)	S	
9	SSKSTSGGTAALGCLVKDYFP				E	
7	EPVTVSWNSGALTSGVHTFPA				Q	
	VLQSSGLYSLSSVVTVPSSSL				I	
	GTQTYICNVNHKPSNTKVDKK				D	
	VEPKSCDKTHTCPPCPAPEaa				N	
	GGPSVFLFPPKPKDTLMISRT				O	
	PEVTCVVVDVSHEDPEVKFNW				:	
	YVDGVEVHNAKTKPREEQYNS				8	
	TYRVVSVLTVLHQDWLNGKEY				8	
	KCKVSNKALPAPIEKTISKAK				)	
	GQPREPQVYTLPPSREEMTKN					
	QVSLSCAVKGFYPSDIAVEWE					
	SNGQPENNYKTTPPVLDSDGS					

FFLVSKLTVDKSRWQQGNVFS CSVMHEALHNRFTQKSLSLSP GGGGGSGGGGSGGGGSGGGGS GGGGSGGGGSAAEPVEDNsIN FVAMKFIDNTLYFIAEDDENI EQDYFGKLESKLSVIRNLNDQ VLFIDQGNRPLFEDMTDSDsR DNAPRTIFIISKYGDSQPRGL AVTISVKsEKISTLSsENKII SFKEMNPPDNIKDTKSDIIFF QRSVPGHDNKVQFESSSYEGY FLAsEKERDLFKLILKKEDEL GDRSIMFTVQNED (SEQ ID NO:17)						
FUSE-7653	(SEQ ID NO:579)	(SEQ ID NO:75)				(SEQ ID NO:598)
FUSE-7733	(SEQ ID NO:584)	(SEQ ID NO:75)				(SEQ ID NO:603)
FUSE-7734	(SEQ ID NO:585)	(SEQ ID NO:75)				(SEQ ID NO:604)

F	AAEPVEDNVINFVAMKFIDNT	EVQLLESGGGLVQPGGSLRLSCAASG	SYELMQPPSVSVSPGQTARITC	AA	I	YFGKLESKLSV	(SEQ ID
U	LYFIAEDDENIEQDYFGKLES	FSISGYAMGWVRQAPGKGLEFVAGID	SGGGYDGSYYYGWYQQKPGQAP	EP	E	IRNLNDQVLFI	NO:613)
S	KLSVIRNLNDQVLFIDQGNRP	VAGISPDYGAAVKGRSTISRDNSKNT	VTVIYDNTNRPSGIPERFSGSN	VE	Q	DQGNRPLFEDM	
E	LFEDMTDSDVRDNAPRTIFII	LYLQMNSLRAEDTAVYYCAKTVVIEG	SGNTITLTISGVQAEDEADYYC	DN	D	TDSDVRDNAPR	
-	SKYGDSQPRGLAVTISVKVEK	LFSTPYIDAWGQGTLVTVSSASTKGP	GGYDRSGGIFGGGTKLTVLRTV	VI	(	TIFIISKYGDS	
9	ISTLSVENKIISFKEMNPPDN	SVFPLAPSSKSTSGGTAALGCLVKDY	AAPSVFIFPPSDEQLKSGTASV	NF	S	QPRGLAVTISV	
1	IKDTKSDIIFFQRSVPGHDNK	FPEPVTVSWNSGALTSGVHTFPAVLQ	VCLLNNFYPREAKVQWKVDNAL	VA	E	KVEKISTLSVE	
6	VQFESSSYEGYFLAVEKERDL	SSGLYSLSSVVTVPSSSLGTQTYICN	QSGNSQESVTEQDSKDSTYSLS	MK	Q	NKIISFKEMNP	
	FKLILKKEDELGDRSIMFTVQ	VNHKPSNTKVDKKVEPKSCDKTHTCP	STLTLSKADYEKHKVYACEVTH	FT	I	PDNIKDTKSDI	
	NEDGGGGSGGGGSDKTHTCPP	PCPAPELLGGPSVFLFPPKPKDTLMI	QGLSSPVTKSFNRGECGGGGSG	DN	D	IFFQRSVPGHD	
	CPAPELLGGPSVFLFPPKPKD	SRTPEVTCVVVDVSHEDPEVKFNWYV	GGGSGGGGSGGGGSGGGGSGGG	TL	N	NKVQFESSSYE	
	TLMISRTPEVTCVVVDVSHED	DGVEVHNAKTKPREEQYNSTYRVVSV	GSEVQLLESGGGLVQPGGSLRL	YF	O	GYFLAVEKERD	
	PEVKFNWYVDGVEVHNAKTKP	LTVLHQDWLNGKEYKCKVSNKALPAP	SCTASGYTNRLKCMGWFRQAPG	IA	:	LFKLILKKEDE	
	REEQYNSTYRVVSVLTVLHQD	IEKTISKAKGQPREPQVYTLPPSREE	KEREEIASISTGTGNTYYADSV	ED	8	LGDRSIMFTVQ	
	WLNGKEYKCKVSNKALPAPIE	MTKNQVSLWCLVKGFYPSDIAVEWES	KGRFTISRDKSRNTLYLQMsTL	DE	8	NED (SEQ ID	
	KTISKAKGQPREPQVYTLPPS	NGQPENNYKTTPPVLDSDGSFFLYSK	RAEDTAVYYCAADVRPHGTTCH	N	)	NO:99)	
	REEMTKNQVSLSCAVKGFYPS	LTVDKSRWQQGNVFSCSVMHEALHNH	YNSRGQGTQVTVSS (SEQ ID	(S			
	DIAVEWESNGQPENNYKTTPP	YTQKSLSLSPGK (SEQ ID	NO:648)	EQ			
	VLDSDGSFFLVSKLTVDKSRW	NO:640)		ID			
	QQGNVFSCSVMHEALHNRFTQ			NO			
	KSLSLSPGGGGSGGGGSGGG			:7			
	GSNWVkVISDLKKIEDLIQSM			8)			
	HIDATLYTESnVHPSCKVTAM						
	KCFLLELQrISLiSGDASIHD						
	TVqNLIILANNSLSSNGNVTE						
	SGCKECEELEKNIKEFLQSF						
	VHIVQMFINTS (SEQ ID						
	NO:616)						
F	AAEPVEDNVINFVAMKFIDNT	EVQLVESGGGLVQAGGSLRLSCAASG		AA	I	(SEQ ID	(SEQ ID
U	LYFIAEDDENIEQDYFGKLES	RPFSNYGMGWFRQAPGKKREFVAGIS		EP	E	NO:99)	NO:613)
S	KLSVIRNLNDQVLFIDQGNRP	WSGGSTDYADSVKGRFTISRDNAKNT		VE	Q		
E	LFEDMTDSDVRDNAPRTIFII	VYLQMNSLKPEDTAVYYCAAVFSGAE		DN	D		
-	SKYGDSQPRGLAVTISVKVEK	TAYYPSDDYDYWGQGTQVTVSSGGGG		VI	(		
9	ISTLSVENKIISFKEMNPPDN	SGGGGSGGGGSGGGGSEVQLLESGGG		NF	S		
2	IKDTKSDIIFFQRSVPGHDNK	LVQPGGSLRLSCTASGYTNRLKCMGW		VA	E		
3	VQFESSSYEGYFLAVEKERDL	FRQAPGKEREEIASISTGTGNTYYAD		MK	Q		
	FKLILKKEDELGDRSIMFTVQ	SVKGRFTISRDKSRNTLYLQMNTLRA		FT	I		
	NEDGGGGSGGGGSDKTHTCPP	EDTAVYYCAADVRPHGTTCHYNSRGQ		DN	D		

CPAPEaaGGPSVFLFPPKPKD	GTQVTVSSEPKSSDKTHTCPPCPAPE		TL N		
TLMISRTPEVTCVVVDVSHED	aaGGPSVFLFPPKPKDTLMISRTPEV		YF O		
PEVKFNWYVDGVEVHNAKTKP	TCVVVDVSHEDPEVKFNWYVDGVEVH		IA :		
REEQYNSTYRVVSVLTVLHQD	NAKTKPREEQYNSTYRVVSVLTVLHQ		ED 8		
WLNGKEYKCKVSNKALPAPIE	DWLNGKEYKCKVSNKALPAPIEKTIS		DE 8		
KTISKAKGQPREPQVYTLPPS	KAKGQPREPQVYTLPPSREEMTKNQV		N)		
REEMTKNQVSLSCAVKGFYPS	SLWCLVKGFYPSDIAVEWESNGQPEN		(S		
DIAVEWESNGQPENNYKTTPP	NYKTTPPVLDSDGSFFLYSKLTVDKS		EQ		
VLDSDGSFFLVSKLTVDKSRW	RWQQGNVFSCSVMHEALHNHYTQKSL		ID		
QQGNVFSCSVMHEALHNRFTQ	SLSPGK (SEQ ID NO:641)		NO		
KSLSLSPGGGGSGGGGSGGG			:7		
GSNWVkVISDLKKIEDLIQSM			8)		
HIDATLYTESnVHPSCKVTAM					
KCFLLELQrISLiSGDASIHD					
TVqNLIILANNSLSSNGNVTE					
SGCKECEELEEKNIKEFLQSF					
VHIVQMFINTS (SEQ ID NO:617)					
F EPKSSDKTHTCPPCPAPELLG	(SEQ ID NO:640)	(SEQ ID NO:648)			
U GPSVFLFPPKPKDTLMISRTP					
S EVTCVVVDVSHEDPEVKFNWY					
E VDGVEVHNAKTKPREEQYNST					
- YRVVSVLTVLHQDWLNGKEYK					
9 CKVSNKALPAPIEKTISKAKG					
2 QPREPQVYTLPPSREEMTKNQ					
6 VSLSCAVKGFYPSDIAVEWES					
NGQPENNYKTTPPVLDSDGSF					
FLVSKLTVDKSRWQQGNVFSC					
SVMHEALHNRFTQKSLSLSPG					
K (SEQ ID NO:1)					
F AAEPVEDNVINFVAMKFIDNT	(SEQ ID NO:639)	(SEQ ID NO:647)	AA I	(SEQ ID NO:99)	(SEQ ID NO:603)
U LYFIAEDDENIEQDYFGKLES			EP E		
S KLSVIRNLNDQVLFIDQGNRP			VE Q		
E LFEDMTDSDVRDNAPRTIFII			DN D		
- SKYGDSQPRGLAVTISVKVEK			VI (		
9 ISTLSVENKIISFKEMNPPDN			NF S		
4 IKDTKSDIIFFQRSVPGHDNK			VA E		
3 VQFESSSYEGYFLAVEKERDL			MK Q		

FKLILKKEDELGDRSIMFTVQ			FI	I			
NEDGGGGSGGGGSDKTHTCPP			DN	D			
CPAPEaaGGPSVFLFPPKPKD			TL	N			
TLMISRTPEVTCVVVDVSHED			YF	O			
PEVKFNWYVDGVEVHNAKTKP			IA	:			
REEQYNSTYRVVSVLTVLHQD			ED	8			
WLNGKEYKCKVSNKALPAPIE			DE	8			
KTISKAKGQPREPQVYTLPPS			N	)			
REEMTKNQVSLSCAVKGFYPS			(S				
DIAVEWESNGQPENNYKTTPP			EQ				
VLDSDGSFFLVSKLTVDKSRW			ID				
QQGNVFSCSVMHEALHNRFTQ			NO				
KSLSLSPGGGGSGGGGSGGG			:7				
GSNWVkVISDLKKIEDLIQSM			8)				
HIDATLYTESnVHPSCKVTAM							
KCFLLELQrISLgSGDASIHD							
TVgNLIILANNSLSSNGNVTE							
SGCKECEELEEKNIKEFLQSF							
VHIVQMFINTS (SEQ ID NO:618)							
F EPKSSDKTHTCPPCPAPEAAG	AAEPVEDNVINFVAMKFIDNTLYFIA		AA	I	(SEQ ID NO:99)		(SEQ ID NO:603)
U GPSVFLFPPKPKDTLMISRTP	EDDENIEQDYFGKLESKLSVIRNLND		EP	E			
S EVTCVVVDVSHEDPEVKFNWY	QVLFIDQGNRPLFEDMTDSDVRDNAP		VE	Q			
E VDGVEVHNAKTKPREEQYNST	RTIFIISKYGDSQPRGLAVTISVKVE		DN	D			
- YRVVSVLTVLHQDWLNGKEYK	KISTLSVENKIISFKEMNPPDNIKDT		VI	(			
9 CKVSNKALPAPIEKTISKAKG	KSDIIFFQRSVPGHDNKVQFESSSYE		NF	S			
4 QPREPQVYTLPPSREEMTKNQ	GYFLAVEKERDLFKLILKKEDELGDR		VA	E			
4 VSLSCAVKGFYPSDIAVEWES	SIMFTVQNEDGGGGSGGGGSDKTHTC		MK	Q			
NGQPENNYKTTPPVLDSDGSF	PPCPAPEaaGGPSVFLFPPKPKDTLM		FI	I			
FLVSKLTVDKSRWQQGNVFSC	ISRTPEVTCVVVDVSHEDPEVKFNWY		DN	D			
SVMHEALHNRFTQKSLSLSPG	VDGVEVHNAKTKPREEQYNSTYRVVS		TL	N			
K (SEQ ID NO:19)	VLTVLHQDWLNGKEYKCKVSNKALPA		YF	O			
	PIEKTISKAKGQPREPQVYTLPPSRE		IA	:			
	EMTKNQVSLWCLVKGFYPSDIAVEWE		ED	8			
	SNGQPENNYKTTPPVLDSDGSFFLYS		DE	8			
	KLTVDKSRWQQGNVFSCSVMHEALHN		N	)			
	HYTQKSLSLSPGGGGSGGGGSGGGG		(S				
	SNWVkVISDLKKIEDLIQSMHIDATL		EQ				

	YTESnVHPSCKVTAMKCFLLELQrIS LgSGDASIHDTVqNLIILANNSLSSN GNVTESGCKECEELEEKNIKEFLQSF VHIVQMFINTS (SEQ ID NO:642)		ID NO :78)			
F U S E - 9 6 6	(SEQ ID NO:19)	(SEQ ID NO:641)			I (SEQ ID E NO:99) Q D (S E) N O : 8 8) S Q D I N O T P S	
F U S E - 9 6 7	AAEPVEDNVINFVAMKFIDNT LYFIAEDDENIEQDYFGKLES KLSVIRNLNDQVLFIDQGNRP LFEDMTDSDVRDNAPRTIFII SKYGDSQPRGLAVTISVKVEK ISTLSVENKIISFKEMNPPDN IKDTKSDIIFFQRSVPGHDNK VQFESSSYEGYFLAVEKERDL FKLILKKEDELGDRSIMFTVQ NEDGGGGSGGGGSDKTHTCPP CPAPEaaGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPS REEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPP VLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNRFTQ KSLSLSPGK (SEQ ID NO:619)	(SEQ ID NO:641)			(SEQ ID NO:613)	
F U S	EPKSsDKTHTCPPCPAPEaaG GPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWY					

E VDGVEVHNAKTKPREEQYNST	AVTLDESGGGLQTPGGALSLVCKASG	QSALTQPSSVSANPGETVKITC	AA		YFGKLkSKLSV	(SEQ ID
- YRVVSVLTVLHQDWLNGKEYK	FDFSSHEMQWVRQAPGKGLEFVAGIG	SGGSYNYGWFQQKSPGSAPVTV	EP		IRNLNDQVLFI	NO:608)
9 CKVSNKALPAPIEKTISKAKG	NMGSFTVYGAAVKGRATISRDNGQST	IYSNNQRPSDIPSRFSGSTSGS	VE		DQGNRPLFEDM	
6 QPREPQVYTLPPSREEMTKNQ	VRLQLNNLRAEDTGTYYCARDFYNSY	TSTLTITGVQADDEAIYYCGSW	DN		TDSDVRDNAPR	
8 VSLSCAVKGFYPSDIAVEWES	GCSASFIDAWGHGTEVIVSSASTKGP	DDSSYVGIFGAGTTLTVLGQPK	VI		TIFIIShYgDS	
NGQPENNYKTTPPVLDSDGSF	SVFPLAPSSKSTSGGTAALGCLVKDY	AAPSVTLFPPSSEELQANKATL	NF		QPRGMAVTISV	
FLVSKLTVDKSRWQQGNVFSC	FPEPVTVSWNSGALTSGVHTFPAVLQ	VCLISDFYPGAVTVAWKADSSP	VA		KVEKISTLSVE	
SVMHEALHNRFTQKSLSLSPG	SSGLYSLSSVVTVPSSSLGTQTYICN	VKAGVETTTPSKQSNNKYAASS	MK		NKIISFKEMNP	
GGGGSGGGGSGGGGSNWVkVI	VNHKPSNTKVDKKVEPKSCDKTHTCP	YLSLTPEQWKSHRSYSCQVTHE	FI		PDNIKDTKSDI	
SDLKKIEDLIQSMHIDATLYT	PCPAPEAAGGPSVFLFPPKPKDTLMI	GSTVEKTVAPTECSGGGGSGGG	DN		IFFQRSVPGHD	
ESnVHPSCKVTAMKCFLLELQ	SRTPEVTCVVVDVSHEDPEVKFNWYV	GSGGGGSGGGGSGGGGSGGGS	TL		NKMQFESSSYE	
rISLiSGDASIHDTVqNLIIL	DGVEVHNAKTKPREEQYNSTYRVVSV	EVQLLESGGGLVQPGGSLRLSC	YF		GYFLAVEKERD	
ANNSLSSNGNVTESGCKECEE	LTVLHQDWLNGKEYKCKVSNKALPAP	TASGYTNRLKCMGWFRQAPGKE	IA		LFKLILKKEDE	
LEEKNIKEFLQSFVHIVQMFI	IEKTISKAKGQPREPQVYTLPPSREE	REEIASISTGTGNTYYADSVKG	En		LGDRSIMFTVQ	
NTS (SEQ ID NO:620)	MTKNQVSLWCLVKGFYPSDIAVEWES	RFTISRDKSRNTLYLQMSTLRA	DE		NED (SEQ ID	
F AAEPVEDNVINFVAMKFIDNT	NGQPENNYKTTPPVLDSDGSFFLYSK	EDTAVYYCAADVRPHGTTCHYN	N		NO:110)	
U LYFIAENDENGGGSYFGKLKS	LTVDKSRWQQGNVFSCSVMHEALHNH	SRGQGTQVTVSS (SEQ ID	(S			
S KLSVIRNLNDQVLFIDQGNRP	YTQKSLSLSPGK (SEQ ID	NO:649)	EQ			
E LFEDMTDSDVRDNAPRTIFII	NO:643)		ID			
- SHYGDSQPRGMAVTISVKVEK			NO			
1 ISTLSVENKIISFKEMNPPDN			:8			
1 IKDTKSDIIFFQRSVPGHDNK			0)			
4 MQFESSSYEGYFLAVEKERDL						
5 FKLILKKEDELGDRSIMFTVQ						
NEDEPKSSDKTHTCPPCPAPE						
AAGGPSVFLFPPKPKDTLMIS						
RTPEVTCVVVDVSHEDPEVKF						
NWYVDGVEVHNAKTKPREEQY						
NSTYRVVSVLTVLHQDWLNGK						
EYKCKVSNKALPAPIEKTISK						
AKGQPREPQVYTLPPSREEMT						
KNQVSLSCAVKGFYPSDIAVE						
WESNGQPENNYKTTPPVLDSD						
GSFFLVSKLTVDKSRWQQGNV						
FSCSVMHEALHNRFTQKSLSL						
SPGGGGSGGGGSGGGGSNWV						
NVISDLKKIEDLIQSMHIDAT						
LYTESNVHPSCKVTAMKCFLL						

	ELQRISLISGDASIHDTVQNL ISLANNSLSSNGNVTESGCKE CEELEEKNIKEFLQSFVHIVQ MFINTS (SEQ ID NO:621)					
FIG. 1144	(SEQ ID NO:621)	AVTLDESGGGLQTPGGALSLVCKASG FDFSSHEMQWVRQAPGKGLEFVAGIG NMGSFTVYGAAVKGRATISRDNGQST VRLQLNNLRAEDTGTYYCARDFYNSY GCSASFIDAWGHGTEVIVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGKGGGGSGGGGSGGGG SGGGGSQVQLQESGGGSVQAGGSLRL SCAASGYIYNNNYMGWFRQAPGKERE GVATIYVGGGSTYYADSVKGRFTISR DNTKNTLYLQMNSLKPEDTAMYYCAA GLVSRPDWVLLARAYTNWGQGTLVTV SS (SEQ ID NO:644)	(SEQ ID NO:649)	(S EQ ID NO		
FIG. 1147	(SEQ ID NO:19)	(SEQ ID NO:643)	(SEQ ID NO:649)			

FUSE-1148 (SEQ ID NO:19)	(SEQ ID NO:644)	(SEQ ID NO:649)				
AVTLDESGGGLQTPGGALSLV		QSALTQPSSVSANPGETVKITC				
CKASGFDFSSHEMQWVRQAPG		SGGSYNYGWFQQKSPGSAPVTV				
KGLEFVAGIGNMGSFTVYGAA		IYSNNQRPSDIPSRFSGSTSGS				
VKGRATISRDNGQSTVRLQLN		TSTLTITGVQADDEAIYYCGSW				
NLRAEDTGTYYCARDFYNSYG		DDSSYVGIFGAGTTLTVLGQPK				
CSASFIDAWGHGTEVIVSSAS		AAPSVTLFPPSSEELQANKATL				
TKGPSVFPLAPSSKSTSGGTA		VCLISDFYPGAVTVAWKADSSP				
ALGCLVKDYFPEPVTVSWNSG		VKAGVETTTPSKQSNNKYAASS				
ALTSGVHTFPAVLQSSGLYSL		YLSLTPEQWKSHRSYSCQVTHE				
SSVVTVPSSSLGTQTYICNVN		GSTVEKTVAPTECS (SEQ ID				
HKPSNTKVDKKVEPKSCDKTH		NO:650)				
TCPPCPAPELLGGPSVFLFPP						
KPKDTLMISRTPEVTCVVVDV						
SHEDPEVKFNWYVDGVEVHNA						
KTKPREEQYNSTYRVVSVLTV						
LHQDWLNGKEYKCKVSNKALP						
APIEKTISKAKGQPREPQVYT						
LPPSREEMTKNQVSLTCLVKG						
FYPSDIAVEWESNGQPENNYK						
TTPPVLDSDGSFFLYSKLTVD						
KSRWQQGNVFSCSVMHEALHN						
HYTQKSLSLSPGK (SEQ ID NO:622)						
FUSE-1148 (SEQ ID NO:621)	EVQVQQSGPELVKPGTSVKISCKTSG	DILMTQSPSAMSVSLGDTVSIT	(SEQ ID NO:8 0)		(SEQ ID NO:110)	(SEQ ID NO:608)
	YTFTEYTMHWVKQSHGESLEWIGGIV	CHASQGISSYIGWLQQKPGKSF				
	PNNGATNYNQKFRGKATLTVDKSSST	KGLIYHGTNLEDGVPSRFSGSG				
	AYMELRSLTSEDSAVYYCARRGLLWH	SGADYSLTISSLESEDFADYYC				
	FAYWGQGTLVTVSAASTKGPSVFPLA	IQYAQFPFTFGSGTKLEIKRTV				
	PSSKSTSGGTAALGCLVKDYFPEPVT	AAPSVFIFPPSDEQLKSGTASV				

1 2 4	VSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQV SLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK (SEQ ID NO:645)	VCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGECGGGGSG GGGSGGGGSGGGGSEVQLLESG GGLVQPGGSLRLSCTASGYTNR LKCMGWFRQAPGKEREEIASIS TGTGNTYYADSVKGRFTISRDK SRNTLYLQMNTLRAEDTAVYYC AADVRPHGTTCHYNSRGQGTQV TVSS (SEQ ID NO:652)				
F U S E - 1 1 2 5	(SEQ ID NO:19) (SEQ ID NO:645)	(SEQ ID NO:652)				
F U S E - 1 1 2 6	AAEPVEDNVINFVAMKFIDNT LYFIAENDENGGGSYFGKLKS KLSVIRNLNDQVLFIDQGNRP LFEDMTDSDVRDNAPRTIFII SHYGDSQPRGMAVTISVKVEK ISTLSVENKIISFKEMNPPDN IKDTKSDIIFFQRSVPGHDNK MQFESSSYEGYFLAVEKERDL FKLILKKEDELGDRSIMFTVQ NEDEPKSSDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMT KNQVSLSCAVKGFYPSDIAVE	(SEQ ID NO:652)	(SEQ ID NO:80)		(SEQ ID NO:110)

WESNGQPENNYKTTPPVLDSD GSFFLVSKLTVDKSRWQQGNV FSCSVMHEALHNRFTQKSLSL SPGK (SEQ ID NO:263)						
FEPKSSDKTHTCPPCPAPEAAG UGPSVFLFPPKPKDTLMISRTP SEVTCVVVDVSHEDPEVKFNWY EVDGVEVHNAKTKPREEQYNST -YRVVSVLTVLHQDWLNGKEYK 1CKVSNKALPAPIEKTISKAKG 1QPREPQVYTLPPSREEMTKNQ 2VSLSCAVKGFYPSDIAVEWES 7NGQPENNYKTTPPVLDSDGSF FLVSKLTVDKSRWQQGNVFSC SVMHEALHNRFTQKSLSLSPG GGGGSGGGGSGGGGSNWVNVI SDLKKIEDLIQSMHIDATLYT ESNVHPSCKVTAMKCFLLELQ RISLISGDASIHDTVQNLISL ANNSLSSNGNVTESGCKECEE LEEKNIKEFLQSFVHIVQMFI NTS (SEQ ID NO:634)	(SEQ ID NO:645)	(SEQ ID NO:652)				(SEQ ID NO:608)
F (SEQ ID NO:621) U S E - 1 1 3 6	EVQLVESGGGLVQAGGSLRLSCAASG RPFSNYAMGWFRQAPGKEREFVAAIS WSGGSTSYADSVKGRFTISRDNAKNT VYLQMSSPKPEDTAIYYCAAQFSGAD YGFGRLGIRGYEYDYWGQGTQVTVSS GGGGSGGGGSGGGGSGGGGSEVQLLE SGGGLVQPGGSLRLSCTASGYTNRLK CMGWFRQAPGKEREEIASISTGTGNT YYADSVKGRFTISRDKSRNTLYLQMS TLRAEDTAVYYCAADVRPHGTTCHYN SRGQGTQVTVSSEPKSSDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMT	(SEQ ID NO:80)		(SEQ ID NO:110)	(SEQ ID NO:608)

	KNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO:646)					
FUSE-11137	(SEQ ID NO:634)	(SEQ ID NO:646)				(SEQ ID NO:608)
FUSE-11138	(SEQ ID NO:263)	(SEQ ID NO:646)		(SEQ ID NO:80)	(SEQ ID NO:110)	
FUSE-11139	(SEQ ID NO:19)	(SEQ ID NO:646)				

Table 3A.1 Polypeptide sequences relating to embodiments involving IL15/IL18 fusion proteins

Note: polypeptide 1 is the "hole" heavy chain; Polypeptide 2 is the "knob" heavy chain; Polypeptide 3 is the light chain.

	Polypeptide 1	Poly-peptide	Poly-peptide

		2	3
FUSE - 1062	DVQLQESGGGLVQAGGSLTLSCAASGVTYSHYWLGWFRQAPGKEREGVATIYANDGTTYYADSVKGRFTVSLDNAKNTLYLQMN RLTPEDTAMYYCALRVNDGSWFSPLQHYHYNYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:623)		
FUSE - 1063	DVQLQESGGGSVQAGGSLRLSCAASGNPYSHYWLGWFRQTPGKEREGVAVIYTGDGDTTYYTDSVKGRFTVSQDNGKNTLYLQ MNNLKPEDTAMYYCALRGNDGSWFSPLQPGHYTYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:624)		
FUSE - 1064	DVQLQESGGGLVQPGGSLRLSCAASGVTDSNGCMGWFRQAPGKERERVASIDSDGQTLYADSVKGRFTISQDNTENTLYLQMNT LKPEDTATYYCAAGATSQPGFSCRILGPGWYTYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:625)		
FUSE - 1065	DVQLQESGGGSVQAGGSLRLSCAVSGYPYSRYWLGWFRQAPGKEREGVATIYTDDGATYYADSVKGRFTVSQDNAKNTLYLQMN SLKPEDTAMYYCALRGNDGTWFSVLQPGHYTVWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:626)		
FUSE - 1072	DVQLQESGGGSVQAGGSLRLSCAASGVTYSHYWLAWFRQAPGKEREGVATIYTNDGTTYYGDSVKGRFTISLDNPKNTLYLQMN SLTPEDTAMYYCALRGNDGSWFSPLQPYHYNYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:627)		
FUSE - 1073	DVQLQESGGGPVQSGGSQRLSCVYSGVTDSNGCMGWFRQAPGKERERVASIDSYGQTLYADSVKGRFTISQDNTENTLYLQMNT LKPEDTATYYCAAGATSQPGFSCRVLGPGWYTYWGQGTQVTVSIEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:628)		
FUSE - 1074	DVQLQESGGGSVQAGGSLRLSCAASGVTYSHYWLGWFRQAPGKEREGVATIYANDGTTYYADSVKGRFTVSLDNAKNTLYLQMN RLTPEDTAMYYCALRVNDGSWFSPLQHYHYNYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:629)		
FUSE -	DVQLQESGGGSVQAGGSLRLSCVASGYSYSHYWLGWFRQAPGKEREGIAVIYTNDGTTYYGDSVKGRFTVSQDNAKNTLYLQMN SLKPEDTAMYYCALRANDGSWFSPLQPGYYTYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT		

1075	PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:630)			
FUSE - 1076	DVQLQESGGGFVQSGGSLRLSCEASGVTVTNGCMAWFRQAPGKERERVASIDEDGQTLYADSVKGRFTISQDNTENTLYLQMNT LKPEDMATYYCAAGARSQPGFSCRILGPGWYTYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:631)			
FUSE - 1077	DVQLQESGGGSVQAGGSLRLSCAVSAYTYGRGCMGWFRQAPGKEREGVAGVDIVGTPTYADSIKGRFTISRDNTKNILYLEMNS LKPEDTAMYYCGAALRPYGGSCLRLGHFDSWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:632)			
FUSE - 1078	QVQLQQSGAELARPGASVKLSCKASGFTFTDHYINWVKQRTGQGLEWIGQIYPGDGNTYYNEKFKGKATLTADKSSSTAYMQLS SLTSEDSAVYFCAFNYGDYTIDFWGQGTSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:633)			(SEQ ID NO:651)
FUSE - 1184	DVQLQESGGGSVQAGGSLRLSCRASGWATNCMGWFRQAPGKQRERVALIDNDGRTIYAESVKGRFTISQENARKALYLQMNSLK PEDTAMYYCAADLDICWVVKDRSFTYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK (SEQ ID NO:638)			
FUSE - 1128	DVQLQESGGGSVQAGGSLRLSCAASGDTSRTYTLMAWFRQAPGKEREGVATTNKWGATTYADSVKGRFTISLDNAKNALYLQMNS LKPEDTAMYYCAAGRDYYANLDPDPRLYAYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO:635)			
FUSE - 1129	DVQLQESGGGSVQAGGSLKLSCAASGYTYSFYCMGWFRQAPGTRKEREGVAVIDADGRTTYADSAKGRFTVSQDEAKEHLYLQM NNLKPEDTAMYYCAAYDCYSGSKWGDSDSFGAWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:636)			
FUSE - 1130	DVQLQESGGGSVQAGGSLRLSCAASRATAYMGWFRQAPGKEREGVACIDSFGSTSFADSVKGRFTISQDRTKNTVVLQMNSLKP EDTAMYYCAARISGENGYIGYCASGAVGPSDYVRWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV			

	FSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:637)		

DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYRYHTFGTG TKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC (SEQ ID NO:651)

Sequences for Fusion Proteins in Figure 12A and 12BB

FUSE-739	QVQLQESGGGSVQAGGSLTLSCVISGDTTSTVYMGWFRQAPGEEREGIASIYGADDSPYYADSVKGRFTIFQDNAKRTVVLQMNSLKPEDTGTYYCAA TTIHFVVGGPWLLAAPNYKYWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:653)
FUSE-740	QVQLQESGGGSVTTGGSLRLSCVASRDPYYMAWFRQAPGKEREGVAAIHSGRTYYAESVKSRFTISRDNAKNTAYLQMNSLRPEDTAMYYCAADLRYD GSWSATKDYHYWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:654)
FUSE-741	QVQLQESGGGSVQAGGSLRLSCAASGYTYSCMGWFRQAPGKEREAVATINTESGTTYYLDSVKGRFTISKDNTKNTLYLQMNSLKPEDTAMYYCAALD GYCGTAAYVRPVSYRYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:655)
FUSE-742	QVQLQESGGGSVQAGGSLRLSCAASRDTYYRGWFRQAPGKEREGVAAIYLGDTYYADSVKGRFTISQDSAKNTVFLEMNSLKVEDTAMYYCAADKKYG SKWYTNDYHYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:656)
FUSE-743	QVQLQESGGESVQAGGSLRLSCAASGYINGGNYMGWFRQAPGKEREGVAAIYIHGRSTYYADSVKGRFTISRDNAKNMVYLQMNSLKPEDTAMYYCAT GLVSRPDWVLLARGYNAWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:657)
FUSE-744	QVQLQESGGGSVQAGGSLRLSCAASGYIRGKMCVGWFRQTPGKEREVVGTVYTGSGAISYGDSVEGRFTISQDNARNTLYLQMNSLSPEDTAMYYCAA TRSVVLNSAGVCVTVPGWYNYWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:658)
FUSE-745	QVQLQESGGGSVQAGGSLRLSCAASGTIYSYYCMGWFRQSPGKEREGVAGIDSYGNIRYSDSAKGRFTISKDNTNNILYLQMNSLEPEDTAMYYCAAE PRRCAYLNVDDRILPALFYDSWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV

	EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:659)
FUSE-746	QVQLQESGGGSVQAGGSLRLSCAASGYTNSINYMAWFRQAPGKEREGVARIYTRDGSAEYLADSVVGRFTISQVNVKNTVFLEMTSLKPEDTAMYYCA AAAGASAYGVLNKDHYNYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:660)
FUSE-747	QVQLQESGGGSVQAGGSLRLSCAGSGYTYSSYSMGWFRQDPGKEREGVAAMNARGDNPYYADSVKGRFTISRDNAKKTLYLQMNSLKPEDTAMYYCAA ARAEPSTVVVSAKGYNYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:661)
FUSE-748	QVQLQESGGGSVQAGGSLRLSCAASGYIYNNNYMGWFRQAPGKEREGVATIYVGGGSTYYADSVKGRFTISRDNTKNTLYLQMNSLKPEDTAMYYCAA GLVSRPDWVLLARAYTNWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:662)
FUSE-749	QVQLQESGGGSVQAGGSLRLSCATSGYPYIGPCMTWFRQAPGKEREWVAFIYKDDGATYYSDSVNGRFTISTDNANNALYLQMNSLKPEDTAVYYCAA SPPSPRTTCYTGASYYNYWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:663)
FUSE-750	QVQLQESGGGSVQAGGSLRLSCAASGYIGGKMCVGWFRQTPGKEREWVGTVYTGSGAISYGDSVEGRFTISQDNARNTLYLQMNSLSPEDTAMYYCAA TRSVVLNSAGVCVTVPGWYNYWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:664)
FUSE-751	QVQLQESGGGSVQAGGSLNLSCTASGYTIGSFSMAWFRQAPGKEREGVAYINTGGVPYYLDSVKGRATISRDNAADTLYLHMNSLKPEDTALYYCAAR PGLGESAWYIDARAYNYWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:665)
FUSE-752	QVQLQESGGGSVQAGGSLRLSCATSERTYTQNWMGWFRQSPGKEREGVAVINTGSGLTAYVDSVKGRFTISKDNTKSTVYLQMNSLKPDDTAMYYCAA EVQGGVLIATGYRYWGQGTLVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:666)
FUSE-753	QVQLQESGGGSVQAGGSLRLSCTTSRFKINDGSVGWFRQAPGKEREGVASIYADGGGISYATDSVKGRFTISQDNSANTVYLQMNSLKPEDTAMYYCAA LALRRYGPLDPREFRFWGQGTQVTVSSEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:667)
4D6	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCVVSGDTYNLNRMAWFRQAPGKEREGVAGTGINDITTAYADSVKGRFTVSNDHAKKSLY LQMNNLKPEDTAMYYCAARSVYLTAPSLRPESYNYWGQGTLVTVSS (SEQ ID NO:668)
4C9	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCAASGYIYGNNYMGWFRQAPGKEREGLATIYINGASTYYADSVKGRFTISRDNTKNTL YLQMNSLKPEDTAMYYCAAGLVSRPDWVLLARAYNNWGQGTLVTVSS (SEQ ID NO:669)

3H8	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCEASGYINSNNYMGWFRQAPGKEREGVAVKYIHGPSTYYADSVKGRFIISQDNAKNMV YLQMNTLKPEDTAMYYCAAGLVSRPDWVLLARGYNNWGQGTQVTVSS (SEQ ID NO:670)
3F8	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQDGGSLRLSCVVSGNTYNLNRMAWFRQAPGKEREGVAGTGINDITGYADSVKGRFTVSVDSAEKTLY LQMNLLKPEDTAMYYCAARSVRLTAPSLRPESYTYWGQGTLVTVSS (SEQ ID NO:671)
5E10	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCVVSGNTYNLNRMAWFRQAPGKEREGVAGTGINDITAYADSVKGRFTVSSDHATKTLH LQMNSLKPEDTGMYYCAARSVYLTAPSLRPESYNYWGQGTQVTVSS (SEQ ID NO:672)
5E7	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCVVSGYTYNLNRMAWFRQAPGKEREGVASTGINDITEYADSVKGRFTVSTDAVKNTLY LQMNSLKPDDTAMYYCAARSVRQTVPSLRPESYTYWGQGTQVTVSS (SEQ ID NO:673)
5B9	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCVVSGNTYNLNRMAWFRQAPGKEREGVAGTGINDITGYADSVKGRFTVSVDSAEKKLY LQMNSLKPEDTAMYYCAARSVRLTAPSLRPESYTYWGQGTQVTVSS (SEQ ID NO:674)
2C8	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCTTSRFKINDGSVGWFRQAPGKEREGVASVYADGGGISYADSVKGRFTTISQDNSANTV YLQMNSLKPEDTAMYYCAALALRRYGPLDPREFRFWGQGILVTVSS (SEQ ID NO:675)
3A6	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQAGGSLRLSCAASGYIGGKMCVGWFRQTPGKEREWVGTVYTGSGAISYGDSVEGRFTISQDNARNTL YLQMNSLSPEDTAMYYCAATRSVVLNSAGVCVTVPGWYNYWGQGTQVTVSS (SEQ ID NO:676)
1B8	MGSTAILGLLLAVLQGGRAQVQLQESGGGSVQPGGSLTLSCRASEYSMSFIGWFRQAPGKEREKVATIYTRSGRTYYADSVRGRFTISQDNAKNTAYL QMNSLKPEDTAMYYCAAGSYGGPWSALDSRHFNYWGQGTLVTVSS (SEQ ID NO:677)

[0245] Various embodiments provide for a polynucleotide encoding an IL-15 variant of the present invention described herein. For example, the nucleic acid sequences may encode in a 5' to 3' direction, the IL-15 variant or the IL-15 fusion protein.

[0246] Various embodiments provide for a polynucleotide encoding an IL-15 fusion protein of the present invention described herein. For example, the nucleic acid sequences may encode in a 5' to 3' direction, the IL-15 variant.

[0247] As such, the polynucleotide encoding an IL-15 fusion protein of the present invention comprises polynucleotide 1, polynucleotide 2 and polynucleotide 3 listed in Table 2A. In other embodiments, the polynucleotide encoding an IL-15 fusion protein of the present invention comprises polynucleotide 1, polynucleotide 2 and polynucleotide 3 listed in Table 2A, except the nucleotides encoding the linker encodes a different linker.

[0248] For example, the linker can be a flexible linker that is generally about 10 to 25 amino acids in length. Additional examples of linkers include but are not limited to a dimer of two amino acids, a tri-mer of three amino acids, or a peptide selected from the group consisting of T, PT, MPT, S, GS, GGS, GGGS (SEQ ID NO:235), ($GGGGX_\lambda$ (SEQ ID NO:236))n, and ($X_\lambda GGGG$ (SEQ ID NO:317))n wherein X_λ is Q, A, E or S and n=1-5 or an integer larger than 5. In some embodiments, the amino acid linker has the amino acid sequence of (GGGGS (SEQ ID NO:237))n where n is an integer between 1 and 5, thereby an amino acid linker of 25 amino acids or shorter in length. As such, the nucleotides encoding the linker in polynucleotide 2 will encode one of these aforementioned linkers instead.

[0249] Exemplary polynucleotides are in Table 2A.

Table 2A Polynucleotides (IL-15 embodiments)

Note: Polynucleotide 1 encodes the "hole" heavy chain; Polynucleotide 2 encodes the "knob" heavy chain, a linker, and an IL-15 variant, Polynucleotide 3 encodes the light chain.

	Polynucleotide 1	Polynucleotide 2	Polynucleotide 3
F	CAGGTTCAGCTCGTCGAAAGCGGAGGAG	CAGGTTCAGCTCGTCGAAAGCGGAGGAGGTCGTTCAGCCAGGAAGATCCCT	GAAATCGTGCTGACCCAGT
U	GAGTCGTTCAGCCAGGAAGATCCCTCAG	CAGGCTCGACTGTAAGGCCTCCGGAATCACCTTCTCCAACTCCGGAATGCACT	CCCCCGCTACCTTGAGTCT
S	GCTCGACTGTAAGGCCTCCGGAAATCACC	GGGTCAGGCAGGCTCCTGGAAAGGACTGGAGTGGGTGGCTGTGATTTGGTAC	GTCCCCTGGAGAGAGAGCC
E	TTCTCCAACTCCGGAATGCACTGGGTCA	GACGGCTCCAAGAGATACTACGCCGACTCTGTGAAGGGCCGGTTCACTATCTC	ACCCTGTCTTGTAGGGCTA
-	GGCAGGCTCCTGGAAAAGGACTGGAGTG	CAGGGACAACTCCAAGAACACACTGTTCCTGCAGATGAACTCCCTGCGGGCTG	GTCAGTCCGTGAGCAGCTA
6	GGTGGCTGTGATTTGGTACGACGGCTCC	AGGATACTGCCGTTTATTACTGCGCCACCAACGATGACTACTGGGGACAGGGA	TCTGGCCTGGTATCAGCAG
9	AAGAGATACTACGCCGACTCTGTGAAGG	ACCTTGGTGACCGTGAGTAGCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	AAGCCCGGCCAGGCTCCTA
6	GCCGGTTCACTATCTCCAGGGACAACTC	GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	GGTTGTTGATTTACGATGC
	CAAGAACACACTGTTCCTGCAGATGAAC	TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	CTCCAACAGAGCCACCGGA
	TCCCTGCGGGCTGAGGATACTGCCGTTT	ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	ATCCCTGCACGGTTCTCCG
	TCCCCCTGGCACCCTCCTCCAAGAGCAC	CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	TCGCCGTTTACTACTGCCA
	CTCTGGGGGCACAGCGGCCCTGGGCTGC	TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGACGTGAGCCACGAA	GCAGTCCTCCAACTGGCCC
	CTGGTCAAGGACTACTTCCCCGAACCGG	GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	AGGACATTCGGTCAGGGCA
	TGACGGTGTCGTGGAACTCAGGCGCCCT	CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	CCAAGGTGGAGATCAAGCG
	GACCAGCGGCGTGCACACCTTCCCGGCT	TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	TACGGTGGCTGCACCATCT
	GTCCTACAGTCCTCAGGACTCTACTCCC	GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	GTCTTCATCTTCCCGCCAT
	TCAGCAGCGTGGTGACCGTGCCCTCCAG	AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	CTGATGAGCAGTTGAAATC
	CAGCTTGGGCACCCAGACCTACATCTGC	TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	TGGAACTGCCTCTGTTGTG
	AACGTGAATCACAAGCCCAGCAACACCA	GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	TGCCTGCTGAATAACTTCT
	AGGTGGACAAGAAAGTTGAGCCCAAATC	CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	ATCCCAGAGAGGCCAAAGT
	TTGTGACAAAACTCACACATGCCCACCG	CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	ACAGTGGAAGGTGGATAAC
	TGCCCAGCACCTGAAGCCGCAGGGGAC	CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	GCCCTCCAATCGGGTAACT
	CGTCAGTCTTCCTCTTCCCCCAAAACC	TGGTGGTGGCGGATCAGGTGGGGAGGCTCTGGTGGAGGCGGTAGTGGATGGG	CCCAGGAGATGTGTCACAGA
	CAAGGACACCCTCATGATCTCCCGGACC	TTAACGTGATCAGCGACTTGAAGAAGATCGAGGATTTGATCCAGAGCATGCAC	GCAGGACAGCAAGGACAGC
	CCCGAGGTCACATGCGTGGTGGACG	ATCGACGCTACTTTGTATACTGAGAGTAACGTGCACCCCTCCTGCAAGGTTAC	ACCTACAGCCTCAGCAGCA
	TGAGCCACGAAGACCCTGAGGTCAAGTT	TGCTATGAAGTGTTTCCTGCTCGAACTGCAGAGAATCTCCCTGGGCTCCGGAG	CCCTGACGCTGAGCAAAGC
	CAACTGGTACGTGGACGGCGTGGAGGTG	ATGCAAGCATCCATGACACCGTGCAGAACTTGATTATCCTGGCTAACAACTCC	AGACTACGAGAAACACAAA
	CATAATGCCAAGACAAAGCCGCGGGAGG	CTGTCCTCCAACGGCAACGTGACCGAATCCGGATGCAAAGAGTGCGAGGAGCT	GTCTACGCCTGCGAAGTCA

AGCAGTACAACAGCACGTACCGTGTGGT CAGCGTCCTCACCGTCCTGCACCAGGAC TGGCTGAATGGCAAGGAGTACAAGTGCA AGGTCTCCAACAAAGCCCTCCCAGCCCC CATCGAGAAAACCATCTCCAAAGCCAAA GGGCAGCCCCGAGAACCACAGGTGTACA CCCTGCCCCCATCCCGGGAGGAGATGAC CAAGAACCAGGTCAGCCTGAGCTGCGCC GTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCC GGAGAACAACTACAAGACCACGCCTCCC GTGCTGGACTCCGACGGCTCCTTCTTCC TCGTGAGCAAGCTCACCGTGGACAAGAG CAGGTGGCAGCAGGGGAACGTCTTCTCA TGCTCCGTGATGCATGAGGCTCTGCACA ACAGGTTCACGCAGAAGAGCCTCTCCCT GTCTCCGGGTAAA (SEQ ID NO:678)	CGAAGAGAAAAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA TGTTCATCAACACCTCC (SEQ ID NO:681)	CCCATCAGGGCCTGAGTTC GCCCGTCACAAAGAGCTTC AACAGGGGAGAGTGT (SEQ ID NO:188)
FUSE-7642	CAGGTTCAGCTCGTCGAAAGCGGAGGAG GAGTCGTTCAGCCAGGAAGATCCCTCAG GCTCGACTGTAAGGCCTCCGGAATCACC TTCTCCAACTCCGGAATGCACTGGGTCA GGCAGGCTCCTGGAAAAGGACTGGAGTG GGTGGCTGTGATTTGGTACGACGGCTCC AAGAGATACTACGCCGACTCTGTGAAGG GCCGGTTCACTATCTCCAGGGACAACTC CAAGAACACACTGTTCCTGCAGATGAGC AGTTTGAGGGCCGAGGACACCGCTGTTT ACTACTGCGCAACCAACGATGACTACTG GGGGCAGGGAACCCTGGTTACAGTGAGT AGCGCTAGCACCAAGGGCCCATCGGTCT TCCCCCTGGCACCCTCCTCCTCCAAGACAC CTCTGGGGGCACAGCGGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAACCGG TGACGGTGTCGTGGAACTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAG	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	(SEQ ID NO:188)

CAGCTTGGGCACCCAGACCTACATCTGC	TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
AACGTGAATCACAAGCCCAGCAACACCA	GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
AGGTGGACAAGAAAGTTGAGCCCAAATC	CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
TTGTGACAAAACTCACACATGCCCACCG	CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
TGCCCAGCACCTGAAGCCGCAGGGGAC	CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
CGTCAGTCTTCCTCTTCCCCCAAAACC	TGGTGGTGGCGGATCAGGTGGGGAGGCTCTGGTGGAGGCGGTAGTGGATGGG	
CAAGGACACCCTCATGATCTCCCGGACC	TTAACGTGATTTCTGACCTGAAGAAGATCGAGGATCTGATTCAGAGTATGCAC	
CCCGAGGTCACATGCGTGGTGGTGGACG	ATTGATGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC	
TGAGCCACGAAGACCCTGAGGTCAAGTT	AGCAATGAAGTGCTTCCTGCTCGAACTGCAGAGAATCTCCCTGAAGTCCGGCG	
CAACTGGTACGTGGACGGCGTGGAGGTG	ACGCCTCCATCCATGACACAGTCCAGAACCTGATCATCCTGGCAAACAACTCC	
CATAATGCCAAGACAAAGCCGCGGGAGG	CTGAGCTCCAACGGCAACGTGACCGAGTCCGGTTGCAAAGAATGCGAGGAGCT	
AGCAGTACAACAGCACGTACCGTGTGGT	GGAGGAGAAAAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
CAGCGTCCTCACCGTCCTGCACCAGGAC	TGTTCATCAACACCTCC (SEQ ID NO:682)	
TGGCTGAATGGCAAGGAGTACAAGTGCA		
AGGTCTCCAACAAAGCCCTCCCAGCCCC		
CATCGAGAAAACCATCTCCAAAGCCAAA		
GGGCAGCCCCGAGAACCACAGGTGTACA		
CCCTGCCCCCATCCCGGGAGGAGATGAC		
CAAGAACCAGGTCAGCCTGAGCTGCGCC		
GTCAAAGGCTTCTATCCCAGCGACATCG		
CCGTGGAGTGGGAGAGCAATGGGCAGCC		
GGAGAACAACTACAAGACCACGCCTCCC		
GTGCTGGACTCCGACGGCTCCTTCTTCC		
TCGTGAGCAAGCTCACCGTGGACAAGAG		
CAGGTGGCAGCAGGGGAACGTCTTCTCA		
TGCTCCGTGATGCATGAGGCTCTGCACA		
ACAGGTTCACGCAGAAGAGCCTCTCCCT		
GTCTCCGGGTAAA (SEQ ID NO:679)		
FUSE-7 6 5 (SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
	CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
	GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
	GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
	CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
	AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
	ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
	GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
	TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	

		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTGGATGGG	
		TTAACGTGATTTCTGACCTGAAGAGATCGAGGATCTGATTCAGAGTATGCAC	
		ATTGATGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC	
		AGCAATGAAGTGCTTCCTGCTCGAACTGCAGAGAATCTCCCTGAAGTCCGGCG	
		ACGCCTCCATCCATGACACAGTCCAGAACCTGATCATCCTGGCAAACAACTCC	
		CTGAGCTCCAACGGCAACGTGACCGAGTCCGGTTGCAAAGAATGCGAGGAGCT	
		GGAGGAGAAAAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCTCC (SEQ ID NO:683)	(SEQ ID NO:188)
FIG. 766	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	
		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	

	TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
	GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
	CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
	TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
	GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
	AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
	TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
	GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
	CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
	CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
	CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
	TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTGGATGGG	
	TTAACGTGATTTCTGACCTGAAGAAGATCGAGGATCTGATTCAGAGTATGCAC	
	ATTGATGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC	
	AGCAATGAAGTGCTTCCTGCTCGAACTGCAGAGAATCTCCCTGAAGTCCGGCG	
	ACGCCTCCATCCATGACACAGTCCAGAACCTGATCATCCTGGCAAACAACTCC	
	CTGAGCTCCAACGGCAACGTGACCGAGTCCGGTTGCAAAGAATGCGAGGAGCT	
	GGAGGAGAAAAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
	TGTTCATCAACACCTCC (SEQ ID NO:684)	
FUSE-767	(SEQ ID NO:679)	(SEQ ID NO:188)
	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	
	CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
	GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
	GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
	CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
	AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
	ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
	GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
	TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
	ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
	CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
	TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
	CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
	CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
	TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGACGTGAGCCACGAA	
	GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
	CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
	TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
	GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	

		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTGGATGGG	
		TTAACGTGATTTCTGACCTGAAGAAGATCGAGGATCTGATTCAGAGTATGCAC	
		ATTGATGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC	
		AGCAATGAAGTGCTTCCTGCTCGAACTGCAGAAGATCAGCCTGAAGTCCGGCG	
		ACGCCTCCATCCATGACACAGTCCAGAACCTGATCATCCTGGCAAACAACTCC	
		CTGAGCAGCAACGGCAACGTGACCGAGTCCGGTTGCAAAGAATGCGAGGAACT	
		GGAAGAGAAGAACATCAAGGAGTTCCTCCAGAGTTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCAGC (SEQ ID NO:685)	
FIG. 768	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	

		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTGGATGGG TTAACGTGATTTCTGACCTGAAGAAGATCGAGGATCTGATTCAGAGTATGCAC ATTGATGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC AGCAATGAAGTGCTTCCTGCTCGAACTGCAGAGAATCTCCCTGAAGTCCGGCG ACGCCTCCATCCATGACACAGTCCAGAACCTGATCATCCTGGCAAACAACTCC CTGAGCTCCAACGGCAACGTGACCGAGTCCGGTTGCAAAGAATGCGAGGAGCT GGAGGAGAAAAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA TGTTCATCAACACCTCC (SEQ ID NO:686)	(SEQ ID NO:188)
FUSE-772	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT CAGGCTGGACTGCCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG TGAACGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGGGCTCCGGCG

		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCATCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCTCC (SEQ ID NO:687)	
FIG. 7	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGCCG	
		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGAGGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAAAGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGGGCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCATCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCTCC (SEQ ID NO:688)	
FIG. 7	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)

US-E-7-7-4		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAACGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGGGCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCTCCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCTCC (SEQ ID NO:689)	(SEQ ID NO:188)
FUSE-1-7-7-8	CAGGTTCAACTCGTCGAGTCAGGCGGAG	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	
	GAGTCGTTCAACCAGGAAGAAGCCTCAA	CAAACTCGACTGCAAAGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
	ACTCGACTGCAAAGCAAGCGGCATCACC	GGGTCAGGCAGGCACCTGGAAAAGGAGACTGGAGTGGGTGGCCGTGATCTGGTAC	
	TTCAGCAACAGCGGAATGCACTGGGTGA	GACGGATCAAAGAGGTACTACGCCGACAGTGTCAAAGGCAGGTTTACTATCTC	
	GACAAGCACCCGGAAAGGGACTGGAGTG	TAGGGACAACAGCAAGAACACACTGTTCCTGCAGATGAGCTCCCTGAGGGCCG	
	GGTTGCTGTTATTTGGTACGATGGCAGC	AGGATACCGCTGTTTACTACTGCGCAACCAACGACGACTACTGGGGCCAGGGA	

0	AAAAGATACTACGCCGACAGTGTGAAAG
2	GCAGATTTACAATCTCAAGGGACAACAG
	CAAGAACACACTGTTCCTGCAGATGAGC
	AGCCTGAGAGCCGAGGATACCGCAGTTT
	ACTACTGCGCCACCAACGACGACTACTG
	GGGACAAGGAACCCTGGTGACCGTGAGT
	AGCGCTAGCACCAAGGGCCCATCGGTCT
	TCCCCCTGGCACCCTCCTCCTCCAAGAGCAC
	CTCTGGGGCACAGCGGCCCTGGGCTGC
	CTGGTCAAGGACTACTTCCCCGAACCGG
	TGACGGTGTCGTGGAACTCAGGCGCCCT
	GACCAGCGGCGTGCACACCTTCCCGGCT
	GTCCTACAGTCCTCAGGACTCTACTCCC
	TCAGCAGCGTGGTGACCGTGCCCTCCAG
	CAGCTTGGGCACCCAGACCTACATCTGC
	AACGTGAATCACAAGCCCAGCAACACCA
	AGGTGGACAAGAAAGTTGAGCCCAAATC
	TTGTGACAAAACTCACACATGCCCACCG
	TGCCCAGCACCTGAAGCCGCAGGGGGAC
	CGTCAGTCTTCCTCTTCCCCCCAAAACC
	CAAGGACACCCTCATGATCTCCCGGACC
	CCCGAGGTCACATGCGTGGTGGTGGACG
	TGAGCCACGAAGACCCTGAGGTCAAGTT
	CAACTGGTACGTGGACGGCGTGGAGGTG
	CATAATGCCAAGACAAAGCCGCGGGAGG
	AGCAGTACAACAGCACGTACCGTGTGGT
	CAGCGTCCTCACCGTCCTGCACCAGGAC
	TGGCTGAATGGCAAGGAGTACAAGTGCA
	AGGTCTCCAACAAAGCCCTCCCAGCCCC
	CATCGAGAAAACCATCTCCAAAGCCAAA
	GGGCAGCCCCGAGAACCACAGGTGTACA
	CCCTGCCCCCATCCCGGGAGGAGATGAC
	CAAGAACCAGGTCAGCCTGAGCTGCGCC
	GTCAAAGGCTTCTATCCCAGCGACATCG
	CCGTGGAGTGGGAGAGCAATGGGCAGCC
	GGAGAACAACTACAAGACCACGCCTCCC
	GTGCTGGACTCCGACGGCTCCTTCTTCC
	TCGTGAGCAAGCTCACCGTGGACAAGAG

ACCCTGGTTACTGTGAGCTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT
GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG
TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG
ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC
CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA
TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG
CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC
CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA
TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC
CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG
TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA
AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA
TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC
CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA
CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG
TTCTGGTGGAGGAGGTTCTGGAGGTGGTGGATCAGGAGGAGGAGGAGGATGGG
TTAACGTGATTTCTGACCTGAAGAAGATCGAGGATCTGATTCAGAGTATGCAC
ATTGCTGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC
AGCAATGAAGTGCTTCCTGTTGGAGCTGCAGAGGATCTCCCTGGGCTCCGGTG
ATGCAAGCATCCACGACACTGTGCAGAACCTCATTATCCTGGCTAACAACTCC
CTGAGCAGCAACGGCAACGTGACCGAGTCCGGTTGCAAAGAGTGCGAGGAGCT
CGAAGCCAAGAATATTAAGGAGTTTCGTGCAGAGTTTCGTGCACATCGTGCAGA
TGTTCATCAACACCTCC (SEQ ID NO:690)

CAGGTGGCAGCAGGGGAACGTCTTCTCA TGCTCCGTGATGCATGAGGCTCTGCACA ACAGGTTCACGCAGAAGAGCCTCTCCCT GTCTCCGGGTAAA (SEQ ID NO:680)		
F (SEQ ID NO:680)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
U	CAAACTCGACTGCAAAGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
S	GGGTCAGGCAGGCACCTGGAAAAGGAGACTGGAGTGGGTGGCCGTGATCTGGTAC	
E	GACCGATCAAAGAGGTACTACGCCGACAGTGTCAAAGGCAGGTTTACTATCTC	
-	TAGGGACAACAGCAAGAACACACTGTTCCTGCAGATGAGCTCCCTGAGGGCCG	
8	AGGATACCGCTGTTTACTACTGCGCAACCAACGACGACTACTGGGGCCAGGGA	
0	ACCCTGGTTACTGTGAGCTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
3	GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
	TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
	ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
	CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
	TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
	CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
	CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCA	
	TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGACGTGAGCCACGAA	
	GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
	CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
	TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
	GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
	AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
	TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
	GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
	CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
	CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
	CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
	TTCTGGTGGAGGAGGTTCTGGAGGTGGTGGATCAGGAGGAGGAGGAGGATGGG	
	TTAACGTGATTTCTGACCTGAAGAAGATCGAGGATCTGATTCAGAGTATGCAC	
	ATTGATGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC	
	AGCAATGAAGTGCTTCCTGCTCGAACTGCAGAGAATCTCCCTGGGTAGCGGCG	
	ACGCTTCCATCCATGACACCGTCCAGAACCTGATCATCCTGGCAAACAACTCC	
	CTGAGCAGCAACGGCAACGTGACCGAGTCCGGTTGCAAAGAGTGCGAGGAGCT	
	GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCCTCGTGCACATCGTGCAGA	
	TGTTCATCAACACCTCC (SEQ ID NO:691)	

FIG. 804	(SEQ ID NO:680)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
		CAAACTCGACTGCAAAGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTCAGGCAGGCACCTGGAAAAGGACTGGAGTGGGTGGCCGTGATCTGGTAC	
		GACGGATCAAAGAGGTACTACGCCGACAGTGTCAAAGGCAGGTTTACTATCTC	
		TAGGGACAACAGCAAGAACACACTGTTCCTGCAGATGAGCTCCCTGAGGGCCG	
		AGGATACCGCTGTTTACTACTGCGCAACCACGACGACTACTGGGGCCAGGGA	
		ACCCTGGTTACTGTGAGCTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TTCTGGTGGAGGAGGTTCTGGAGGTGGTGGATCAGGAGGAGGAGGATGGG	
		TTAACGTGATTTCTGACCTGAAGAAGATCGAGGATCTGATTCAGAGTATGCAC	
		ATTGATGCTACACTGTATACAGAGAGCAACGTGCACCCCAGCTGTAAGGTGAC	
		AGCAATGCAGTGCTTCCTGTCCGAGTTGCAGAGAATCTCCCTGGGCTCCGGCG	
		ACGCTAGTATCCATGACACCGTGCAGAACCTCACCATCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCTACGTCACCGAGTCCGGTTGCAAGGAGTGCGAGGAGCT	
		GGAAGCCAAGAACATCAAGGAGTTCCTGCAGAGTTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCAGT (SEQ ID NO:692)	
FIG. 805	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	

8		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
4		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
6		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAACGTGATCTCCGACCTGAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGATCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCTCCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCTCC (SEQ ID NO:693)	
F	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
U		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
S		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
E		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
-		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
8		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
4		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
7		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	

	(SEQ ID NO:679)	CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	(SEQ ID NO:188)
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAAAGTGATCTCCGACCTGAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGATCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCTCCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
		TGTTCATCAACACCTCC (SEQ ID NO:694)	
FUSE-8488		CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	
		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
		AGGACACCGCGTTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	

		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAACGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGATCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCTCCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGACCA	
		TGTTCATCAACACCTCC (SEQ ID NO:695)	(SEQ ID NO:188)
FUSE-849	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	
		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	

		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAACGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGATCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCTCCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA	
		TGTTCATCAGGACCTCC (SEQ ID NO:696)	
FUSE-850	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
		CAGGCTGGACTGCCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCCAGGTTCACCATCAG	
		CAGGGACAATAGCCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	

		TGGTGGTGGCGGATCAGGTGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAACGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGATCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCTCCCTGGCCAACAACTCC	
		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT	
		GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGACCA	
		TGTTCATCAGGACCTCC (SEQ ID NO:697)	
FIG. 8	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT	(SEQ ID NO:188)
		CAGGCTGGACTGCAAGGCATCAGGCATCACATTCTCCAACTCCGGCATGCACT	
		GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC	
		GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG	
		CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG	
		AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA	
		ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT	
		GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG	
		TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG	
		ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC	
		CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA	
		TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG	
		CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC	
		CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA	
		TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGACGTGAGCCACGAA	
		GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC	
		CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG	
		TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG	
		GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA	
		AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCGGGAGGAGA	
		TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC	
		GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC	
		CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA	
		CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG	
		CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG	
		TGGTGGTGGCGGATCAGGTGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG	
		TGAAAGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC	
		ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC	
		CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGATCTCCGGCG	
		ACGCCTCCATCCACGACACCGTGCAGAACCTGATCATCCTGGCCAACAACTCC	

		CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA TGTTCATCAACACCTCC (SEQ ID NO:698)	
FIG. 85	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCT CAGGCTGGACTGCAAGGCATCAGGCATCACACATTCTCCAACTCCGGCATGCACT GGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCTGGTAC GATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAG CAGGGACAATAGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGAGGGCCG AGGACACCGCCGTTTATTACTGCGCCACCAACGACGACTACTGGGGCCAGGGA ACCTTGGTGACCGTGAGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCT GGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGC CGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA TGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCG TCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA TGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG TGGTGGTGGCGGATCAGGTGGGGAGGAGGCTCTGGTGGAGGCGGTAGTAACTGGG TGCTGGTGATCTCCGACCTGAAAAAGATCGAGGACCTGATCCAGTCCATGCAC ATCGACGCCACCCTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGGTGAC CGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCCCTGATCTCCGGCG ACGCCTCCATCCACGACACCGTGCAGAACCTGATCATCCTGGCCAACAACTCC CTGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAGCT GGAGGAGAAGAACATCAAGGAGTTCCTGCAGTCCTTCGTGCACATCGTGCAGA TGTTCATCAACACCTCC (SEQ ID NO:699)	(SEQ ID NO:188)

Table 2B Polynucleotides

Note: Polynucleotide 1 encodes the heavy chain; Polynucleotide 2, while left blank, is identical to Polynucleotide 1; Polynucleotide 3 encodes the light chain.

	Polynucleotide 1	Poly-nucleotide 2	Poly-nucleotide 3
FUSE-691	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTCAGCCAGGAAGGAGTCTCAGGCTGGACTGCAAGGCATCAGG CATCACATTCTCCAACTCCGGCATGCACTGGGTGAGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAGTGATCT GGTACGATGGAAGCAAAAGATACTACGCCGACAGCGTCAAGGGCAGGTTCACCATCAGCAGGGACAATAGCAAGAAC ACCCTGTTCTTGCAGATGAATAGCTTGAGGGCCGAGGACACCGCCGTGTATTACTGCGCTACCAACGACGACTACTG GGGCCAGGGAACCCTGGTTACCGTGAGTAGCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCA AGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG AACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG CGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCA AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGCCGCA GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACATG CGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATG CCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAA AGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCA GCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAG CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC TCTCCCTGTCTCCGGGTAAA (SEQ ID NO:129)		(SEQ ID NO:188)

Table 3B – Polynucleotide Sequences – IL18/IL15

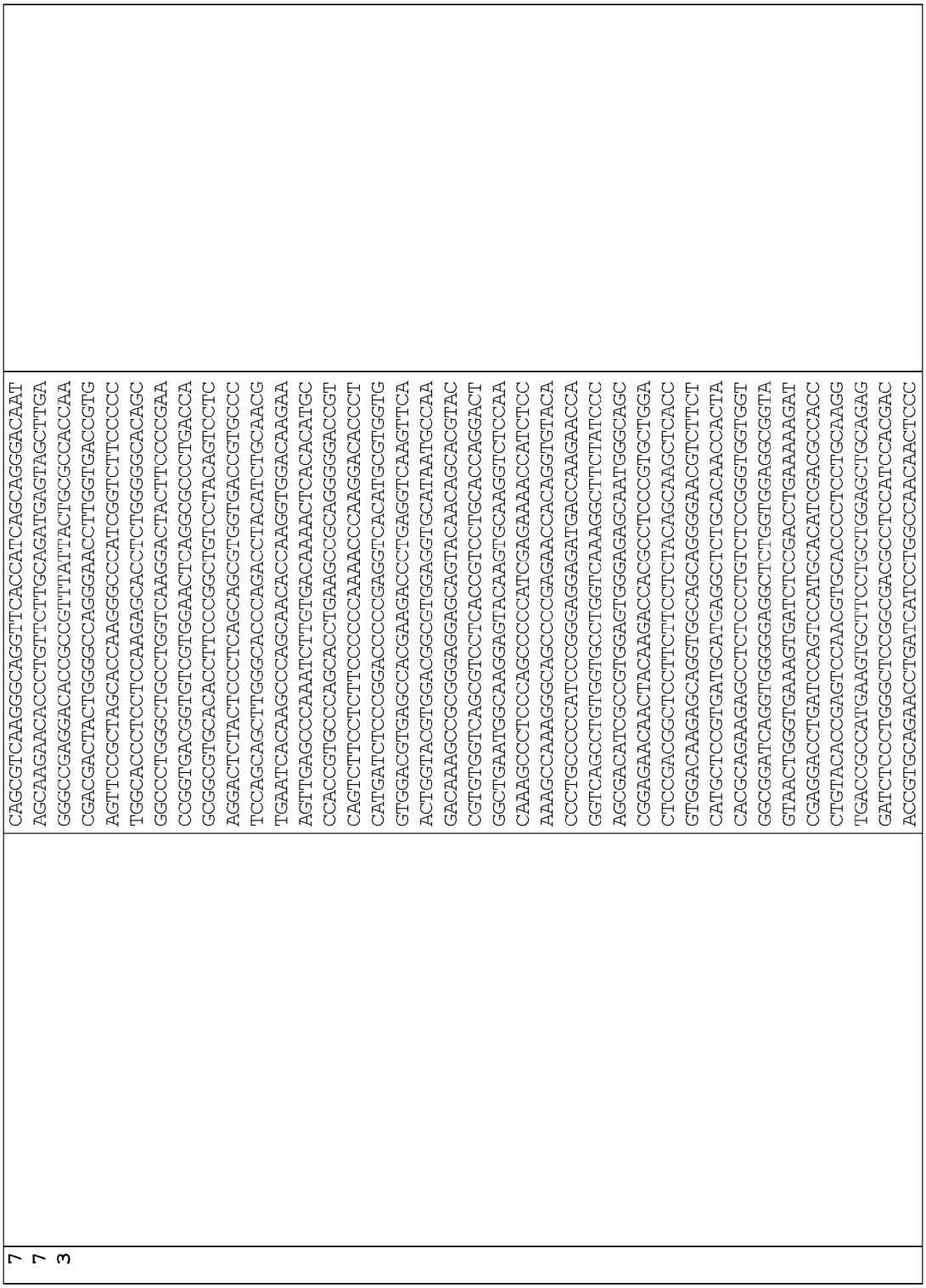
Note: Polynucleotide 1 encodes the "hole" heavy chain; Polynucleotide 2 encodes the "knob" heavy chain; either polynucleotide 1 or polynucleotide 2 encodes a linker, an IL-18 variant, and an IL-15 variant; Polynucleotide 3 encodes the light chain.

	Polynucleotide 1	Polynucleotide 2	Polynucleotide 3
FUSE-	GAGGTGCAGCTGGTGGAGAGCGGGGGGGGACTG GTGCAGGCTGGAGGAAGCCTGAGACTGAGCTGT GCCGCCAGCGGGAGAACATTTAGCAGCTACGCC ATGGGATGGTTTAGACAGGCCCCCGGAAAAGAG AGAGAGTTCGTGGTGGCCATCAACTGGAGCAGC	GAGGTGCAGGTGCAGCAGAGCGGACCAGAGCTGGTGA AGCCAGGAACAAGCGTGAAGATTAGCTGTAAGACAAG CGGATATACATTCACAGAGTATACAATGCACTGGGTG AAGCAGAGCCATGGCGAGAGTCTGGAATGGATCGGAG GAATTGTGCCCAACAACGGAGCCACCAACTACAACCA	GACATTCTGATGACCCAGTCCCCCTCCGC CATGTCCGTGAGCCTGGGCGACACCGTGA GCATCACCTGCCACGCCTCCCAGGGCATC TCCTCCTATATCGGCTGGCTGCAGCAGAA ACCCGGCAAGTCCTTCAAGGGCCTGATCT

3	GGAAGCACATACTACGCCGACAGTGTGAAGGGA	GAAATTCAGGGGCAAAGCCACCCTGACCGTGGACAAG	ACCACGGCACCAATCTGGAGGACGGCGTG
2	AGATTCACCATCAGCAGACACAACGCCAAGAAC	TCCAGCAGCACCGCATACATGGAGCTGAGGAGCCTGA	CCCTCCCGGTTCTCCGGATCTGGCTCTGG
0	ACCATGTACCTGCAGATGAACAGCCTGAAGCCT	CCAGCGAAGACTCCGCCGTGTACTACTGCGCCAGAAG	CGCCGACTACTCCCTGACTATCTCCTCCC
	GAAGACACCGCCGTGTACTATTGCGCCGCCGGC	GGGCCTGCTGTGGCACTTCGCCTATTGGGGCCAGGGA	TGGAATCCGAAGACTTCGCCGACTACTAC
	TACCAGATCAATAGCGGAAACTACAACTTCAAA	ACCCTGGTGACCGTGTCCGCCGCTAAGACCACCCCCC	TGCATCCAGTACGCCCAGTTTCCCTTCAC
	GACTACGAGTATGACTACTGGGGGCAGGGCACC	CCTCCGTGTATCCCCTGGCCCCTGGAAGTGCCGCCCA	CTTCGGCTCCGGCACCAAGCTGGAGATCA
	CAGGTGACCGTGAGCAGCGAGCCCAAATCTAGC	GACAAACTCCATGGTGACCCTGGGCTGTCTGGTGAAA	AGAGAGCAGATGCTGCACCAACTGTATCC
	GACAAAACTCACACATGCCCACCGTGCCCAGCA	GGATACTTCCCCGAACCGTGACCGTGACCTGGAACT	ATCTTCCCACCATCCAGTGAGCAGTTAAC
	CCTGAAGCCGCAGGGGGACCGTCAGTCTTCCTC	CCGGCTCCCTGAGCTCCGGCGTGCACACCTTTCCCGC	ATCTGGAGGTGCCTCAGTCGTGTGCTTCT
	TTCCCCCCAAAACCCAAGGACACCCTCATGATC	CGTGCTGCAGTCCGACCTGTATACCCTGTCTTCCTCC	TGAACAACTTCTACCCCAAAGACATCAAT
	TCCCGGACCCCCGAGGTCACATGCGTGGTGGTG	GTGACCGTGCCCTCCTCCACCTGGCCCTCCGAGACTG	GTCAAGTGGAAGATTGATGGCAGTGAACG
	GACGTGAGCCACGAAGACCCTGAGGTCAAGTTC	TGACCTGCAATGTGGCCACACCCGCTTCCTCCACCAA	ACAAAATGGCGTCCTGAACAGTTGGACTG
	AACTGGTACGTGGACGGCGTGGAGGTGCATAAT	GGTGGACAAGAAAATCGTGCCCCGCGACTGCGACAAA	ATCAGGACAGCAAAGACAGCACCTACAGC
	GCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC	ACTCACACATGCCCACCGTGCCCAGCACCTGAAGCCG	ATGAGCAGCACCCTCACGTTGACCAAGGA
	AGCACGTACCGTGTGGTCAGCGTCCTCACCGTC	CAGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACC	CGAGTATGAACGACATAACAGCTATACCT
	CTGCACCAGGACTGGCTGAATGGCAAGGAGTAC	CAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTC	GTGAGGCCACTCACAAGACATCAACTTCA
	AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCC	ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTG	CCCATTGTCAAGAGCTTCAACAGGAATGA
	CCCATCGAGAAAACCATCTCCAAAGCCAAAGGG	AGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGT	GTGT (SEQ ID NO:732)
	CAGCCCCGAGAACCACAGGTGTACACCCTGCCC	GCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC	
	CCATCCCGGGAGGAGATGACCAAGAACCAGGTC	AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCC	
	AGCCTGAGCTGCGCCGTCAAAGGCTTCTATCCC	TGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG	
	AGCGACATCGCCGTGGAGTGGGAGAGCAATGGG	CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG	
	CAGCCGGAGAACAACTACAAGACCACGCCTCCC	AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC	
	GTGCTGGACTCCGACGGCTCCTTCTTCCTCGTG	CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT	
	AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG	GACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTCAAA	
	CAGGGGAACGTCTTCTCATGCTCCGTGATGCAT	GGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA	
	GAGGCTCTGCACAACCGGTTCACGCAGAAGAGC	GCAATGGGCAGCCGGAGAACAACTACAAGACCACGCC	
	CTCTCCCTGTCTCCGGGTAAA (SEQ ID	TCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC	
	NO:700)	AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGG	
		GGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT	
		GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT	
		CCGGGTAAA (SEQ ID NO:724)	
F	(SEQ ID NO:678)	(SEQ ID NO:681)	(SEQ ID NO:188)
U			
S			
E			
-			
6			

96			
FUSE-6997	CAGGTTCAGCTCGTCGAAAGCGGAGGAGGAGTC	(SEQ ID NO:681)	(SEQ ID NO:188)
	GTTCAGCCAGGAAGATCCCTCAGGCTCGACTGT		
	AAGGCCTCCGGAATCACCTTCTCCAACTCCGGA		
	ATGCACTGGGTCAGGCAGGCTCCTGGAAAAGGA		
	CTGGAGTGGGTGGCTGTGATTTGGTACGACGGC		
	TCCAAGAGATACTACGCCGACTCTGTGAAGGGC		
	CGGTTCACTATCTCCAGGGACAACTCCAAGAAC		
	ACACTGTTCCTGCAGATGAACTCCCTGCGGGCT		
	GAGGATACTGCCGTTTATTACTGCGCCACCAAC		
	GATGACTACTGGGGACAGGGAACCTTGGTGACC		
	GTGAGTAGCGCTAGCACCAAGGGCCCATCGGTC		
	TTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT		
	GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG		
	GACTACTTCCCCGAACCGGTGACGGTGTCGTGG		
	AACTCAGGCGCCCTGACCAGCGGCGTGCACACC		
	TTCCCGGCTGTCCTACAGTCCTCAGGACTCTAC		
	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC		
	AGCTTGGGCACCCAGACCTACATCTGCAACGTG		
	AATCACAAGCCCAGCAACACCAAGGTGGACAAG		
	AAAGTTGAGCCCAAATCTTGTGACAAAACTCAC		
	ACATGCCCACCGTGCCCAGCACCTGAAGCCGCA		
	GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAA		
	CCCAAGGACACCCTCATGATCTCCCGGACCCCC		
	GAGGTCACATGCGTGGTGGTGGACGTGAGCCAC		
	GAAGACCCTGAGGTCAAGTTCAACTGGTACGTG		
	GACGGCGTGGAGGTGCATAATGCCAAGACAAAG		
	CCGCGGGAGGAGCAGTACAACAGCACGTACCGT		
	GTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC		
	TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC		
	TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAA		
	ACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA		
	CCACAGGTGTACACCCTGCCCCCATCCCGGGAG		
	GAGATGACCAAGAACCAGGTCAGCCTGAGCTGC		
	GCCGTCAAAGGCTTCTATCCCAGCGACATCGCC		
	GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC		
	AACTACAAGACCACGCCTCCCGTGCTGGACTCC		

GACGGCTCCTTCTTCCTCGTGAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC TTCTCATGCTCCGTGATGCATGAGGCTCTGCAC AACAGGTTCACACAGAAGAGCTTGAGCTTGAGC CCCGGCGGAGGCGGGGGATCTGGGGGAGGAGGT AGTGGAGGCGGAGGATCCGGCGGTGGAGGTTCT GGTGGTGGGGGCAGTGGGGGTGGCGGATCAGCT GCTGAACCTGTGGAAGATAATTCTATTAACTTC GTGGCCATGAAGTTCATCGATAATACTCTGTAC TTCATCGCCGAAGATGATGAAATATCGAGCAG GATTACTTCGGCAAACTGGAAAGCAAACTGAGC GTGATCAGGAACCTCAACGACCAGGTGCTGTTC ATCGATCAGGGCAACAGGCCCCTCTTTGAGGAC ATGACCGACAGCGATAGCAGGGACAACGCACCT AGGACAATCTTCATCATCTCCAAGTACGGCGAC TCCCAGCCCAGAGGATTGGCTGTGACAATTTCC GTGAAATCCGAGAGATCTCCACCCTGTCCAGC GAGAACAAGATCATCTCCTTCAAGGAGATGAAC CCCCCCGACAACATCAAGGACACAAAGTCCGAC ATCATCTTCTTCCAGCGCTCCGTGCCCGGTCAT GATAACAAGGTCCAGTTCGAGTCCAGCTCCTAC GAGGGATACTTTCTGGCCTCAGAGAAGGAAAGG GACCTGTTCAAACTCATCCTGAAGAAGGAGGAC GAGCTGGGGGACAGAAGCATCATGTTCACCGTC CAGAATGAGGAC (SEQ ID NO:130)		
FUSE-7765	(SEQ ID NO:679)	(SEQ ID NO:683)	(SEQ ID NO:188)
FUSE-	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTC AGCCAGGAAGGAGTCTCAGGCTGGACTGCAAGGCATC AGGCATCACATTCTCCAACTCCGGCATGCACTGGGTG AGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAG TGATCTGGTACGATGGAAGCAAAAGATACTACGCCGA	(SEQ ID NO:188)


773
CAGCGTCAAGGGCAGGTTCACCATCAGCAGGGACAAT
AGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGA
GGGCCGAGGACACCGCCGTTTTATTACTGCGCCACCAA
CGACGACTACTGGGGCCAGGGAACCTTGGTGACCGTG
AGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCC
TGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGC
GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
CCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCA
GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTC
AGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA
AGTTGAGCCCAAATCTTGTGACAAAACTCACACATGC
CCACCGTGCCCAGCACCTGAAGCCGCAGGGGGACCGT
CAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCT
CATGATCTCCCGGACCCCCGAGGTCACATGCGTGGTG
GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCA
ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA
GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
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GGCGGATCAGGTGGGGAGGCTCTGGTGGAGGCGGTA
GTAACTGGGTGAAAGTGATCTCCGACCTGAAAAAGAT
CGAGGACCTGATCCAGTCCATGCACATCGACGCCACC
CTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGG
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ACCGTGCAGAACCTGATCATCCTGGCCAACACTCCC

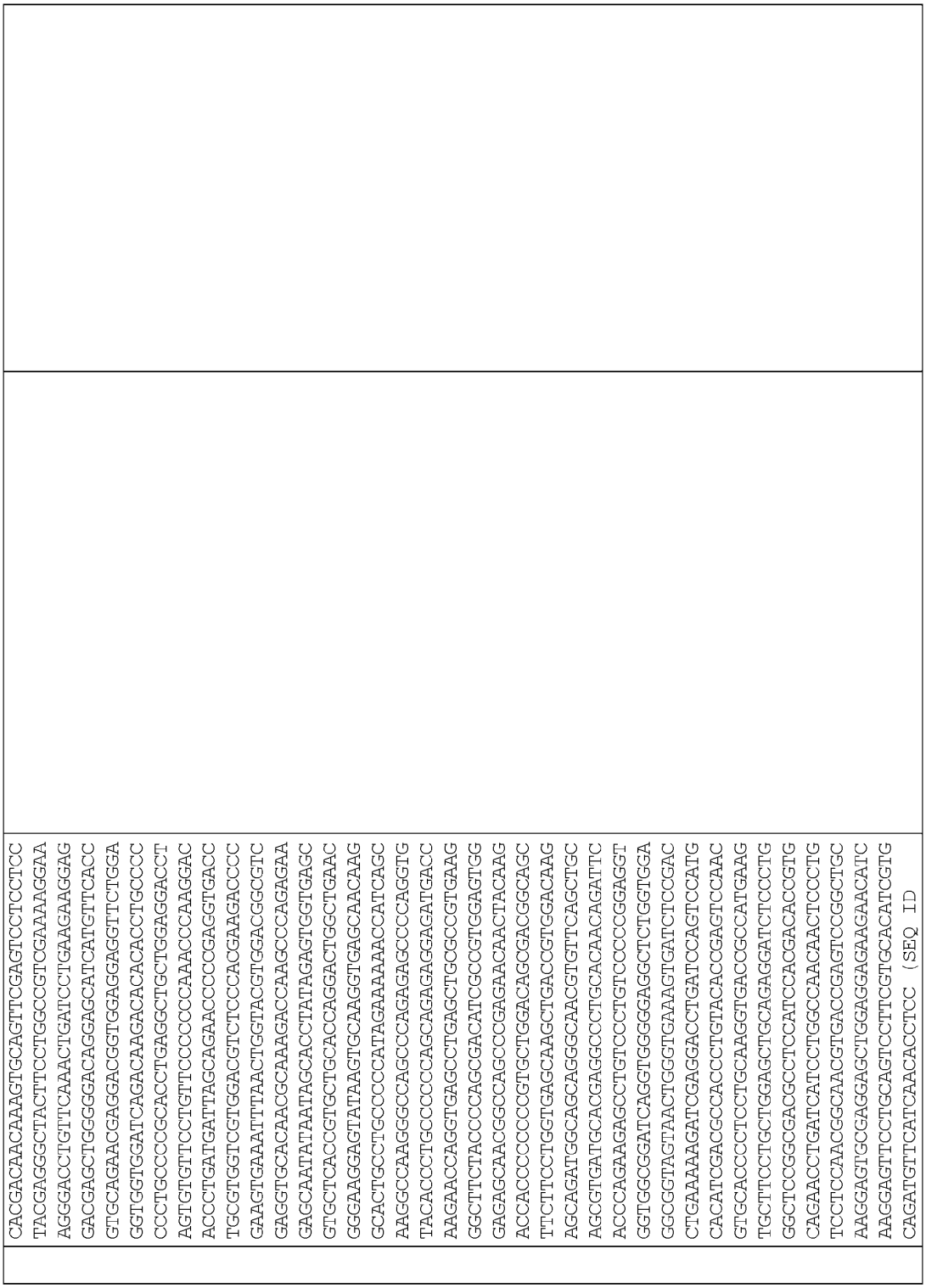
		TGTCCTCCAACGGCAACGTGACCGAGTCCGGCTGCAA GGAGTGCGAGGAGCTGGAGGAGAAGAACATCAAGGAG TTCCTGCAGTCCTTCGTGCACATCGTGCAGATGTTCA TCAACACCTCC (SEQ ID NO:688)	
FUSE-7774	(SEQ ID NO:679)	CAGGTTCAGCTCGTCGAGTCAGGCGGAGGAGTCGTTC AGCCAGGAAGGAGTCTCAGGCTGGACTGCAAGGCATC AGGCATCACATTCTCCAACTCCGGCATGCACTGGGTG AGGCAGGCACCTGGAAAAGGATTGGAGTGGGTGGCAG TGATCTGGTACGATGGAAGCAAAAGATACTACGCCGA CAGCGTCAAGGGCAGGTTCACCATCAGCAGGGACAAT AGCAAGAACACCCTGTTCTTGCAGATGAGTAGCTTGA GGGCCGAGGACACCGCCGTTTATTACTGCGCCACCAA CGACGACTACTGGGGCCAGGGAACCTTGGTGACCGTG AGTTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCC TGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGC GGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA CCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCA GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTC AGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAA AGTTGAGCCCAAATCTTGTGACAAAACTCACACATGC CCACCGTGCCCAGCACCTGAAGCCGCAGGGGGACCGT CAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCT CATGATCTCCCGGACCCCCGAGGTCACATGCGTGGTG GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCA ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACA CCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA GGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC CGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGA CTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCT	(SEQ ID NO:188)

		CATGCTCCGTGATGCATGAGGCTCTGCACAACCACTA	
		CACGCAGAAGAGCCTCTCCCTGTCTCCGGGTGGTGGT	
		GGCGGATCAGGTGGGGAGGCTCTGGTGGAGGCGGTA	
		GTAACTGGGTGAACGTGATCTCCGACCTGAAAAAGAT	
		CGAGGACCTGATCCAGTCCATGCACATCGACGCCACC	
		CTGTACACCGAGTCCAACGTGCACCCCTCCTGCAAGG	
		TGACCGCCATGAAGTGCTTCCTGCTGGAGCTGCAGAG	
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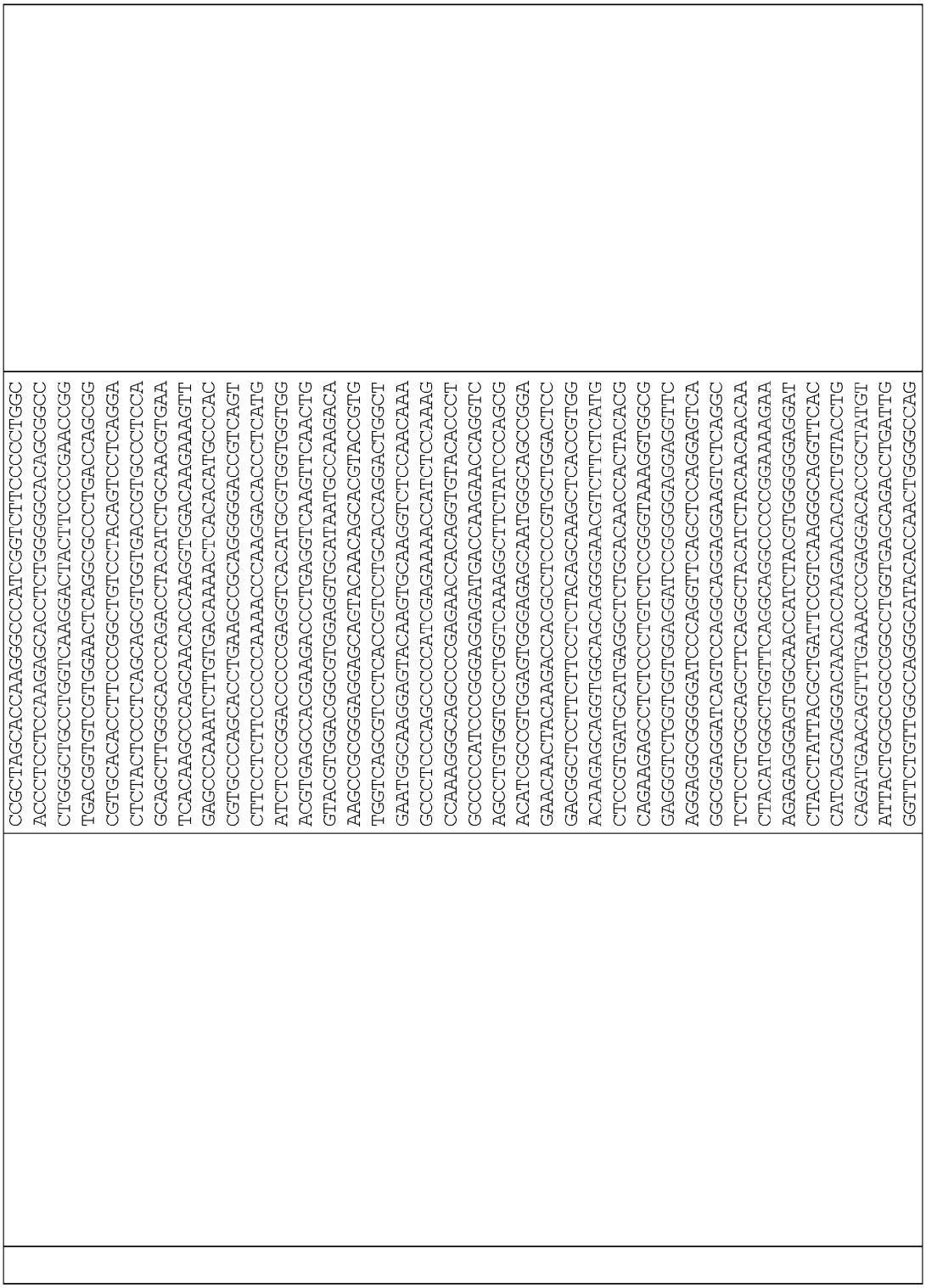
	NO:703)	
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FUSE-9666	(SEQ ID NO:132)	(SEQ ID NO:726)	
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4 CCAAGAACCATCTTCATCATCTCTCACTACGGC	GGGCCGAGGACACCGGAACCTATTACTGTGCAAGGGA	TACACTGACCATCACCGGCGTCCAGGCTG	
5 GACTCCCAGCCAAGGGGAATGGCTGTGACAATC	CTTTTTACAACTCCTACGGATGCAGTGCCTCCTTCATC	ACGATGAGGCTATCTATTACTGCGGCTCC	
TCCGTGAAGGTGGAGAAGATTTCCACCCTGTCC	GACGCATGGGGCCACGGAACCGAGGTTATCGTGTCCT	TGGGACGACTCCTCCTACGTTGGAATCTT	
GTTGAGAACAAGATCATCTCCTTCAAAGAGATG	CCGCTAGCACCACAAGGGCCCATCGGTCTTCCCCCTGGC	CGGCGCTGGTACCACCCTCACCGTCTTGG	
AACCCCCCCGACAACATCAAAGACACCAAATCC	ACCCTCCTCCAAGAGCACCTCTGGGGCACAGCGGCC	GTCAGCCCAAGGCTGCCCCCTCGGTCACT	
GACATCATCTTCTTCCAGCGCAGCGTGCCTGGG	CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG	CTGTTCCCGCCCTCCTCTGAGGAGCTTCA	
CATGATAACAAAATGCAGTTCGAAAGCTCCTCC	TGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGG	AGCCAACAAGGCCACACTGGTGTGTCTCA	
TACGAGGGATACTTCCTCGCCGTCGAAAAGGAA	CGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGA	TAAGTGACTTCTACCCGGGAGCCGTGACA	
AGGGACCTGTTCAAGCTGATCCTGAAGAAGGAG	CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCA	GTGGCCTGGAAGGCAGATAGCAGCCCCGT	
GATGAATTGGGGATAGGAGCATCATGTTCACC	GCAGCTTGGGCACCCAGACCTACATCATCTGCAACGTGAA	CAAGGCGGGAGTGGAGACCACACCACCCT	
GTCCAGAACGAAGACGAGCCTAAATCCTCCGAC	TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT	CCAAACAAAGCAACAACAAGTACGCGGCC	
AAGACACACACCTGCCCCCCCCTGCCCCGCACCT	GAGCCCAAATCTTGTGACAAAACTCACACATGCCCAC	AGCAGCTACCTGAGCCTGACGCCTGAGCA	
GAGGCTGCTGGAGGACCTAGTGTGTTCCTGTTC	CGTGCCCAGCACCTGAAGCCGCAGGGGGACCGTCAGT	GTGGAAGTCCCACAGGAGCTACAGTTGCC	
CCCCCCAAACCCAAGGACACCCTGATGATTTAGC	CTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATG	AGGTCACGCATGAAGGGAGCACCGTGGAG	
AGAACCCCCGAGGTGACCTGCGTGGTCGTGGAC	ATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGG	AAGACAGTGGCCCCTACAGAATGTTCAGG	
GTCTCCCACGAAGACCCCGAAGTGAAATTTAAC	ACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG	AGGCGGGGGATCTGGGGGAGGAGGTAGTG	

TGGTACGTGGACGGCGTCGAGGTGCACAACGCA	GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA	GAGGCGGAGGATCCGGCGGTGGAGGTTCT
AAGACCAAGCCCAGAGAAGAGCAATATAATAGC	AAGCCGCGGAGGAGCAGTACAACAGCACGTACCGTG	GGTGGTGGGGGCAGTGGGGGTGGCGGATC
ACCTATAGAGTGGTGAGCGTGCTCACCGTGCTG	TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCT	AGAAGTGCAGCTGCTGGAGTCCGGAGGAG
CACCAGGACTGGCTGAACGGGAAGGAGTATAAG	GAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA	GATTGGTGCAGCCTGGAGGAAGCCTGAGG
TGCAAGGTGAGCAACAAGGCACTGCCTGCCCCC	GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAG	CTGTCTTGTACCGCCTCCGGATACACCAA
ATAGAAAAAACCATCAGCAAGGCCAAGGGCCAG	CCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT	CAGGCTGAAGTGCATGGGATGGTTTAGGC
CCCAGAGAGCCCCAGGTGTACACCCTGCCCCCC	GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC	AGGCCCCTGGCAAGGAGAGGGAGGAAATC
AGCAGAGAGGAGATGACCAAGAACCAGGTGAGC	AGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCG	GCTAGCATTAGCACCGGAACAGGAAACAC
CTGAGCTGCGCCGTGAAGGGCTTCTACCCCAGC	ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA	CTACTACGCCGACAGCGTGAAAGGCAGAT
GACATCGCCGTGGAGTGGGAGAGCAACGGCCAG	GAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC	TCACAATCTCCAGGGACAAGAGTAGGAAC
CCCGAGAACAACTACAAGACCACCCCCCCCGTG	GACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG	ACACTGTATCTGCAGATGAGCACACTGAG
CTGGACAGCGACGGCAGCTTCTTCCTGGTGAGC	ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG	GGCCGAGGACACCGCAGTTTACTATTGCG
AAGCTGACCGTGGACAAGAGCAGATGGCAGCAG	CTCCGTGATGCATGAGGCTCTGCACAACCACTACACG	CCGCCGACGTTAGGCCCCATGGAACAACA
GGCAACGTGTTCAGCTGCAGCGTGATGCACGAG	CAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ	TGCCACTACAACTCCAGGGACAGGGAAC
GCCCTGCACAACAGGTTCACCCAGAAGAGCCTG	ID NO:728)	CCAGGTGACCGTTTCAAGC (SEQ ID
AGCCTGAGCCCCGGCGGTGGTGGCGGATCAGGT		NO:734)
GGGGGAGGCTCTGGTGGAGGCGGTAGTAACTGG		
GTGAACGTGATCTCCGACCTGAAAAAGATCGAG		
GACCTGATCCAGTCCATGCACATCGACGCCACC		
CTGTACACCGAGTCCAACGTGCACCCCTCCTGC		
AAGGTGACCGCCATGAAGTGCTTCCTGCTGGAG		
CTGCAGAGGATCTCCCTGATCTCCGGCGACGCC		
TCCATCCACGACACCGTGCAGAACCTGATCTCC		
CTGGCCAACAACTCCCTGTCCTCCAACGGCAAC		
GTGACCGAGTCCGGCTGCAAGGAGTGCGAGGAG		
CTGGAGGAGAAGAACATCAAGGAGTTCCTGCAG		
TCCTTCGTGCACATCGTGCAGATGTTCATCAAC		
ACCTCC (SEQ ID NO:706)		
F (SEQ ID NO:706)	GCTGTTACCCTGGATGAGTCCGGGGAGGATTGCAGA	(SEQ ID NO:734)
U	CCCCTGGAGGAGCTCTGTCTTTGGTGTGTAAGGCCTC	
S	TGGATTTGATTTTTCCTCTCATGAAATGCAGTGGGTG	
E	AGGCAGGCCCCCGGAAAAGGATTGGAGTTTGTGGCTG	
-	GGATCGGAAACATGGGATCTTTCACCGTGTATGGGGC	
1	AGCAGTGAAGGGCAGGGCTACAATCTCTAGGGACAAC	
1	GGCCAGAGCACCGTGAGGTTGCAGCTGAACACAACTTGA	
4	GGGCCGAGGACACCGGAACCTATTACTGCGCAAGGGA	
6	CTTTTACAACTCCTACGGGTGCTCCGCCTCCTTCATC	
	GACGCTTGGGGACACGGAACCGAGGTGATCGTGAGCT	


CCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGC
ACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC
CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
TGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGG
CGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGA
CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCA
GCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCAC
CGTGCCCAGCACCTGAAGCCGCAGGGGGACCGTCAGT
CTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATG
ATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGTGG
ACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTG
TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCT
GAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA
GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAG
CCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT
GCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
AGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCG
ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA
GAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC
GACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGTAAAGGTGGCG
GAGGGTCTGGTGGTGGAGGATCCGGGGGAGGAGGTTC
AGGAGGCGGGGGATCCCAGGTTCAGCTCCAGGAGTCA
GGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGC
TCTCCTGCGCAGCTTCAGGCTACATCTACAACAACAA
CTACATGGGCTGGTTCAGGCAGGCCCCCGGAAAAGAA
AGAGAGGGAGTGGCAACCATCTACGTGGGGGGAGGAT
CTACCTATTTACGCTGATTCCGTCAAGGGCAGGTTCAC
CATCAGCAGGGACAACACCAAGAACACACACTGTACCTG
CAGATGAACAGTTTGAAACCCGAGGACACCGCTATGT
ATTACTGCGCCGGCCTGGTGAGCAGACCTGATTG
GGTTCTGTTGGCCAGGGCATACACCAACTGGGGCCAG

		GGAACCCTGGTGACAGTGTCTAGC (SEQ ID NO:729)	
FUSE-1147	(SEQ ID NO:132)	(SEQ ID NO:728)	(SEQ ID NO:734)
FUSE-1148	(SEQ ID NO:132)	(SEQ ID NO:729)	(SEQ ID NO:734)
FUSE-11480	GCTGTTACCCTGGATGAGTCCGGGGGAGGATTG		CAGTCTGCACTCACCCAGCCATCCTCCGT
	CAGACCCCTGGAGGAGCTCTGTCTTTGGTGTGT		CTCCGCTAACCCAGGAGAAACCGTCAAAA
	AAGGCCTCTGGATTTGATTTTTCCTCTCATGAA		TCACCTGCTCCGGCGGCTCCTACAACTAC
	ATGCAGTGGGTGAGGCAGGCCCCCGGAAAAGGA		GGATGGTTCCAGCAGAAATCCCCCGGCTC
	TTGGAGTTTGTGGCTGGGATCGGAAACATGGGA		CGCACCTGTTTACCGTGATTTTACTCCAATA
	TCTTTCACCGTGTATGGGGCAGCAGTGAAGGGC		ATCAGAGGCCTTCCGACATCCCCTCCAGA
	AGGGCTACAATCTCTAGGGACAACGGCCAGAGC		TTCTCCGGCTCCACCTCCGGTTCCACCTC
	ACCGTGAGGTTGCAGCTGAACAACTTGAGGGCC		TACACTGACCATCACCGGCGTCCAGGCTG
	GAGGACACCGGAACCTATTACTGCGCAAGGGAC		ACGATGAGGCTATCTATTACTGCGGCTCC
	TTTTTACAACTCCTACGGGTGCTCCGCCTCCTTC		TGGGACGACTCCTCCTACGTTGGAATCTT
	ATCGACGCTTGGGGACACGGAACCGAGGTGATC		CGGCGCTGGTACCACCCTCACCGTCTTGG
	GTGAGCTCCGCTAGCACCAAGGGCCCATCGGTC		GTCAGCCCAAGGCTGCCCCCTCGGTCACT
	TTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT		CTGTTCCCGCCCTCCTCTGAGGAGCTTCA
	GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG		AGCCAACAAGGCCACACTGGTGTGTCTCA
	GACTACTTCCCCGAACCGGTGACGGTGTCGTGG		TAAGTGACTTCTACCCGGGAGCCGTGACA
	AACTCAGGCGCCCTGACCAGCGGCGTGCACACC		GTGGCCTGGAAGGCAGATAGCAGCCCCGT
	TTCCCGGCTGTCCTACAGTCCTCAGGACTCTAC		CAAGGCGGAGTGGAGACCACACACCCT
	TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC		CCAAACAAAGCAACAACAAGTACGCGGCC

AGCTTGGGCACCCAGACCTACATCTGCAACGTG AATCACAAGCCCAGCAACACCAAGGTGGACAAG AAAGTTGAGCCCAAATCTTGTGACAAAACTCAC ACATGCCCACCGTGCCCAGCACCTGAACTCCTG GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAA CCCAAGGACACCCTCATGATCTCCCGGACCCCC GAGGTCACATGCGTGGTGGTGGACGTGAGCCAC GAAGACCCTGAGGTCAAGTTCAACTGGTACGTG GACGGCGTGGAGGTGCATAATGCCAAGACAAAG CCGCGGGAGGAGCAGTACAACAGCACGTACCGT GTGGTCAGCGTCCTCACCGTCCTGCACCAGGAC TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAA ACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA CCACAGGTGTACACCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCC GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC AACTACAAGACCACGCCTCCCGTGCTGGACTCC GACGGCTCCTTCTTCCTCTACAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC TTCTCATGCTCCGTGATGCATGAGGCTCTGCAC AACCACTACACGCAGAAGAGCCTCTCCCTGTCT CCGGGTAAA (SEQ ID NO:707)			AGCAGCTACCTGAGCCTGACGCCTGAGCA GTGGAAGTCCCACAGGAGCTACAGTTGCC AGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCTACAGAATGTTCA (SEQ ID NO:735)
F U S E - 1 1 2 4	(SEQ ID NO:706)	GAGGTTCAGGTGCAGCAGAGCGGACCAGAGTTGGTGA AGCCAGGAACAAGCGTGAAGATTAGTTGCAAGACAAG CGGGTATACATTTACAGAGTACACAATGCACTGGGTG AAGCAGAGCCACGGTGAGAGCCTCGAATGGATCGGAG GAATCGTGCCTAACAACGGAGCCACCAACTACAACCA GAAATTCAGGGGGAAAGCCACACTGACCGTGGACAAA AGCAGCAGCACCGCCTACATGGAACTGCGCAGCCTGA CCAGCGAGGACTCTGCTGTTTACTACTGCGCCAGAAG GGGCCTCCTGTGGCATTTTTGCCTATTGGGGCCAGGGG ACCCTCGTGACCGTTTCCGCTGCTAGCACCAAGGGCC CATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCAC CTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACT CAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGC	GACATCCTGATGACCCAGTCCCCCTCCGC TATGTCCGTGTCTCTGGGAGACACCGTGT CCATCACTTGCCACGCCTCCCAGGGAATC TCCTCCTACATCGGATGGCTGCAGCAGAA ACCCGGCAAGTCCTTCAAAGGTCTGATTT ATCAGCGGCACCAACCTCGAAGATGGTGTT CCTTCCCGCTTCTCCGGTTCCGGTTCTGG AGCTGACTACTCCCTGACTATCTCCTCCC TGGAATCTGAGGACTTCGCCGATTACTAC TGCATCCAGTATACGCCCCAGTTTCCCTTCAC CTTCGGTTCCCGGCACCAAGCTGGAAATCA AGCGTACGGTGGCTGCACCATCTGTCTTC ATCTTCCCGCCATCTGATGAGCAGTTGAA ATCTGGAACTGCCTCTGTTGTGTGCCTGC

	TGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC	TGAATAACTTCTATCCCAGAGAGGCCAAA
	GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGA	GTACAGTGGAAGGTGGATAACGCCCTCCA
	CCTACATCTGCAACGTGAATCACAAGCCCAGCAACAC	ATCGGGTAACTCCCAGGAGAGTGTCACAG
	CAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGAC	AGCAGGACAGCAAGGACAGCACCTACAGC
	AAAACTCACACATGCCCACCGTGCCCAGCACCTGAAG	CTCAGCAGCACCCTGACGCTGAGCAAAGC
	CCGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCAAA	AGACTACGAGAAACACAAAGTCTACGCCT
	ACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAG	GCGAAGTCACCCATCAGGGCCTGAGTTCG
	GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACC	CCCGTCACAAAGAGCTTCAACAGGGGAGA
	CTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGA	GTGTGGTGCCGAGGGTCTGGTGGTGGAG
	GGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG	GATCCGGGGAGAGGTTCAGTGTGGAGGCGGG
	TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCG	GGAAGTGAGGTTCAGTTGTTGGAGTCCGG
	TCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA	GGGCGGATTGGTGCAGCCTGGAGGAAGCC
	GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC	TGAGGTTGAGCTGTACAGCAAGCGGTAC
	GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAG	ACAAACAGGTTGAAGTGTATGGGGTGGTT
	AACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGA	CAGGCAGGCACCCGGAAAGGAAAGGGAGG
	GATGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTC	AGATTGCTAGTATCAGCACCGGAACCGGA
	AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGG	AATACCTACTACGCCGACAGTGTGAAGGG
	AGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC	CAGATTCACAATCAGCAGGACAAGAGCA
	GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTC	GGAACACCCTGTATCTGCAGATGAACACA
	TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC	CTGAGGGCCGAGGACACCGCAGTGTACTA
	AGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGC	CTGCGCAGCCGACGTGAGGCCACATGGAA
	TCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG	CAACCTGCCACTACAACTCCAGGGGCCAG
	TCTCCGGGTAAA (SEQ ID NO:730)	GGAACCCAGGTCACAGTTTCCTCC (SEQ ID NO:737)
FUSE-1125	(SEQ ID NO:132)	(SEQ ID NO:737)
	(SEQ ID NO:730)	(SEQ ID NO:737)
FUSE-2	GCTGCTGAACCTGTGGAAGATAATGTGATCAAT	
	TTCGTGGCCATGAAGTTCATTGACAACACCCTG	
	TACTTCATCGCTGAAAACGATGAGAACGGCGGA	
	GGATCTTACTTCGGAAAACTGAAATCCAAGCTG	
	AGCGTGATCAGGAACCTGAACGATCAGGTGCTG	

1 1 2 6	TTCATCGACCAGGGCAATAGGCCCCTGTTCGAG GATATGACAGACTCCGACGTGAGGGACAACGCT CCAAGAACCATCTTCATCATCTCTCACTACGGC GACTCCCAGCCAAGGGGAATGGCTGTGACAATC TCCGTGAAGGTGGAGAAGATTTCCACCCTGTCC GTTGAGAACAAGATCATCTCCTTCAAAGAGATG AACCCCCCCGACAACATCAAAGACACCAAATCC GACATCATCTTCTTCCAGCGCAGCGTGCCTGGG CATGATAACAAAATGCAGTTCGAAAGCTCCTCC TACGAGGGATACTTCCTCGCCGTCGAAAAGGAA AGGGACCTGTTCAAGCTGATCCTGAAGAAGGAG GATGAATTGGGGATAGGAGCATCATGTTCACC GTCCAGAACGAAGAGACGAGCCTAAATCCTCCGAC AAGACACACACCTGCCCCCCCTGCCCCGCACCT GAGGCTGCTGGAGGACCTAGTGTGTTCCTGTTC CCCCCCAAACCCAAGGACACCCTGATGATTAGC AGAACCCCCGAGGTGACCTGCGTGGTCGTGGAC GTCTCCCACGAAGACCCCGAAGTGAAATTTAAC TGGTACGTGGACGGCGTCGAGGTGCACACAACGCA AAGACCAAGCCCAGAGAAGAGCAATATAATAGC ACCTATAGAGTGGTGAGCGTGCTCACCGTGCTG CACCAGGACTGGCTGAACGGGAAGGAGTATAAG TGCAAGGTGAGCAACAAGGCACTGCCTGCCCCC ATAGAAAAACCATCAGCAAGGCCAAGGGCCAG CCCAGAGAGCCCCAGGTGTACACCCTGCCCCCC AGCAGAGAGGAGATGACCAAGAACCAGGTGAGC CTGAGCTGCGCCGTGAAGGGCTTCTACCCCAGC GACATCGCCGTGGAGTGGGAGAGCAACGGCCAG CCCGAGAACAACTACAAGACCACCCCCCCGTG CTGGACAGCGACGGCAGCTTCTTCCTGGTGAGC AAGCTGACCGTGGACAAGAGCAGATGGCAGCAG GGCAACGTGTTCAGCTGCAGCGTGATGCACGAG GCCCTGCACAACAGGTTCACCCAGAAGAGCCTG AGCCTGAGCCCCGGCAAG (SEQ ID NO:298)	(SEQ ID NO:730)	(SEQ ID NO:737)
F U S	GAGCCTAAATCCTCCGACAAGACACACACCTGC CCCCCCTGCCCCGCACCTGAGGCTGCTGGAGGA CCTAGTGTGTTCCTGTTCCCCCCCAAACCCAAG		

E GACACCCTGATGATTAGCAGAACCCCCGAGGTG **-** ACCTGCGTGGTCGTGGACGTCTCCCACGAAGAC **1** CCCGAAGTGAAATTTAACTGGTACGTGGACGGC **1** GTCGAGGTGCACAACGCAAAGACCAAGCCCAGA **2** GAAGAGCAATATAATAGCACCTATAGAGTGGTG **7** AGCGTGCTCACCGTGCTGCACCAGGACTGGCTG AACGGGAAGGAGTATAAGTGCAAGGTGAGCAAC AAGGCACTGCCTGCCCCCATAGAAAAAACCATC AGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAG GTGTACACCCTGCCCCCCAGCAGAGAGGAGATG ACCAAGAACCAGGTGAGCCTGAGCTGCGCCGTG AAGGCTTCTACCCCAGCGACATCGCCGTGGAG TGGGAGAGCAACGGCCAGCCCGAGAACAACTAC AAGACCACCCCCCCCGTGCTGGACAGCGACGGC AGCTTCTTCCTGGTGAGCAAGCTGACCGTGGAC AAGAGCAGATGGCAGCAGGGCAACGTGTTCAGC TGCAGCGTGATGCACGAGGCCCTGCACAACAGG TTCACACAGAAGAGCTTGAGCTTGAGCCCCGGC GGTGGTGGCGGATCAGGTGGGGGAGGCTCTGGT GGAGGCGGTAGTAACTGGGTGAACGTGATCTCC GACCTGAAAAAGATCGAGGACCTGATCCAGTCC ATGCACATCGACGCCACCCTGTACACCGAGTCC AACGTGCACCCCTCCTGCAAGGTGACCGCCATG AAGTGCTTCCTGCTGGAGCTGCAGAGGATCTCC CTGATCTCCGGCGACGCCTCCATCCACGACACC GTGCAGAACCTGATCTCCCTGGCCAACAACTCC CTGTCCTCCAACGGCAACGTGACCGAGTCCGGC TGCAAGGAGTGCGAGGAGCTGGAGGAGAAGAAC ATCAAGGAGTTCCTGCAGTCCTTCGTGCACATC GTGCAGATGTTCATCAACACCTCC (SEQ ID NO:719)		
F (SEQ ID NO:706) **U** **S** **E** **-** **1** **1**	GAAGTGCAGCTGGTGGAGAGCGGAGGAGGATTGGTGC AGGCAGGAGGAAGCCTGAGGCTGAGTTGTGCCGCTAG TGGAAGGCCATTTAGTAATTACGCAATGGGGTGGTTT AGGCAGGCTCCTGGCAAGGAGAGGGAGTTCGTGGCTG CTATTTCCTGGAGTGGGGGAGCACATCCTACGCTGA TTCCGTGAAGGGACGGTTTACCATCTCCAGGGACAAT GCTAAGAACACAGTGTACCTGCAGATGAGCAGCCCCA	

3 6		AGCCCGAGGATACCGCTATCTATTATTGCGCCGCCCA GTTTTCCGGCGCCGATTATGGATTCGGCAGGTTGGGA ATCAGGGGATACGAATACGATTACTGGGGCCAGGGAA CCCAGGTGACCGTTTCTAGCGGTGGCGGAGGGTCTGG TGGTGGAGGATCCGGGGGAGGAGGTTCAGGAGGCGGG GGAAGTGAAGTGCAGCTGCTGGAGTCCGGTGGAGGAT TGGTGCAGCCTGGAGGAAGCCTGAGGCTGTCTTGTAC CGCCTCCGGTTATACAAACAGGCTGAAGTGCATGGGA TGGTTTAGGCAGGCCCCCGGAAAAGAGAGGGAGGAAA TCGCCAGCATTAGTACCGGCACAGGAAACACCTACTA CGCCGACAGTGTCAAGGGCAGATTCACAATCTCCCGG GACAAGAGCAGGAACACCCTGTACCTGCAGATGAGCA CACTGAGAGCCGAAGATACTGCTGTTTACTATTGCGC CGCCGACGTGAGGCCCCACGGTACAACATGTCACTAC AACAGCAGGGACAGGGCACCCAGGTGACAGTTTCCA GCGAACCTAAATCCTCCGACACAAGACCCACACCTGCCC CCCTTGCCCCGCTCCTGAAGCTGCTGGAGGCCCTTCA GTGTTTCTGTTTCCTCCCAAGCCCAAGGACACACTGA TGATTTCCAGAACCCCCGAGGTGACATGCGTGGTGGT GGACGTGAGCCACGAAGATCCCGAGGTGAAGTTTAAT TGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGA CCAAACCCCGCGAGGAGCAGTATAATAGCACCTACAG AGTGGTGAGCGTGCTGACCGTGCTGCATCAGGACTGG CTGAACGGCAAGGAATACAAATGCAAGGTGAGCAACA AGGCCCTGCCTGCCCCTATCGAGAAGACCATCAGCAA AGCCAAGGGACAGCCAAGAGAACCCCAGGTGTACACC CTGCCCCCCAGCAGAGAAGAAATGACCAAGAACCAGG TGAGCCTGTGGTGCCTGGTGAAAGGCTTCTACCCCAG CGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCA GAGAACAACTACAAGACAACACCCCCCGTGCTGGACA GCGACGGCTCTTTCTTCCTGTACAGTAAACTGACCGT GGACAAATCAAGGTGGCAGCAGGGCAACGTGTTCAGC TGCTCCGTGATGCACGAGGCCCTGCACAACCACTATA CCCAGAAGAGCCTGTCCCTGAGCCCCGGCAAG (SEQ ID NO:731)	
F U S	(SEQ ID NO:719)	(SEQ ID NO:731)	

E-1137			
FUSE-1138	(SEQ ID NO:298)	(SEQ ID NO:731)	
FUSE-1139	(SEQ ID NO:132)	(SEQ ID NO:731)	

Table 3B.1 Polynucleotide Sequences relating to embodiments involving IL15/IL18 fusion proteins

	Polynucleotide 1	Poly-nucleotide 2	Poly-nucleotide 3
FUSE-1137	GACGTTCAGCTGCAGGAATCCGGAGGAGGATTGGTGCAGGCAGGAGGATCTTTGACATTGAGCTGCGCCGCCTCCGGAGTTACATA		
FUSE-1138	TTCCCACTATTGGTTGGGATGGTTTAGGCAGGCACCCGGAAAAGAGAGGAGGGAGTTGCAACCATTTACGCCAACGACGGACCA		
FUSE-1139	CCTACTACGCTGATAGTGTGAAGGGAAGGTTCACCGTGTCCTTGGACAATGCCAAGAACACCTTGTACCTGCAGATGAACAGGCTG		
E-1137	ACCCCCGAGGATACCGCTATGTACTACTGCGCCCTCCGGGTTAACGACGGATCTTGGTTTTCCCCCCTGCAGCACTACCACTACAA		
FUSE-1138	CTACTGGGGCCAGGGCACCCAGGTTACAGTTTCTTCCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCAC		
FUSE-1139	CTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCCGAGGTCACA		
E-1137	TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC		
FUSE-1138	AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG		

2	AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAG CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:708)		
FUSE - 1 0 6 3	GACGTTCAGCTGCAGGAATCCGGAGGAGGATCTGTGCAGGCAGGAGGATCTTTGAGGCTGTCCTGCGCTGCATCCGGAAACCCTTA CAGCCACTATTGGTTGGATGGTTCAGGCAGACACCCGGTAAGGAGAGGAGGGAGTTGCTGTTATTTACACCGGAGACGGGATG GAACCACCTACTATACCGACAGTGTGAAAGGCAGGTTCACCGTTTCCCAGGATAACGGAAAGAACACCCTGTACCTGCAGATGAAC AATCTGAAGCCCGAGGACACAGCCATGTACTACTGCGCACTCAGGGCAACGAGAAGTTGGTTCAGCCCTTTGCAGCCCGGACA CTACACATACTGGGGCCAGGGAACACAGGTTACCGTTAGTTCCGAGCCCAAATCTGCTGAACAAAACTCACACATGCCCACCGTGCC CAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAG GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTA TCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG GCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAG GCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:709)		
FUSE - 1 0 6 4	GACGTTCAGCTGCAGGAATCCGGAGGAGGATTGGTGCAGCCAGGAGGATCTTTGAGGCTGTCCTGCGCTGCATCCGGAGTTACAGA CTCCAATGGGTGCATGGGCTGGTTTAGGCAGGCCCCTGGAAAGGAAAGGGAGAGAGTGGCTAGTATTGACAGCGACGGACAGACCC TGTACGCCGACTCCGTGAAAGGAAGGTTCACCATCTCCCAGGACAACACAGAGAACACCCTCTATCTGCAGATGAACACCCTGAAG CCTGAAGACACAGCCACCTACTATTGCGCCGCCGGAGCTACCTCTCAGCCTGGATTCTTCTTGCCCGGATCTTTGGGACCCGGATGGTA CACATACTGGGGCCAGGGAACCCAGGTGACAGTTAGTTCCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAG CACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCC CAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:710)		
FUSE - 1 0	GACGTTCAGCTGCAGGAATCCGGAGGAGGATCTGTGCAGGCAGGAGGATCTTTGAGGCTGTCCTGCGCTGCATCCGGAAACCCTTA CAGCAGGTACTGGCTGGGATGGTTTAGGCAGGCCCCTGGAAAGGAGAGGGAGGGAGTTGCTACCATTTACACCGACGACGGCGCTA CATACTACGCCGATTCTGTCAAGGGCCGGTTCACAGTGTCCCAGGATAACGCTTGTACCTGCAGATGAACAGCCTG AAGCCCGAAGACACAGCCATGTACGTGTGTGCCTGAGGGGAAACGACGGAACATGGTTCTCTGTGCTGCAGCCCGGACACTATAC CGTGTGGGGACAGGGAACCCAGGTCACCGTGTCCTCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCAC CTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACA TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC		

6 5	AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG		
	AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA		
	CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAG		
	CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT		
	TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG		
	CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:711)		
F	GATGTGCAGCTGCAGGAGAGCGGAGGAGGAAGTGTGCAGGCTGGAGGAAGCCTGAGGCTGAGTTGCGCAGCTAGTGGAGTGACCTA		
U	CTCCCACTACTGGTTGGCCTGGTTTAGGCAGGCCCCTGGAAAAGAGAGGGAGGGAGTGGCAACAATTTACACCAATGATGGAACCA		
S	CATACTACGCGGATTCTGTGAAGGGAGGTTCACAATTAGCCTGGACAACCCCAAGAACACACTGTACCTGCAGATGAACAGCCTG		
E	ACCCCTGAGGACACCGCCATGTACTACTGCGCCCTCAGAGGCAACGACTCATGGTTCTCCCCCTTGCAGCCTTACCACTACAA		
-	CTACTGGGGCCAGGGCACCCAGGTCACAGTTTCTTCCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCAC		
1	CTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACA		
0	TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC		
7	AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG		
2	AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA		
	CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAG		
	CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT		
	TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG		
	CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:712)		
F	GATGTGCAGCTGCAGGAGAGCGGAGGAGGACCTGTTCAGAGCGGAGGAAGCCAGAGGCTGAGCTGTGTGTACTCCGGGGTGACCGA		
U	CTCCAACGGATGTATGGGATGGTTCAGGCAGGCCCCTGGAAAAGAGAGGGAGAGGGTGGCTAGTATCGACAGCTACGGACAGACAT		
S	TGTATGCCGACAGTGTGAAGGGGAGGTTCACAATTAGCCAGGATAACACAGAGAACACATTGTACCTGCAGATGAACAGCCTGAAG		
E	CCCGAGGACACAGCCACCTACTACTGCGCTGCCGGAGCTACATCCCAGCCAGGATTCAGCTGCCAGGTCGCTCGGACCAGGATGGTA		
-	CACCTACTGGGGCCAGGGAACCCAGGTGACAGTCAGTATCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAG		
1	CACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTC		
0	ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA		
7	GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA		
3	AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA		
	CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCC		
	CAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT		
	CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT		
	CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:713)		
F	GATGTGCAGCTGCAGGAGAGCGGAGGAGGAAGTGTGCAGGCTGGAGGAAGCCTGAGGCTGAGTTGCGCAGCTAGTGGAGTGACCTA		
U	CTCCCACTACTGGTTGGGGTGGTTTAGGCAGGCCCCTGGAAAAGAGAGGGAGGGAGTGGCAACAATTTACGCCAATGATGGAACCA		
S	CATACTACGCCGATTCTGTGAAGGGGCGGTTCACAGTCAGCTTGGATAATGCCAAGAACACACTGTACCTGCAGATGAACAGGCTG		
E	ACCCCCGAGGACACCGCTATGTACTACTGCGCCCTGAGGGTGAACGACGGGTCTTGGTTCTCCCCCCTGCAGCATTACCACTACAA		
-	CTACTGGGGCCAGGGCACACAGGTGACCGTTTCTTCCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCAC		
1	CTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACA		

0 7 4	TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAG CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:714)		
F U S E - 1 0 7 5	GATGTGCAGCTGCAGGAGAGCGGAGGAGGGAAGTGTGCAGGCTGGAGGAAGCCTGAGGCTGAGTTGCGTGGCTTCCGGTTACTCCTA CTCCCACTACTGGCTGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACA CATACTACGGGGATTCCGTGAAGGCAGGTTCACTGTGTCCCAGGACAACGCCAAGAACACCCTGTATCTGCAGATGAACTCCCTG AAGCCCGAGGACACCGCCATGTACTACTGCGCACTCCGCGCTAACGACGGCTCATGGTTCTCCCCTCTGCAGCCAGGATACTACAC ATACTGGGGCCAGGGCACCCAGGTGACAGTTTCTTCCGAGCCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCAC CTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACA TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAG CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:715)		
F U S E - 1 0 7 6	GATGTGCAGCTGCAGGAGAGCGGAGGAGGGACCTGTTCAGAGCGGAGGAAGCCTGAGGTTGAGCTGCGAGGCTTCCGGTGTGACCGT GACAAACGGCTGTATGGCCTGGTTCAGGCAGGCCCCTGGAAAAGAGAGGAGGGAGAGGTGGCTAGTATCGACGAGGACGGCCAGACCC TGTATGCCGATTCCGTGAAGGGAAGGTTCACCATCTCTCAGGACAACACCGAGAACACCCTGTATCTCCAGATGAACACACTGAAG CCTGAAGATATGGCTACTTACTACTGCGCCGCTGGGGCCAGATCCCAGCCTGGATTCTCTTGCCGGATCTTTGGGACCCGGCTGGTA CACATATTGGGGCCAGGGAACCCAGGTGACCGTCTCTTCTGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAG CACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCA AGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCC CAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT CCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:716)		
F U S E -	GATGTGCAGCTGCAGGAGAGCGGAGGAGGGAAGTGTGCAGGCTGAGGCTGAGTTGCGCACAGTGTCCGCTTACACCTA CGGCAGGGGATGTATGGGATGGTTTAGGCAGGCCCCTGGCAAAGAAAGAGAGGGAGTGGCTGGCGTGGACATTGTGGGAACCCCTA CCTACGCCGACTCCATCAAGGGAAGGTTCACCATCAGCAGGGATAACACAAAGAACATCCTCTACCTGGAGATGAACAGCCTGAAG CCTGAAGATACTGCTATGTACTACTGCGGCGCCGCCCTGAGGCCTTATGGAGGATCTTGCCTCAGTTGGCCACTTCGACAGCTG GGGACAGGGAACACAGGTGACCGTGAGCTCAGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAC		

1 0 7 7	TCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACATGCGTG GTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCC GCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG TACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACAT CGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAC CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:717)		(SEQ ID NO:736)
F U S E - 1 0 7 8	CAGGTTCAGCTCCAGCAGTCAGGCGCAGAGCTCGCTAGACCAGGAGCTTCAGTCAAACTCTCCTGCAAGGCCTCCGGCTTCACATT CACCGACCACTACATCAACTGGTCAAACAGAGGACCGGCCAGGGCTTGGAGTGGATTGGACACGATTTACCCCGGCGATGGCAACA CCTACTACAACGAGAAATTCAAGGCAAAGCCACCCTGACAGCCGACAAATCCAGCACAGCATACATGCAGTTGAGCTCCCTG ACCAGCGAAGATTCTGCTGTGTACTTCTGCGCCCCCAACTACGGCGACTACACCATCGACTTCTGGGGCCAGGGAACCTCCGTGAC AGTGTCAAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGG GCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCG GCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTG CAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGT GCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCC GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCG ACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:718)		
F U S E - 1 1 8 4	GATGTGCAGCTGCAGGAGAGCGGAGGAGGAAGTGTGCAGGCTGGAGGAAGCCTGAGGCTGAGTTGCAGGGCATCCGGATGGGCTAC CAACTGTATGGGATGGTTTAGGCAGGCCCCCGGAAAGCAGAGGGAGAGTTGCTCTGATTGACAACGACGGGAGGACAATCTACG CAGAGAGCGTGAAGGGGAGGTTTACCATCTCCCAGGAGAACGCAAGGAAGGCACTCTATTTGCAGATGAACAGCCTGAAGCCCGAA GATACTGCTATGTACTACTGCGCCGCCGACCTGGATATCTGCTGGGTTGTGAAGGACAGGAGCTTCACATACTGGGGCCAGGGAAC CCAGGTCACCGTTTCTTCCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGAC CGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACATGCGTGGTGGACGTG AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCA GTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCT CCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC CCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGC TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG AAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:723)		
F	GACGTGCAGCTGCAGGAGTCCGGAGGAGGAAGCGTGCAGGCTGGAGGAAGCCTGAGGCTGTCCTGCGCTGCTTCCGGAGACACCTC		

U	CAGGACCTATCTGATGGCCTGGTTTAGGCAGGCCCCCGGAAAGAAAGGGAGGGAGTGGCTACAACCAACAAATGGGGCGCCACCA		
S	CATACGCCGACTCTGTGAAAGGAAGGTTCACCATCTCCCTGGACAACGCCAAGAACGCACTGTACCTGCAGATGAACAGCCTGAAA		
E	CCTGAAGATACTGCTATGTACTACTGCGCCGCCGGCAGAGATTACTATGCAAACCTGGACCCCGACCCCAGGCTGTGTACGCTTACTG		
-	GGGACAGGGAACCCAGGTGACAGTGTCTAGCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACGTGCCCAGCACCTGAAC		
1	TCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACATGCGTG		
1	GTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCC		
2	GCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA		
8	AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG		
	TACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACAT		
	CGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC		
	TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAC		
	CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:720)		
F	GACGTGCAGCTGCAGGAGTCCGGAGGAGGAAGCGTGCAGGCTGGAGGAAGCCTGAAACTGTCCTGCGCTGCTTCCGGATACACCTA		
U	TTCCTTTTACTGCATGGGATGGTTTAGGCAGGCCCCCGGAACAAGAAAAGAGAGGAGGGAGTGGCCGTCATTGACGCTGATGGAA		
S	GGACCACCTACGCCGATAGCGCAAAGGGAAGGTTTACCGTGAGCCAGGACGAGGCTAAAGAGCACCTCTACCTGCAGATGAATAAC		
E	CTGAAGCCTGAAGATACAGCAATGTACTACTGCGCCGCCTACGATTGTTACAGTGGAAGTAAGTGGGGCGACTCCGACTCCTTTGG		
-	AGCTTGGGGACAGGGAACCCAGGTGACCGTGTCCTCCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCCAGCAC		
1	CTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACA		
1	TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC		
2	AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG		
9	AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA		
	CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAG		
	CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT		
	TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG		
	CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:721)		
F	GACGTGCAGCTGCAGGAGTCCGGAGGAGGAAGCGTGCAGGCTGGAGGAAGCCTGAGGCTGTCCTGCGCTGCTTCCAGGGCTACCGC		
U	TTATATGGGATGGTTTAGGCAGGCCCCAGGGAAGGAAAGAGAGGAGGGAGTGGCTTGCATCGACTCCTTTGGATCTACCAGCTTCGCCG		
S	ACTCCGTGAAGGGAAGATTCACAATCTCACAGGACAGGACAAAAAACACCGTGGTGCTGCAGATGAACTCCCTGAAGCCCGAGGAC		
E	ACCGCCATGTACTACTGCGCCGCCAGGATCTCCGGCGAGAACGGCTACATCGGCTACTGCGCCTCCGGCGCCGTGGGCCCCTCCGA		
-	CTACGTGAGGTGGGGCCAGGGCACCCAGGTGACCGTGTCCTCCGAGCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCC		
1	CAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAG		
1	GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC		
3	CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATG		
O	GCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA		
	GAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTA		
	TCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG		
	GCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAG		
	GCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:722)		

GATATCGTGATGTCCCAGTCCCCCTCCTCCCTGGCTGTTTCTGTTGGAGAGAAAGTGACCATGTCCTGCAAGAGCTCCCAGTCCCTGCTGTACAGCTCAAACCAGA

AGAACTACCTGGCCTGGTATCAGCAGAAACCCGGCCAGTCCCCCAAATTGTTGATTTACTGGGCCAGTACCCGGGAGAGCGGAGTTCCTGATCGCTTCACCGGCTC

CGGATCAGGAACCGATTTCACTCTGACTATCTCCTCAGTGAAGGCTGAGGACCTGCGCCGTGTACTACTGCCAGCAGTACTACAGATATCACACATTTGGCACCGGC

ACCAAACTGGAGCTGAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGA

ATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCAC

CTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGTTCGCCCGTCACAAAG

AGCTTCAACAGGGGAGAGTGT (SEQ ID NO:736)

Sequences encoding the fusion proteins in Figure 12A and Figure 12B

F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAAGTCTCACACTCCTGCGTCATCAGCGGCGACACAACCTCCACAGTCTACAT
U	GGGCTGGTTCAGGCAGGCACCCGGAGAAGAAAGGGAGGGAATTGCTTCCATTTACGGAGCTGACGACTCCCCCTACTACGCCGATTCCGTGAAGGGACGAT
S	TCACCATCTTTCAGGACAACGCCAAGAGAACAGTTGTCTTGCAGATGAACAGTCTGAAGCCCGAGGACACCGGCACCTACTACTGCGCTGCTACCACCATT
E	CATTTTGTCGTGGGCGGGCCCTGGCTCTTGGCTGCTCCTAACTACAAGTACTGGGGACAGGGCACCCTGGTGACCGTTTCTTCTTCCGAGCCCAAATCTGCTGA
-	CAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCC
7	GGACCCCCGAGGTCACATGCGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG
3	ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAA
9	GGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGG
	AGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
	AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
	CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:738)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAAGTCTCACACTCCTGCGTCATCAGCGGCGACACAACCTCCACAGTCTACAT
U	CAGGCAGGCACCCGGCAAAGAGAGAAGGGCGTGGCTGCTATCCACAGTGGGAGAACATATTATGCCGAATCAGTGAAGTCCAGGTTTACCATCTCCCGCG
S	ACAACGCCAAAAACACCGCCTACCTGCAGATGAACTCCCTGAGGCCCGAGGACACCGCCGTGTACTACTGCGCCGCCGACCTGAGGTACGACGGCTCCTGG
E	TCCGCCACCCAAGGACTACCACTACTGGGGCCAGGGCACCCTGGTGACCGTTTCCTCCGAGCCCCAAATCTGCTGACAAAACTCACACATGCCCACCGTGCCC
-	AGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGG
7	TGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
4	AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCC
O	CATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCC
	TGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTG
	GACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT
	GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:739)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAAGTCTCAGGCTTCCTGCGCAGCTTCAGGCTACACATACTCCTGCATGGGCTG

U	GTTCAGGCAGGCCCCTGGAAAGGAAAGGGAGGCTGTGGCAACAATTAACACTGAGTCCGGCACCACATACTACTTGGACTCCGTTAAAGGCAGATTCACCA
S	TCTCCAAGGACAACACCAAGAACACACTGTACCTGCAGATGAACTCCCTGAAACCCGAGGACACAGCCATGTACTACTGCGCCGCCCTCGACGGATACTGC
E	GGAACAGCTGCATACGTGAGGCCCGTGAGCTACCGATACTGGGGACAGGGAACCCAGGTCACCGTGTCATCCGAGCCCAAATCTGCTGACAAAACTCACAC
-	ATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGG
7	TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGG
4	GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
1	AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCA
	AGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
	ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
	GATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:740)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCGCAGCTTCAAGGGACACATACTACAGGGGCTGGTT
U	CAGGCAGGCTCCCGGAAAAGAGAGGAAGGCGTGGCTGCTATCTACCTCGGGGACACATACTATGCCGACACGGTGAAGGGGAGATTCACCATCAGCCAGG
S	ACAGCGCAAAGAACACCGTGTTCTTGGAGATGAACAGCTTGAAGGTGGAGGATACCGCCATGTACTATTGCGCCGCCGACACAAGAAGTACGGCAGCAAGTGG
E	TACACAAACGACTACCACTACTGGGGGCAGGGCACCCAGGTTACAGTGAGTTCCGAGCCCAAATCTGACAAAACTCACACATGCCCACCGTGCCCAGC
-	ACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGG
7	ACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC
4	ACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCAT
2	CGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGA
	CCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGAC
	TCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCA
	CAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:741)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGAAAGCGTCCAGGCAGGAGGATCACTCAGGCTCTCCTCCGCAGCATCAGGCTACATCAACGGCGGCAACTACAT
U	GGGCTGGTTTAGGCAGGCTCCTGGCAAAGAAAGGGAGGGAGTGGCCGCAATCTACATCCACGGACGCTCTACCTACTACGCCGACAGTGTCAAAGGCAGGT
S	TCACAATCTCCAGGGACAACGCCAAGAACATGGTGTATCTGCAGATGAACAGCCTGAAGCCCGAAGATACTGCTATGTACTACTGCGCCACCGGCCTGGTT
E	TCCAGGCCTGATTGGGTCCTCCTGGCCAGAGGATACAACGCCTGGGGACAGGGAACCCTGGTGACTGTTTCCTCCGAGCCCAAATCTGCTGACAAAACTCA
-	CACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCG
7	AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
4	CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
3	CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGA
	CCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
	ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
	CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:742)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGAAGTCTCAGGCTCTCCTGCGCAGCTTCAGGCTACATCAGGGCCAAAATGTGCGT
U	GGGCTGGTTCAGGCAGACACCCGGAAAAGAGAGGAGGTGGTGGGGACAGTGTACACCGGAAGCGGAGCTATCTCCTACGGTGATAGTGTGGAGGGCAGGT
S	TCACAATCTCCCAGGATAATGCCAGGAACACACTGTACTTGCAGATGAACTCCCTGAGTCCTGAGGATACCGCCATGTACTACTGTGCCGCCACCAGGTCC
E	GTGGTGTTGAACAGTGCCGGCGTGTGCGTTACCGTGCCTGGATGGTACAACTACTGGGGCCAGGGCACCCTGGTGACAGTTTCTTCCGAGCCCAAATCTGC
-	TGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT
7	CCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC

4	AAGACAAAGCCGCGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
4	CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCC
	GGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
	AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGT
	CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:743)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCGCAGCTTCAGGCACAATCTCCTACTGCATGCAT
U	GGGCTGGTTCAGGCAGTCACCCGGAAAAGAAAGGGAGGGGGTGGCAGGTATCGACTCCTACGGAAACATTCGGTACTCTGACTCCGCAAAGGGACGCTTCA
S	CCATCTCCAAGGACAACACCAACAACATCCTCTACCTCCAGATGAACAGCCTGGAGCCTGAAGATACTGCTATTGTACTACTGCGCCGCCGCGAACCCAGAAGG
E	TGTGCATACCTCAACGTGGACGACAGAATTTTGCCCGCCCTGTTCTACGACTCCTGGGACAGGCAACCCAGGTCACAGTGTCCTCCGAGCCCAAATCTGC
-	TGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT
7	CCCGGACCCCCGAGGTCACATGCGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC
4	AAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
5	CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCC
	GGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
	AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGT
	CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:744)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCGCAGCTTCAGGCTACACAAACTCCATCAACTACAT
U	GGCCTGGTTCAGACAGGCCCCCGGAAAAGAAAGGGAAGGCGTGGCAAGGATTTACACCAGGGACGGTCTGCAGAATACTTGGCCGATTCCGTTGTCGGCC
S	GGTTCACCATCTCCCAGGTTAATGTGAAAAACACCGTGTTCCTGGAGATGACCTCCCTGAAGCCCGAGGACACCGCCATGTACTACTGCGCCGCCGCCGCC
E	GGCGCCTCCGCCTACGGCGTGCTGAACAAGGACCACTACAACTACTGGGGCCAGGGCACCCAGGTGACCGTGTCCTCCGAGCCCAAATCTGCTGACAAAAC
-	TCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
7	CCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG
4	CCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC
6	CAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA
	TGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
	AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
	CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:745)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCGCAGCTTCAGGATCAGGCTACACATACTCCTCCTACAGCAT
U	GGGTTGGTTCAGGCAGGACCCCGGAAAAGAGAGGGAGGGAGTGGCTGCTATGAACGCCAGGGGAGACAACCCATATTTACGCCGACAGTGTCAAAGGCAGGT
S	TCACCATCTCCAGGGACAATGCTAAGAAAACTTTATATCTGCAGATGAACAGCCTGAAGCCCGAGGACACCGCCATGTACTACTGCGCTGCCGCTAGGGCT
E	GAACCAAGCACAGTGGTCGTGAGCGCCAAGGGATACAATTACTGGGGCCAGGGCACCCAGGTGACCGTTTCTTCTGAGCCCAAATCTGCTGACAAAACTCA
-	CACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCG
7	AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
4	CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
7	CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGA
	CCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
	ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
	CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:746)

F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCGCAGCTTCAGGCTACATCTACAACACAACTACAT
U	GGGCTGGTTCAGGCAGGCCCCCGGAAAAGAAAGAGAGGAGTGGCAACCATCTACGTGGGGGGAGGATCTACCTATTTACGCTGATTCCGTCAAGGGCAGGT
S	TCACCATCAGCAGGACAACACCAAGAACACACTGTACCTGCAGATGAACAGTTTGAAACCCGAGACACCGCTATGTATTACTGCGCCGCCGGCCTGGTG
E	AGCAGACCTGATTGGGTTCTGTTGGCCAGGGCATACACCAACTGGGGCCAGGAACCCTGGTGACAGTGTCTAGCGAGCCCAAATCTGCTGACAAAACTCA
-	CACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCG
7	AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
4	CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
8	CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGA
	CCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
	ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
	CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAA (SEQ ID NO:747)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCGCAACATCCGGCTACCCATACATCGGCCCCTGCAT
U	GACCTGGTTCAGGCAGGCTCCTGGCAAAGAGAGGAGTGGGTGGCTTTCATTTACAAGGACGACGGGGCCACCTACTACAGTGACTCCGTTAACGGCCGCT
S	TCACAATTTCCACCGACAACGCCAACAACGCATTGTACCTGCAGATGAACAGCCTGAAGCCCGAGGACACCGCAGTGTATTATTGTGCCGCCTCCCCCCCC
E	TCCCCTAGAACACATGTTACACCGGCGCCTCCTACTACAACTACTGGGGGCAGGGCACCCAGGTCACAGTTTCTTCCGAGCCCAAATCTGCTGACAAAAC
-	TCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
7	CCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG
4	CCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC
9	CAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA
	TGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
	AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
	CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:748)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTCCGCAGCTTCAGGCTACATCGGCGGAAAAATGTGCGT
U	GGGCTGGTTCAGGCAGACCCCCGGCAAGGAGAGGAGGTGGTGGGCACCGTGTACACCGGCTCCGGCGCCATCTCCTACGGCGACTCCGTGGAGGGCAGGT
S	TCACCATCTCCCAGGACAACGCCAGGAACACCCTGTACCTGCAGATGAACTCCCTGTCCCCCGAGGACACCGCCATGTACTACTGCGCCGCCGCCACCAGGTCC
E	GTGGTGCTGAACTCCGCCGGCGTGTGCGTGACCGTGGTACAACTACTGGGGCCAGGGCACCCTGGTGACCGTGTCCTCCGAGCCCAAATCTGC
-	TGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT
7	CCCGGACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC
5	AAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
O	CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCC
	GGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
	AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGT
	CTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:749)
F	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAACCTCTCCTGCACAGCATCAGGCTACACAATCGGCCAGCTTCAGCAT
U	GGCTTGGTTCAGGCAGGCCCCCGGAAAAGAGAGGAGGGAGGTTGCTTACATCAACACCGGCGGAGTGCCCTACTACCTCGACTCAGTGAAGGGAAGGGCCA
S	CCATCAGCAGGGACAACGCTGCTGACACATGAACTCCTTGAAGCCCGAGGACACCGCCCTGTATTACTGCGCTGCCCGACCTGGACTC
E	GGCGAATCTGCTTGGTACATCGACGCCAGGGCCTACAATTACTGGGGCCAGGGAACCCTGGTGACCGTTTCTTCTTCCGAGCCCAAATCTGCTGACAAAACTCA
-	CACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCG

7 5 1	AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
	CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
	CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGA
	CCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
	ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
	CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:750)
F U S E - 7 5 2	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCGCAACATCCGAGAGGACATACACACAGAACTGGAT
	GGGCTGGTTCAGGCAGAGCCCCGGAAAAGAGGAGGGTGTGGCTGTGATTAACACCGGAAGCGGGCTGACCGCCTACGTTGATTCCGTGAAGGGAAGGT
	TTACAATTTCCAAGGACAACACCAAGAGCACCGTGTACCTGCAGATGAACAGCTTGAAGCCCGACACACCGCCATGTACTATTGCGCCGCCGAGGTGCAG
	GGCGGAGTTTTGATTGCCACCGGCTACAGATATTGGGGCCAGGGAACCCTGGTGACCGTGTCTAGTGAGCCCAAATCTGCTGACAAAACTCACACATGCCC
	ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACAT
	GCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG
	CAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT
	CCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACC
	AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
	CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
	TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:751)
F U S E - 7 5 3	CAGGTTCAGCTCCAGGAGTCAGGCGGAGGATCAGTCCAGGCAGGAGGAAGTCTCAGGCTCTCCTGCACAACATCCAGGTTCAAAATCAACGACGGCTCCGT
	CGGCTGGTTCAGGCAGGCTCCTGGAAAAGAGAGGAGGGGGTGGCTTCCATCTACGCTGATGGAGGAGGCATCAGCTATCCGTGAAGGGCAGGT
	TCACCATTTCTCAGGATAACTCCGCCAACACCGTGTACCTGCAGATGAACAGCTTGAAGCCCGAAGATACTGCTATGTATTACTGCGCCGCCCTGGCCTTG
	CGCAGATATGGACCTCTGGACCCCAGGGAGTTCAGGTTTTGGGGCCAGGGAACCCAGGTGACCGTGTCTAGTGAGCCCAAATCTGCTGACAAAACTCACAC
	ATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGG
	TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGG
	GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
	AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCA
	AGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
	ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
	GATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:752)
4 D 6	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTGTCCTGCAGGAGGCGCGCCCAGGTCCAACTGCAGGAGTCTGGGGGAGGCTCGGTGCAGGCTGG
	AGGGTCTCTGAGACTCTCCTGTGTAGTCTCTGGAGACACCTACAATTTGAACCGCATGGCCTGTTCCGCCAGGCTCCAGGAAAGGAGCGCCGAGGGCGTCG
	CAGGTACTGGAATTAATGATATTACAGCATACGCGGACTCCGTGAAGGGCCGATTCACGGTCTCCAATGACCACGCCAAGAAAAGTCTGTATCTGCAAATG
	AACAACCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCCCGATCCGTGTATCTTACAGCTCCATCTCTCCGGCCGGAGTCTTATAACTACTGGGG
	CCAGGGCACCCTGGTCACCGTCTCCTCA (SEQ ID NO:753)
4 C 9	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTGTCCTGCAGGAGGCGCGCCCAGGTCCAACTGCAGGAGTCTGGAGGAGGCTCGGTGCAGGCTGG
	AGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATACATCTACGGTTAACAACTACATGGGCTGGTTCCGCCAGGGAAGGAGCGCGAGGGGCTCG
	CAACTATTTATATTAATGGTGCTAGCACATACTATGCCGACTCCGTGAAGGGCCGATTCACCATCTCCCGAGACAACCACAGAATACACTGTATCTGCAA
	ATGAACAGCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCAGGTCTCGTCTCACGCCCAGATTGGGTCCTCTTGGCGCGGGCATATAACACTG
	GGGCCAGGGGACCCTGGTCACCGTCTCCTCA (SEQ ID NO:754)

3	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTGGGGGAGGCTCGGTGCAGGCTGG
H	AGGGTCTCTGAGACTCTCCTGTGAAGCCTCTGGATACATTAACAGTAATAACTACATGGGCTGGTTCCGCCAGGCTCCAGGGAAGGAGCGCGAGGGGGTCG
8	CAGTTAAATATATTCATGGGCCTAGTACATACTATGCCGACTCCGTGAAGGGCCGATTTTATCATCTCCCAAGACAACGCCAAGAAATATGGTGTATCTGCAA
	ATGAACACCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGCAGGTCTCGTCTCCCGGCCGGATTGGGTGCTCTTGGCGCGGGGATATAACAACTG
	GGGTCAGGGGACCCAGGTCACCGTCTCCTCA (SEQ ID NO:755)
3	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTGGGGGAGGCTCGGTGCAGGATGG
F	AGGGTCTCTGAGACTCTCCTGTGTAGTCTCTGGAAACACCTACAATTTGAACCGCATGGCCTGGTTCCGCCAGGCTCCAGGAAAAGAGCGCGAGGGCGTCG
8	CAGGTACTGGAATTAATGATATTACAGGGTACGCAGACTCCGTGAAGGGCCGATTCACGGTCTCCGTAGACAGCGCCGAGAAAACGCTGTATCTACAAATG
	AACTTGCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCCCGATCCGTTCCTGCGGCTTACAGCTCCAATCTCTCCGGCCGGAGTCTTATACGTACTGGGG
	CCAGGGCACCCTGGTCACCGTCTCCTCA (SEQ ID NO:756)
5	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTCGGTGCAGGCTGG
E	AGGGTCTCTGAGACTCTCCTGTGTAGTCTCTGGAAACACCTACAATTTGAACCGCATGGCCTGGTTCCGCCAGGCTCCAGGAAAGGAGCGCGAGGGCGTCG
1	CAGGTACTGGAATTAATGATATTACAGCGTACGCGGACTCCGTGAAGGGCCGATTCACGGTCTCCAGTGACCACGCCACGAAAACTCTGCATCTGCAAATG
O	AACAGCCTGAAACCTGAGGACACTGGGATGTACTACTGTGCGGCCCGATCCGTTCCTGCGGCCGGAGTCTTATAACTACTGGGG
	CCAGGGGACCCAGGTCACCGTCTCCTCA (SEQ ID NO:757)
5	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTCGGTGCAGGCTGG
E	AGGGTCTCTGAGACTCTCCTGTGTAGTCTCTGGATACACCTACAATTTGAACCGCATGGCCTGGTTCCGCCAGGCTCCAGGAAAGGAGCGCGAGGGCGTCG
7	CAAGTACTGGAATTAATGATATTACAGAATACGCAGACTCCGTGAAGGGCCGATTCACGGTCTCCACAGACGCCGTCAAGAACACTCTGTATCTGCAAATG
	AACAGCCTGAAACCTGACGACACTGCCATGTACTACTGTGCGGCCCGATCCGTTCCTGAGGCAGACAGTCCCGTCTCTCCGGCCGGAGTCTTATACGTACTGGGG
	CCAGGGCACCCAGGTCACCGTCTCCTCA (SEQ ID NO:758)
5	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTCGGTGCAGGCTGG
B	AGGGTCTCTGAGACTCTCCTGTGTAGTCTCTGGAAACACCTACAATTTGAACCGCATGGCCTGGTTCCGCCAGGCTCCAGGAAAAGAGCGCGAGGGCGTCG
9	CAGGTACTGGAATTAATGATATTACAGGGTACGCAGACTCCGTGAAGGGCCGATTCACGGTCTCCGTAGACAGCGCCGAGAAAAAGCTGTATCTACAAATG
	AACAGCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCCCGATCCGTTCCTACAGCTCCATCTCTCCGGCCGGAGTCTTATACGTACTGGGG
	CCAGGGCACCCAGGTCACCGTCTCCTCA (SEQ ID NO:759)
2	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTCGGTGCAGGCTGG
C	AGGGTCTCTGAGACTCTCCTGTACAACCTCTAGATTCAAGATCAATGACGGCTCAGTGGGCTGGTTCCGCCAGGCTCCAGGGAAAGAGCGCGAGGGGGTCG
8	CATCTGTTTATGCTGATGGTGGTGGTATATCCTATGCCGACTCCGTGAAGGGCCGATTCACCATCTCCCAAGACAACTCCGCGAACACGGTGTATCTGCAA
	ATGAACAGCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCGCTAGCTTTGCGCCGGTATGGGCCCTTGGACCCCAGGGAATTTAGGTTCTGGGG
	CCAGGGGATCCTGGTCACCGTCTCCTCA (SEQ ID NO:760)
3	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTCGGTGCAGGCTGG
A	AGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATACATCGGCGGAAAAATGTGCGTGGTTGGTTCCGCCAGACTCCAGGGAAGGAGCGCGAGGTTGTCG
6	GAACAGTTTACACTGGTAGTGGTGCAATATCGTATGGCGACTCCGTGGAGGGCCGATTCACCATCTCCCAAGACAACGCCAGGAACACGCTGTATCTGCAA
	ATGAACAGCCTGAGCCCTGAGGACACCGCCATGTACTACTGTGCGCTACCCGGAGCGTGGTGTCTGCGTCACAGTCCCCGGTTG
	GTATAACTACTGGGGCCAGGGACCCAGGTCACCGTCTCCTCA (SEQ ID NO:761)
1	ATGGGGTCTACCGCCATCCTTGGCCTCCTGCTCGCTGTCCTGCAGGAGGGCGCGCCCAGGTCCAACTGCAGGAGTCTGGGGGAGGCTCGGTGCAGCCTGG
B	AGGGTCTCTGACACTCTCCTGTAGAGCCTCAGAATACAGTATGAGCTTCATAGGGTGGTTCCGCCAGGCTCCAGGGAAGGAGCGCGAGAAGGTCGCAACTA
8	TTTATACTCGTAGTGGTCGCACATACTATGCCGACTCCGTGCGGGCCGATTCACCATCTCCCAAGACAACGCCAAGAACACGGCGTATCTGCAAATGAAC

AGCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCAGGGTCCCTACGGAGGTCCCTGGTCCGGCTCTAGACTCACGTCACTTCAACTACTGGGGCCA GGGGACCCTGGTCACCGTCTCCTCA (SEQ ID NO:762)

Table 4. Expression Yield of the proteins

	Yield		Yield		Yield		
FUSE-320	291 mg/L	**FUSE-1145**	232 mg/L	**FUSE-750**	451 mg/L	**FUSE-1109**	76 mg/L
FUSE-691	207 mg/L	**FUSE-1146**	328 mg/L	**FUSE-751**	469 mg/L	**FUSE-1124**	45 mg/L
FUSE-694	67 mg/L	**FUSE-1147**	232 mg/L	**FUSE-752**	351 mg/L	**FUSE-1125**	229 mg/L
FUSE-696	59 mg/L	**FUSE-1148**	286 mg/L	**FUSE-753**	553 mg/L	**FUSE-1126**	83 mg/L
FUSE-697	61 mg/L	**FUSE-599**	108 mg/L	**FUSE-1054**	407 mg/L	**FUSE-1127**	66 mg/L
FUSE-765	197 mg/L	**FUSE-739**	134 mg/L	**FUSE-1062**	67 mg/L	**FUSE-1128**	53 mg/L
FUSE-773	99 mg/L	**FUSE-740**	373 mg/L	**FUSE-1063**	57 mg/L	**FUSE-1129**	379 mg/L
FUSE-774	131 mg/L	**FUSE-741**	438 mg/L	**FUSE-1064**	516 mg/L	**FUSE-1130**	379 mg/L
FUSE-916	206 mg/L	**FUSE-742**	330 mg/L	**FUSE-1065**	37 mg/L	**FUSE-1136**	149 mg/L
FUSE-923	429 mg/L	**FUSE-743**	440 mg/L	**FUSE-1072**	136 mg/L	**FUSE-1137**	171 mg/L
FUSE-926	113 mg/L	**FUSE-744**	231 mg/L	**FUSE-1073**	503 mg/L	**FUSE-1138**	176 mg/L
FUSE-943	185 mg/L	**FUSE-745**	92 mg/L	**FUSE-1074**	155 mg/L	**FUSE-1139**	177 mg/L
FUSE-944	205 mg/L	**FUSE-746**	273 mg/L	**FUSE-1075**	144 mg/L	**FUSE-1177**	133 mg/L
FUSE-966	232 mg/L	**FUSE-747**	329 mg/L	**FUSE-1076**	453 mg/L	**FUSE-1179**	103 mg/L
FUSE-967	196 mg/L	**FUSE-748**	495 mg/L	**FUSE-1077**	612 mg/L	**FUSE-1184**	551 mg/L
FUSE-968	198 mg/L	**FUSE-749**	281 mg/L	**FUSE-1078**	330 mg/L	**FUSE-1229**	205 mg/L

IL-18 details and embodiments

[0250] The contents of International Application No. PCT/US2023/071663, filed August 4, 2023, entitled, "IL-18 Fusion Proteins and Methods of Producing IL-18," and International Application No. PCT/US2024/027248, filed May 1, 2024, entitled, "Methods of Using IL-18 Fusion Proteins" are incorporated by reference herein in their entirety as though fully set forth.

[0251] Described herein are IL-18 variants that can be used in the multi-cytokine fusion proteins of the invention. Described herein are also IL-18 fusion proteins comprising IL-18 variants that can be used in the methods relating to administering separate fusion proteins.

[0252] Various embodiments provide one or more fusion proteins, each comprising (i) an IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, and (ii) a first protein capable of translocating into an endoplasmic reticulum (ER) including a cytosolic or nuclear protein engineered to translocate into an endoplasmic reticulum (ER) by means of addition of signal peptide/leader sequence onto the N-terminus of such an engineered protein, or a fragment of said proteins. Preferably, the protein capable of translocating into an ER has an amino acid sequence which initiates transport of a protein (e.g., the IL-18 or its fragment, variant, or a fragment of its variant) across the membrane of the endoplasmic reticulum. In various embodiments, the fusion protein comprises further an amino acid linker. For example, the amino acid linker can be between (a) the protein capable of translocating into an endoplasmic reticulum (ER) and (b) the propeptide (or variant) or IL-18 (or variant).

[0253] In some embodiments, the one or more fusion proteins do not comprise an IL-18 propeptide or its variant. An "IL-18 propeptide", or "propeptide" or "PP" in this invention, may also be used interchangeably, which describes an amino acid sequence linked to IL-18 or IL-18 variant in an IL-18 precursor or IL-18 variant precursor, and which upon removal renders a mature IL-18 or its fragment, or IL-18 variant or its fragment thereof. For example, an IL-18 propeptide may have a sequence of amino acid residues 1-36 of Uniprot ID Q14116.

[0254] In some embodiments, the one or more fusion proteins also include a propeptide (PP) or its variant. Examples of propeptide variants are provided herein, including those in Table 6B. This can inactivate IL-18 or IL-18 variant, and so a propeptide directly or indirectly is linked to the IL-18 or IL-18 variant forms a precursor IL-18 or precursor IL-18 variant. Preferably, the PP or its variant is on the N-terminus end relative to IL-18 (or its fragment, variant, or a fragment of its variant) in the fusion protein. In some embodiments, the one or more fusion proteins also include a cleavage site, which is preferably based on a peptide substrate sensitive to enzymatic/protease cleavage. The cleavage site may be positioned within the PP, between the PP or its variant (if present) and the IL-18 (or its fragment, variant, or a fragment of its variant); or may be positioned between the protein capable of translocating into an ER and the PP (if present); or may be positioned between the protein capable of translocating into an ER and the IL-18 or its fragment, variant, or a fragment of its variant, especially in the absence of a PP. In some embodiments, when PP is present, the cleavage site is positioned within the PP. In further embodiments, the one or more fusion proteins include (i) an IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, (ii), a propeptide (PP) or its variant, which inactivates IL-18, and a cleavage site.

[0255] Examples of propeptide variants include a polypeptide having AAEPVEDNX_1INFVAMKFIDNTLYFIAEDDEN wherein X_1 is any amino acid except cysteine (SEQ ID NO:238). In various embodiments, X_1 is alanine, valine, isoleucine, leucin, methionine, phenylalanine, tyrosine or tryptophan (SEQ ID NO:239). In various embodiments, X_1 is valine (SEQ ID NO:78). In various embodiments, X1 is serine, threonine, asparagine, or glutamine (SEQ ID NO:240). In various embodiments, X_1 is serine (SEQ ID NO:76).

[0256] In various embodiments, the fusion protein does not comprise a polypeptide consisting of the sequence X_1-X_2-X_3-X_4 between (i) the propeptide or propeptide variant, and (ii) the mature IL-18 or mature IL-18 variant, wherein X_1 is L or absent, X_2 is E or absent, X_3 is S or absent, and X_4 is D or absent.

[0257] In various aspects of the fusion proteins, the IL-18 (or its fragment, variant, or fragment of its variant) is linked by a polypeptide bond to the first protein capable of translocating into the ER. The fusion proteins may have a variety of configurations. Preferably, the N-terminus of the IL-18 (or its fragment, variant, or a fragment of its variant) is linked by a polypeptide bond directly or indirectly to the C-terminus of the first protein capable of translocating in the ER.

[0258] Yet in other embodiments, the C-terminus of the IL-18 (or its fragment, variant, or a fragment of its variant) is linked by a polypeptide bond directly or indirectly to the N-terminus of the first protein capable of translocating in the ER. As a nonlimiting example, the IL-18-variant (or IL-18, a fragment of IL-18, a fragment of the

IL-18 variant) is fused to the N-terminus of a knob of a knob-into-hole heterodimeric IgG1 protein with or without a propeptide (pp).

[0259] In further embodiments, where the C-terminus of the IL-18 is linked to the N-terminus of the first protein capable of translocating in (or through) the ER, a second protein capable of translocating through the ER is often fused to the N-terminus of the IL-18 to mediate masking. It is contemplated that a fusion protein further comprises (iii) a second protein capable of translocating into/through an ER, or a "scaffold" such as heat shock proteins (HSPs) that may not translocate through the ER. In some embodiments, if HSP (nuclear protein) or a cytosolic protein is fused to the N-terminus of the IL-18 to mediate masking, it often requires a signal peptide fused to the N-terminus of the "scaffold" to mediate transport to the ER; and, if the scaffold is fused to the C-terminus of IL-18 to serve to stabilize the complex, then a second protein capable of translocating through the ER is often fused to the N-terminus of the IL-18 to mediate masking. Hence, in some embodiments, the IL-18 (or its fragment, variant, or a fragment of its variant) is at the C-terminus of the fusion protein; in some embodiments, the N-terminus of the IL-18 (or its fragment, variant, or a fragment of its variant) is on the C-terminus end relative to the first protein capable of translocating in the ER, and the C-terminus of the IL-18 (or its fragment, variant, or a fragment of its variant) is on the N-terminus end relative to the second protein capable of translocating in the ER. The "first" or "second" protein capable of translocating in an ER is used as a relative reference. One or more exemplary amino acid sequences of each component of the fusion protein are shown in Tables 1 and 4.

[0260] Some embodiments provide that the first protein/polypeptide capable of translocating into an ER comprises an immunoglobulin heavy chain constant region. In some embodiments, the immunoglobulin heavy chain constant region comprises an immunoglobulin heavy chain constant region domain selected from the group consisting of a CH2 domain, a CH3 domain, and a CH4 domain, or a combination thereof. In some embodiments, wherein the immunoglobulin heavy chain constant region comprises a CH2 domain and a CH3 domain. In some embodiments, the immunoglobulin heavy chain constant region lacks at least a CH1 domain. In some embodiments, the immunoglobulin heavy chain constant region is a human immunoglobulin heavy chain constant region. In some embodiments, the immunoglobulin heavy chain constant region is an immunoglobulin heavy chain constant region present in the same species as the IL-18. In other embodiments, the immunoglobulin heavy chain constant region is an immunoglobulin heavy chain constant region present in the same species as an organism with which a nucleic acid molecule encoding the fusion protein or a precursor of the fusion protein is transformed or transfected. Further embodiments provide that the fusion protein lacks an immunoglobulin variable domain (V_H).

[0261] In various embodiments, the IL-18 (or its fragment, variant, or fragment of its variant) is identical (in sequence) to that of a human origin, and the immunoglobulin heavy chain constant region comprises a hinge region, and a CH2 domain or a CH3 domain, and more preferably comprises a hinge region and both a CH2 domain and a CH3 domain.

[0262] In various embodiments, the IL-18 (or its fragment, variant, or fragment of its variant) is at least 95%, 90%, or 85% identical (in sequence) to that of a human origin, but with amino acid substitutions or other modifications that reduces affinity of the IL-18 (or its fragment, variant, or fragment of its variant) for IL-18BP. It is contemplated that immunoglobulin heavy chain constant regions suitable for the invention may be derived from immunoglobulins belonging to any of the five immunoglobulin classes referred to in the art as IgA (Igα), IgD (Igδ), IgE (Igε), IgG (Igγ), and IgM (Igμ). However, immunoglobulin heavy chain constant regions from the IgG class are preferred. Furthermore, the immunoglobulin heavy chain constant regions may be derived from any of the IgG antibody subclasses referred to in the art as IgG1, IgG2, IgG3, and IgG4. Immunoglobulin heavy chain constant region domains have cross-homology among the immunoglobulin classes. For example, the CH2 domain of IgG is homologous to the CH2 domain of IgA and IgD, and to the CH3 domain of IgM and IgE. Preferred immunoglobulin heavy chain constant regions include protein domains corresponding to a CH2 region and a CH3 region of IgG, or functional portions or derivatives thereof. Further description of immunoglobulin heavy chain constant regions is discussed in detail in U.S. Pat. No. 5,541,087, and U.S. Pat. No. 5,726,044, which are incorporated by reference herein.

[0263] In multiple embodiments, the protein/polypeptide to be fused with the IL-18 (or its fragment, variant, or a fragment of its variant) is a dimer of two immunoglobulin heavy chain constant regions/chains, optionally cross-linked by a pair of disulfide bonds between cysteines on adjacent hinge regions. In some embodiments, a hinge region may have an upper hinge domain, a core hinge domain, and a lower hinge domain. In some embodiments, an upper portion of the hinge domain may include or remove the cysteine that is known to form a disulfide bond with the light chain or a fab, resulting in sequences such as EPKSC (SEQ ID NO:241) or EPKSS (SEQ ID NO:242) or EPKSA (SEQ ID NO:243). For example, fusion proteins including IgG1-based ER translocating protein, except FUSE-501, FUSE-503, and FUSE-509, may have removed cysteine from the hinge region, e.g., EPKSS (SEQ ID NO:242) in IgG1-based ER translocating protein, except for FUSE-507 (FUSE-507 has EPKSA (SEQ ID NO:243) in the hinge region). A hinge region may also contain a core hinge domain, such as comprising a sequence CPPCP (SEQ ID NO:244) or a variant where the cysteine is replaced. A hinge region may further include a lower hinge domain, such as comprising a sequence APELLGGP (SEQ ID NO:245) or APEAAGGP (SEQ ID NO:246). In another example, FUSE-509 has an IgG4-based ER-translocating protein, using a hinge region as depicted in Chiu et al., *Antibodies* 2019, *8*(4), 55, 2019. While constructs including immunoglobulin hinge regions are preferred, as depicted in the drawings, the invention contemplates that crosslinking at other positions may be chosen as desired. Furthermore, in some cases, two or more monomers may associate non-covalently to produce dimers or multimers. In various aspects wherein the protein/polypeptide is a dimer of two immunoglobulin heavy chain constant regions/chains, the IL-18 (or its fragment, variant, or a fragment of its variant) is linked to one, and only one, of the two (or more) immunoglobulin heavy chain constant regions/chains. In the case of a wild type IgG-Fc that forms a homodimer, in various instances, a IL-18 is placed on the C-terminus of each monomer of the Fc, thereby having two IL-18 placed on the C-terminus of the Fc. In some instances, a heterodimer may form (e.g., in purification step) when one wild-type Fc fused to one IL-18 is mixed with another wild-type Fc not fused to IL-18. In other aspects, an IL-18 (or its fragment, variant, or a fragment

of its variant) is linked each of the two (or more) immunoglobulin heavy chain constant regions/chains in the fusion protein.

[0264] In some embodiments, two arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can be heterodimerized by creating "knobs-in-holes" (KiH) mutations in the CH3 domain. This structural feature in the polypeptide arms allows for assembly of two half antibodies (e.g., Fc heterodimer; and VH–CH and VL–CL domains). For example, a heteromultimer (including a heterodimer) may comprise a first polypeptide and a second polypeptide each comprising a CH3 domain, wherein the polypeptides meet at an engineered interface within the CH3 domain, and the first polypeptide contains an engineered protuberance ("knob") in the interface with at least one contact residue replaced with an import residue having a larger side chain volume than the original residue, and the second polypeptide contains an engineered cavity ("hole") in the interface with at least one contact residue replaced with an import residue having a smaller side chain volume than the original residue. In some embodiments, the engineered interface of a heteromultimer includes at least two protuberance-into-cavity mutant pairs. Volumes and accessible surface areas of each amino acid are described in A. A. Zamyatnin, *Prog. Biophys. Mol. Biol.* 24: 107-123, 1972 and C. Chothia, *J. Mol. Biol.* 105: 1-14, 1975. For example, import residues for the formation of a protuberance can be arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W); and preferably the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine. As another example, import residues for the formation of a cavity can be alanine (A), serine (S), threonine (T) and valine (V); and preferably the original residue for the formation of the cavity has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. For example, a T366W mutation in CH3 domain for the "knob"/protuberance chain, and a T366S/L368A/Y407V mutation in CH3 domain for the "hole"/cavity chain. Additionally, the KiH configuration may be coupled further mutations to permit S-S disulfide linkage between the two chains. In various aspects wherein the protein/polypeptide is a heterodimer of a the KiH configuration, the IL-18 (or its fragment, variant, or a fragment of its variant) is linked to one, and only one, of the two (or more) immunoglobulin heavy chain constant regions/chains (i.e. knob or hole).

[0265] In some embodiments, the two or more arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can contain another symmetric-to-asymmetric steric complementarity design (e.g., HA-TF, ZW1), a charge-to-charge swap interaction (DD-KK), a charge-to-steric complementarity swap plus additional long-range electrostatic interaction (e.g., EW-RVT), or an isotype strand swap design (e.g., strand-exchange engineered domain (SEED)), or Xmab, 7.8.60, Electrostatic Steering, A107, or Duobody, so as to form heterodimers/heteromultimers. Further description of these configurations and exemplary mutations/residues are seen in *Front Immunol.* 2016; 7: 394.

[0266] In additional embodiments, suitable proteins capable of translocating into the ER or a fragment thereof of the fusion protein is a globular protein, immunoglobular protein, or a fragment thereof. In various embodiments, suitable proteins capable of translocating into the ER or a fragment thereof of the fusion protein is a short

polypeptide or protein engineered with a signal peptide for translocating into the ER. In some embodiments, suitable proteins capable of translocating into the ER or a fragment thereof of the fusion protein is a short polypeptide or protein being about 2 kDa or no greater than 250 kDa. As examples, the short polypeptide or protein is about 2-5 kDa, about 6-10kDa, about 11-20 kDa, about 21-30 kDa, about 31-40 kDa, about 41-50 kDa, about 51-75 kDa, about 76-100 kDa, about 101-125 kDa, about 126-150 kDa, about 151-175 kDa, about 176-200 kDa, about 201-225 kDa, or about 256-250 kDa. As further examples, the short polypeptide is 2, 3, or 4 amino acids, or flexible linkers or amino acid chain of 2-5, 6-10, 11-15, 16-20, 21-25, 26-30 amino acids. As further examples, the short polypeptide is 1, 2, 3, or 4 amino acids, or flexible linkers or amino acid chain of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, or 35 amino acids. Additional examples of these short polypeptide include but are not limited to a dimer of two amino acids, a tri-mer of three amino acids, a 4-mer of four amino acids, a 5-mer of five amino acids, or a peptide selected from the group consisting of T, PT, MPT, S, GS, GGS, GGGS (SEQ ID NO:235), and (GGGGX$_\lambda$ (SEQ ID NO:236))n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or an integer larger than 5. In some embodiments, the amino acid linker has the amino acid sequence of (GGGGS (SEQ ID NO:237))n where n is an integer between 1 and 5, thereby an amino acid linker of 25 amino acids or shorter in length. Additional examples include (X$_\lambda$GGGG (SEQ ID NO:317))$_n$ wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX$_\lambda$GGG (SEQ ID NO:318))$_n$ wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGX$_\lambda$GG (SEQ ID NO:319))$_n$ wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGGX$_\lambda$G (SEQ ID NO:320))$_n$ wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5. Still additional examples include (X$_\lambda$GGG)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX$_\lambda$GG)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGX$_\lambda$G)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGGX$_\lambda$)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5. Still additional examples include (X$_\lambda$GG)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX$_\lambda$G)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GGX$_\lambda$)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments. Still additional examples include (X$_\lambda$G)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, (GX$_\lambda$)n wherein X$_\lambda$ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5.

[0267] In additional embodiments, suitable proteins capable of translocating in an ER can be a globular protein, human serum albumin (HSA), beta2microglobulin, transferrin, fragment antigen-binding region (Fab region), VHH antibody, single-chain variable fragment (scFv), anticalin, designed ankyrin repeat protein (DARPin), a binding domain thereof, and a fragment thereof. Additional suitable proteins capable of translocating in an ER can include type I transmembrane proteins or a fragment thereof, or type II transmembrane proteins or a fragment thereof.

[0268] In various embodiments, the fusion protein comprising the short polypeptide or protein and the IL-18 or IL-18 variant, or fragments thereof, further comprises a second proteins capable of translocating into the ER or a fragment thereof. The second protein capable of translocating into the ER or a fragment thereof can be an Fc domain or HSA, beta2microglobulin, transferrin, fragment antigen-binding region (Fab region), VHH antibody, single-chain

variable fragment (scFv), anticalin, designed ankyrin repeat protein (DARPin), a binding domain thereof, and a fragment thereof, or type I transmembrane proteins or a fragment thereof, or type II transmembrane proteins or a fragment thereof as described herein. Figures 50A and 53A (first three from left to right) are nonlimiting examples of such fusion proteins.

[0269] In yet other embodiments, the fusion protein further comprises a protein that cannot naturally translocate into the ER such as nuclear or cytosolic proteins fused to the N-terminus of IL-18. For said proteins, a signal peptide (which may be termed a leader sequence), such as Ig-kappa leader sequence (e.g., METDTLLLWVLLLWVPGSTG (SEQ ID NO:247)) in FUSE-499, or one or more other signal peptides including but not limited to those derived from human albumin and human azurocidin, *see* Kober et al. *Biotechnol Bioeng.* 2013 Apr;110(4):1164-73 is fused to the N-terminus of the non ER translocating protein. For example, the signal peptide may be on the N-terminus end of the propeptide or of the IL-18 (or its fragment, variant, or a variant of its fragment). A further example of a protein capable of translocating in/into/through ER may be a protein engineered with a signal peptide, e.g., on the N-terminus end. As an example, Hsp70 is a nuclear protein, but can be engineered to be an ER-translocating protein when fused or linked with a signal peptide on the N-terminus of Hsp70. In various embodiments, the addition of an N-terminal signal peptide, such as the Ig-kappa leader sequence, is in place of a Fc, globular protein, or HSS that'd otherwise be present in a fusion protein disclosed herein.

[0270] In some embodiments, the fusion protein (e.g., a masked IL-18) further comprises a tumor targeting fragment, e.g., a fragment that targets cell surface proteins including but not limited to a tumor associated antigen (TAA). For example, FUSE-517 as shown in FIG. 51G is a masked IL-18 fusion protein that also comprises an anti-EGFR antibody fragment, e.g., Fab of cetuximab. One or more antigen-targeting (preferably tumor antigen-targeting) fragments of known antibodies are conceived to be compatible with the fusion protein system disclosed herein.

[0271] In some embodiments, the fusion protein (e.g., a masked IL-18) comprises an activation receptor targeting fragment, e.g., a fragment that targets activation receptors on cell surface including but not limited to CD16 on natural killer cell surface. Activation receptor targeting fragment can be one as described herein or one known in the art as of the filing date of the present invention. Activation receptors include immunoreceptor tyrosine-based activation motif (ITAM)-associated receptors, such as CD16 and NKp46. Activation receptors also include those participating in spontaneous NK cell activation, such as NKp46 (CD335), NKp30 (CD337), NKp44 (CD336), NKG2D (CD314), DNAM-1 (CD226), 2B4 (CD244), LFA-1 (CD11a-CD18), and CD2. In some embodiments, the fusion protein (e.g., a masked IL-18) comprises both an activation receptor targeting fragment and a tumor targeting fragment. Examples of anti-CD16 fragments include but are not limited to CH2 domains of IgG1, CH2 domain of IgG4. In some embodiments, the fusion protein (e.g., a masked IL-18) comprises a polypeptide fragment that targets an immune checkpoint, e.g., fragment that targets an immune checkpoint expressed on T cell. For example, FUSE-694 as shown in FIG. 23 is a masked IL-18 fusion protein that also comprises an anti-PD1 fragment. Example immune checkpoints include but are not limited to PD-1, PD-L1, CTLA-4, LAG-3. One or more immune checkpoint-targeting fragments of

known antibodies are conceived to be compatible with the fusion protein system disclosed herein. Examples of anti-PD1 fragments include fragments (E.g. Fab, Fv) from pembrolizumab, nivolumab, pidilizumab, AMP-514, spartalizumab, cemiplimab, AK105, BCD-100, BI 754091, JS001, LZM009, MGA012, Sym021, TSR-042, MGD013, AK104, XmAb20717, tislelizumab, or PF-06801591. Additional examples of anti-PD1 fragments also include fragments (e.g., Fab, Fv) from vopratelimab, camrelizumab, sintilimab, AMP-224, AMP-514, and Acrixolimab. Thus, for example, the fusion protein comprises an anti-PD1 fragment from an anti-PD1 antibody (e.g., Fab, Fv), an IL-18 variant, and a protein capable of translocating into an ER (e.g., a Fc domain), and optionally, a linker.

[0272] In some embodiments, the fusion protein (e.g., a masked IL-18) comprises a targeting polypeptide, wherein the targeting polypeptide targets a protein on the same surface as the IL-18 RC. Examples of such proteins include but are not limited to CD16, γ9 TCR, δ2 TCR or δ1 TCR, NKp46, CD137, CD40 or NKG2D. Targeting polypeptide to CD16, γ9 TCR, δ2 TCR or δ1 TCR, NKp46, CD137, CD40 or NKG2D can be one as described herein or one known in the art as of the filing date of the present invention. In some embodiments, fusion protein (e.g., a masked IL-18) comprises a targeting polypeptide, wherein the targeting polypeptide targets a protein on a cell that does not contain an IL-18RC. In these embodiments, the IL-18 fusion protein will need to be delivered close to the IL-18R complex for a cis or density effect to result in the interaction between the IL-18 fusion protein and the IL-18R complex. For example, TAA targeted IL-18 fusion protein could get to interact with the IL-18R complex on T cells if (a) the fusion protein bridged T cells with TAA+ cells or (b) the fusion protein was combined with another protein that bridged T cells with TAA+ cells or (c) the fusion protein bound to a TAA+ cell that naturally interacted with T cell via a secondary means (e.g. TCR/MHC interaction). In other examples, the fusion protein could be delivered to fibroblasts or other accessory cells in the tumor microenvironment and released by proteases such that it can act on IL-18R+ T or NK cells at a distance.

[0273] Exemplary targeting polypeptides include those noted in Table 18 or fragments thereof. Of those listed as the antigen-binding antibodies, their VHH, Fab regions, or single-chain variable fragments (scFv) can be used as the antigen-binding site of the multispecific antibodies disclosed herein.

[0274] In some embodiments, enterokinase is used for site-specific cleavage of recombinant fusion proteins containing an accessible enterokinase recognition site. For example, enterokinase can specifically cleaves after the C-terminal end of the lysine residue at its cleavage site, Asp-Asp-Asp-Asp-Lys (SEQ ID NO:87). Therefore, the fragment produced from this cleavage reaction does not inherit any residues from the DDDDK (SEQ ID NO:87) recognition sequence. Additionally, DDDDK (SEQ ID NO:87) is a part of the octapeptide FLAG tag (DYKDDDDK (SEQ ID NO:248)), which can be utilized as a fusion tag for recognition by antibody, and for detection of fusion protein with Western blot analysis, as well as for purification of the fusion protein by Anti-FLAG affinity chromatography.

[0275] Preferably, a cleavage site can be based on peptide substrates sensitive to other enzymes, especially proteases highly expressed in tumor microenvironment, such as granzyme B, granzyme A, granzyme M, granzyme K, matrix metalloproteinase (MMP) 1/2/9/14 or other MMPs. Of note, granzymes are usually only upregulated in inflamed

tumors. For example, a substrate sequence for granzyme B can be Ile–Glu–Xaa–Asp↓Xaa–Gly (SEQ ID NO:249) with the cleavage at the Asp↓Xaa peptide bond. Alternatively, a substrate sequence for granzyme B can also be Ile–Glu–Xaa–Asp↓, with the cleavage at the C-terminus end of Asp, and Xaa can be Gln (SEQ ID NO:88) or another amino acid.

[0276] Several immune cells can release granzyme, such as T cells, NK cells, neutrophils, and mast cells. In several embodiments, a fusion protein comprising (a) a polypeptide fragment that targets an immune checkpoint expressed on an immune cell and/or a polypeptide fragment that targets an activation receptor on NK cell, and (b) a tumor targeting fragment, is effective for bringing the immune cell (e.g., T cell, NK cell) to the tumor, which can result in the release of granzymes that release IL-18. For example, an IL-18 fusion protein comprising a polypeptide fragment targeting an immune checkpoint protein can reverse exhaustion of NK and/or T cells that then are capable of releasing more granzymes.

[0277] In additional embodiments, the fusion protein comprises a cleavage site recognized by a serine protease, a cysteine protease, an aspartate protease, a threonine protease, a glutamic acidprotease, a metalloproteinase, a gelatinase, or an asparagine peptide lyase. In some embodiments, the protease cleavage site is recognized by a Cathepsin B, a Cathepsin C, a Cathepsin D, a Cathepsin E, a Cathepsin K, a Cathepsin L, a kallikrein, ahKl, a hK10, a hK15, a plasmin, a collagenase, a Type IV collagenase, a stromelysin, a Factor Xa, a chymotrypsin-like protease, a trypsin-like protease, an elastase-like protease, a subtilisinlike protease, an actinidain, a bromelain, a calpain, a caspase, a caspase-3, a Mir 1-CP, a papain, a HIV-1 protease, a HSV protease, a CMV protease, a chymosin, a renin, a pepsin, a matriptase, a legumain, a plasmepsin, a nepenthesin, a metalloexopeptidase, a metalloendopeptidase, a matrix metalloprotease (MMP), a MMP1, a MMP2, a MMP3, a MMP8, a MMP9, a MMP10, a MMP11, a MMP12, a MMP13, a MMP14, an ADAM10, an ADAM17, an ADAM12, an urokinase plasminogen activator (uPA), an enterokinase, a prostate-specific target (PSA, hK3), an interleukin-1β converting enzyme, a thrombin, a FAP (FAP-α), a dipeptidyl peptidase, or dipeptidyl peptidase IV (DPPIV/CD26), a type II transmembrane serine protease (TTSP), a neutrophil elastase, a cathepsin G, a proteinase 3, a neutrophil serine protease 4, a mast cell chymase, a mast cell tryptase, a dipeptidyl peptidase, and a dipeptidyl peptidase IV (DPPIV/CD26). Nonlimiting examples of cleavage sites are included in Table 6B. As a particular example, IEQD can be used.

[0278] It is contemplated that a variant, fragment, or a fragment of a variant of the IL-18 is suitable, and in some embodiments preferred, for the composition of the fusion protein. For example, a variant of mature IL-18 can have one, two, three, four, five, or more amino acid substitutions compared to the wild type mature IL-18. For example, one or more cysteines in the IL-18 or its propeptide are replaced with a natural or non-natural amino acid, such as from Cys to Ser, Ala, or Val, so as to reduce aggregation of the molecule in the fusion protein. Additional examples include cysteine to threonine, asparagine, or glutamine; cysteine to alanine, isoleucine, leucine methionine, phenylalanine, tyrosine, or tryptophan; cysteine to phenylalanine, alanine, aspartic acid, or asparagine; or cysteine to threonine, glutamine, aspartic acid, phenylalanine, isoleucine or histidine.

[0279] A variant of IL-18 may have a 95%, 90%, 85%, 83%, 80%, 75%, 70%, 65% or at least 60% sequence identity to wild type IL-18. In some embodiments, a variant of IL-18 may have at least 60% and at most 83% sequence identity to wild type IL-18. In some embodiments, a variant of IL-18 may have between 60%-64%, 65%-69%, 70%-74%, 75%-89%, or 80%-83% sequence identity to wild type IL-18. In some embodiments, the variant of IL-18 in the fusion protein is released as an about 15 kDa functional fragment (e.g., on the electrophoresis gels as tested) when cleaved at a cleavage site of the fusion protein. (It is conceived that the released protein may be mature IL-18 which would normally run at 18 kDa but may appear as about 15 kDa due to the ladder being used or the specific polyacrylamide percentage in a gel.) A fragment of IL-18 may have a 95%, 90%, 85%, 83%, 80%, 75%, 70%, 65%, or at least 60% sequence identity (and/or length) to wild type IL-18. In some embodiments, an IL-18 fragment produced by the fusion protein disclosed herein, especially after protease cleavage of the fusion protein, is less than 85% (e.g., about 83%, about 83%-80%, about 80%-75%, about 75%-70%, or about 70%-65%) in size compared to natural/wild-type mature IL-18; for example, an IL-18 fragment of about 15 kDa in size, preferably having comparable binding affinity for IL-18Ra/b as the wild type mature IL-18, is fused to a propeptide (or PP variant) and an ER translocating protein (with or without mutations), and the fusion protein also includes a protease cleavage site, such that upon protease cleavage, a small IL-18 fragment (e.g., about 15 kDa in size), is released. Preferably, this small IL-18 fragment maintains the natural binding affinity for IL-18Ra/b and an equal or lower binding affinity relative to IL-18Ra/b for IL-18BP. Preferably a variant, fragment, or a fragment of a variant of the IL-18 is capable of binding IL-18R and forming complex, so as to activate proinflammatory programs and/or NF-κB pathway. In some embodiments, the variant, fragment, or a fragment of a variant of the IL-18 is capable of having an increased binding affinity (e.g., 150%, 140%, 130%, 120%, 110%, or at least 100% relative to wild type IL-18) and/or inducing the biological activity of at least 150%, 140%, 130%, 120%, 110%, 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10%, compared to wild type IL-18. In some embodiments, the variant, fragment, or a fragment of a variant of the IL-18 is capable of having an increased binding affinity at 120%, 110%, or at least 100% relative to wild type IL-18, and/or inducing the biological activity of at 120%, 110%, 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, or 10%, compared to wild type IL-18.In additional embodiments, a variant, fragment, or a fragment of a variant of the IL-18 has diminished binding to IL-18 binding protein (IL-18BP), as compared to a wild type IL-18.

[0280] In some embodiments, the IL-18 or its fragment or variant cleaved from the fusion protein has at least 1,000, 2,000, 3,000, 5,000, 10,000, 30,000, 50,000, 70,000, 80,000, 90,000, or 100,000-fold increase in biological activity (e.g., binding with IL-18R to form IL-18/IL-18Rα/β complex and induce downstream signaling), compared to an uncleaved form in the fusion protein especially with propeptide. In further embodiments, the IL-18 or its fragment or variant cleaved from the fusion protein has a comparable biological activity, or within about 10, 20, 30, 40, or 50-fold difference in the biological activity, compared to recombinant human mature IL-18.

[0281] In some embodiments, the IL-18 or its fragment or variant cleaved from the fusion protein has a binding affinity for its IL-18R complex with an equilibrium dissociation constant (KD) of about 18 nM, (e.g., 18 nM ±

0.3 nM, 18 nM ± 0.5 nM, 18 nM ± 1.0 nM). In some embodiments, the IL-18 or its fragment or variant cleaved from the fusion protein has a binding affinity for its IL-18R complex which is about the same, or at least 100%, 95%, or 90%, compared to that of the wild type IL-18. In some embodiments, the IL-18 or its fragment or variant cleaved from the fusion protein has a binding affinity for its IL-18R complex which is greater than that of the wild type IL-18, e.g., a binding affinity that is at least 105%, 110% compared to that of the wild type IL-18, or having a KD value at least 10% or 20% smaller than that of wild type IL-18. Preferably, the IL-18 or its fragment or variant cleaved from the fusion protein has a reduced binding affinity for IL-18BP, compared to that of the wild type IL-18. For example, in some instances, the IL-18 or its fragment or variant cleaved from the fusion protein has a KD with IL-18BP of 18 nM or greater, such that it has a lower binding affinity to IL-18BP than to IL-18R. In some instances, the IL-18 or its fragment or variant cleaved from the fusion protein has a KD with IL-18BP of 18 nM or greater, whereas the wild type IL-18 has a KD with IL-18BP of about 0.4 nM. It is also conceived that KD may vary depending on instrument and protocol setup.

[0282] In various embodiments, the fusion protein comprises a first polypeptide or protein capable of translocating into an endoplasmic reticulum (ER) or a fragment thereof; and an interleukin 18 (IL-18), a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, wherein the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant is on the C-terminus end of the fusion protein relative to the first polypeptide or protein capable of translocating into the ER. In various embodiments, the first polypeptide is not the wild-type IL-18 propeptide. In various embodiments, the protein capable of translocating into an endoplasmic reticulum (ER) or a fragment thereof is not the wild-type IL-18 propeptide. In addition to these features, additional features of the fusion protein are discussed herein.

[0283] In some embodiments, the fusion protein comprises an IL-18 variant. In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDVRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGH DNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:250) with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDVRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGH DNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:250). In various embodiments, the one to five amino acid substitutions is one amino acid substitution. In other embodiments, the one to five amino acid substitutions are two amino acid substitutions. In other embodiments, the one to five amino acid substitutions are three amino acid substitutions. In other embodiments, the one to five amino acid substitutions are four amino acid substitutions. In other embodiments, the one to five amino acid substitutions are five amino acid substitutions. In various embodiments, the IL-18 variant comprises no more than five amino acid substitutions, with the exception of substituting cysteines. In various embodiments, the IL-18 variant has an amino acid sequence comprising

or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHD NKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251) with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHD NKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251).

[0284] In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHD NKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251) with one or more amino acid substitutions at positions C74, C104, C112, and C164, and one to five amino acid substitutions at positions E42, M87, K89, M96, and M149.

[0285] In various embodiments, the amino acid substitutions at one or more of C74, C104, C112, and C164 are each independently substituted to valine, alanine or serine. In various embodiments, the one to five amino acid substitutions are one or more of: E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; or M149V or M149I. In various embodiments, the one to five amino acid substitutions are E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; and M149V or M149I.

[0286] In various embodiments, the fusion protein comprises an IL-18 variant selected from Table 6B. In various embodiments, the fusion protein comprising the IL-18 variant selected from Table 6B, further comprises a propeptide having an amino acid sequence selected from Propeptide column in Table 6B, and optionally from the same row as the IL-18 variant. In various embodiments, the fusion protein comprising the IL-18 variant selected from Table 6B and propeptide selected from Table 6B, further comprises a cleavage peptide selected from Table 6B, and optionally from the same row as the IL-18 variant and propeptide. As a particular example, IEQD (SEQ ID NO:88) can be used.

[0287] In various embodiments, IL-18 variant is an IL-18 variant disclosed in U.S. Patent 7,524,488, U.S. Patent Publication No. 2019/0070262, U.S. Patent Publication No. 2021/0015891, or PCT Publication No. WO 2022/038417, the IL-18 variants and sequences of each of these patent or publications of which are hereby incorporated by reference as though fully set forth.

[0288] In various embodiments, the fusion protein further comprises a targeting polypeptide. In some embodiments, the targeting polypeptide targets a protein on a cell surface, wherein the cell surface also has an IL-18 RC or the cell is capable of expressing the IL-18 RC. In various embodiments, the fusion protein binds to a cell having an IL-18 RC or capable of expressing the IL-18 RC upon activation of the cell, and activates the IL-18 RC signal.

[0289] In other embodiments, the targeting polypeptide targets a protein on a cell surface that does not have an IL-18 RC or the cell is not capable of expressing the IL-18 RC. The cell not having the IL-18 RC on its surface or not capable of expressing the IL-18 RC is in close proximity to a cell expressing the IL-18 RC or is capable of expressing the IL-18 RC. In other instances, the fusion protein can bring the cell not having the IL-18 RC on its surface or not capable of expressing the IL-18 RC into close proximity to a cell expressing the IL-18 RC or is capable of expressing the IL-18 RC.

[0290] In various embodiments, the targeting polypeptide comprises a tumor associated antigen binding domain.

[0291] In various embodiments, the fusion protein further comprises a binding domain for a protein expressed on immune cells. In various embodiments, the fusion protein further comprises a binding domain for a protein expressed on immune cells that express IL-18 receptor complex or on immune cells that upon activation express the IL-18 receptor complex.

[0292] In various embodiments, the fusion protein further comprises an antibody or antibody fragment. This enables the fusion protein to bind to a tumor cell, or to an immune cell or stromal cell in a tumor tissue, or to a tumor draining lymph node, or other secondary lymphoid organ. Examples of antibody fragments include Fc fragment, Fab fragment, Fv fragment, as well as others discussed herein.

[0293] In various embodiments, the fusion protein further comprises a masking domain. In some of these embodiments, the masking domain provides protection for IL-18, and IL-18 cannot be released by a protease. In some of these embodiments, a mature IL-18 or mature IL-18 variant can be released from a masking domain by a protease. In various embodiments, the protease is granzyme, which can be released from an immune cell. Examples of immune cells include but are not limited to an NK cell, a T cell, a neutrophil, or a mast cell. In various embodiments, the protease is a metalloprotease, which the metalloprotease can be expressed in a tumor microenvironment or tumor draining lymph node. Further examples of protease and types of granzymes are described herein. In various embodiments, the mature IL-18 increases the activity of NK cells or T cells, and optionally the activity being one or more of proliferation, survival, and cytotoxicity.

[0294] In various embodiments, the fusion protein further comprises half-life extending molecule. A nonlimiting example of a half-life extending molecule is a half-life extending polypeptide; for example, human serum albumin (HSA) or an HSA-binding fragment. In various embodiments, the fusion protein has reduced activity as compared to wild-type IL-18 when not bound to a cell having the IL-18 RC. In various embodiments, the reduced activity is at least a 75% reduction in activity as compared to wild-type IL-18.

[0295] In some embodiments, a mature IL-18 variant on the C-terminus of the mask is attenuated. For example, mature IL-18-mut13A is 100 fold attenuated.

[0296] In various embodiments, the fusion protein comprises polypeptide 1 and polypeptide 2 selected from Table 6A. In various embodiments, the fusion protein further comprises polypeptide 3 selected from Table 6A. In

various embodiments, polypeptide 1 and polypeptide 2, and optionally polypeptide 3 is selected from the same row of Table 6A.

[0297] In various embodiments, the fusion protein comprises polypeptide 1 selected from Table 6A, wherein polypeptide 1 comprises HSA.

[0298] In various embodiments, the fusion protein does not comprise an IL-18 variant disclosed in U.S. Patent 7,524,488, U.S. Patent Publication No. 2019/0070262, U.S. Patent Publication No. 2021/0015891, or PCT Publication No. WO 2022/038417, the IL-18 variants and sequences of each of these patent or publications of which are hereby incorporated by reference as though fully set forth.

[0299] Various embodiments of the invention include propeptide variants. In various embodiments, the propeptide variant has the following amino acid sequence: AAEPVEDNX_1INFVAMKFIDNTLYFIAEDDEN wherein X_1 is any amino acid except cysteine (SEQ ID NO:238). In various embodiments, X_1 is alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine or tryptophan (SEQ ID NO:239). In various embodiments, X_1 is valine (SEQ ID NO:78). In various embodiments, X1 is serine, threonine, asparagine, or glutamine (SEQ ID NO:240). In various embodiments, X_1 is serine (SEQ ID NO:76).

[0300] Various embodiments include for IL-18 variants to be used in the multi-cytokine fusion proteins of the present invention. In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of SEQ ID NO:250 with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDVRDNAPRTIFIISMYKDSQPRGMAVTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGH DNKMQFESSSYEGYFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:250). In various embodiments, the one to five amino acid substitutions is one amino acid substitution. In other embodiments, the one to five amino acid substitutions are two amino acid substitutions. In other embodiments, the one to five amino acid substitutions are three amino acid substitutions. In other embodiments, the one to five amino acid substitutions are four amino acid substitutions. In other embodiments, the one to five amino acid substitutions are five amino acid substitutions. In various embodiments, the IL-18 variant comprises no more than five amino acid substitutions, with the exception of substituting cysteines.

[0301] In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHD NKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251) with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD

SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHD NKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251).

[0302] In various embodiments, the IL-18 variant has an amino acid sequence comprising or consisting of amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHD NKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251) with one or more amino acid substitutions at positions C74, C104, C112, and C164 , and one to five amino acid substitutions at positions E42, M87, K89, M96, and M149, of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTD SDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHD NKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:251). In various embodiments, the amino acid substitutions at one or more of C74, C104, C112, and C164 are each independently substituted to valine, alanine or serine.

[0303] In various embodiments, the one to five amino acid substitutions are one or more of: E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; or M149V or M149I. In various embodiments, the one to five amino acid substitutions are E42K, E42R, E42A, E42H, or E42Q;M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; and M149V or M149I.

[0304] In various embodiments, the IL-18 variant is selected from the "Mature IL18 variant" column in Table 6B.

[0305] In various embodiments, the IL-18 variant further comprises an IL-18 propeptide or IL-18 propeptide variant.

[0306] In various embodiments, the IL-18 variant further comprises an IL-18 propeptide variant having the following amino acid sequence: AAEPVEDNX_1INFVAMKFIDNTLYFIAEDDEN wherein X_1 is any amino acid except cysteine (SEQ ID NO:238). In various embodiments, X_1 is alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine or tryptophan (SEQ ID NO:239). In various embodiments, X_1 is valine (SEQ ID NO:78). In various embodiments, X_1 is serine, threonine, asparagine, or glutamine (SEQ ID NO:240). In various embodiments, X_1 is serine (SEQ ID NO:76). In various embodiments, the IL-18 variant is selected from the "Mature IL18 variant" column of Table 6B, and further comprises a propeptide having an amino acid sequence selected from "Propeptide" column in Table 6B, and optionally from the same row as the IL-18 variant.

[0307] In various embodiments, the IL-18 variant further comprises an IL-18 propeptide variant, and a cleavage peptide. In various embodiments, the cleavage peptide selected from Table 6B. As a particular example, IEQD can be used.

[0308] In various embodiments, a fusion protein comprising an IL-18 variant selected from Table 6B, a propeptide selected from Table 6B, and a cleavage peptide selected from Table 6B, and optionally from the same row as the IL-18 variant and propeptide.

[0309] In various embodiments, the IL-18 variant is not an IL-18 variant disclosed in U.S. Patent 7,524,488, U.S. Patent Publication No. 2019/0070262, U.S. Patent Publication No. 2021/0015891, or PCT Publication No. WO 2022/038417, the IL-18 variants and sequences of each of these patent or publications of which are hereby incorporated by reference as though fully set forth.

Table 6A. Amino acid sequences of respective components in exemplary fusion proteins ("FUSE"). Some of "Polypeptide 1" sequences, i.e., first polypeptide arm sequences, are identical in FUSE-422, FUSE-423, FUSE-424, FUSE-441, FUSE-442, FUSE-462, FUSE-480, FUSE-481, FUSE-484, FUSE-485, FUSE-486, FUSE-487, FUSE-499, FUSE-500, FUSE-505, FUSE-516, FUSE-517, FUSE-545, FUSE-546, FUSE547, FUSE5-556, FUSE-583-587, FUSE-599-602, FUSE-645, FUSE-686, FUSE-756-758, FUSE-775, FUSE-874-876, and FUSE-878-892.

	Polypeptide 1	Polypeptide 2	Polypeptide 3
FUSE-422	(SEQ ID NO:1)	(SEQ ID NO:20)	
FUSE-423	(SEQ ID NO:1)	(SEQ ID NO:21)	
FUSE-424	(SEQ ID NO:1)	(SEQ ID NO:22)	
FUSE-441	(SEQ ID NO:1)	(SEQ ID NO:23)	
FUSE-442	(SEQ ID NO:1)	(SEQ ID NO:24)	
FUSE-462	(SEQ ID NO:1)	(SEQ ID NO:25)	
FUSE-480	(SEQ ID NO:1)	(SEQ ID NO:26)	
FUSE-481	(SEQ ID NO:1)	(SEQ ID NO:27)	
FUSE-484	(SEQ ID NO:1)	(SEQ ID NO:28)	
FUSE-485	(SEQ ID NO:1)	(SEQ ID NO:29)	
FUSE-486	(SEQ ID NO:1)	(SEQ ID NO:30)	
FUSE-487	(SEQ ID NO:1)	(SEQ ID NO:31)	
FUSE-499	(SEQ ID NO:1)	(SEQ ID NO:32)	
FUSE-500	(SEQ ID NO:1)	(SEQ ID NO:33)	
FUSE-501	(SEQ ID NO:2)		
FUSE-502	(SEQ ID NO:3)		
FUSE-503	(SEQ ID NO:4)		
FUSE-504	(SEQ ID NO:5)		
FUSE-505	(SEQ ID NO:1)	(SEQ ID NO:34)	
FUSE-507	(SEQ ID NO:6)		
FUSE-508	(SEQ ID NO:7)		
FUSE-509	(SEQ ID NO:8)		
FUSE-510	(SEQ ID NO:9)		
FUSE-516	(SEQ ID NO:1)	(SEQ ID NO:35)	
FUSE-517	(SEQ ID NO:1)	(SEQ ID NO:36)	SEQ ID NO:73
FUSE-545	(SEQ ID NO:1)	(SEQ ID NO:37)	
FUSE-546	(SEQ ID NO:1)	(SEQ ID NO:38)	
FUSE-547	(SEQ ID NO:1)	(SEQ ID NO:39)	
FUSE-555	(SEQ ID NO:10)	(SEQ ID NO:40)	SEQ ID NO:73
FUSE-556	(SEQ ID NO:1)	(SEQ ID NO:41)	

FUSE-557	(SEQ ID NO:1	(SEQ ID NO:254)	SEQ ID NO:73
FUSE-583	(SEQ ID NO:1)	(SEQ ID NO:42)	
FUSE-584	(SEQ ID NO:1)	(SEQ ID NO:43)	
FUSE-585	(SEQ ID NO:1)	(SEQ ID NO:44)	
FUSE-586	(SEQ ID NO:1)	(SEQ ID NO:45)	
FUSE-587	(SEQ ID NO:1)	(SEQ ID NO:46)	
FUSE-599	(SEQ ID NO:1)	(SEQ ID NO:47)	
FUSE-600	(SEQ ID NO:1)	(SEQ ID NO:48)	
FUSE-601	(SEQ ID NO:1)	(SEQ ID NO:49)	
FUSE-602	(SEQ ID NO:1)	(SEQ ID NO:50)	
FUSE-624	(SEQ ID NO:10)	(SEQ ID NO:51)	SEQ ID NO:73
FUSE-625	(SEQ ID NO:11)	(SEQ ID NO:52)	SEQ ID NO:74
FUSE-626	(SEQ ID NO:1)	(SEQ ID NO:257)	
FUSE-627	(SEQ ID NO:12)	(SEQ ID NO:53)	
FUSE-645	(SEQ ID NO:1)	(SEQ ID NO:54)	
FUSE-658	(SEQ ID NO:13)	SEQ ID NO:41	
FUSE-659	(SEQ ID NO:14)	SEQ ID NO:41	
FUSE-660	(SEQ ID NO:15)	SEQ ID NO:41	
FUSE-669	(SEQ ID NO:10)	SEQ ID NO:254	SEQ ID NO:73
FUSE-670	(SEQ ID NO:255)		SEQ ID NO:73
FUSE 675	(SEQ ID NO:256)		
FUSE-676	(SEQ ID NO:267)		
FUSE-682	(SEQ ID NO:258)	SEQ ID NO:35	
FUSE-683	(SEQ ID NO:259)	(SEQ ID NO:272)	
FUSE-684	SEQ ID NO:259	(SEQ ID NO:273)	
FUSE-685	(SEQ ID NO:1)	(SEQ ID NO:275)	
FUSE-686	(SEQ ID NO:1)	(SEQ ID NO:55)	
FUSE-691	(SEQ ID NO:16)		SEQ ID NO:75
FUSE-694	(SEQ ID NO:17)	(SEQ ID NO:56)	SEQ ID NO:75
FUSE-756	(SEQ ID NO:1)	(SEQ ID NO:57)	
FUSE-757	(SEQ ID NO:1)	(SEQ ID NO:58)	
FUSE-758	(SEQ ID NO:1)	(SEQ ID NO:59)	
FUSE-775	(SEQ ID NO:1)	(SEQ ID NO:60)	
FUSE-782	(SEQ ID NO:18)	(SEQ ID NO:61)	SEQ ID NO:75
FUSE-783	(SEQ ID NO:18)	(SEQ ID NO:62)	SEQ ID NO:75
FUSE-827	(SEQ ID NO:19)	(SEQ ID NO:63)	
FUSE-832	(SEQ ID NO:260)	(SEQ ID NO:276)	
FUSE-874	(SEQ ID NO:1)	(SEQ ID NO:64)	
FUSE-875	(SEQ ID NO:1)	(SEQ ID NO:65)	
FUSE-876	(SEQ ID NO:1)	(SEQ ID NO:66)	
FUSE-882	(SEQ ID NO:261)	(SEQ ID NO:277)	SEQ ID NO:280
FUSE-887	(SEQ ID NO:1)	(SEQ ID NO:67)	
FUSE-888	(SEQ ID NO:1)	(SEQ ID NO:68)	
FUSE-889	(SEQ ID NO:1)	(SEQ ID NO:69)	
FUSE-890	(SEQ ID NO:1)	(SEQ ID NO:70)	
FUSE-891	(SEQ ID NO:1)	(SEQ ID NO:71)	
FUSE-892	(SEQ ID NO:1)	(SEQ ID NO:72)	
FUSE-895	(SEQ ID NO:261)	(SEQ ID NO:278)	SEQ ID NO:280
FUSE-952	SEQ ID NO:260	(SEQ ID NO:279)	SEQ ID NO:281
FUSE-1015	(SEQ ID NO:262)	SEQ ID NO:279	SEQ ID NO:281

FUSE-1017	SEQ ID NO:18	SEQ ID NO:276	SEQ ID NO:75
FUSE-1041	SEQ ID NO:260	SEQ ID NO:56	SEQ ID NO:75
FUSE-1042	SEQ ID NO:262	SEQ ID NO:56	SEQ ID NO:75
FUSE-1044	(SEQ ID NO:263)	SEQ ID NO:56	SEQ ID NO:75
FUSE-1109	(SEQ ID NO:264)	SEQ ID NO:56	SEQ ID NO:75
FUSE-1110	(SEQ ID NO:265)	SEQ ID NO:56	SEQ ID NO:75
FUSE-1111	(SEQ ID NO:266)	SEQ ID NO:56	SEQ ID NO:75
FUSE-1112	(SEQ ID NO:274)	SEQ ID NO:56	SEQ ID NO:75
FUSE-1113	(SEQ ID NO:268)	SEQ ID NO:279	SEQ ID NO:281
FUSE-1116	(SEQ ID NO:269)	SEQ ID NO:56	SEQ ID NO:75
FUSE-1176	(SEQ ID NO:270)	-	SEQ ID NO:282
FUSE-1177	(SEQ ID NO:271)	SEQ ID NO:56	SEQ ID NO:75
FUSE-1179	GGGGS***YFGKLKSKLSVIRNL******NDQVLFIDQGNRPLFEDMTD******SDVRDNAPRTIFIISMYDDS******QPRGRAVTISVKVEKISTLS******VENKIISFKEMNPPDNIKDT******KSDIIFFQRSVPGHDNKMQF******ESSSYEGYFLAVEKERDLFK******LILKKEDELGDRSIMFTVQN*****ED***EPKSSDKTHTCPPCPAPE*AAGGPSVFLFPPKPKDTLMI*SRTPEVTCVVVDVSHEDPEV*KFNWYVDGVEVHNAKTKPRE*EQYNSTYRVVSVLTVLHQDW*LNGKEYKCKVSNKALPAPIE*KTISKAKGQPREPQVYTLPP*SREEMTKNQVSLSCAVKGFY*PSDIAVEWESNGQPENNYKT*TPPVLDSDGSFFLVSKLTVD*KSRWQQGNVFSCSVMHEALH*NRFTQKSLSLSPG* (SEQ ID NO:321)	**QVQLVESGGGVVQPGRSLRL****DCKASGITFSNSGMHWVRQA****PGKGLEWVAVIWYDGSKRYY****ADSVKGRFTISRDNSKNTLF****LQMNSLRAEDTAVYYCATND****DYWGQGTLVTVSSASTKGPS****VFPLAPSSKSTSGGTAALGC****LVKDYFPEPVTVSWNSGALT****SGVHTFPAVLQSSGLYSLSS****VVTVPSSSLGTQTYICNVNH****KPSNTKVDKKV***EPKSCDKTH**TCPPCPAPEAAGGPSVFLFP**PKPKDTLMISRTPEVTCVVV**DVSHEDPEVKFNWYVDGVEV**HNAKTKPREEQYNSTYRVVS**VLTVLHQDWLNGKEYKCKVS**NKALPAPIEKTISKAKGQPR**EPQVYTLPPSREEMTKNQVS**LWCLVKGFYPSDIAVEWESN**GQPENNYKTTPPVLDSDGSF**FLYSKLTVDKSRWQQGNVFS**CSVMHEALHNHYTQKSLSLS**PGK* (SEQ ID NO:56)	**SEQ ID NO:75**
FUSE-1229	GGGGS***YFGKLQSKLSVIRNL******NDQVLFIDQGNRPLFEDMTD******SDVRDNAPRTIFIISMYDDS******QPRGKAVTISVKVEKISTLS******VENKIISFKEMNPPDNIKDT******KSDIIFFQRSVPGHDNKMQF******ESSLYEGYFLAVEKERDLFK******LILKKEDELGDRSIMFTVQN*****ED***EPKSSDKTHTCPPCPAPE*AAGGPSVFLFPPKPKDTLMI*SRTPEVTCVVVDVSHEDPEV*KFNWYVDGVEVHNAKTKPRE*EQYNSTYRVVSVLTVLHQDW*LNGKEYKCKVSNKALPAPIE*KTISKAKGQPREPQVYTLPP*SREEMTKNQVSLSCAVKGFY*PSDIAVEWESNGQPENNYKT*	**QVQLVESGGGVVQPGRSLRL****DCKASGITFSNSGMHWVRQA****PGKGLEWVAVIWYDGSKRYY****ADSVKGRFTISRDNSKNTLF****LQMNSLRAEDTAVYYCATND****DYWGQGTLVTVSSASTKGPS****VFPLAPSSKSTSGGTAALGC****LVKDYFPEPVTVSWNSGALT****SGVHTFPAVLQSSGLYSLSS****VVTVPSSSLGTQTYICNVNH****KPSNTKVDKKV***EPKSCDKTH**TCPPCPAPEAAGGPSVFLFP**PKPKDTLMISRTPEVTCVVV**DVSHEDPEVKFNWYVDGVEV**HNAKTKPREEQYNSTYRVVS**VLTVLHQDWLNGKEYKCKVS**NKALPAPIEKTISKAKGQPR**	**SEQ ID NO:75**

	TPPVLDSDGSFFLVSKLTVD KSRWQQGNVFSCSVMHEALH NRFTQKSLSLSPGK (SEQ ID NO:322)	*EPQVYTLPPSREEMTKNQVS LWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLS PGK* (SEQ ID NO:56)	

For FUSE1179 and 1229: (1) regular font = short peptide; (2) ***italicized bold font*** = IL-18 variant; (3) *italicized font* = Fc hole; (4) *italicized and underlined font* = Fc Knob; (5) **bold font** = Fab

Polypeptide 3 sequences

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINS
VESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:73)

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISS
LQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTE (SEQ ID NO:74)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISS
LEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:75)

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISS
LQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:280)

DIVMTQGTLPNPVPSGESVSITCRSSKSLLYSDGKTYLNWYLQRPGQSPQLLIYWMSTRASGVSDRFSGSGSGTDFT
LKISGVEAEDVGIYYCQQGLEFPTFGGGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO:281)

GGGGSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISKYGDSQPRGLAVTISVKVEKIS
TLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKVQFESSSYEGYFLAVEKERDLFKLILKKEDELGDRSI
MFTVQNEDGGGGSGGGGSGGGGSGGGGSDIVMTQGTLPNPVPSGESVSITCRSSKSLLYSDGKTYLNWYLQRPGQSP
QLLIYWMSTRASGVSDRFSGSGSGTDFTLKISGVEAEDVGIYYCQQGLEFPTFGGGTKLELKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC (SEQ ID NO:282)

Table 6B. Sequences for the propeptide, cleavage and mature IL18 variant in Table 6A.

	Propeptide	Cleavage	Mature IL18 variant
FUSE-422	AAEPVEDNSINFVAMKFIDNTLY FIAEDDEN (SEQ ID NO:76)	DDDDK (SEQ ID NO:87)	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISKYGDSQPRGLA VTISVKSEKISTLSSENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKVQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:97)
FUSE-423	AAEPVEDNAINFVAMKFIDNTLY FIAEDDEN (SEQ ID NO:77)	SEQ ID NO:87	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDARDNAPRTIFIISKYGDSQPRGLA VTISVKAEKISTLSAENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKVQFESSSYEG YFLAAEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:98)
FUSE-424	AAEPVEDNVINFVAMKFIDNTLY FIAEDDEN (SEQ ID NO:78)	SEQ ID NO:87	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISKYGDSQPRGLA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKVQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:99)
FUSE-441		SEQ ID NO:87	SEQ ID NO:99
FUSE-442	SEQ ID NO:78	SEQ ID NO:87	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYKDSQPRGMA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:100)
FUSE-462	SEQ ID NO:78	IEQD (SEQ ID NO:88)	SEQ ID NO:99
FUSE-480	SEQ ID NO:76	SEQ ID NO:87	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDSRDNAPRTIFIISMYKDSQPRGMA VTISVKSEKISTLSSENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLASEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:101)
FUSE-481	SEQ ID NO:77	SEQ ID NO:87	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDARDNAPRTIFIISMYKDSQPRGMA VTISVKAEKISTLSAENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAAEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:102)
FUSE-484	SEQ ID NO:78	SEQ ID NO:87	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISKYGDSGARGLA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFERDVPGHSGKVQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:103)
FUSE-485	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:100
FUSE-486	SEQ ID NO:78	GPLGVR (SEQ ID NO:89)	SEQ ID NO:100
FUSE-487	SEQ ID NO:78	VHMPLGFLGP (SEQ ID NO:90)	SEQ ID NO:100

FUSE -499	SEQ ID NO:78	LESD (SEQ ID NO:91)	SEQ ID NO:100
FUSE -500			SEQ ID NO:100
FUSE -501	SEQ ID NO:78	SEQ ID NO:87	SEQ ID NO:100
FUSE -502	SEQ ID NO:78	SEQ ID NO:87	SEQ ID NO:99
FUSE -503		SEQ ID NO:87	SEQ ID NO:100
FUSE -504		SEQ ID NO:87	SEQ ID NO:99
FUSE -505		SEQ ID NO:87	SEQ ID NO:100
FUSE -507	SEQ ID NO:78	SEQ ID NO:87	SEQ ID NO:100
FUSE -508		SEQ ID NO:87	SEQ ID NO:100
FUSE -509	SEQ ID NO:78	SEQ ID NO:87	SEQ ID NO:100
FUSE -510		SEQ ID NO:87	SEQ ID NO:100
FUSE -516	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -517	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -545			SEQ ID NO:99
FUSE -546			YFGKLkSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFTISMYgDSQPRGMA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:104)
FUSE -547			YFGKLrSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFTISMYgDSQPRGMA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:105)
FUSE -555	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE			YFGKLaSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYaDSQPRGMA

-583			VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:106)
FUSE -584			YFGKLhSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYgDSQPRGMA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:107)
FUSE -585			YFGKLrSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYeDSQPRGMA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:108)
FUSE -586			YFGKLqSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYaDSQPRGMA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:109)
FUSE -587	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:100
FUSE -599			(SEQ ID NO:110)
FUSE -600			YFGKLkSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISkYgDSQPRGMA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:111)
FUSE -601			YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIIShYaDSQPRGiA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKiQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:112)
FUSE -602			YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISKYaDSQPRGiA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKiQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:113)
FUSE -624	SEQ ID NO:78	KPLGLQARVVGG GGTIEQD (SEQ ID NO:92)	SEQ ID NO:99
FUSE -625	SEQ ID NO:78	SEQ ID NO:92	SEQ ID NO:99
FUSE -626	SEQ ID NO:78		SEQ ID NO:99
FUSE -627	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -645	SEQ ID NO:76	SEQ ID NO:88	SEQ ID NO:97
FUSE -658	SEQ ID NO:78	IGNRS (SEQ ID NO:93)	SEQ ID NO:99

FUSE -659	SEQ ID NO:78	KPLGLQARVV (SEQ ID NO:94)	SEQ ID NO:99
FUSE -660	SEQ ID NO:78	IEQDGGSIGNRS GGGGKPLGLQAR VVGGGGTIEQD (SEQ ID NO:95)	SEQ ID NO:99
FUSE -670	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -676	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -682	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -683	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -684	SEQ ID NO:78	SEQ ID NO:88	SEQ ID NO:99
FUSE -685	SEQ ID NO:76		SEQ ID NO:97
FUSE -686	AAEPVEDNsINFVAMKFIDNTLY FIAEnDEN (SEQ ID NO:79)	QDIE (SEQ ID NO:96)	SEQ ID NO:97
FUSE -694	SEQ ID NO:76	SEQ ID NO:88	SEQ ID NO:97
FUSE -756		SEQ ID NO:88	SEQ ID NO:100
FUSE -757		SEQ ID NO:88	SEQ ID NO:100
FUSE -758		SEQ ID NO:88	SEQ ID NO:100
FUSE -775	SEQ ID NO:80		SEQ ID NO:99
FUSE -782	SEQ ID NO:79		SEQ ID NO:97
FUSE -783	SEQ ID NO:80		SEQ ID NO:99
FUSE	SEQ ID NO:79		SEQ ID NO:97

-827			
FUSE -832			SEQ ID NO:99
FUSE -874			SEQ ID NO:100
FUSE -875			SEQ ID NO:100
FUSE -876			SEQ ID NO:100
FUSE -882	SEQ ID NO:80		SEQ ID NO:99
FUSE -887	AAEPVEDNtINFVAMKFIDNTLY FIAEDDENLESD (SEQ ID NO:81)		SEQ ID NO:101
FUSE -888	AAEPVEDNqINFVAMKFIDNTLY FIAEDDENLESD (SEQ ID NO:82)		SEQ ID NO:101
FUSE -889	AAEPVEDNdINFVAMKFIDNTLY FIAEDDENLESD (SEQ ID NO:83)		SEQ ID NO:102
FUSE -890	AAEPVEDNfINFVAMKFIDNTLY FIAEDDENLESD (SEQ ID NO:84)		SEQ ID NO:102
FUSE -891	AAEPVEDNlINFVAMKFIDNTLY FIAEDDENLESD (SEQ ID NO:85)		SEQ ID NO:100
FUSE -892	AAEPVEDNhINFVAMKFIDNTLY FIAEDDENLESD (SEQ ID NO:86)		SEQ ID NO:100
FUSE -895	SEQ ID NO:80		SEQ ID NO:99
FUSE - 1015	SEQ ID NO:80		SEQ ID NO:99
FUSE - 1017	SEQ ID NO:131		SEQ ID NO:99

FUSE - 1042	SEQ ID NO:80		SEQ ID NO:99
FUSE - 1044	SEQ ID NO:80		SEQ ID NO:110
FUSE - 1109			YFGKLqSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYdDSQPRGkA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNkMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:283)
FUSE - 1110			YFGKLqSKLSVIRNLNDQVLkIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYdDSQPRGkA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNkMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:284)
FUSE - 1111			YFGKLqSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYdDSQPRGkA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNeMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:285)
FUSE - 1112			YFGKLkSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYdDSQPRGrA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNkMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:286)
FUSE - 1113			SEQ ID NO:99
FUSE - 1116			YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISKYGDSQPRGLA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHRNkVQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:287)
FUSE - 1176			SEQ ID NO:99
FUSE - 1177			YFGKLqSKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDVRDNAPRTIFIISMYdDSQPRGkA VTISVKVEKISTLSVENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNkMQFESSSYEG YFLAVEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO:283)

Table 7. Nucleotide sequences encoding respective polypeptides of Table 6A.

	Polynucleotide 1	Polynucleotide 2	Polynucleotide 3
FUSE-422	(SEQ ID NO:114)	(SEQ ID NO:133)	
FUSE-423	(SEQ ID NO:114)	(SEQ ID NO:134)	
FUSE-424	(SEQ ID NO:114)	(SEQ ID NO:135)	
FUSE-441	(SEQ ID NO:114)	(SEQ ID NO:136)	
FUSE-442	(SEQ ID NO:114)	(SEQ ID NO:137)	
FUSE-462	(SEQ ID NO:114)	(SEQ ID NO:138)	
FUSE-480	(SEQ ID NO:114)	(SEQ ID NO:139)	
FUSE-481	(SEQ ID NO:114)	(SEQ ID NO:140)	
FUSE-484	(SEQ ID NO:114)	(SEQ ID NO:141)	
FUSE-485	(SEQ ID NO:114)	(SEQ ID NO:142)	
FUSE-486	(SEQ ID NO:114)	(SEQ ID NO:143)	
FUSE-487	(SEQ ID NO:114)	(SEQ ID NO:144)	
FUSE-499	(SEQ ID NO:114)	(SEQ ID NO:145)	
FUSE-500	(SEQ ID NO:114)	(SEQ ID NO:146)	
FUSE-501	(SEQ ID NO:115)		
FUSE-502	(SEQ ID NO:116)		
FUSE-503	(SEQ ID NO:117)		
FUSE-504	(SEQ ID NO:118)		
FUSE-505	(SEQ ID NO:114)	(SEQ ID NO:147)	
FUSE-507	(SEQ ID NO:119)		
FUSE-508	(SEQ ID NO:120)		
FUSE-509	(SEQ ID NO:121)		
FUSE-510	(SEQ ID NO:122)		
FUSE-516	(SEQ ID NO:114)	(SEQ ID NO:148)	
FUSE-517	(SEQ ID NO:114)	(SEQ ID NO:149)	SEQ ID NO:186
FUSE-545	(SEQ ID NO:114)	(SEQ ID NO:150)	
FUSE-546	(SEQ ID NO:114)	(SEQ ID NO:151)	
FUSE-547	(SEQ ID NO:114)	(SEQ ID NO:152)	
FUSE-555	(SEQ ID NO:123)	(SEQ ID NO:153)	SEQ ID NO:186
FUSE-556	(SEQ ID NO:114)	(SEQ ID NO:154)	
FUSE-557	(SEQ ID NO:114)	(SEQ ID NO:288)	SEQ ID NO:186
FUSE-583	(SEQ ID NO:114)	(SEQ ID NO:155)	
FUSE-584	(SEQ ID NO:114)	(SEQ ID NO:156)	
FUSE-585	(SEQ ID NO:114)	(SEQ ID NO:157)	
FUSE-586	(SEQ ID NO:114)	(SEQ ID NO:158)	
FUSE-587	(SEQ ID NO:114)	(SEQ ID NO:159)	
FUSE-599	(SEQ ID NO:114)	(SEQ ID NO:160)	
FUSE-600	(SEQ ID NO:114)	(SEQ ID NO:161)	
FUSE-601	(SEQ ID NO:114)	(SEQ ID NO:162)	
FUSE-602	(SEQ ID NO:114)	(SEQ ID NO:163)	
FUSE-624	(SEQ ID NO:123)	(SEQ ID NO:164)	SEQ ID NO:186
FUSE-625	(SEQ ID NO:124)	(SEQ ID NO:165)	SEQ ID NO:187
FUSE-626	(SEQ ID NO:114)	(SEQ ID NO:289)	
FUSE-627	(SEQ ID NO:125)	(SEQ ID NO:166)	
FUSE-645	(SEQ ID NO:114)	(SEQ ID NO:167)	
FUSE-658	(SEQ ID NO:126)	(SEQ ID NO:154)	
FUSE-659	(SEQ ID NO:127)	(SEQ ID NO:154)	
FUSE-660	(SEQ ID NO:128)	(SEQ ID NO:154)	

FUSE-669	(SEQ ID NO:114)	SEQ ID NO:288	SEQ ID NO:186
FUSE-670	(SEQ ID NO:290)	-	SEQ ID NO:186
FUSE-675	(SEQ ID NO:291)	-	-
FUSE-676	(SEQ ID NO:292)	-	-
FUSE-682	(SEQ ID NO:293)	(SEQ ID NO:148)	-
FUSE-683	(SEQ ID NO:294)	(SEQ ID NO:307)	-
FUSE-684	(SEQ ID NO:294)	(SEQ ID NO:308)	SEQ ID NO:186
FUSE-685	(SEQ ID NO:114)	(SEQ ID NO:309)	-
FUSE-686	(SEQ ID NO:114)	(SEQ ID NO:168)	-
FUSE-691	(SEQ ID NO:129)		SEQ ID NO:188
FUSE-694	(SEQ ID NO:130)	(SEQ ID NO:169)	SEQ ID NO:188
FUSE-756	(SEQ ID NO:114)	(SEQ ID NO:170)	
FUSE-757	(SEQ ID NO:114)	(SEQ ID NO:171)	
FUSE-758	(SEQ ID NO:114)	(SEQ ID NO:172)	
FUSE-775	(SEQ ID NO:114)	(SEQ ID NO:173)	
FUSE-782	(SEQ ID NO:131)	(SEQ ID NO:174)	SEQ ID NO:188
FUSE-783	(SEQ ID NO:131)	(SEQ ID NO:175)	SEQ ID NO:188
FUSE-827	(SEQ ID NO:132)	(SEQ ID NO:176)	
FUSE-832	(SEQ ID NO:295)	(SEQ ID NO:314)	-
FUSE-874	(SEQ ID NO:114)	(SEQ ID NO:177)	
FUSE-875	(SEQ ID NO:114)	(SEQ ID NO:178)	
FUSE-876	(SEQ ID NO:114)	(SEQ ID NO:179)	
FUSE-882	(SEQ ID NO:296)	(SEQ ID NO:310)	SEQ ID NO:311
FUSE-887	(SEQ ID NO:114)	(SEQ ID NO:180)	
FUSE-888	(SEQ ID NO:114)	(SEQ ID NO:181)	
FUSE-889	(SEQ ID NO:114)	(SEQ ID NO:182)	
FUSE-890	(SEQ ID NO:114)	(SEQ ID NO:183)	
FUSE-891	(SEQ ID NO:114)	(SEQ ID NO:184)	
FUSE-892	(SEQ ID NO:114)	(SEQ ID NO:185)	
FUSE-895	(SEQ ID NO:296)	(SEQ ID NO:315)	SEQ ID NO:311
FUSE-952	(SEQ ID NO:295)	(SEQ ID NO:316)	SEQ ID NO:312
FUSE-1015	(SEQ ID NO:297)	(SEQ ID NO:316)	SEQ ID NO:312
FUSE-1017	(SEQ ID NO:131)	SEQ ID NO:314	SEQ ID NO:188
FUSE-1041	(SEQ ID NO:295)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1042	(SEQ ID NO:297)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1044	(SEQ ID NO:298)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1109	(SEQ ID NO:299)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1110	(SEQ ID NO:300)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1111	(SEQ ID NO:301)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1112	(SEQ ID NO:302)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1113	(SEQ ID NO:303)	SEQ ID NO:316	SEQ ID NO:312
FUSE-1116	(SEQ ID NO:304)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1176	(SEQ ID NO:305)	-	SEQ ID NO:313
FUSE-1177	(SEQ ID NO:306)	SEQ ID NO:169	SEQ ID NO:188
FUSE-1179	(SEQ ID NO:323)	(SEQ ID NO:169)	SEQ ID NO:188
FUSE-1229	(SEQ ID NO:324)	(SEQ ID NO:169)	SEQ ID NO:188

[0310] Table 20. Yields of exemplary fusion proteins.

	Yield		**Yield**		**Yield**		**Yield**

FUSE-422	191 mg/L	**FUSE-504**	135 mg/L	**FUSE-600**	79 mg/L	**FUSE-889**	223 mg/L
FUSE-423	214 mg/L	**FUSE-505**	162 mg/L	**FUSE-601**	86 mg/L	**FUSE-890**	190 mg/L
FUSE-424	198 mg/L	**FUSE-507**	557 mg/L	**FUSE-602**	64 mg/L	**FUSE-891**	181 mg/L
FUSE-441	386 mg/L	**FUSE-508**	34 mg/L	**FUSE-691**	69 mg/L	**FUSE-892**	371 mg/L
FUSE-442	197 mg/L	**FUSE-509**	537 mg/L	**FUSE-694**	67 mg/L	**FUSE-1145**	232 mg/L
FUSE-462	304 mg/L	**FUSE-510**	32 mg/L	**FUSE-756**	46 mg/L	**FUSE-1146**	328 mg/L
FUSE-480	130 mg/L	**FUSE-516**	215 mg/L	**FUSE-757**	96 mg/L	**FUSE-1147**	232 mg/L
FUSE-481	199 mg/L	**FUSE-517**	150 mg/L	**FUSE-758**	64 mg/L	**FUSE-1148**	286 mg/L
FUSE-484	164 mg/L	**FUSE-545**	144 mg/L	**FUSE-775**	79 mg/L		
FUSE-485	348 mg/L	**FUSE-546**	143 mg/L	**FUSE-782**	41 mg/L		
FUSE-486	400 mg/L	**FUSE-547**	135 mg/L	**FUSE-783**	99 mg/L		
FUSE-487	205 mg/L	**FUSE-583**	86 mg/L	**FUSE-827**	207 mg/L		
FUSE-499	410 mg/L	**FUSE-584**	124 mg/L	**FUSE-874**	246 mg/L		
FUSE-500	253 mg/L	**FUSE-585**	136 mg/L	**FUSE-875**	137 mg/L		
FUSE-501	147 mg/L	**FUSE-586**	107 mg/L	**FUSE-876**	216 mg/L		
FUSE-502	159 mg/L	**FUSE-587**	228 mg/L	**FUSE-887**	155 mg/L		
FUSE-503	44 mg/L	**FUSE-599**	76 mg/L	**FUSE-888**	149 mg/L		

[0312] Table 5. Amino acid sequences for Fc heterodimerization including different versions of knobs-into-holes of respective polypeptide arms; for example, as scaffolding polypeptide or forming (part of) the protein capable of translocating to the ER, for exemplary fusion proteins.

	First Polypeptide Arm	Second Polypeptide Arm
7.8.60	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTDNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLMSDGSFFLASKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:189)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPRRPRVYTLPPSRDELTKNQVSLVCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSVLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:190)
7.8.60 CH3	GQPREPQVYTLPPSRDELTDNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLMSDGSFFLASKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:191)	GQPRRPRVYTLPPSRDELTKNQVSLVCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSVLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:192)
A107	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTENQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:193)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDNLTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:194)
A107 CH3	GQPREPQVYTLPPSRDELTENQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:195)	GQPREPQVYTLPPSRDNLTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:196)
DD-KK	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:197)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDKLTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:198)
DD-KK CH3	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYDTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:199)	GQPREPQVYTLPPSRDKLTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:200)
Duobody	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:201)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFLLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:202)
Duobody CH3	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:203)	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFLLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:204)
Electros	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN

tatic Steering CH3	PENNYKTTPPVLDSDGSFFLYSNLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:205)	NYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:206)
Electros tatic Steering	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSNLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:207)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:208)
EW-RVT	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTENQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:193)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPRVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:209)
EW-RVT (2)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTENQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:210)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPRVYTLPPCRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:211)
EW-RVT (2) CH3	GQPREPQVCTLPPSRDELTENQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:212)	GQPREPRVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:213)
EW-RVT CH3	GQPREPQVYTLPPSRDELTENQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSWLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:195)	GQPREPRVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLVSDGSFTLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:214)
HA-TF (1)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVHLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFALYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:215)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVTTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:216)
HA-TF (1) CH3	GQPREPQVYTLPPSRDELTKNQVHLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFALYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:217)	GQPREPQVTTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:218)
Knob-in-hole (1)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:219)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:220)
Knob-in-	GQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ	GQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN

hole (1) CH3	PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:221)	NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:222)
Knob-in-hole (2)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:223)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:224)
Knob-in-hole (2) CH3	GQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:225)	GQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:226)
XmAb	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCDVSGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:227)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDQLTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:228)
XmAb CH3	GQPREPQVYTLPPSRDELTKNQVSLTCDVSGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:229)	GQPREPQVYTLPPSRDQLTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:230)
ZW1	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:231)	PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYVYLPPSRDELTKNQVSLLCLVKGFYPSDIAVEWESN GQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:232)
ZW1 CH3	GQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:233)	GQPREPQVYVYLPPSRDELTKNQVSLLCLVKGFYPSDIAVEWESNGQPEN NYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK (SEQ ID NO:234)

ROR1 details and embodiments

[0313] The contents of International Application No. PCT/US2023/070587, filed July 20, 2023, entitled Receptor Tyrosine Kinase-Like Orphan Receptor I (ROR1)-Specific VHH Antibodies and Multispecific Antibodies Thereof as Immune Cell Engagers are incorporated by reference herein as though fully disclosed.

[0314] Disclosed herein include antibodies (e.g., isolated antibodies), or antigen-binding fragments thereof, that immunospecifically bind to ROR1 that can be used with the mutli-cytokine fusion proteins of the present invention. Also described herein include multispecific antibody construct that can be used in the methods involving administering separate fusion protein/constructs.

[0315] The inventors have identified human ROR1-specific VHH produced in camels in response to stimulation by *human* ROR1, (thereby termed as VHH derived from Bactrian camels). These human ROR1-stimulated, camel-derived VHH include:

2A11 (a polypeptide comprising a polypeptide having an amino acid sequence of QVQLQESGGGSVPAGGSLRLSCAASGSTYSANCMGWFRQAPGKEREEVASMSIRSGRTYYSDSVKGRFTI SQDGSKNTLYLQLNSLKAEDTALYYCAAAYGGSRCVYNYRGQGTQVTVSS, SEQ ID NO: 331), and

5A1 (a polypeptide comprising a polypeptide having an amino acid sequence of QVQLQESGGGSVQAGGSLKLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTF SQDKVKNTVYLQMNTLKPDDTGMYYCAADVRPDGTTCHYNSGGQGTQVTVSS, SEQ ID NO:332).

[0316] An isolated anti-ROR1 (or ROR1-specific) antibody, or ROR1-binding fragment thereof, can comprise a VHH which comprises a polypeptide having an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:331. In some embodiments, a VHH antibody (or nanobody) is provided, which can comprise, or consist of, the polypeptide having an amino acid sequence of SEQ ID NO:331.

[0317] In some embodiments, a polypeptide is provided comprising one or more complementarity-determining regions (CDRs) for binding human ROR1, and these CDRs are identified/present in 2A11, wherein CDR1 of 2A11 consists of a polypeptide having an amino acid sequence of GSTYSANC (SEQ ID NO:325), CDR2 of 2A11 consists of a polypeptide having an amino acid sequence of MSIRSGRTY (SEQ ID NO:326), and CDR3 of 2A11 consists of a polypeptide having an amino acid sequence of AYGGSRCVYNY (SEQ ID NO:327).

[0318] In some embodiments, a polypeptide is provided comprising one or more of a variant of the polypeptide having an amino acid sequence of SEQ ID NO:325, a variant of the polypeptide having an amino acid sequence of SEQ ID NO:326, and a variant of the polypeptide having an amino acid sequence of SEQ ID NO:327, wherein each of the variants comprises one or more deletions, additions, or substitutions of an amino acid residue compared to respective "wild-type" polypeptides having the amino acid sequence of SEQ ID NOs: 325, 326, or 327, respectively. In various embodiments, the substitutions are conservative substitutions. Preferably, a variant of the polypeptide having an amino acid sequence of SEQ ID NO:325, a variant of the polypeptide having an amino acid

sequence of SEQ ID NO:326, and/or a variant of the polypeptide having an amino acid sequence of SEQ ID NO:327 are capable of binding human ROR1, more preferably still capable of immunospecifically binding human ROR1, or possess a binding affinity to ROR1 that is at least 90%, 80%, 70%, 60%, or 50% compared to respective "wild-type" CDR polypeptides present in 2A11. Preferably, one or both of the cysteine residues in the CDRs of 2A11 (i.e., cysteine residues in SEQ ID NO:325 and in SEQ ID NO:327) is unchanged, in a polypeptide that is a variant of 2A11 or in a polypeptide that is a variant of the polypeptide having an amino acid sequence of SEQ ID NO:325, 326, or 327. Hence, in some embodiments, a polypeptide comprising one or more of a variant of the polypeptide having an amino acid sequence of SEQ ID NO:325, a variant of the polypeptide having an amino acid sequence of SEQ ID NO:326, and a variant of the polypeptide having an amino acid sequence of SEQ ID NO:327, still maintains the cysteine residues as those in the polypeptide having an amino acid sequence of SEQ ID NO:325 and in the polypeptide having an amino acid sequence of SEQ ID NO:327. Alternatively, a polypeptide can be a variant of 2A11, wherein one or both of the cysteine residues in the CDRs of 2A11 is replaced with a natural or non-natural amino acid that contains a cross-linking functional group, so as to support formation of inter-/intra-molecular bonds; examples of these amino acids including but not limited to p-benzoyl-L-phenylalanine (Bpa), azide, and haloalkane. Preserving the capability to form disulfide and/or intramolecular bonds at those residue locations where cysteine is present in the CDR of 2A11 is indicated to be important, as data in FIG. 62G demonstrates that replacing the cysteine residues with valine significantly weakens the binding affinity of 2A11 to antigen.

[0319] In some embodiments, the amino acids in the non-CDR portion of 2A11 are modified, so as to generate a humanized version of 2A11 (comprising the CDRs of SEQ ID NOs: 325, 326, and 327 and a framework/non-CDR portion that is humanized), so as to better suit applications in human subjects. Therefore, various embodiments provide a humanized, anti-ROR1 antibody or fragment thereof.

[0320] In additional embodiments, an anti-ROR1 (or ROR1-specific) heavy chain antibody is provided, which comprises (1) an antigen binding portion being a VHH comprising a polypeptide having an amino acid sequence of SEQ ID NO:331, or being a VHH comprising the polypeptides having amino acid sequences of SEQ ID NOs: 325, 326, and 327, and (2) a Fc-domain of an Ig heavy chain. Preferably, the heavy chain antibody further comprises a hinge domain of an Ig heavy chain, so that the VHH is linked to the hinge and the Fc-domains of the Ig heavy chain. The disclosed anti-ROR1 heavy chain antibody, or an antigen-binding fragment thereof, include all isotypes, IgA, IgD, IgE, IgG and IgM, and synthetic multimers of the four-chain immunoglobulin (Ig) structure, as well as the IgY isotype generally found in hen or turkey serum and hen or turkey egg yolk. In some embodiments, the anti-ROR1 heavy chain antibody comprises (1) an antigen binding portion being a VHH comprising a polypeptide having the amino acid sequence of SEQ ID NO:331, or a VHH comprising CDRs having amino acid sequences of SEQ ID NOs: 325, 326, and 327, and (2) the hinge and Fc-domains of an IgG heavy chain, wherein the IgG can be any of the subclasses, IgG1, IgG2, IgG3, and IgG4.

[0321] An isolated anti-ROR1 (or ROR1-specific) antibody, or ROR1-binding fragment thereof, can alternatively comprise a VHH comprising a polypeptide having an amino acid sequence that is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:331. In some embodiments, a VHH antibody (or nanobody) is provided, which can comprise, or consist of, a polypeptide having the amino acid sequence of SEQ ID NO:331.

[0322] In some embodiments, a polypeptide is provided comprising one or more complementarity-determining regions (CDRs) of 5A1, wherein CDR1 of 5A1 consists of a polypeptide having the amino acid sequence of GYTNRLKC (SEQ ID NO:328), CDR2 of 5A1 consists of a polypeptide having the amino acid sequence of ISTGTGNTY (SEQ ID NO:329), and CDR3 of 5A1 consists of a polypeptide having the amino acid sequence of DVRPDGTTCHYNS (SEQ ID NO:330).

[0323] In some embodiments, a polypeptide is provided comprising one or more of a variant of the polypeptide having the amino acid sequence of SEQ ID NO:328, a variant of the polypeptide having the amino acid sequence of SEQ ID NO:329, and a variant of the polypeptide having the amino acid sequence of SEQ ID NO:330, wherein each of the variants comprises one or more deletions, additions, or substitutions of an amino acid residue compared to respective "wild-type" polypeptide having the amino acid sequences of SEQ ID NOs: 328, 329, or 330. In various embodiments, the substitutions are conservative substitutions. Preferably, a variant of the polypeptide having the amino acid sequence of SEQ ID NO:328, a variant of the polypeptide having the amino acid sequence of SEQ ID NO:329, and/or a variant of the polypeptide having the amino acid sequence of SEQ ID NO:330 are capable of binding human ROR1, more preferably still capable of immunospecifically binding human ROR1, or possess a binding affinity to human ROR1 that is at least 90%, 80%, 70%, 60%, or 50% compared to respective "wild-type" CDRs present in 5A1. Preferably, one or both of the cysteine residues in the CDRs of 5A1 (i.e., cysteine residues in SEQ ID NO:328 and in SEQ ID NO:330) are unchanged, in a polypeptide that is a variant of 5A1 or in a polypeptide that is a variant of the polypeptide having an amino acid sequence of SEQ ID NO:328, 329, or 330. Hence, in some embodiments, a polypeptide comprising one or more of a variant of SEQ ID NO:328, a variant of SEQ ID NO:329, and a variant of SEQ ID NO:330 still maintains the cysteine residues as those in SEQ ID NO:328 and SEQ ID NO:330. Alternatively, a polypeptide can be a variant of 5A1, wherein one or both of the cysteine residues in the CDRs of 5A1 is replaced with a natural or non-natural amino acid that contains a cross-linking functional group, so as to support formation of inter-/intra-molecular bonds; examples of these amino acids including but not limited to p-benzoyl-L-phenylalanine (Bpa), azide, and haloalkane. Preserving the capability to form disulfide and/or intramolecular bonds at those residue locations is indicated to be important, as data in FIG. 62F demonstrates that replacing the cysteine residues with valine significantly weakens the binding affinity of 5A1 to antigen.

[0324] In some embodiments, the amino acids in the non-CDR portion of 5A1 are modified, so as to generate a humanized version of 5A1 (comprising the CDRs whose amino acid sequences are SEQ ID NOs: 328, 329,

330, and a framework/non-CDR portion that is humanized), so as to better suit applications in human subjects. Exemplary humanized VHH derived from 5A1 include:

5A1-H1 (a polypeptide having an amino acid sequence of EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKERELASISTGTGNTYYADSVKGRFTIS RDNSKNTLYLQMNSLKAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS, SEQ ID NO:335),

5A1-H2 (a polypeptide having an amino acid sequence of QVQLQESGGGLVQPGGSLRLSCTASGYTNRLKCMGWVRQAPGKEREEVATISTGTGNTYYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAMYYCAADVRPDGTTCHYNSGGQGTQVTVSS, SEQ ID NO:336),

5A1-H3 (a polypeptide having an amino acid sequence of EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVSTISTGTGNTYYADSVKGRFTI SQDKSKNTLYLRMNSLRAEDTALYYCAADVRPDGTTCHYNSGGQGTQVTVSS, SEQ ID NO:337),

5A1-H4 (a polypeptide having an amino acid sequence of EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTIS RDNSRNTLYLQMKTLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTQVTVSS, SEQ ID NO:338), and

5A1-H5 (a polypeptide having an amino acid sequence of EVQLVESGGGLVQPGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTF SRDNSKNTLYLQMNSLRAEDTAVYYCAADVRPDGTTCHYNSGGQGTQVTVSS, SEQ ID NO:339).

[0325] Additional exemplary humanized VHH are shown in Table 14.

[0326] In additional embodiments, an anti-ROR1 (or ROR1-specific) heavy chain antibody is provided, which comprises (1) an antigen binding portion (i) being a VHH comprising a polypeptide having the amino acid sequence of SEQ ID NO:332, or (ii) being a VHH comprising CDRs which are polypeptides having amino acid sequences of SEQ ID NOs: 328, 329, and 330, or (iii) being a VHH comprising a polypeptide having the amino acid sequence of any one of SEQ ID NO:335-339, or (iv) comprising one or more of (i), (ii) and (iii), and (2) a Fc-domain of an Ig heavy chain. Preferably, the heavy chain antibody further comprises a hinge domain of an Ig heavy chain, so that the VHH is linked to the hinge and the Fc-domains of the Ig heavy chain. The disclosed anti-ROR1 heavy chain antibody, or an antigen-binding fragment thereof, include all isotypes, IgA, IgD, IgE, IgG and IgM, and synthetic multimers of the four-chain immunoglobulin (Ig) structure, as well as the IgY isotype generally found in hen or turkey serum and hen or turkey egg yolk. In some embodiments, the anti-ROR1 heavy chain antibody comprises (1) an antigen binding portion being a VHH having the amino acid sequence of SEQ ID NO:332, or being a VHH comprising CDRs whose amino acid sequences are SEQ ID NOs: 328, 329, and 330, or being a VHH having the amino acid sequence of any one of SEQ ID NOs:335-339, and (2) the hinge and Fc-domains of an IgG heavy chain, wherein the IgG can be any of the subclasses, IgG1, IgG2, IgG3, and IgG4.

[0327] Polypeptides are also provided comprising (1) two or more repeats of a polypeptide having an amino acid sequence of SEQ ID NO:331, (2) two or more repeats of a polypeptide having an amino acid sequence of SEQ ID

NO:332, (3) one or more repeats of a polypeptide having an amino acid sequence of SEQ ID NO:331 and one or more repeats of a polypeptide having an amino acid sequence of SEQ ID NO:332, (4) two or more repeats of a polypeptide having an amino acid sequence of any one of SEQ ID NOs:335-339, or (5) one or more repeats of a first polypeptide having an amino acid sequence of one of SEQ ID NOs:335-339 and one or more repeats of a second polypeptide having an amino acid sequence of one of SEQ ID NOs:335-339, where the first and the second polypeptides have different amino acid sequences. In some embodiments, a polypeptide comprises a tandem sequence of a first VHH and a second VHH, wherein the first VHH and the second VHH can be the same or different. The amino acid sequence of the first VHH and the amino acid sequence of the second VHH can be individually selected from any one of SEQ ID NOs:331 and 332, or selected from any one of SEQ ID NOs:335-339. In one aspect, the first VHH and the second VHH are the same and both have the amino acid sequence of SEQ ID NO:331; in another aspect, the first VHH and the second VHH are the same and both have the amino acid sequence of SEQ ID NO:332; in yet another aspect, the first VHH and the second VHH have the amino acid sequence of SEQ ID NO:331 and/or a variant of the polypeptide having an amino acid sequence of SEQ ID NO:331; in yet another aspect, the first VHH and the second VHH have the amino acid sequence of SEQ ID NO:332 and/or a variant of the polypeptide having the amino acid sequence of SEQ ID NO:332; or in an alternative aspect, the first VHH may have the amino acid sequence of SEQ ID NO:331 or may be a variant of the polypeptide having the amino acid sequence of SEQ ID NO:331, and the second VHH may have the amino acid sequence of SEQ ID NO:332 or may be a variant of the polypeptide having the amino acid sequence of SEQ ID NO:332. In further embodiments, the polypeptide comprising the tandem sequence further comprises a linker, wherein the first VHH and the second VHH are connected with a linker. The linker may be on the N-terminal end of the VHH closer to the N-terminus of the Fc, or alternatively on the C-terminal end.

[0328] Various embodiments provide for a polypeptide, comprising: a polypeptide having a complementarity-determining region (CDR) 1, a polypeptide having a CDR2, and a polypeptide having a CDR3 selected from Table 15A, wherein CDR1, CDR2 and CDR3 are selected from the same row in Table 15A. Various embodiments provide for a polypeptide, comprising: a polypeptide having a complementarity-determining region (CDR) 1, a polypeptide having a CDR2, and a polypeptide having a CDR3 selected from Table 15B, wherein CDR1, CDR2 and CDR3 are selected from the same row in Table 15B. Various embodiments provide for a polypeptide, comprising: a variant of the polypeptide having the polypeptide having the CDR1, the polypeptide having the CDR2, and the polypeptide having the CDR3 selected from Table 15A, wherein the variant of the polypeptide having the CDR1 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having the CDR1, wherein the variant of the polypeptide having the CDR2 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having the CDR2, and wherein the variant of the polypeptide having the CDR3 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having the CDR3, wherein CDR1, CDR2 and CDR3 are selected from the same row in Table 15A. Various embodiments provide for a polypeptide, comprising: a variant of the polypeptide having the polypeptide having the CDR1, the polypeptide having the CDR2, and the polypeptide having the CDR3 selected from Table 15B, wherein

the variant of the polypeptide having the CDR1 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having the CDR1, wherein the variant of the polypeptide having the CDR2 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having the CDR2, and wherein the variant of the polypeptide having the CDR3 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having the CDR3, wherein CDR1, CDR2 and CDR3 are selected from the same row in Table 15B. Various embodiments provide for a polypeptide, comprising: a polypeptide having a CDR1 of SEQ ID NO:328, a polypeptide having a CDR2 of SEQ ID NO:401, and a polypeptide having a CDR3 of SEQ ID NO:330. Various embodiments provide for a polypeptide, comprising: a polypeptide having a CDR1 of SEQ ID NO:397, a polypeptide having a CDR2 of SEQ ID NO:401, and a polypeptide having a CDR3 of SEQ ID NO:408. Various embodiments provide for a polypeptide, comprising: a polypeptide having a CDR1 of SEQ ID NO:397, a polypeptide having a CDR2 of SEQ ID NO:401, and a polypeptide having a CDR3 of SEQ ID NO:409. Various embodiments provide for a polypeptide, comprising: a polypeptide having a CDR1 of SEQ ID NO:397, a polypeptide having a CDR2 of SEQ ID NO:329, and a polypeptide having a CDR3 of SEQ ID NO:408. Various embodiments provide for a polypeptide, comprising: a polypeptide having a CDR1 of SEQ ID NO:397, a polypeptide having a CDR2 of SEQ ID NO:329, and a polypeptide having a CDR3 of SEQ ID NO:409. In various embodiments, the substitutions are conservative substitutions. Preferably, variants of the CDR1, CDR2 or CDR3 are capable of binding human ROR1, more preferably still capable of immunospecifically binding human ROR1, or possess a binding affinity to ROR1 that is at least 90%, 80%, 70%, 60%, or 50% compared to respective CDR polypeptides. In various embodiments, the polypeptide further comprises framework region (FWR) 1, framework region 2, framework region 3, and framework region 4 selected from Table 15B, and wherein FWR1, FWR2, FWR3, and FWR4 are selected from the same row in Table 15B In various embodiments, the polypeptide having CDR1, CDR2, CDR3, further comprises a FWR1, FWR2, FWR3 and FWR4 from a human IgG. In various embodiments, the polypeptide having a variant of CDR1, a variant of CDR2, a variant of CDR3, further comprises a FWR1, FWR2, FWR3 and FWR4 from a human IgG. In various embodiments, the polypeptide is a VHH polypeptide selected from Table 14. In various embodiments, the polypeptide is a variant of a VHH polypeptide selected from Table 14, wherein the variant comprises one or more deletions, additions or a substitutions of an amino acid residues of the polypeptide. In various embodiments, the variant comprises up to 5 deletions, additions or a substitutions of an amino acid residues of the polypeptide. In various embodiments, the variant comprises up to 1, 2 3 or 4 deletions, additions or a substitutions of an amino acid residues of the polypeptide...In various embodiments, the variant is at least 95% identical to the polypeptide selected from Table 14. In various embodiments, the variant is at least 96%, 97%, 98%, or 99% identical to the polypeptide selected from Table 14. In various embodiments, the variant is at least 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to its corresponding polypeptide selected from Table 14. In various embodiments, the polypeptide is a VHH polypeptide having an amino acid sequence of SEQ ID NO:340, SEQ ID NO:384, SEQ ID NO:363, or SEQ ID NO:364. In various embodiments, the polypeptide is a variant of a VHH polypeptide having an amino acid sequence of SEQ ID NO:340, SEQ ID NO:384, SEQ ID NO:363, or SEQ ID NO:364, wherein the variant comprises one or more deletions, additions or a

substitutions of an amino acid residues of the polypeptide. In various embodiments, the variant comprises up to 5 deletions, additions or a substitutions of an amino acid residues of the polypeptide. In various embodiments, the variant comprises up to 1, 2 3 or 4 deletions, additions or a substitutions of an amino acid residues of the polypeptide...In various embodiments, the variant is at least 95% identical to the VHH polypeptide having an amino acid sequence of SEQ ID NO:340, SEQ ID NO:384, SEQ ID NO:363, or SEQ ID NO:364. In various embodiments, the variant is at least 96%, 97%, 98%, or 99% identical to the VHH polypeptide having an amino acid sequence of SEQ ID NO:340, SEQ ID NO:384, SEQ ID NO:363, or SEQ ID NO:364. In various embodiments, the variant is at least 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to its VHH polypeptide having an amino acid sequence of SEQ ID NO:340, SEQ ID NO:384, SEQ ID NO:363, or SEQ ID NO:364...

[0329] The isolated antibody, or antigen-binding fragment thereof, can preferably bind to a region of the ROR1 extracellular domain, and therefore bind to cells with surface expression of ROR1.

[0330] The anti-ROR1 VHH (nanobody), heavy chain antibodies, and/or antigen-binding fragments thereof, or polypeptides containing the anti-ROR1 VHH or CDRs thereof, as disclosed above, may be derived from any species by recombinant means. For example, the VHH, heavy chain antibodies or antigen-binding fragments may be mouse, rat, goat, horse, swine, bovine, chicken, rabbit, camelid, donkey, human, or chimeric versions thereof. Particularly, the VHH, heavy chain antibodies or antigen-binding fragments may be camelid or humanized version thereof. For use in administration to humans, non-human derived antibodies or antigen-binding fragments may be genetically or structurally altered to be less antigenic upon administration to the human patient.

[0331] Also provided are polynucleotide sequences encoding the disclosed anti-ROR1 VHH, the disclosed anti-ROR1 heavy chain antibodies, and/or the disclosed polypeptides containing the anti-ROR1 VHH or CDRs thereof. For example, vectors encoding the sequence of 2A11, 5A1, or humanized versions of 2A11 or 5A1 can be expressed in various transfection-compatible cell types, not limited to camel cells or human cells. Also, vectors encoding any one of the sequences noted in Table 14 can be expressed in various transfection-compatible cell types, not limited to camel cells or human cells. Vectors comprising the polynucleotides are also provided. The vectors can be expression vectors, such as recombinant expression vectors. The expression vector may contain one or more additional sequences such as, but not limited, to regulatory sequences (e.g., promoter, enhancer), selection markers, and polyadenylation signals. Vectors for transforming a wide variety of host cells are well known and include, but are not limited to, plasmids, phagemids, cosmids, baculoviruses, bacmids, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), as well as other bacterial, yeast and viral vectors. Also provided are cells expressing, and capable of expressing, the disclosed vectors. These cells may be mammalian cells (such as 293F cells, CHO cells), insect cells (such as Sf7 cells), yeast cells, plant cells, or bacteria cells (such as E. coli). The disclosed antibodies may also be produced by hybridoma cells.

Multispecific Antibodies and Multispecific Antigen-Binding Fragments

[0332] Also disclosed herein are multispecific (including bispecific or trispecific) antibodies, or multispecific antigen-binding fragments thereof, which bind to at least ROR1 and CD3 (anti-ROR1/anti-CD3 antibodies),or bind to at least ROR1 and an activation receptor expressed on an immune cell (including but not limited to CD3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, NKp46, and NKG2D). In various embodiments, isolated bispecific antibodies, or bispecific antigen-binding fragments thereof, are provided which bind to ROR1 and CD3 (anti-ROR1/anti-CD3 bispecific antibodies).

[0333] In various embodiments, the anti-ROR1/anti-CD3 antibodies have at least a first antigen-binding site that immunospecifically binds ROR1 (ROR1 arm) and a second antigen-binding site that immunospecifically binds CD3 (CD3 arm). Exemplary anti-ROR1/anti-CD3 antibodies of these embodiments are shown in FIG. 58A and 58E.

[0334] In some embodiments, the anti-ROR1/anti-CD3 antibodies have at least a first arm that is ROR1-specific and a second arm that is bispecific, wherein the first arm that is ROR1-specific comprises a first antigen-binding site that immunospecifically binds ROR1, and the second arm that is bispecific comprises an antigen-biding site that immunospecifically binds CD3 and a second antigen-binding site that immunospecifically binds ROR1. Exemplary anti-ROR1/anti-CD3 antibodies of these embodiments are shown in FIG. 58B and 58C.

[0335] In various embodiments, the anti-ROR1/anti-NKG2D antibodies have at least a first antigen-binding site that immunospecifically binds ROR1 (ROR1 arm) and a second antigen-binding site that immunospecifically binds NKG2D (NKG2D arm).

[0336] In some embodiments, the anti-ROR1/anti-NKG2D antibodies have at least a first arm that is ROR1-specific and a second arm that is bispecific, wherein the first arm that is ROR1-specific comprises a first antigen-binding site that immunospecifically binds ROR1, and the second arm that is bispecific comprises an antigen-biding site that immunospecifically binds NKG2D and a second antigen-binding site that immunospecifically binds ROR1.

[0337] In various embodiments, the anti-ROR1/anti-CD16 antibodies have at least a first antigen-binding site that immunospecifically binds ROR1 (ROR1 arm) and a second antigen-binding site that immunospecifically binds CD16 (CD16 arm).

[0338] In some embodiments, the anti-ROR1/anti-CD16 antibodies have at least a first arm that is ROR1-specific and a second arm that is bispecific, wherein the first arm that is ROR1-specific comprises a first antigen-binding site that immunospecifically binds ROR1, and the second arm that is bispecific comprises an antigen-biding site that immunospecifically binds CD16 and a second antigen-binding site that immunospecifically binds ROR1.

[0339] In various embodiments, the anti-ROR1/anti-NKp46 antibodies have at least a first antigen-binding site that immunospecifically binds ROR1 (ROR1 arm) and a second antigen-binding site that immunospecifically binds NKp46 (NKp46 arm).

[0340] In some embodiments, the anti-ROR1/anti-NKp46 antibodies have at least a first arm that is ROR1-specific and a second arm that is bispecific, wherein the first arm that is ROR1-specific comprises a first antigen-

binding site that immunospecifically binds ROR1, and the second arm that is bispecific comprises an antigen-biding site that immunospecifically binds NKp46 and a second antigen-binding site that immunospecifically binds ROR1.

[0341] In further embodiments, isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, are provided, which contains at least one ROR1-binding site, at least one CD3-binding site, and a Fc-domain of an Ig heavy chain, and optionally a hinge domain of the Ig heavy chain. The Ig heavy chain can be any one of isotypes IgA, IgD, IgE, IgG and IgM, and synthetic multimers of the four-chain immunoglobulin (Ig) structure, as well as the IgY isotype generally found in hen or turkey serum and hen or turkey egg yolk. Preferably, the Ig heavy chain is a IgG heavy chain, which can be any of the subclasses, IgG1, IgG2, IgG3, and IgG4.

[0342] Hence, in some embodiments, anti-ROR1/anti-CD3 antibodies have at least (1) a first antigen-binding site specific for ROR1, (2) a second antigen-binding site at least specific for CD3, or a bispecific antigen-binding site for ROR1 and CD3, and (3) the hinge and Fc domains of two or more chains of Ig, which can be dimerized or multimerized, wherein at least one heaving chain of the Ig may further comprise an antigen-binding site specific for ROR1, preferably on the C-terminus of the Ig heavy chain.

[0343] Hence, in other embodiments, anti-ROR1/anti-NKG2D antibodies have at least (1) a first antigen-binding site specific for ROR1, (2) a second antigen-binding site at least specific for NKG2D, or a bispecific antigen-binding site for ROR1 and NKG2D, and (3) the hinge and Fc domains of two or more chains of Ig, which can be dimerized or multimerized, wherein at least one heaving chain of the Ig may further comprise an antigen-binding site specific for ROR1, preferably on the C-terminus of the Ig heavy chain.

[0344] Hence, in other embodiments, anti-ROR1/anti-CD16 antibodies have at least (1) a first antigen-binding site specific for ROR1, (2) a second antigen-binding site at least specific for CD16 , or a bispecific antigen-binding site for ROR1 and CD16 , and (3) the hinge and Fc domains of two or more chains of Ig, which can be dimerized or multimerized, wherein at least one heaving chain of the Ig may further comprise an antigen-binding site specific for ROR1, preferably on the C-terminus of the Ig heavy chain.

[0345] Hence, in other embodiments, anti-ROR1/anti-NKp46 antibodies have at least (1) a first antigen-binding site specific for ROR1, (2) a second antigen-binding site at least specific for NKp46 , or a bispecific antigen-binding site for ROR1 and NKp46 , and (3) the hinge and Fc domains of two or more chains of Ig, which can be dimerized or multimerized, wherein at least one heaving chain of the Ig may further comprise an antigen-binding site specific for ROR1, preferably on the C-terminus of the Ig heavy chain.

[0346] Alternatively, isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, are provided, which contains at least one ROR1-binding site, at least one CD3-binding site, and a scaffold protein such as human serum albumin. Alternatively, isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, are provided, which contains at least one ROR1-binding site, at least one NKG2D-binding site, and a scaffold protein such as human serum albumin. Alternatively, isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, are provided, which contains at least one ROR1-binding site, at least one CD16-binding site, and a

scaffold protein such as human serum albumin. Alternatively, isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, are provided, which contains at least one ROR1-binding site, at least one NKp46 -binding site, and a scaffold protein such as human serum albumin.

[0347] The isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, can comprise:

a) a first antigen-binding site that immunospecifically binds ROR1, the first antigen-binding site comprising one or more of:

2A11 (the peptide having an amino acid sequence of SEQ ID NO:331),

a variant of 2A11 disclosed above,

5A1 (the peptide having an amino acid sequence of SEQ ID NO:332),

a variant of 5A1 disclosed above (such as a polypeptide having an amino acid sequence of any one of SEQ ID NOs:335-339),

three, two, or at least one of the CDR1 (having an amino acid sequence of SEQ ID NO:325), CDR2 (having an amino acid sequence of SEQ ID NO:326), and CDR3 (having an amino acid sequence of SEQ ID NO:327) of 2A11,

one or more variants of the three, two, or at least one of the CDR1 (having an amino acid sequence of SEQ ID NO:325), CDR2 (having an amino acid sequence of SEQ ID NO:326), and CDR3 (having an amino acid sequence of SEQ ID NO:327) of 2A11,

three, two, or at least one of the CDR1 (having an amino acid sequence of SEQ ID NO:328), CDR2 (having an amino acid sequence of SEQ ID NO:329), and CDR3 (having an amino acid sequence of SEQ ID NO:330) of 5A1, and

one or more variants of the three, two, or at least one of the CDR1 (having an amino acid sequence of SEQ ID NO:328), CDR2 (having an amino acid sequence of SEQ ID NO:329), and CDR3 (having an amino acid sequence of SEQ ID NO:330) of 5A1; and

b) a second antigen-binding site that immunospecifically binds CD3, CD16, NKp46, or NKG2D.

[0348] In various embodiments, the second antigen-binding site immunospecifically binds to CD3. In various embodiments, the antigen-binding site that immunospecifically binds to NKG2D.

[0349] In other embodiments, the isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, can comprise:

a1) a first antigen-binding site comprising a polypeptide selected from Table 14, OR

a2) a first antigen-binding site comprising CDR1, CDR2, and CDR3 selected from Table 15A, OR

a3) a first antigen-binding site comprising CDR1, CDR2, and CDR3 selected from Table 15B; and

b) a second antigen-binding site that immunospecifically binds to CD3, CD16, NKp46, or NKG2D.

[0350] In various embodiments, the second antigen-binding site immunospecifically binds to CD3. In various embodiments, the antigen-binding site that immunospecifically binds to NKG2D.

[0351] In other embodiments, the isolated multispecific antibodies, or multispecific antigen-binding fragments thereof, can comprise:

a1) a first antigen-binding site comprising a variant of CDR1, a variant of CDR2, and a variant of CDR3 selected from Table 15A, OR

a2) a first antigen-binding site comprising a variant of CDR1, a variant of CDR2, and a variant of CDR3 selected from Table 15B; and

b) a second antigen-binding site that immunospecifically binds to CD3, CD16, NKp46, or NKG2D.

[0352] In various embodiments, the multispecific antibody comprises two or more VHH domains, or two or more single-chain variable fragments (scFv), capable of binding a tumor-associated antigen (TAA), wherein the two or more VHH domains are each independently the one or more first polypeptides, wherein the one or more first polypeptides comprise a polypeptide selected from Table 14.

[0353] In various embodiments, the multispecific antibody comprises two or more VHH domains, or two or more single-chain variable fragments (scFv), capable of binding a tumor-associated antigen (TAA), wherein the two or more VHH domains are each independently the one or more first polypeptides, wherein the one or more first polypeptides comprise a polypeptide having a sequence as set forth in SEQ ID NO:335-339.

[0354] In various embodiments, the multispecific antibody comprises two or more VHH domains, or two or more single-chain variable fragments (scFv), capable of binding a tumor-associated antigen (TAA), wherein the two or more VHH domains are each independently the one or more first polypeptides, wherein the one or more first polypeptides comprise a polypeptide having a sequence as set forth in SEQ ID NO:335, 338-394.

[0355] Variants of CDR1, CDR2, and CDR3 are as discussed herein. In various embodiments, the first antigen-binding site further comprises a FWR1, FWR2, FWR3 and FWR4 selected from Table 15B. In various embodiments, the first antigen-binding site further comprises a FWR1, FWR2, FWR3 and FWR4 from a human IgG. In various embodiments, the second antigen-binding site immunospecifically binds to CD3. In various embodiments, the antigen-binding site that immunospecifically binds to NKG2D.

[0356] Suitable antigen-binding sites can be in the form of a VHH, scFv, Fab, $(Fab')_2$, one or more CDRs, or a fusion (tandem sequence optionally connected via a linker) of one or more of the VHH, scFv, Fab, $(Fab')_2$, or CDRs. Suitable antigen-binding sites that immunospecifically bind to ROR1 include any of the above disclosed anti-ROR1 antibodies or ROR1-binding fragments thereof. Suitable antigen-binding sites that immunospecifically bind to CD3 can be derived from CD3 antibodies disclosed in one or more publications including but not limited to U.S. Pat. No. 8,236,308, U.S. Patent App. Pub. Nos. 2010/0260668, 2013/0018174, 2012/0321626, 2013/0060011, 2013/0058936, 2013/0078249, and 2013/0058937.

[0357] In some embodiments, the antigen-binding site of the multispecific antibodies disclosed herein is connected to an Ig heavy chain constant region that contains a mutation. In some aspects, "knob-in-hole" (KiH) mutations are present in the CH3 domains of two arms (or chains) of Ig heavy chain constant regions, so that the two

can heterodimerize. This structural feature in the polypeptide arms allows for assembly of two half antibodies (e.g., Fc heterodimer; and VH–CH and VL–CL domains). For example, a heteromultimer (including a heterodimer) may comprise a first polypeptide and a second polypeptide each comprising a CH3 domain, wherein the polypeptides meet at an engineered interface within the CH3 domain, and the first polypeptide contains an engineered protuberance ("knob") in the interface with at least one contact residue replaced with an import residue having a larger side chain volume than the original residue, and the second polypeptide contains an engineered cavity ("hole") in the interface with at least one contact residue replaced with an import residue having a smaller side chain volume than the original residue. In some embodiments, the engineered interface of a heteromultimer includes at least two protuberance-into-cavity mutant pairs. Volumes and accessible surface areas of each amino acid are described in A. A. Zamyatnin, *Prog. Biophys. Mol. Biol.* 24: 107-123, 1972 and C. Chothia, *J. Mol. Biol.* 105: 1-14, 1975. For example, import residues for the formation of a protuberance can be arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W); and preferably the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine. As another example, import residues for the formation of a cavity can be alanine (A), serine (S), threonine (T) and valine (V); and preferably the original residue for the formation of the cavity has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. For example, a T366W mutation in CH3 domain for the "knob"/protuberance chain, and a T366S/L368A/Y407V mutation in CH3 domain for the "hole"/cavity chain. Additionally, the KiH configuration may be coupled further mutations to permit S-S disulfide linkage between the two chains.

[0358] In some embodiments, the two or more arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can contain another symmetric-to-asymmetric steric complementarity design (e.g., HA-TF, ZW1), a charge-to-charge swap interaction (DD-KK), a charge-to-steric complementarity swap plus additional long-range electrostatic interaction (e.g., EW-RVT), or an isotype strand swap design (e.g., strand-exchange engineered domain (SEED)), or Xmab, 7.8.60, Electrostatic Steering, A107, or Duobody, so as to form heterodimeris/heteromultimers. Further description of these configurations and exemplary mutations/residues are seen in, for example, *Front Immunol.* 2016; 7: 394.

[0359] Further embodiments provide that a multispecific antibody may further comprise one or more linkers, such as amino acid linkers. In some embodiments, at least one linker is between the Fc or the HSA and at least one of the antigen-binding sites. In some embodiments, at least one linker is positioned between two antigen-binding sites.

[0360] In some embodiments, a multispecific antibody comprises (1) a "knob-in-hole" IgG Fc domains with (2) a CD3-specific Fab on the N-terminus of the "knob" chain (or the "hole" chain) of the IgG Fc domain and (3) two ROR1-specific VHH fused in tandem, optionally connected via a linker (VHH-linker-VHH), on the N-terminus of the "hold" chain (or the "knob chain, respectively) of the IgG Fc domain. The two ROR1-specific VHH fused in tandem herein may be two 2A11, two 5A1, or one 2A11 and one 5A1, two variants of 2A11, or two variants of 5A1.

Exemplary multispecific antibodies of these embodiments include fusion proteins of FUSE-211, and FUSE-393, whose amino acid sequences are shown in Table 8. In some embodiments, the Fc domain is selected from Table 17A

[0361] In some embodiments, a multispecific antibody comprises (1) a "knob-in-hole" IgG Fc domains with (2) a CD3-specific Fab (comprising one constant and one variable domain of each of the CD3-specific heavy and the CD3-specific light chain) on the N-terminus of the "knob" chain (or the "hole" chain) of the IgG Fc domain, (3) a first ROR1-specific VHH fused to the N-terminus of the "hole" chain (or the "knob" chain, respectively), and (4) a second ROR1-specific VHH fused to the C-terminus of the CD3-specific light chain. The first and second ROR1-specific VHH can be identical or different, independently selected from 2A11, 5A1, or a variant of 2A11 or a variant of 5A1. Exemplary multispecific antibodies of these embodiments include fusion protein FUSE-394, whose amino acid sequence is shown in Table 8. In some embodiments, the Fc domain is selected from Table 17A.

[0362] EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVARIRSKYNN YATYYADSVKDRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAYWGQGTMVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKV (SEQ ID NO:532) is an exemplary heavy chain of an anti-CD3 Fab, and SEQ ID NO:479 is an exemplary light chain of an anti-CD3 Fab. Tin various embodiments, the anti-CD3 Fab is constant and sometimes present on the knob Fc and sometimes present on the hole Fc.

[0363] In some embodiments, a multispecific antibody comprises (1) a "knob-in-hole" IgG Fc domains with (2) a CD3-specific Fab (comprising one constant and one variable domain of each of the CD3-specific heavy and the CD3-specific light chain) fused to the N-terminal of the "knob" chain (or the "hole" chain) of the IgG Fc domain, (3) two ROR1-specific VHH fused in tandem to the N-terminus of the "hole" chain (or the "knob" chain, respectively) of the IgG Fc domain, and (4) a third specific VHH fused to the C-terminus of the CD3-specific light chain. The two, fused-in-tandem ROR1-specific VHH and the third ROR1-specific VHH may be identical or different, each independently selected from 2A11, 5A1, or a variant of 2A11 or a variant of 5A1. In other embodiments, the first and second ROR1-specific VHH can be identical or different, independently selected from Table 14. In some embodiments, the Fc domain is selected from Table 17A.

[0364] In some embodiments, a multispecific antibody comprises (1) a "knob-in-hole" IgG Fc domains with (2) a CD3-specific Fab fused to the N-terminus of the "knob" chain (or the "hole" chain) of the IgG Fc domains, (3) a first ROR1-specific VHH fused to the N-terminus of the "hole" chain (or the "key" chain, respectively) of the IgG Fc domains, and (4) a second ROR1-specific VHH fused to the N-terminus of the CD3-specific light chain of the Fab. The first and second ROR1-specific VHH can be identical or different, independently selected from 2A11, 5A1, or a variant of 2A11 or a variant of 5A1. In other embodiments, the first and second ROR1-specific VHH can be identical or different, independently selected from Table 14. In some embodiments, the Fc domain is selected from Table 17A.

[0365] In some embodiments, a multispecific antibody comprises (1) a "knob-in-hole" IgG Fc domains with (2) a CD3-specific Fab fused to the N-terminus of the "knob" chain (or the "hole" chain) of the IgG Fc domains,

(3) a first ROR1-specific scFV fused to the N-terminus of the "hole" chain (or the "knob" chain, respectively) of the IgG Fc domains, and (4) a second ROR1-specific scFV fused to the C-terminus of the "hole" (or the "knob" chain, respectively) of the IgG Fc domains. The first and second ROR1-specific scFv can be identical or different. In some embodiments, the Fc domain is selected from Table 17A.

[0366] In some embodiments, a multispecific antibody comprises (1) a "knob-in-hole" IgG Fc domains with (2) a CD3-specific Fab fused to the N-terminus of the "hole" chain (or the "knob" chain) of the IgG Fc domains, (3) a first ROR1-specific VHH fused to the N-terminus of the "knob" chain (or the "hole" chain) of the IgG Fc domains, and (4) a second ROR1-specific VHH fused to the C-terminus of the CD3-specific light chain of the Fab. The first and second ROR1-specific VHH can be identical or different, independently selected from 2A11, 5A1, or a variant of 2A11 or a variant of 5A1. In other embodiments, the first and second ROR1-specific VHH can be identical or different, independently selected from Table147. In some embodiments, the Fc domain is selected from Table 17A.

[0367] In various embodiments, the disclosed multispecific antibodies (e.g., at least with immunospecificity towards ROR1 and CD3) can bind to ROR1 with an apparent binding affinity that generally increases (i.e., corresponding to a binding EC_{50} – a concentration of the antibody that results in 50% of maximum binding to a certain population of ROR1-expressing target cells – that generally decreases) with an increase in the cell surface density of ROR1 of the target cells. That is, the multispecific antibodies can in various embodiments have a greater binding avidity towards ROR1-expressing cells with a higher expression level of ROR1 than towards cells with a lower or zero expression level of ROR1.

[0368] In various embodiments, a system comprising the disclosed multispecific antibodies (e.g., at least with immunospecificity towards ROR1 and CD3) and a population of CD3-positive immune effector cells or a CD3-positive fraction of mononuclear cells or a population of mononuclear cells comprising a CD3-positive fraction can induce cytotoxicity against ROR1-positive cells, i.e., ROR1-expressing tumor cells.

[0369] In some embodiments, the multispecific antibodies comprising an antigen-binding site that immunospecifically binds CD3 can bind CD3-epsilon on primary human T cells and/or primary cynomolgus T cells. In some embodiments, the multispecific antibodies comprising an antigen-binding site that immunospecifically binds CD3 activates cytotoxicity of primary human CD3+ T cells and/or primary cynomolgus CD3+ T cells. In general, CD8+ T cells are cytotoxic whereas CD4+ T cells primary secrete cytokines to "help" CD8+ T cells. In some instances, CD4+ T cells have also been reported to be cytotoxic (David Oh, et al., *Immunity*, Volume 54, Issue 12, 14 December 2021, Pages 2701-2711). Cytotoxicity generally is associated with killing of target cells via the perforin/Granzyme pathway that activates Caspase 3 and subsequent target cell apoptosis. Other mechanisms of target killing include but are not limited to activation of target cell express Fas, TNF-R, and DR4. TCEs directly induce cytotoxicity and may also indirectly induce the other said mechanisms of target killing (Sandra Ross et al., *PLoS One*, 2017 Aug 24;12(8):e0183390).

[0370] In some embodiments, the disclosed multispecific antibodies can induce cytotoxicity against ROR1-positive cells, i.e., ROR1-expressing tumor cells, in the presence of a population of CD3-positive immune effector cells, a CD3-positive fraction of mononuclear cells, or a population of mononuclear cells comprising a CD3-positive fraction. In some embodiments, the disclosed multispecific antibodies can induce a greater cytotoxicity against ROR1-positive cells that have a higher expression level of ROR1 than against ROR1-negative cells or ROR1-positive cells that have a lower expression level of ROR1, when in the presence of a population of CD3-positive immune effector cells, a CD3-positive fraction of mononuclear cells, or a population of mononuclear cells comprising a CD3-positive fraction.

[0371] Further embodiments provide that in place of, or in addition to, having CD3-specificity, a multispecific antibody of the invention may have a specificity against another activation receptor and/or costimulatory receptor and/or co-activation receptor typically expressed on an immune cell, besides having a specificity against ROR1. For example, an activation receptor can be expressed on a T cell, such that the multispecific antibody may be a T cell engager that immunospecifically binds to the activation receptor on the T cell and activates the cytotoxicity or a cytokine expression property of the T cell. Alternatively, an activation receptor can be expressed on a natural killer (NK) cell, such that the multispecific antibody may be a NK cell engager that immunospecifically binds to the activation receptor on the NK cell and activates the cytotoxicity of the NK cell. Additionally, an activation receptor may be expressed on multiple immune cells such as T cells, NK cells and Dendritic cells (DC). Hence, the multispecific antibodies may be trifunctional immune cell engagers, or trispecific antibodies, which bind to two or more activation receptors (such as two or more of CD3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, NKp46, CD137 and CD40), thereby inducing activity of the immune cells, and also bind to ROR1-positive cells, thereby directing the immune cell activity towards the ROR-positive cells.

[0372] Exemplary activation receptors that can be immunospecifically bound by an antigen-binding site of the multispecific antibodies disclosed herein include, but are not limited to CD16, γ9 TCR, δ2 TCR or δ1 TCR, NKp46, CD137, CD40 or NKG2D. Exemplary antigen-binding fragments against said activation and/or costimulatory and/or co-activation receptors include scFv, VH, VL, VHH, Fab, etc., described in various publications including U.S. Pat. No. 9,035,026 and Gauthier et al., 2019, *Cell* 177, 1701–1713. Additional exemplary antigen-binding antibodies and/or fragments against said activation and/or costimulatory and/or co-activation receptors include those noted in Table 18. Of those listed as the antigen-binding antibodies, their VHH or Fab regions can be used as the antigen-binding site of the multispecific antibodies disclosed herein.

[0373] Additional embodiments provide ROR1-specific antibody-drug conjugates (ADCs), which comprises an anti-ROR1 antibody, or an ROR1-binding fragment thereof, disclosed herein, and a drug unit that is conjugated or bound to the anti-ROR1 antibody or ROR1-binding fragment thereof. Optionally, a linker unit is positioned between the anti-ROR1 antibody or ROR1-binding fragment thereof and the drug unit, and the linker unit may be an amino acid linker or a chemical moiety linker. Exemplary drug units can be cytotoxic agents such as antitumor drugs or chemotherapy agents, including small molecules as well as siRNA.

[0374] In additional embodiments, the multispecific antibody, multispecific antigen-binding fragment, or composition comprising the same may also contain a further therapeutic agent/therapy, as necessary for the particular disorder being treated. Preferably, the multispecific antibody or multispecific antigen-binding fragment thereof and the further therapeutic agent have complementary activities that do not adversely affect each other. In some embodiments, the further therapeutic agent/therapy is a chemotherapeutic agent or a radiation therapy. Combined administration of the disclosed multispecific antibodies or multispecific antigen-binding fragments thereof and the other therapeutic agent may be simultaneous, separate or sequential, in any order. For simultaneous administration, the agents may be administered as one composition or as separate compositions, as appropriate.

[0375] The dose of the multispecific antibody, multispecific antigen-binding fragment, or composition comprising the same depends on the desired effect, the duration of the treatment, and the route of administration used. For example, a therapeutically effective amount of a multispecific antibody disclosed herein includes one or more doses, wherein a dose is in the range of about 10-50 mg, 50-100 mg, 100-150 mg, 150-200 mg, 100-200 mg, 200-300 mg, 300-400 mg, 400-500 mg, 500-600 mg, 600-700 mg, 700-800 mg, 800-900 mg, 900-1000 mg, 1000-1100 mg, 1100-1200 mg, 1200-1300 mg, 1300-1400 mg, 1400-1500 mg, 1500-1600 mg, 1600-1700 mg, 1700-1800 mg, 1800-1900 mg, 1900-2000 mg, 2000-2100 mg, 2100-2200 mg, 2200-2300 mg, 2300-2400 mg, 2400-2500 mg, 2500-2600 mg, 2600-2700 mg, 2700-2800 mg, 2800-2900 mg or 2900-3000 mg. In another embodiment, a therapeutically effective amount of a multispecific antibody disclosed herein includes one or more doses, wherein a dose is in the range of 0.001-0.005 mg/kg, 0.005-0.01 mg/kg, 0.01-0.02 mg/kg, 0.02-0.04 mg/kg, 0.04-0.06 mg/kg, 0.06-0.08 mg/kg, 0.08-1 mg/kg, 1-5 mg/kg, 5-10 mg/kg, 10-15 mg/kg, 15-20 mg/kg, 20-25 mg/kg, 25-30 mg/kg, 30-35 mg/kg, 35-40 mg/kg, 40-45 mg/kg, 45-50 mg/kg, 10-50 mg/kg, 50-100 mg/kg, 100-150 mg/kg, 150-200 mg/kg, 100-200 mg/kg, 200-300 mg/kg, 300-400 mg/kg, 400-500 mg/kg, 500-600 mg/kg, 600-700 mg/kg, 700-800 mg/kg, 800-900 mg/kg, 900-1000 mg/kg, 1000-1100 mg/kg, 1100-1200 mg/kg, 1200-1300 mg/kg, 1300-1400 mg/kg, 1400-1500 mg/kg, 1500-1600 mg/kg, 1600-1700 mg/kg, 1700-1800 mg/kg, 1800-1900 mg/kg, 1900-2000 mg/kg, 2000-2100 mg/kg, 2100-2200 mg/kg, 2200-2300 mg/kg, 2300-2400 mg/kg, 2400-2500 mg/kg, 2500-2600 mg/kg, 2600-2700 mg/kg, 2700-2800 mg/kg, 2800-2900 mg/kg or 2900-3000 mg/kg. In some embodiments, a therapeutically effective amount includes two, three, or more doses administered on a daily, weekly, biweekly, monthly, quarterly, or yearly frequency; or continued when evaluation of the severity of the disease shows improvement, compared to before the last dose, until the disease is successfully treated or the subject shows no symptoms or signs of the disease.

[0376] Table 8. Amino acid sequences of exemplary fusion proteins. Polypeptide 1 comprises the 'hole' of the KiH Fc; Polypeptide 2 comprises the 'knob' of the KiH Fc; and Polypeptide 3 comprises the light chain. The VHH may be included in Polypeptide 1, polypeptide 2 and/or polypeptide 3 with a linker between them. In this table the anti-CD3 domain is on the N-terminal of the knob Fc or hole Fc.

	Polypeptide 1	Polypeptide 2	Polypeptide 3
FUSE-211	(SEQ ID NO:481)	(SEQ ID NO:478)	(SEQ ID NO:479)
FUSE-393	(SEQ ID NO:482)	(SEQ ID NO:478)	(SEQ ID NO:479)

FUSE-394	(SEQ ID NO:483)	(SEQ ID NO:478)	(SEQ ID NO:480)
FUSE-399	(SEQ ID NO:484)	(SEQ ID NO:478)	(SEQ ID NO:479)
FUSE-489	(SEQ ID NO:477)	(SEQ ID NO:485)	(SEQ ID NO:486)
FUSE-494	(SEQ ID NO:477)	(SEQ ID NO:487)	(SEQ ID NO:488)
FUSE-497	(SEQ ID NO:477)	(SEQ ID NO:489)	(SEQ ID NO:490)
FUSE-498	(SEQ ID NO:477)	(SEQ ID NO:491)	(SEQ ID NO:492)
FUSE-536	(SEQ ID NO:477)	(SEQ ID NO:493)	(SEQ ID NO:494)

Table 9. Polynucleotide sequences encoding the fusion proteins corresponding to those in Table 8.

	Encoding Polypeptide 1	**Encoding Polypeptide 2**	**Encoding Polypeptide 3**
FUSE-211	(SEQ ID NO:499)	(SEQ ID NO:496)	(SEQ ID NO:497)
FUSE-393	(SEQ ID NO:500)	(SEQ ID NO:496)	(SEQ ID NO:497)
FUSE-394	(SEQ ID NO:501)	(SEQ ID NO:496)	(SEQ ID NO:498)
FUSE-399	(SEQ ID NO:502)	(SEQ ID NO:496)	(SEQ ID NO:497)
FUSE-489	(SEQ ID NO:495)	(SEQ ID NO:503)	(SEQ ID NO:504)
FUSE-494	(SEQ ID NO:495)	(SEQ ID NO:505)	(SEQ ID NO:506)
FUSE-497	(SEQ ID NO:495)	(SEQ ID NO:507)	(SEQ ID NO:508)
FUSE-498	(SEQ ID NO:495)	(SEQ ID NO:509)	(SEQ ID NO:510)
FUSE-536	(SEQ ID NO:495)	(SEQ ID NO:511)	(SEQ ID NO:512)

Table 10. Amino acid sequences of exemplary VHH.

2A11	QVQLQESGGGSVPAGGSLRLSCAASGSTYSANCMGWFRQAPGKEREEVASMSIRSGRTYYSDSVKGRFTIS QDGSKNTLYLQLNSLKAEDTALYYCAAAYGGSRCVYNYRGQGTQVTVSS (SEQ ID NO:331)
5A1	QVQLQESGGGSVQAGGSLKLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTFS QDKVKNTVYLQMNTLKPDDTGMYYCAADVRPDGTTCHYNSGGQGTQVTVSS (SEQ ID NO:332)
5A1-H1	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKERELASISTGTGNTYYADSVKGRFTISR DNSKNTLYLQMNSLKAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS (SEQ ID NO:335)
5A1-H2	QVQLQESGGGLVQPGGSLRLSCTASGYTNRLKCMGWVRQAPGKEREEVATISTGTGNTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAMYYCAADVRPDGTTCHYNSGGQGTQVTVSS (SEQ ID NO:336)
5A1-H3	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVSTISTGTGNTYYADSVKGRFTIS QDKSKNTLYLRMNSLRAEDTALYYCAADVRPDGTTCHYNSGGQGTQVTVSS (SEQ ID NO:337)
5A1-H4	EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTIS RDNSRNTLYLQMKTLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTQVTVSS (SEQ ID NO:338)
5A1-H5	EVQLVESGGGLVQPGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTFS RDNSKNTLYLQMNSLRAEDTAVYYCAADVRPDGTTCHYNSGGQGTQVTVSS (SEQ ID NO:339)
2A11-CV	QVQLQESGGGSVPAGGSLRLSCAASGSTYSANvMGWFRQAPGKEREEVASMSIRSGRTYYSDSVKGRFTIS QDGSKNTLYLQLNSLKAEDTALYYCAAAYGGSRvVYNYRGQGTQVTVSS (SEQ ID NO:513)
5A1-CV	QVQLQESGGGSVQAGGSLKLSCTASGYTNRLKvMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTFS QDKVKNTVYLQMNTLKPDDTGMYYCAADVRPDGTTvHYNSGGQGTQVTVSS (SEQ ID NO:514)

Table 11. Polynucleotide sequences encoding the VHH shown in Table 10.

2A11	(SEQ ID NO:333)
5A1	(SEQ ID NO:334)
5A1-H1	(SEQ ID NO:515)

5A1-H2	(SEQ ID NO:516)
5A1-H3	(SEQ ID NO:517)
5A1-H4	(SEQ ID NO:518)
5A1-H5	(SEQ ID NO:519)
2A11-CV	(SEQ ID NO:520)
5A1-CV	(SEQ ID NO:521)

Table 12. Yield of expressions as demonstrated in Examples for some fusion proteins.

	Yield
FUSE-211	360 mg/L
FUSE-393	458 mg/L
FUSE-394	455 mg/L
FUSE-489	253 mg/L
FUSE-497	213 mg/L
FUSE-498	117 mg/L

Table 13. Melting temperature (Tm) of exemplary fusion proteins.

	Tm
FUSE-210	70.5°C
FUSE-211	70.4°C
FUSE-394	70.6°C

Table 14. Additional exemplary VHH sequences used in in accordance with various embodiments of the invention. All sequences are humanized sequences except for SEQ ID NO:7 and SEQ ID NO:8.

	Framework region	VHH
FUSE-112	camel	QVQLQESGGGSVPAGGSLRLSCAASGSTYSANCMGWFRQAPGKEREEVASMSIRSGRTYYSDSVKGRFTISQDGSKNTLYLQ LNSLKAEDTALYYCAAAYGGSRCVYNYRGQGTQVTVSS(SEQ ID NO:331)
FUSE-179	camel	QVQLQESGGGSVQAGGSLKLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTFSQDKVKNTVYLQ MNTLKPDDTGMYYCAADVRPDGTTCHYNSGGQGTQVTVSS(SEQ ID NO:332)
FUSE-488	camel	QVQLQESGGGSVQAGGSLKLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTFSQDKVKNTVYLQ MNTLKPDDTGMYYCAADVRPDGTTCHYNSGGQGTQVTVSS(SEQ ID NO:332)
FUSE-497	GenBank AWH66715	EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTISRDNSRNTLYLQ MKTLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTQVTVSS(SEQ ID NO:338)
FUSE-498	Fusion Abs	EVQLVESGGGLVQPGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTFSRDNSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSGGQGTQVTVSS(SEQ ID NO:339)
FUSE-524	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKERELASISTGTGNTYYADSVKGRFTISRDNSKNTLYLQM NSLKAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS(SEQ ID NO:335)
FUSE-525	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKERELvASISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS(SEQ ID NO:340)
FUSE-537	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGSTYSANCMGWFRQAPGKERELASMSIRSGRTYYADSVKGRFTISRDNSKNTLYLQM NSLKAEDTAVYYCAAAYGGSRCVYNYRGQGTLVTVSS(SEQ ID NO:341)
FUSE-559	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIASISTGTGNTYYADSVKGRFTISRDKSRNTLYLQ MNTLRAEDTAVYYCAADVRPHGTTCHYNSRGQGTQVTVSS(SEQ ID NO:342)
FUSE-560	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGSTYSANCMGWFRQAPGKERELASMSIRSGRTYYADSVKGRFTISRDSSKNTLYLQM NSLKAEDTAVYYCAAAYGGSRCVYNYRGQGTLVTVSS(SEQ ID NO:343)
FUSE-561	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGSTYSANCMGWFRQAPGKERELASMSIRSGRTYYADSVKGRFTISRDSSKNTLYLQM NSLRAEDTAVYYCAAAYGGSRCVYNYRGQGTLVTVSS(SEQ ID NO:344)
FUSE-562	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGSTYSANCMGWFRQAPGKERELASMSIRSGRTYYSDSVKGRFTISRDGSKNTLYLQM NSLKAEDTAVYYCAAAYGGSRCVYNYRGQGTLVTVSS(SEQ ID NO:345)
FUSE-563	Fusion Abs	EVQLLESGGGLVQPGGSLKLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTISQDRSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTLVTVSS(SEQ ID NO:346)
FUSE-569	Fusion Abs	QVQLVESGGGLVQPGGSLRLSCSASGYTNRLKCMGWFRQAPGKERELASISTGTGNTYYADSVKGRFTISRDNSKNTLYLQM NSLRADDTAVYYCAADVRPDGTTCHYNSWGQGTQVTVSS(SEQ ID NO:347)
FUSE-570	Fusion Abs	QVQLVESGGGLVQPGGSLRLSCSASGYTNRLKCMGWFRQAPGKEREEIATISSGTGNTYYADSVKGRFTFSRDNSKNTLYLQ MNSLRAEDTGMYYCAADVRPDGTTCHYNSGGQGTQVTVSS(SEQ ID NO:348)
FUSE-589	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGSTYSANCMGWFRQAPGKERELASMSIRSGRTYYADSVKGRFTISQDGSKNTLYLQM

		NSLKAEDTAVYYCAAAYGGSRCVYNYRGQGTLVTVSS (SEQ ID NO:349)
FUSE-590	GenBank AWH66715	EVQLLESGGGLVQRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIASISTGTGNTYYSDSVKGRFTISRDKSRNTLYLQ MKSLRAEDTAVYYCAADVRPRGTTCHYNSRGQGTQVTVSS (SEQ ID NO:350)
FUSE-591	GenBank AWH66715	EVQLLESGGGLVPRGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTISQDKSRNTLYLQ MNSLKAEDTAVYYCAADVRPKGTTCHYNKRGQGTQVTVSS (SEQ ID NO:351)
FUSE-592	GenBank AWH66715	EVQLLESGGGLVPRGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSRNTLYLQ LNSLRAEDTAVYYCAADVRPRGTTCHYNSRGQGTQVTVSS (SEQ ID NO:352)
FUSE-593	GenBank AWH66715	EVQLLESGGGLVQRGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEIASISTGTGNTYYSDSVKGRFTISRDKSRNTLYLQ LNSLKAEDTAVYYCAADVRPKGTTCHYNSRGQGTQVTVSS (SEQ ID NO:353)
FUSE-594	GenBank AWH66715	EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIASISTGTGNTYYADSVKGRFTISQDKSRNTLYLQ MNSLKAEDTAVYYCAADVRPRGTTCHYNSRGQGTQVTVSS (SEQ ID NO:354)
FUSE-595	GenBank AWH66715	EVQLLESGGGLVQRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTISRDKSRNTLYLQ MKSLKAEDTAVYYCAADVRPKGTTCHYNSRGQGTQVTVSS (SEQ ID NO:355)
FUSE-596	GenBank AWH66715	EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIASISTGTGNTYYSDSVKGRFTISRDKSRNTLYLK LKSLRAEDTAVYYCAADVRPDGTTCHYNSRGQGTQVTVSS (SEQ ID NO:356)
FUSE-597	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKERELASISTGTGNTYYADSVKGRFTISRDGSKNTLYLQM NSLRAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS (SEQ ID NO:357)
FUSE-598	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKERELASISTGTGNTYYADSVKGRFTISQDGSKNTLYLQM NSLRAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS (SEQ ID NO:358)
FUSE-654	GenBank AWH66715	EVQLLESGGGLVPRGGSLkLSCTASGYTNRLKCMGWFRQAPGKEREEIASISTGTGNTYYADSVKGRFTISQDKSRNTLYLQ MNSLKAEDTAVYYCAADVRPRGTTCHYNSRGQGTQVTVSS (SEQ ID NO:359)
FUSE-655	GenBank AWH66715	EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIASISTGTGNTYYADSVKGRFTISQDKSRNTLYLQ MsSLKAEDTAVYYCAADVRPRGTTCHYNSRGQGTQVTVSS (SEQ ID NO:360)
FUSE-661	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGSQRSANCMGWFRQAPGKDRELASMSIRSGRTYYADSVKGRFTISRDSSKNTLYLQM SSLKAEDTAVYYCAAAYGGSRCVYNYRGQGTLVTVSS (SEQ ID NO:361)
FUSE-662	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGVGYSANCMGWFRQAPGKDRELASMSIRSGRTYYADSVKGRFTISRDSSKNTLYLQM SSLKAEDTAVYYCAAAYGGSRCVYNYRGQGTLVTVSS (SEQ ID NO:362)
F22158101 h5A1-BI-001	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGFTCHYNSRGQGTLVTVSS (SEQ ID NO:363)
F22158102 h5A1-BI-002	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGTTCHYNKRGQGTLVTVSS (SEQ ID NO:364)
F22158103 h5A1-BI-003	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGTTCHYNKRGQGTLVTVSS (SEQ ID NO:365)
F22158104 h5A1-BI-004	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGTTCHYNKRGQGTLVTVSS (SEQ ID NO:366)
F22158105 h5A1-BI-005	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGFTCHYNKRGQGTLVTVSS (SEQ ID NO:367)

F2158106 h5A1-BI-006	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGRTCHYNSRGQGTLVTVSS(SEQ ID NO:368)
F2158107 h5A1-BI-007	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGRTCHYNSRGQGTLVTVSS(SEQ ID NO:369)
F2158108 h5A1-BI-008	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGRTCHYNSRGQGTLVTVSS(SEQ ID NO:370)
F2158109 h5A1-BI-009	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNSYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGRTCHYNKRGQGTLVTVSS(SEQ ID NO:371)
F2158110 h5A1-BI-010	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGRTCHYNKRGQGTLVTVSS(SEQ ID NO:372)
F2158111 h5A1-BI-011	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPFGTTCHYNSRGQGTLVTVSS(SEQ ID NO:373)
F2158112 h5A1-BI-012	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPFGRTCHYNSRGQGTLVTVSS(SEQ ID NO:374)
F2158113 h5A1-BI-013	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPFGTTCHYNKRGQGTLVTVSS(SEQ ID NO:375)
F2158114 h5A1-BI-014	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPFGRTCHYNSRGQGTLVTVSS(SEQ ID NO:376)
F2158115 h5A1-BI-015	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPFGTTCHYNKRGQGTLVTVSS(SEQ ID NO:377)
F2158116 h5A1-BI-016	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPHGTTCHYNSRGQGTLVTVSS(SEQ ID NO:378)
F2158117 h5A1-BI-017	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPHGTTCHYNSRGQGTLVTVSS(SEQ ID NO:379)
F2158118 h5A1-BI-018	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPHGRTCHYNSRGQGTLVTVSS(SEQ ID NO:380)
F2158119 h5A1-BI-019	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPHGRTCHYNSRGQGTLVTVSS(SEQ ID NO:381)
F2158120 h5A1-BI-020	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPHGTTCHYNKRGQGTLVTVSS(SEQ ID NO:382)
F2158121 h5A1-BI-021	GenBank AWH66715	EVQLLESGGGLVQPGGSLRLSCAASGYTNRIKCMGWFRQAPGKEREEVASISTGTGNAYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPHGTTCHYNKRGQGTLVTVSS(SEQ ID NO:383)
BI-HU9-HK h5A1	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKERELVASISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS(SEQ ID NO:340)
BI-HU9-HK-1 h5A1	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKEREEVASISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLKAEDTAVYYCAADVRPDGTTCHYNSRGQGTLVTVSS(SEQ ID NO:384)
BI-HU9-VH9	GenBank	EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTISRDNSRNTLYLQ

h5A1	AWH66715	MKTLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTQVTVSS(SEQ ID NO:338)
BI-HU9-VH9-1 h5A1	GenBank AWH66715	EVQLLESGGGLVPRGGSLRLSCTASGYTNRLKCMGWFRQAPGKEREEIATISTGTGNTYYADSVKGRFTISQDKSRNTLYLQ MKTLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTQVTVSS(SEQ ID NO:385)
BI-HU9-VHH11 h5A1 Fc	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGFTFRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:386)
BI-HU9-VHH12 h5A1 Fc	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:387)
BI-HU9-VHH13 h5A1	IGHV3-23	EVQLLESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:388)
BI-HU9-VHH21 h5A1	IGHV3-30	QVQLVESGGGVVQPGGSLRLSCAASGFTFRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:389)
BI-HU9-VHH22 h5A1	IGHV3-30	QVQLVESGGGVVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:390)
BI-HU9-VHH23 h5A1	IGHV3-30	QVQLVESGGGVVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:391)
BI-HU9-VHH31 h5A1	IGHV3-66	EVQLVESGGGLVQPGGSLRLSCAASGFTVRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:392)
BI-HU9-VHH32 h5A1	IGHV3-66	EVQLVESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:393)
BI-HU9-VHH33 h5A1	IGHV3-66	EVQLVESGGGLVQPGGSLRLSCAASGYTNRLKCMGWFRQAPGKGLEEVATISTGTGNTYYADSVKGRFTISQDKSKNTLYLQ MNSLRAEDTAVYYCAADVRPDGTTCHYNSWGQGTTVTVSS(SEQ ID NO:394)

Table 15A CDRs

	CDR1	**CDR2**	**CDR3**
FUSE-112	GSTYSANC (SEQ ID NO:325)	SIRSGR(SEQ ID NO:400)	AYGGSRCVYNY (SEQ ID NO:327)
FUSE-179	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-488	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-497	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-498	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-524	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-525	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)

FUSE-537	GSTYSANC(SEQ ID NO:325)	IRSGR(SEQ ID NO:402)	AYGGSRCVYNY(SEQ ID NO:327)
FUSE-559	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPHGTTCHYNS(SEQ ID NO:404)
FUSE-560	GSTYSANC(SEQ ID NO:325)	IRSGR(SEQ ID NO:402)	AYGGSRCVYNY(SEQ ID NO:327)
FUSE-561	GSTYSANC(SEQ ID NO:325)	IRSGR(SEQ ID NO:402)	AYGGSRCVYNY(SEQ ID NO:327)
FUSE-562	GSTYSANC(SEQ ID NO:325)	IRSGR(SEQ ID NO:402)	AYGGSRCVYNY(SEQ ID NO:327)
FUSE-563	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-569	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-570	GYTNRLKC(SEQ ID NO:328)	SSGTGN(SEQ ID NO:403)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-589	GSTYSANC(SEQ ID NO:325)	IRSGR(SEQ ID NO:402)	AYGGSRCVYNY(SEQ ID NO:327)
FUSE-590	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPRGTTCHYNS(SEQ ID NO:405)
FUSE-591	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPKGTTCHYNK(SEQ ID NO:406)
FUSE-592	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPRGTTCHYNS(SEQ ID NO:405)
FUSE-593	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPKGTTCHYNS(SEQ ID NO:407)
FUSE-594	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPRGTTCHYNS(SEQ ID NO:405)
FUSE-595	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPKGTTCHYNS(SEQ ID NO:407)
FUSE-596	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-597	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-598	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
FUSE-654	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPRGTTCHYNS(SEQ ID NO:405)
FUSE-655	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPRGTTCHYNS(SEQ ID NO:405)
FUSE-661	GSQRSANC(SEQ ID NO:395)	IRSGR(SEQ ID NO:402)	AYGGSRCVYNY(SEQ ID NO:327)
FUSE-662	GVGYSANC(SEQ ID NO:396)	IRSGR(SEQ ID NO:402)	AYGGSRCVYNY(SEQ ID NO:327)
F2158101 h5A1-BI-001	GYTNRIKC(SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS(SEQ ID NO:408)
F2158102 h5A1-BI-002	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNK(SEQ ID NO:409)
F2158103 h5A1-BI-003	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNK(SEQ ID NO:409)
F2158104 h5A1-BI-004	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNK(SEQ ID NO:409)
F2158105 h5A1-BI-005	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNK(SEQ ID NO:410)

F2158106 h5A1-BI-006	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPDGRTCHYNS(SEQ ID NO:411)
F2158107 h5A1-BI-007	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGRTCHYNS(SEQ ID NO:411)
F2158108 h5A1-BI-008	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPDGRTCHYNS(SEQ ID NO:411)
F2158109 h5A1-BI-009	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPDGRTCHYNK(SEQ ID NO:412)
F2158110 h5A1-BI-010	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGRTCHYNK(SEQ ID NO:412)
F2158111 h5A1-BI-011	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPFGTTCHYNS(SEQ ID NO:413)
F2158112 h5A1-BI-012	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPFGRTCHYNS(SEQ ID NO:414)
F2158113 h5A1-BI-013	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPFGTTCHYNK(SEQ ID NO:415)
F2158114 h5A1-BI-014	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPFGRTCHYNS(SEQ ID NO:414)
F2158115 h5A1-BI-015	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPFGTTCHYNK(SEQ ID NO:415)
F2158116 h5A1-BI-016	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPHGTTCHYNS(SEQ ID NO:404)
F2158117 h5A1-BI-017	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPHGTTCHYNS(SEQ ID NO:404)
F2158118 h5A1-BI-018	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPHGRTCHYNS(SEQ ID NO:416)
F2158119 h5A1-BI-019	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPHGRTCHYNS(SEQ ID NO:9416)
F2158120 h5A1-BI-020	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPHGTTCHYNK(SEQ ID NO:417)
F2158121 h5A1-BI-021	GYTNRIKC (SEQ ID NO:397)	STGTGN(SEQ ID NO:401)	DVRPHGTTCHYNK(SEQ ID NO:417)
BI-HU9-HK h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-HK-1 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VH9 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)

BI-HU9-VH9-1 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH11 h5A1 Fc	GFTFRLKC(SEQ ID NO:398)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH12 h5A1 Fc	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH13 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH21 h5A1	GFTFRLKC(SEQ ID NO:398)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH22 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH23 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH31 h5A1	GFTVRLKC(SEQ ID NO:399)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH32 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)
BI-HU9-VHH33 h5A1	GYTNRLKC(SEQ ID NO:328)	STGTGN(SEQ ID NO:401)	DVRPDGTTCHYNS (SEQ ID NO:330)

Table 15B. Framework regions and some alternative CDRs

	FW1	**CDR1**	**FW2**	**CDR2**	**FW3**	**CDR3**	**FW4**
FUSE -112	QVQLQESGGGSVP AGGSLRLSCAAS (SEQ ID NO:421)	GSTYSANC (SEQ ID NO:325)	MGWFRQAPGKERE EVAS (SEQ ID NO:435)	MSIRSGRTY (SEQ ID NO:326)	YSDSVKGRFTISQDG SKNTLYLQLNSLKAE DTALYYCAA (SEQ ID NO:442)	AYGGSRCVYNY (SEQ ID NO:327)	RGQGTQVTVSS (SEQ ID NO:468)
FUSE -179	QVQLQESGGGSVQ AGGSLKLSCTAS (SEQ ID NO:422)	GYTNRLKC (SEQ ID NO:328)	MGWFRQAPGKERE EIAT (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTFSQDK VKNTVYLQMNTLKPD DTGMYYCAA (SEQ ID NO:443)	DVRPDGTTCHYN S (SEQ ID NO:330)	GGQGTQVTVSS (SEQ ID NO:469)
FUSE -488	QVQLQESGGGSVQ AGGSLKLSCTAS (SEQ ID NO:422)	GYTNRLKC (SEQ ID NO:328)	MGWFRQAPGKERE EIAT (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTFSQDK VKNTVYLQMNTLKPD DTGMYYCAA (SEQ ID NO:443)	DVRPDGTTCHYN S (SEQ ID NO:330)	GGQGTQVTVSS (SEQ ID NO:469)

FUSE -497	EVQLLESGGGLVP RGGSLRLSC**T**AS (SEQ ID NO:423)	GYTNRLKC (SEQ ID NO:328)	MG**W**F**R**QAPGK**ERE** **EIA**T (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SRNTLYLQMKTLRAE DTAVYYCA**A** (SEQ ID NO:444)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGT**Q**VTVSS (SEQ ID NO:470)
FUSE -498	EVQLVESGGGLVQ PGGSLRLSC**T**AS (SEQ ID NO:424)	GYTNRLKC (SEQ ID NO:328)	MG**W**F**R**QAPGK**ERE** **EIAT** (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFT**I**SRDN **S**RNTLYLQMKTLRAE DTAVYYCA**A** (SEQ ID NO:444)	DVRPDGTTCHYN S (SEQ ID NO:330)	**G**GQGT**Q**VTVSS (SEQ ID NO:469)
FUSE -524	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MG**W**F**R**QAPGK**ERE** **LA**S (SEQ ID NO:437)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQ**M**NSL**K**AE DTAVYYCA**A** (SEQ ID NO:445)	DVRPDGTTCHYN S (SEQ ID NO:330)	**R**GQGTLVTVSS (SEQ ID NO:471)
FUSE -525	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MG**W**F**R**QAPGKERE **LVAS** (SEQ ID NO:438)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQ**M**NSL**K**AE DTAVYYCA**A** (SEQ ID NO:445)	DVRPDGTTCHYN S (SEQ ID NO:330)	**R**GQGTLVTVSS (SEQ ID NO:471)
FUSE -537	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GSTYSANC (SEQ ID NO:325)	MG**W**F**R**QAPGK**ERE** **LA**S (SEQ ID NO:437)	MSIRSGRTY (SEQ ID NO:326)	YADSVKGRFTISRDN SKNTLYLQ**M**NSL**K**AE DTAVYYCA**A** (SEQ ID NO:445)	AYGGSRCVYNY (SEQ ID NO:327)	**R**GQGTLVTVSS (SEQ ID NO:471)
FUSE -559	EVQLLESGGGLVQ PGGSLRLSC**T**AS (SEQ ID NO:426)	GYTNRLKC (SEQ ID NO:328)	MG**W**F**R**QAPGK**ERE** **EIAS** (SEQ ID NO:439)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRD**K** SRNTLYLQ**M**N**T**LRAE DTAVYYCA**A** (SEQ ID NO:446)	DVRPHGTTCHYN S (SEQ ID NO:404)	**R**GQGT**Q**VTVSS (SEQ ID NO:468)
FUSE -560	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GSTYSANC (SEQ ID NO:325)	MG**W**F**R**QAPGK**ERE** **LA**S (SEQ ID NO:437)	MSIRSGRTY (SEQ ID NO:326)	YADSVKGRFTISRD**S** SKNTLYLQ**M**NSL**K**AE DTAVYYCA**A** (SEQ ID NO:447)	AYGGSRCVYNY (SEQ ID NO:327)	**R**GQGTLVTVSS (SEQ ID NO:471)
FUSE -561	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GSTYSANC (SEQ ID NO:325)	MG**W**F**R**QAPGK**ERE** **LA**S (SEQ ID NO:437)	MSIRSGRTY (SEQ ID NO:326)	YADSVKGRFTISRD**S** SKNTLYLQ**M**NSLRAE DTAVYYCA**A** (SEQ ID NO:448)	AYGGSRCVYNY (SEQ ID NO:327)	**R**GQGTLVTVSS (SEQ ID NO:471)
FUSE -562	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GSTYSANC (SEQ ID NO:325)	MG**W**F**R**QAPGK**ERE** **LA**S (SEQ ID NO:437)	MSIRSGRTY (SEQ ID NO:326)	Y**S**DSVKGRFTISRD**G** SKNTLYLQ**M**NSL**K**AE DTAVYYCA**A** (SEQ ID NO:449)	AYGGSRCVYNY (SEQ ID NO:327)	**R**GQGTLVTVSS (SEQ ID NO:471)
FUSE -563	EVQLLESGGGLVQ PGGSL**K**LSC**T**AS	GYTNRLKC (SEQ ID	MG**W**F**R**QAPGK**ERE** **EIAT**	ISTGTGNTY (SEQ ID	YADSVKGRFTISR**QDR** SKNTLYLQ**M**NSLRAE	DVRPDGTTCHYN S (SEQ ID	WGQGTLVTVSS (SEQ ID

	(SEQ ID NO:427)	(SEQ ID NO:328)	(SEQ ID NO:436)	(SEQ ID NO:329)	DTAVYYCA**A** (SEQ ID NO:450)	(SEQ ID NO:330)	(SEQ ID NO:472)
FUSE -569	QVQLVESGGGLVQ PGGSLRLSCSAS (SEQ ID NO:428)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **LAS** (SEQ ID NO:437)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQ**M**NSLRA**D** DTAVYYCA**A** (SEQ ID NO:451)	DVRPDGTTCHYN S (SEQ ID NO:330)	**W**GQGT**Q**VTVSS (SEQ ID NO:470)
FUSE -570	QVQLVESGGGLVQ PGGSLRLSC**S**AS (SEQ ID NO:428)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EIAT** (SEQ ID NO:436)	ISSGTGNTY (SEQ ID NO:418)	YADSVKGRFT**F**SRDN SKNTLYLQ**M**NSLRAE DT**G**M**Y**YCA**A** (SEQ ID NO:452)	DVRPDGTTCHYN S (SEQ ID NO:330)	**G**GQGT**Q**VTVSS (SEQ ID NO:469)
FUSE -589	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GSTYSANC (SEQ ID NO:325)	M**G**W**F**RQAPGK**ER**E **LAS** (SEQ ID NO:437)	MSIRSGRTY (SEQ ID NO:326)	YADSVKGRFTIS**Q**D**G** SKNTLYLQ**M**NSL**K**AE DTAVYYCA**A** (SEQ ID NO:453)	AYGGSRCVYNY (SEQ ID NO:327)	**R**GQGTLVTVSS (SEQ ID NO:471)
FUSE -590	EVQLLESGGGLV**Q** RGGSLRLSC**T**AS (SEQ ID NO:429)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EIAS** (SEQ ID NO:439)	ISTGTGNTY (SEQ ID NO:329)	**Y**S**D**SVKGRFTISRD**K** SRNTLYLQ**M**K**S**LRAE DTAVYYCA**A** (SEQ ID NO:454)	DVRPRGTTCHYN S (SEQ ID NO:405)	**R**GQGT**Q**VTVSS (SEQ ID NO:468)
FUSE -591	EVQLLESGGGLVP RGGSLRLSCAAS (SEQ ID NO:430)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EIA**T (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SRNTLYLQ**M**N**S**LK**A**E DTAVYYCA**A** (SEQ ID NO:455)	DVRPRGTTCHYN S(SEQ ID NO:405)	**R**GQGT**Q**VTVSS (SEQ ID NO:468)
FUSE -592	EVQLLESGGGLVP RGGSLRLSCAAS (SEQ ID NO:430)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SRNTLYLQ**LNS**LRAE DTAVYYCA**A** (SEQ ID NO:564)	DVRPRGTTCHYN S (SEQ ID NO:405)	**R**GQGT**Q**VTVSS (SEQ ID NO:468)
FUSE -593	EVQLLESGGGLV**Q** RGGSLRLSCAAS (SEQ ID NO:431)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EIAS** (SEQ ID NO:439)	ISTGTGNTY (SEQ ID NO:329)	**Y**S**D**SVKGRFTISRD**K** SRNTLYLQ**LNS**LK**A**E DTAVYYCA**A** (SEQ ID NO:457)	DVRPKGTTCHYN S (SEQ ID NO:407)	**R**GQGT**Q**VTVSS (SEQ ID NO:468)
FUSE -594	EVQLLESGGGLVP RGGSLRLSC**T**AS (SEQ ID NO:423)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EIAS** (SEQ ID NO:439)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SRNTLYLQ**M**N**S**LK**A**E DTAVYYCA**A** (SEQ ID NO:455)	DVRPRGTTCHYN S (SEQ ID NO:405)	**R**GQGT**Q**VTVSS (SEQ ID NO:468)
FUSE -595	EVQLLESGGGLV**Q** RGGSLRLSC**T**AS (SEQ ID NO:429)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EIA**T (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRD**K** SRNTLYLQ**M**K**S**LK**A**E DTAVYYCA**A** (SEQ ID NO:458)	DVRPKGTTCHYN S (SEQ ID NO:407)	**R**GQGT**Q**VTVSS (SEQ ID NO:468)

FUSE-596	EVQLLESGGGLVP RGGSLRLSCTAS (SEQ ID NO:423)	GYTNRLKC (SEQ ID NO:328)	MGWFRQAPGKERE EIAS (SEQ ID NO:439)	ISTGTGNTY (SEQ ID NO:329)	YSDSVKGRFTISRDK SRNTLYLKLKSLRAE DTAVYYCAA (SEQ ID NO:459)	DVRPDGTTCHYN S (SEQ ID NO:330)	RGQGTQVTVSS (SEQ ID NO:468)
FUSE-597	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MGWFRQAPGKERE LAS (SEQ ID NO:437)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDG SKNTLYLQMNSLRAE DTAVYYCAA (SEQ ID NO:460)	DVRPDGTTCHYN S (SEQ ID NO:330)	RGQGTLVTVSS (SEQ ID NO:471)
FUSE-598	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MGWFRQAPGKERE LAS (SEQ ID NO:437)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDG SKNTLYLQMNSLRAE DTAVYYCAA (SEQ ID NO:461)	DVRPDGTTCHYN S (SEQ ID NO:330)	RGQGTLVTVSS (SEQ ID NO:471)
FUSE-654	EVQLLESGGGLVP RGGSLkLSCTAS (SEQ ID NO:432)	GYTNRLKC (SEQ ID NO:328)	MGWFRQAPGKERE EIAS (SEQ ID NO:439)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDK SRNTLYLQMNSLKAE DTAVYYCAA (SEQ ID NO:455)	DVRPRGTTCHYN S (SEQ ID NO:405)	RGQGTQVTVSS (SEQ ID NO:468)
FUSE-655	EVQLLESGGGLVP RGGSLRLSCTAS (SEQ ID NO:423)	GYTNRLKC (SEQ ID NO:328)	MGWFRQAPGKERE EIAS (SEQ ID NO:439)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDK SRNTLYLQMssLKAE DTAVYYCAA (SEQ ID NO:462)	DVRPRGTTCHYN S (SEQ ID NO:405)	RGQGTQVTVSS (SEQ ID NO:468)
FUSE-661	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GSQRSANC (SEQ ID NO:395)	MGWFRQAPGKDRE LAS (SEQ ID NO:440)	MSIRSGRTY (SEQ ID NO:326)	YADSVKGRFTISRDS SKNTLYLQMSSLKAE DTAVYYCAA (SEQ ID NO:463)	AYGGSRCVYNY (SEQ ID NO:327)	RGQGTLVTVSS (SEQ ID NO:471)
FUSE-662	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GVGYSANC (SEQ ID NO:396)	MGWFRQAPGKDRE LAS (SEQ ID NO:440)	MSIRSGRTY (SEQ ID NO:326)	YADSVKGRFTISRDS SKNTLYLQMSSLKAE DTAVYYCAA (SEQ ID NO:463)	AYGGSRCVYNY (SEQ ID NO:327)	RGQGTLVTVSS (SEQ ID NO:471)
F2215810 1 h5A1-BI-001	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	MGWFRQAPGKERE EVAS (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDK SKNTLYLQMNSLKAE DTAVYYCAA (SEQ ID NO:464)	DVRPDGFTCHYN S (SEQ ID NO:408)	RGQGTLVTVSS (SEQ ID NO:471)
F2215810 2 h5A1	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	MGWFRQAPGKERE EVAS (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDK SKNTLYLQMNSLKAE DTAVYYCAA (SEQ ID NO:464)	DVRPDGTTCHYN K (SEQ ID NO:409)	RGQGTLVTVSS (SEQ ID NO:471)

-BI-002							
F221 5810 3 h5A1 -BI-003	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGTTCHYN K (SEQ ID NO:409)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5810 4 h5A1 -BI-004	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGTTCHYN K (SEQ ID NO:409)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5810 5 h5A1 -BI-005	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGFTCHYN K (SEQ ID NO:410)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5810 6 h5A1 -BI-006	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGRTCHYN S (SEQ ID NO:411)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5810 7 h5A1 -BI-007	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGRTCHYN S (SEQ ID NO:411)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5810 8 h5A1 -BI-008	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGRTCHYN S (SEQ ID NO:411)	**R**GQGTLVTVSS (SEQ ID NO:471)

F221 5810 9 h5A1 -BI- 009	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNSY (SEQ ID NO:420)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGRTCHYN K (SEQ ID NO:412)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 0 h5A1 -BI- 010	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGRTCHYN K (SEQ ID NO:412)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 1 h5A1 -BI- 011	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPFGTTCHYN S (SEQ ID NO:413)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 2 h5A1 -BI- 012	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPFGRTCHYN S (SEQ ID NO:414)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 3 h5A1 -BI- 013	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPFGTTCHYN K (SEQ ID NO:415)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 4 h5A1 -BI- 014	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPFGRTCHYN S (SEQ ID NO:414)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811	EVQLLESGGGLVQ PGGSLRLSCAAS	GYTNRIKC (SEQ ID	M**G**W**F**RQAPGK**ER**E **EVAS**	ISTGTGNTY (SEQ ID	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E**	DVRPFGTTCHYN K	**R**GQGTLVTVSS (SEQ ID

5 h5A1 -BI-015	(SEQ ID NO:425)	NO:397)	(SEQ ID NO:435)	NO:329)	DTAVYYCA**A** (SEQ ID NO:464)	(SEQ ID NO:415)	NO:471)
F221 5811 6 h5A1 -BI-016	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPHGTTCHYN S (SEQ ID NO:404)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 7 h5A1 -BI-017	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPHGTTCHYN S (SEQ ID NO:404)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 8 h5A1 -BI-018	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPHGRTCHYN S (SEQ ID NO:416)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5811 9 h5A1 -BI-019	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPHGRTCHYN S (SEQ ID NO:416)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5812 0 h5A1 -BI-020	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPHGTTCHYN K (SEQ ID NO:417)	**R**GQGTLVTVSS (SEQ ID NO:471)
F221 5812 1 h5A1	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRIKC (SEQ ID NO:397)	M**G**W**F**RQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNAY (SEQ ID NO:419)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSL**K**A**E** DTAVYYCA**A** (SEQ ID NO:464)	DVRPHGTTCHYN K (SEQ ID NO:417)	**R**GQGTLVTVSS (SEQ ID NO:471)

-BI-021							
BI-HU9-HK h5A1	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MG**W**FRQAPGK**ER**E **LVAS** (SEQ ID NO:438)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQMNSLK**A**E DTAVYYCA**A** (SEQ ID NO:445)	DVRPDGTTCHYN S (SEQ ID NO:330)	**R**GQGTLVTVSS (SEQ ID NO:471)
BI-HU9-HK-1 h5A1	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MG**W**FRQAPGK**ER**E **EVAS** (SEQ ID NO:435)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDK SKNTLYLQMNSLK**A**E DTAVYYCA**A** (SEQ ID NO:464)	DVRPDGTTCHYN S (SEQ ID NO:330)	**R**GQGTLVTVSS (SEQ ID NO:471)
BI-HU9-VH9 h5A1	EVQLLESGGGLVP RGGSLRLSC**T**AS (SEQ ID NO:423)	GYTNRLKC (SEQ ID NO:328)	MG**W**FRQAPGK**ER**E **E**I**A**T (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SRNTLYLQMKTLRAE DTAVYYCA**A** (SEQ ID NO:444)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGT**Q**VTVSS (SEQ ID NO:470)
BI-HU9-VH9-1 h5A1	EVQLLESGGGLVP RGGSLRLSCTAS (SEQ ID NO:423)	GYTNRLKC (SEQ ID NO:328)	MG**W**FRQAPGK**ER**E **E**I**A**T (SEQ ID NO:436)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDK SRNTLYLQMKTLRAE DTAVYYCA**A** (SEQ ID NO:465)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGT**Q**VTVSS (SEQ ID NO:470)
BI-HU9-VHH1 1 h5A1 Fc	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GFTFRLKC (SEQ ID NO:398)	MG**W**FRQAPGKGLE **EVAT** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO:466)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)
BI-HU9-VHH1 2 h5A1 Fc	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MG**W**FRQAPGKGLE **EVAT** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO:466)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)
BI-HU9-VHH1 3 h5A1	EVQLLESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:425)	GYTNRLKC (SEQ ID NO:328)	MG**W**FRQAPGKGLE **EVAT** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISQDK SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO467)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)
BI-HU9-	QVQLVESGGGVVQ PGGSLRLSCAAS	GFTFRLKC (SEQ ID	MG**W**FRQAPGKGLE **EVAT**	ISTGTGNTY (SEQ ID	YADSVKGRFTISRDN SKNTLYLQMNSLRAE	DVRPDGTTCHYN S (SEQ ID	WGQGTTVTVSS (SEQ ID

VHH21 h5A1	(SEQ ID NO:433)	NO:398)	(SEQ ID NO:441)	NO:329)	DTAVYYCA**A** (SEQ ID NO:466)	NO:330)	NO:473)
BI-HU9-VHH22 h5A1	QVQLVESGGGVVQ PGGSLRLSCAAS (SEQ ID NO:433)	GYTNRLKC (SEQ ID NO:328)	M**GWF**RQAPGKGLE **E**VA**T** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO:466)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)
BI-HU9-VHH23 h5A1	QVQLVESGGGVVQ PGGSLRLSCAAS (SEQ ID NO:433)	GYTNRLKC (SEQ ID NO:328)	M**GWF**RQAPGKGLE **E**VA**T** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO:467)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)
BI-HU9-VHH31 h5A1	EVQLVESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:434)	GFTVRLKC (SEQ ID NO:399)	M**GWF**RQAPGKGLE **E**VA**T** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO:466)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)
BI-HU9-VHH32 h5A1	EVQLVESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:434)	GYTNRLKC (SEQ ID NO:328)	M**GWF**RQAPGKGLE **E**VA**T** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO:466)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)
BI-HU9-VHH33 h5A1	EVQLVESGGGLVQ PGGSLRLSCAAS (SEQ ID NO:434)	GYTNRLKC (SEQ ID NO:328)	M**GWF**RQAPGKGLE **E**VA**T** (SEQ ID NO:441)	ISTGTGNTY (SEQ ID NO:329)	YADSVKGRFTIS**Q**D**K** SKNTLYLQMNSLRAE DTAVYYCA**A** (SEQ ID NO:467)	DVRPDGTTCHYN S (SEQ ID NO:330)	WGQGTTVTVSS (SEQ ID NO:473)

Table 17A. Polypeptide 1 comprises an Fc domain; Polypeptide 2 comprises an Fc domain; and Polypeptide 3 comprises the light chain. The VHH antibody may be included in Polypeptide 1, polypeptide 2 and/or polypeptide 3, and can utilize a linker between them. In this table the anti-CD3 domain is on the N-terminal of the Fc. The "(VHH antibody)" can be selected from Table 14.

	Polypeptide 1	**Polypeptide 2**	**Polypeptide 3**
Mo	(VHH		

no cl on al Fc	antibody)EPKSADKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK (SEQ ID NO:522)		
Ac 13	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAM NWVRQAPGKGLEWVARIRSKYNNYATYYADSVKD RFTISRDDSKNTLYLQMNSLRAEDTAVYYCARHG NFGNSYVSWFAYWGQGTMVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHN RFTQKSLSLSPGK (SEQ ID NO:477)	**(VHH antibody)** EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:523)	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTT SNYANWVQQKPGQAPRGLIGGTNKRAPGTPA RFSGSLLGGKAALTLSGVQPEDEAEYYCALW YSNLWVFGGGTKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADS SPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECSGG GGSGGGSGGGGSGGGGS **(VHH antibody) (SEQ ID NO:524)**

Table 17B. Nucleotide sequences encoding the polypeptides in Table 17A.

	DNA1	DNA2	DNA3
M o n o c l o n a l F c a c 1 3	**(DNA encoding VHH antibody)** GAGCCCAAATCTGCTGACAAAACTCACACATGC CCACCGTGCCCAGCACCTGAACTCCTGGGGGGA CCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAG GACACCCTCATGATCTCCCGGACCCCCGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGAC CCTGAGGTCAAGTTCAACTGGTACGTGGACGGC GTGGAGGTGCATAATGCCAAGACAAAGCCGCGG GAGGAGCAGTACAACAGCACGTACCGTGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC AAAGCCCTCCCAGCCCCCATCGAGAAAACCATC TCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG		

	GTGTACACCCTGCCCCCATCCCGGGAGGAGATG		
	ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC		
	AAAGGCTTCTATCCCAGCGACATCGCCGTGGAG		
	TGGGAGAGCAATGGGCAGCCGGAGAACAACTAC		
	AAGACCACGCCTCCCGTGCTGGACTCCGACGGC		
	TCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC		
	AAGAGCAGGTGGCAGCAGGGAACGTCTTCTCA		
	TGCTCCGTGATGCATGAGGCTCTGCACAACCAC		
	TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT		
	AAA (SEQ ID NO:525)		
A	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGACTG	(DNA encoding VHH antibody)	CAGGCTGTGGTGACCCAGGAGCCCAGCCTGACC
C	GTGCAGCCTGGAGGAAGCCTGAGGCTGTCCTGC	GAGCCCAAATCTAGCGACAAAACTCACACATGC	GTGAGCCCCGGAGGAACAGTGACCCTGACCTGC
1	GCTGCTTCCGGATTTACATTTAGCACATATGCC	CCACCGTGCCCAGCACCTGAAGCCGCCGGGGGA	AGAAGCAGCACCGGCGCCGTGACAACCTCAAAC
3	ATGAACTGGGTGAGGCAGGCCCCTGGAAAAGGT	CCGTCAGTCTTCCTCTTCCCCCAAAACCCAAG	TACGCCAACTGGGTGCAGCAGAAACCTGGACAG
	CTGGAATGGGTGGCTAGGATTCGGTCCAAGTAC	GACACCCTCATGATCTCCCGGACCCCCGAGGTC	GCCCCCAGAGGACTGATCGGCGGAACCAACAAG
	AACAATTACGCCACCTACTATGCCGACAGTGTC	ACATGCGTGGTGGTGGACGTGAGCCACGAAGAC	AGAGCCCCCGGCACCCCCGCCAGATTCAGCGGA
	AAGGACAGGTTTACCATCTCCAGGGACGATTCA	CCTGAGGTCAAGTTCAACTGGTACGTGGACGGC	AGTCTGCTGGGCGGCAAAGCCGCCCTGACCCTG
	AAGAACACCCTGTACCTGCAGATGAACTCCCTG	GTGGAGGTGCATAATGCCAAGACAAAGCCGCGG	AGCGGAGTGCAGCCTGAGGATGAAGCCGAGTAC
	AGGGCCGAGGATACCGCTGTGTATTACTGTGCC	GAGGAGCAGTACAACAGCACGTACCGTGTGGTC	TACTGCGCTCTGTGGTACAGCAACCTGTGGGTG
	CGGCACGGAAATTTCGGGAACTCCTACGTGTCC	AGCGTCCTCACCGTCCTGCACCAGGACTGGCTG	TTCGGAGGAGGCACCAAGCTGACCGTGCTGGGT
	TGGTTCGCATACTGGGGCCAGGGAACAATGGTG	AATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC	CAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTC
	ACTGTGTCCTCCGCTAGCACCAAGGGCCCATCG	AAAGCCCTCCCAGCCCCCATCGAGAAAACCATC	CCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAG
	GTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC	TCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG	GCCACACTGGTGTGTCTCATAAGTGACTTCTAC
	TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC	GTGTACACCCTGCCCCCATCCCGGGAGGAGATG	CCGGGAGCCGTGACAGTGGCCTGGAAGGCAGAT
	AAGGACTACTTCCCCGAACCGGTGACGGTGTCG	ACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTC	AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACC
	TGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC	AAAGGCTTCTATCCCAGCGACATCGCCGTGGAG	ACACCCTCCAAACAAAGCAACAACAAGTACGCG
	ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC	TGGGAGAGCAATGGGCAGCCGGAGAACAACTAC	GCCAGCAGCTACCTGAGCCTGACGCCTGAGCAG
	TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC	AAGACCAGCGCCTCCCGTGCTGGACTCCGACGGC	TGGAAGTCCCACAGGAGCTACAGTTGCCAGGTC
	AGCAGCTTGGGCACCCAGACCTACATCTGCAAC	TCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC	ACGCATGAAGGGAGCACCGTGGAGAAGACAGTG
	GTGAATCACAAGCCCAGCAACACCAAGGTGGAC	AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA	GCCCCTACAGAATGTTCAGGTGGCGGAGGGTCT
	AAGAAAGTTGAGCCCAAATCTTGTGACAAAACT	TGCTCCGTGATGCATGAGGCTCTGCACAACCAC	GGTGGTGGAGGATCAGGGGGTGGAGGTTCAGGA
	CACACATGCCCACCGTGCCCAGCACCTGAAGCC	TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT	GGCGGGGGAAGT
	GCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCA	AAA (SEQ ID NO:526)	(DNA encoding VHH antibody) (SEQ ID NO:527)
	AAACCCAAGGACACCCTCATGATCTCCCGGACC		
	CCCGAGGTCACATGCGTGGTGGTGGACGTGAGC		
	CACGAAGACCCTGAGGTCAAGTTCAACTGGTAC		
	GTGGACGGCGTGGAGGTGCATAATGCCAAGACA		
	AAGCCGCGGGAGGAGCAGTACAACAGCACGTAC		

CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGCCTGAGC TGCGCGGTCAAAGGCTTCTATCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG AACAACTACAAGACCACGCCTCCCGTGCTGGAC TCCGACGGCTCCTTCTTCCTCGTCAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC GTCTTCTCATGCTCCGTGATGCATGAGGCTCTG CACAACAGATTTACGCAGAAGAGCCTCTCCCTG TCTCCGGGTAAA (SEQ ID NO:495)		

Table 18.

Name	Alternative names	Description
Anti-CD3		
muromonab	OKT3 (Ortho Kung T3)	First monoclonal murine antibody to become available for therapy in humans - mouse IgG2a is directed against the CD3 epsilon chain of the CD3/TCR complex
Teplizumab	hOKT3γ1 (Ala-Ala) and MGA031	CDRs granted onto human IgG backbone with two mutations (L234/A, L235A)
Otelixizumab	ChAglyCD3, TRX4, GSK2136525	chimeric and humanized IgG1 bears a single mutation in the γ1 Fc portion (N297/A) to avoid glycosylation and thus inhibit FcR binding
Visilizumab	Nuvion, HuM291	humanized IgG2 antibody rendered non mitogenic by two point mutations in its Fc region (V234/A, V237/A)
Foralumab	28F11-AE; NI-0401	entirely human anti-CD3 mAb whose Fc portion of this human IgG1 was mutated (L234/A, V235E) such that the mAb is non FcR binding in vitro and exhibits only minor cytokine release
Bispecific		
Blinatumomab	AMG 103	linked scFv(s) againt CD19 on tumor cells and CD3 on T cells; no Fc domain; Anti-CD3 comes from murine mAb: L2K-07; published sequences encoding scFv addressing CD3ε (diL2K, the de-immunized version of the mouse monoclonal antibody L2K)
flotetuzumab	MGD006	DART targeting CD3 and CD123
Cibisatamab		1 binding site for CD3e chain and 2 CEA binding sties for cancer cells
Catumaxomab		consists of mouse IgG2 and rat IgG2b and targets CD3 on T cells and EpCAM on tumor cells
Mosunetuzumab		CD20×CD3 T-cell engaging bsAb
All candidates shared the same CD3-specific single chain antibody derived from mouse monoclonal antibody L2K, which is distinct from monoclonal antibodies TR66 and OKT3. L2K was subjected to a procedure that identifies human T helper cell epitopes and removes by point mutations MHC class II anchor amino acids within potential epitopes (Jones et al., 2004). This resulted in "de-immunized" L2K (diL2K)		
(sequence encoding scFv addressing CD3e is disclosed in K. Brischwein et al. / Molecular Immunology 43 (2006) 1129–1143)		
TR66		monoclonal anti-human CD3 epsilon antibody
PIT565		Trispecific: CD19 X CD3 X CD2
Four CD3 mAb-based binders: OKT3, L2K, h38E4.v1 and TRX4		
OKT3		

TRX4		
h38E4.v1		Binds N-terminal portion of CD3e extracellular domain
L2K		L2K, for instance shares over 90% sequence identity with OKT3 and recognizes the same epitope on CD3ε but differs in affinity by about 100-fold
Anti-CD16		
3G8		monoclonal anti-human CD16
US9035026		
HRS-3/**A9**		A9 is anti-CD16 mouse IgG1/λ Mab
Humanization of anti-CD16 Fab		Protein Engineering, Design and Selection, Volume 22, Issue 3, March 2009, Pages 175–188. the CDRs of the anti-CD16 mouse IgG1/λ MAb A9 were grafted onto human Ig sequences (CDRs were transplanted onto a human κ light chain + Affinity maturation via radon mutation in the humanized VH and Vκ domains)
AFM13		Tetravalent bispecific CD30/CD16 (binds CD16A on NK cell surface, thus activating and recruiting NK cells to CD30+ tumor cell)
GTB-3550		Trispecific CD16/IL-15/CD33 (anti-CD16 nanobody, or 1st generation uses scFv anti-CD16)
IPH6501		Antibody-based tetraspecific against NKp46, CD16a, CD122 or IL-2R, and CD20
AFM13: anti-CD16A binder is variable domains from human scFv clone LSIV21		
Other human scFv anti-CD16A clones: LSIV14, LSIII49, LBIII6, LBIII5, LBIII3, 50NI		
Cite: Reusch et al., mAbs 6:3, 727-738, May/June 2014		
Binders for V gamma 9, V delta 2 gene, V delta 1 gene segments in TCR		
LAVA-051		Lava Therapeutics. bispecific gamma delta T cell engagers (bsTCEs): Vγ9Vδ2 T cell receptor occupancy of LAVA-051 increased with LAVA-051 dose increases and peripheral blood Vγ9Vδ2 T cells also expressed higher levels of activation markers after LAVA-051 dosing
		LAVA-051 consists of two humanized single domain antibodies (VHH) linked via a short five a.a. G-S linker, wherein one doamin antibody recognizes the Vδ2 chain of the Vγ9Vδ2 T cell receptor, and the other domain antibody is specific for CD1d.
LAVA-1207		Binds: the Vδ2 chain of Vγ9Vδ2-T cells and prostate specific membrane antigen (PSMA)
Bruin et al., Clinical Immunology, v. 169, 128-138, August 2016		set of Vγ9Vδ2-T cell specific VHH (or nanobody)

Clones: 5C1, 5D3, 5E3, 5G3, 5F5, 5C7, 5D7, 5E7, 5C8, 5B11, 6A1, 6C1, 6H1, 6E3, 6G3, 6H3, 6C4, 6E4, 6H4, 6F6		
US Pat. Nos. 10,501,540 & 11384145		
clone **7A5**: the variable regions of the Vg9gd T-cell–specific antibody (		Construct: Vg9 TCR x Her2/neu; that is, $(Her2)_2xV\gamma9$ bispecific antibody
GDX012	GammaDelta Therapeutics	allogeneic, non-engineered, variable delta 1 (Vδ1) gamma-delta (γδ) T cell therapy
See Pat. Appl. pub.: US20220403025; US20220290101;		
Anti-CD137 (4-1BB/TNFRSF9)		
AGEN2373		by Agenus Inc; mAb IgG1 ; **US Patent # 11,098,117 & 11,242,385**
utomilumab	PF-05082566	Humanized IgG2 mAb; binding site: cysteine-rich domain (CRD)s III and IV
Urelumab	BMS-663513	Human IgG4 mAb; binding site: CRD I; trial by BMS
CTX-471	by Compass Therapeutics	fully human IgG4 mAb
ADG106	by Adagene Suzhou	fully human ligand-blocking, agonistic IgG4
LVGN6051	by Lyvgen Biopharma/Merck Sharp & Dohme	IgG1, improved FcγRIIB binding
ATOR-1017	by Alligator Bioscience AB	IgG4
HOT-1030	by Shanghai Huabo Biopharmaceutical (Huaota)/Eutilex	IgG1, abrogated FcγRIIB binding
EU101	by Eutilex/Zhejiang Huahai Pharmaceutical	IgG
YH004	by Eucure (Beijing) Biopharma	IgG1
ADG206	by Adagene Suzhou	IgG1, enhanced IgG1 binding, 4–1BB binding sites are masked
PE0116	by Shanghai Hyamab Biotech	IgG4
FS120	by F-Star	OX40/CD137 mAb^2 dual agonist bispecific antibody

	Therapeutics Inc.	
cinrebafusp alfa	PRS-343	bispecific 4-1BB (Anticalin®) X HER2
RG7827	RO 7122290	FAP x 4-1BBL antibody fusion protein
INBRX-105	ES101	humanized, bispecific IgG1 targeting PD-L1 X 4-1BB
Acasunlimab	GEN 1046, BNT 311	Fc-silenced IgG1 bispecific PD-L1 X 4-1BB
MCLA-145		bispecific CD137 X PD-L1
Englumafusp alfa	RG6076, RO7227166	CD19 X CD137
Additional bi/multispecific in MAbs. 2023; 15(1): 2167189.		
Anti-CD28		
Theralizumab	TGN1412, CD28-SuperMAB,	CYTOKINE storm failed human safety trial; humanised monoclonal antibody directed against the human CD28
	> Renamed as TAB08	Trial for rheumatoid arthritis
Anti-DNAM-1 (CD226 or PTA1)		
LY3435151		agonistic anti-CD226; NCT04099277 by Eli Lilly; US Pat. # 11,440,959
Anti-NKp46 (NCR1, CD335)		
IPH6501		NKp46-Targeting Tetraspecific Antibody-Based Natural Killer Cell Engager Therapeutic Armed with a Non-Alpha IL-2 Variant (by Innate Pharma)
Trifunctional NKCE		Targeting NKp46, CD16, and a tumor antigen (Cell 177, 1701–1713, June 13, 2019); **US Pat # 10,519,234** (by Innate Pharma)
		Other Innate Pharma owned NKp46 binder patents: **11,001,629, 11,377,492, and 11,267,897**
Anti-NKp30		
	Compass Therapeutics	**US20200109195, US20200079867, particularly SEQ ID NOs: 14 and 18 in US20200109195 and US20200079867.**

Anti-NKG2D		
	NCT05213195 by Zhejiang Univ.	**US20200360437 by Asko (Suzhou)**
		NCT05213195, NCT05247957, NCT04324996, NCT04623944, NCT03415100
Anti-OX40 (CD134; TNFRSF4)		
INCAGN1949		human IgG1
		Anti-OX40 patents : US Patent # 11,472,883, 11,447,557, **11,359,028**, 11,332,536, **11,136,404**, 10,626,181, **10,259,882**
HFB301001		2nd gen. fullly human IgG1 OX40 agonist antibody by HiFiBio
GSK3174998		anti-OX40 mAb
INBRX 106		hexavalent OX40 agonist, active ingredient: hexavalent IgG antibody that targets the human OX40 receptor (TNFRSF4)
FS120	by F-Star Therapeutics Inc.	OX40/CD137 mAb^2 dual agonist bispecific antibody
Ivuxolimab	PF-04518600 or PF 8600	humanized agonistic IgG2 monoclonal antibodies against OX40
BGB-A445		does not block the OX40-OX40L interaction;
MOXR0916	by Genentech	humanized effector-competent agonist IgG1 mAb; completely blocked OX40 binding to OX40L
BMS-986178		fully human IgG1 agonist mAb
Anti-ICOS (CD278, AILIM, CVID1)		
Izuralimab	XmAb23104 by Xencor	bispecific PD1 X ICOS
MEDI-570	by Astrazeneca	
BMS-986226		
KY1044	by Kymab	fully human anti-ICOS IgG1
feladilimab	GSK3359609, INDUCE-1	humanized IgG4 anti-ICOS monoclonal

vopratelimab	JTX-2011 by Jounce Therapeutics	
Acazicolcept		ICOS X CD28
Anti-CD2		
Siplizumab	TCD601, MEDI-507, Hu-BTI-322	humanized monoclonal **anti-CD2** IgG1 (Humanized form of BTI-322 below)
PIT565	by Novartis	Trispecific CD19 X CD3 X CD2 (NCT05397496)
Alefacept		fusion protein composed of the first extracellular domain of LFA-3 (natural ligand of CD2) fused to the human IgG1 hinge, CH2, and CH3 domains
BTI-322	Lo-CD2a	rat anti-human CD2 IgG2b mAb
Anti-CD40L (CD154)		
ruplizumab	BG 9588, hu5c8	humanized IgG1k
Dazodalibep	HZN-4920 by	antagonistic
Dapirolizumab pegol	CDP7657	anti-CD40L antibody fragment with the Fc moiety substituted by polyethylene glycol
Tegoprubart	AT-1501	humanized mAb
Frexalimab	SAR441344	2nd gen. mAb engineered to lack the domain that activated FcgRIIA
Lu AG22515	APB-A1; by Lundbeck	differentiated anti-CD40L antibody
Letolizumab	BMS 986004	humanized mAb (a dimeric fusion protein composed of the C-terminus of the domain antibody (dAb) BMS2h-572-633 targeting CD40L linked to a modified Fc fragment of IgG1)
BIIB063		high affinity MAb, engineered to reduce Fc effector function and avoid half antibody formation in vivo (IgG4P
Anti-CD40 (TNFRSF5)		
CDX-1140		fully human IgG2 agonistic mAb
Sotigalimab	APX005M	humanized IgG1 agonistic mAb
2141-V11		human anti-CD40 antibody with modified Fc domain (IgG1), which may induce durable anti-tumor immunity
selicrelumab	RO7009789 by Roche, or CP-870893	human IgG2 agonistic mAb; CP-870,893

dalnicastobart	LVGN7409	humanized IgG1 mAb
Iscalimab	CFZ533	fully human IGg1 mAb
Dacetuzumab	SGN-40	humanized IgG1 form of S2C6 (a murine anti-human CD40)
BI 655064	by Boehringer Ingelheim	humanised, nondepleting, antagonistic anti-CD40
Bleselumab	ASKP1240	fully human IgG4 mAb
YH003		humanized agonistic CD40 IgG2 mAb
SL-172154	SIRPα-Fc-CD40L	Bifunctional CD47 X CD40
Giloralimab	ABBV-927	agonistic mAb
Lucatumumab	CHIR 12.12 or HCD122	fully human mAb
Mitazalimab	ADC-1013	agonistic human IgG1 mAb
GEN1042		agonistic bispecific CD40 X 4-1BB
FFP104	PG102	humanised
KPL-404		humanized IgG4 antibody engineered to bind CD40 without triggering Fc effector functions
Cifurtilimab	SEA-CD40	humanized IgG1 mAb
Ravagalimab	ABBV-323	antagonistic
ABBV-428		Bispecific CD40 X MSLN
BMS-986090	INX-189	a dimeric anti-human CD40 VH antagonist domain antibody formatted with a human IgG4 Fc
BSI-038		agonistic mAb
MEDI5083		by MedImmune, now Astrazeneca; hexameric CD40L-Fc fusion
RG6189		a FAP-targeted CD40 agonistic bispecific antibody
Inezetamab	AMG 994	an immunoglobulin G1-scFv_L-lambda2 dimer

IL-15 variants and IL-15 fusion proteins

[0377] Various embodiments of the present invention provide for IL-15 variants.

[0378] Various embodiments provide for an interleukin 15 (IL-15) variant comprising the sequence of Formula I:

X_1WVX_4VISDLKKIEDLIQSMHIX_{22}ATLYTESX_{30}VHPSCKVTAMX_{41}CFLX_{45}ELQX_{49}ISLX_{53}SGDASIHDTVX_{64} NL$X_{67}X_{68}$LANNSLSSNGX_{79}VTESGCKECEELEX_{93}KNIKEFLQSX_{103}VHIVX_{108}MFIX_{112}TS, wherein

X_1 is any amino acid,

X_4 is any amino acid,

X_{22} is any amino acid,

X_{30} is any amino acid,

X_{41} is any amino acid,

X_{45} is any amino acid,

X_{49} is any amino acid except V,

X_{53} is any amino acid except E,

X_{64} is any amino acid,

X_{67} is any amino acid,

X_{68} is any amino acid,

X_{79} is any amino acid,

X_{93} is any amino acid,

X_{103} is any amino acid,

X_{108} is any amino acid, and

X_{112} is any amino acid; (SEQ ID NO:533).

[0100] In various embodiments of the IL-15 variant, X_1 is N (SEQ ID NO:534), X_4 is N (SEQ ID NO:535), X_{22} is D (SEQ ID NO:536), X_{30} is D (SEQ ID NO:537), X_{41} is K (SEQ ID NO:538), X_{45} is L (SEQ ID NO:539), X_{64} is E (SEQ ID NO:540), X_{67} is I (SEQ ID NO:541), X_{68} is I (SEQ ID NO:542), X_{79} is N (SEQ ID NO:543), X_{93} is E (SEQ ID NO:544), X_{103} is F (SEQ ID NO:545), X_{108} is Q (SEQ ID NO:546), or X_{112} is N (SEQ ID NO:547),or any combination thereof. In various embodiments, any combination thereof is any two combinations thereof. In various embodiments, any combination thereof is any 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 combinations thereof.

[0101] In various embodiments of the IL-15 variant, X_1 is N (SEQ ID NO:534), X_4 is N (SEQ ID NO:535), X_{22} is D (SEQ ID NO:536), X_{30} is D (SEQ ID NO:537), X_{41} is K (SEQ ID NO:538), X_{45} is L (SEQ ID NO:539), X_{49} is R or K (SEQ ID NO:548), X_{53} is G, K, I or A (SEQ ID NO:549), X_{64} is E (SEQ ID NO:540), X_{67} is I (SEQ ID NO:541), X_{68} is I (SEQ ID NO:542), X_{79} is N (SEQ ID NO:543), X_{93} is E (SEQ ID NO:544), X_{103} is F (SEQ ID NO:545), X_{108} is Q (SEQ ID NO:546), or X_{112} is N (SEQ ID NO:547), or any combination thereof. In various embodiments, any combination thereof is any two combinations thereof. In various embodiments, any combination thereof is any 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 combinations thereof.

[0102] In various embodiments of the IL-15 variant, X_1 is N or G (SEQ ID NO:550), X_4 is N, K or L (SEQ ID NO:551), X_{22} is D or A (SEQ ID NO:552), X_{30} is D or N (SEQ ID NO:553), X_{41} is K or Q (SEQ ID NO:554), X_{45} is L or S (SEQ ID NO:555), X_{49} is R or K (SEQ ID NO:548), X_{53} is G, K, I, or A (SEQ ID NO:549), X_{64} is E or Q (SEQ ID NO:556), X_{67} is I or T (SEQ ID NO:557), X_{68} is I or S (SEQ ID NO:558), X_{79} is N or Y (SEQ ID NO:559), X_{93} is E or A (SEQ ID NO:560), X_{103} is F or L (SEQ ID NO:561), X_{108} is Q or T (SEQ ID NO:562), or X_{112} is N or R (SEQ ID NO:563), or any combination thereof. In various embodiments, any combination thereof is any two combinations thereof. In various embodiments, any combination thereof is any 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 combinations thereof.

[0379] In various embodiments of the IL-15 variant, X_{49} is R or K, and X_{53} is G, K, I or A; (SEQ ID NO:564).

[0380] In various embodiments of the IL-15 variant, X_{30} is N (SEQ ID NO:565).

[0381] In various embodiments of the IL-15 variant, X_{64} is Q (SEQ ID NO:566).

[0382] In various embodiments of the IL-15 variant, X_1 is G (SEQ ID NO:567).

[0383] In various embodiments, the IL-15 variant is a variant selected from Table 1A or Table 3A.

[0384] In various embodiments, the IL-15 variants are not IL-15 variants described in U.S. Patent Application Publ. No. US 2019/0263877, the variants which are herein incorporated by reference.

[0385] Also provided herein are fusion proteins comprising IL-15 variants and a scaffold polypeptide. Various embodiments of the invention provide for a fusion protein, comprising an IL-15 variant as described herein; and a scaffold polypeptide. In various embodiments, the fusion protein comprises two or more IL-15 variants as described herein; and a scaffold polypeptide. In various embodiments, the two or more IL-15 variants can be 3, 4, 5 or 6 IL-15 variants. In various embodiments, the two or more IL-15 variants can be up to 8 IL-15 variants.

[0386] In various embodiments, the fusion protein further comprises one or more immune checkpoint-targeting fragment.

[0387] In various embodiments, the immune checkpoints include but are not limited to PD-1, PD-L1, CTLA-4, LAG-3. In various embodiments, one or more immune checkpoint-targeting fragment comprises immune checkpoint-targeting fragments of known antibodies. Examples of anti-PD1 fragments include fragments (E.g. Fab, Fv) from pembrolizumab, nivolumab, pidilizumab, AMP-224, AMP-514, spartalizumab, cemiplimab, penpulimab (AK105), prolgolimab (BCD-100), ezabenlimab (BI 754091), toripalimab (JS001), lipustobart (LZM009), retifanlimab (MGA012), Sym021, dostarlimab (TSR-042), tebotelimab (MGD013), cadonilimab (AK104), vudalimab (XmAb20717), tislelizumab, or PF-06801591. Additional examples of anti-PD1 fragments also include fragments (e.g., Fab, Fv) from vopratelimab, camrelizumab, sintilimab, AMP-224, AMP-514, and Acrixolimab. Examples of anti-PD-L1 antibodies include but are not limited to garivulimab (BGB-A333), cosibelimab (CK-301), FAZ053, envafolimab (KN035), MDX-1105, betifisolimab (MSB2311), adebrelimab (SHR-1316), atezolizumab, avelumab, durvalumab,

BMS-936559, CK-301, and M7824. Thus, for example, the fusion protein comprises an anti-PD1 fragment from an anti-PD1 antibody (e.g., Fab, Fv), and an IL-15 variant, and optionally, a linker.

[0388] In various embodiments, the IL-15 variant is fused to the C-terminus of the scaffold polypeptide. In other embodiments, the IL-15 variant is fused to the N-terminus of the scaffold polypeptide.

[0389] In still other embodiments, wherein the scaffold polypeptide is an antibody, the IL-15-variant is fused to any one of the C-terminus or N-terminus of the heavy chain or light chain, or fused to any one of the C_H2 domain, or hinge region (e.g., between the C_H2 and C_H1 domains).

[0390] In various embodiments, the scaffold polypeptide is an antibody or a fragment thereof. In various embodiments, the antibody is an IgA, IgM, IgG, or IgE antibody.

[0391] In various embodiments, the antibody is an anti-PD-1 antibody or anti-PD-L1 antibody. Examples of anti-PD-1 antibodies include but are not limited to pembrolizumab, nivolumab, pidilizumab, AMP-224, AMP-514, spartalizumab, cemiplimab, penpulimab (AK105), prolgolimab (BCD-100), ezabenlimab (BI 754091), toripalimab (JS001), lipustobart (LZM009), retifanlimab (MGA012), Sym021, dostarlimab (TSR-042), tebotelimab (MGD013), cadonilimab (AK104), vudalimab (XmAb20717), tislelizumab, PF-06801591, and anti-PD1 antibody expressing pluripotent killer T lymphocytes (PIK-PD-1). Examples of anti-PD-L1 antibodies include but are not limited to garivulimab (BGB-A333), cosibelimab (CK-301), FAZ053, envafolimab (KN035), MDX-1105, betifisolimab (MSB2311), adebrelimab (SHR-1316), atezolizumab, avelumab, durvalumab, BMS-936559, CK-301, and M7824.

[0392] In various embodiments, the scaffold polypeptide is a Fc region or a fragment thereof. In various embodiments, the scaffold polypeptide is a Fc region or a fragment thereof and the scaffold polypeptide does not comprise an Fab.

[0393] In various embodiments, two arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can be heterodimerized by creating "knobs-in-holes" (KiH) mutations in the CH3 domain. This structural feature in the polypeptide arms allows for assembly of two half antibodies (e.g., Fc heterodimer; and VH–CH and VL–CL domains). For example, a heteromultimer (including a heterodimer) may comprise two polypeptides, each comprising a CH3 domain, wherein the polypeptides meet at an engineered interface within the CH3 domain, and the one polypeptide contains an engineered protuberance ("knob") in the interface with at least one contact residue replaced with an import residue having a larger side chain volume than the original residue, and another polypeptide contains an engineered cavity ("hole") in the interface with at least one contact residue replaced with an import residue having a smaller side chain volume than the original residue. In some embodiments, the engineered interface of a heteromultimer includes at least two protuberance-into-cavity mutant pairs. Volumes and accessible surface areas of each amino acid are described in A. A. Zamyatnin, *Prog. Biophys. Mol. Biol.* 24: 107-123, 1972 and C. Chothia, *J. Mol. Biol.* 105: 1-14, 1975. For example, import residues for the formation of a protuberance can be arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W); and preferably the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine. As another example, import

residues for the formation of a cavity can be alanine (A), serine (S), threonine (T) and valine (V); and preferably the original residue for the formation of the cavity has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. For example, a T366W mutation in CH3 domain for the "knob"/protuberance chain, and a T366S/L368A/Y407V mutation in CH3 domain for the "hole"/cavity chain. Additionally, the KiH configuration may be coupled further mutations to permit S-S disulfide linkage between the two chains. In various aspects wherein the protein/polypeptide is a heterodimer of a the KiH configuration, the IL-18 (or its fragment, variant, or a fragment of its variant) is linked to one, and only one, of the two (or more) immunoglobulin heavy chain constant regions/chains (i.e. knob or hole).

[0394] As such, in some embodiments, the Fc region is a knobs-in-hole (KiH) Fc.

[0395] In various embodiments, the IL-15 variant is fused to the knob of the KiH Fc. In various embodiments, the IL-15 variant is fused to the hole of the KiH Fc.

[0396] In various embodiments, the Fc region is an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG1. In various embodiments, Fc region can be a human IgG1, IgG2, or IgG4.

[0397] In some embodiments, the two or more arms (or chains) of immunoglobulin heavy chain constant regions (e.g., Fc polypeptides) can contain another symmetric-to-asymmetric steric complementarity design (e.g., HA-TF, ZW1), a charge-to-charge swap interaction (DD-KK), a charge-to-steric complementarity swap plus additional long-range electrostatic interaction (e.g., EW-RVT), or an isotype strand swap design (e.g., strand-exchange engineered domain (SEED)), or Xmab, 7.8.60, Electrostatic Steering, A107, or Duobody, so as to form heterodimeris/heteromultimers. Further description of these configurations and exemplary mutations/residues are seen in *Front Immunol.* 2016; 7: 394.

[0398] In various embodiments, the scaffold polypeptide of the fusion protein comprises a globular protein, human serum albumin (HSA), beta2microglobulin, transferrin, fragment antigen-binding region (Fab region), VHH antibody, single-chain variable fragment (scFv), anticalin, designed ankyrin repeat protein (DARPin), a binding domain thereof, or a fragment thereof.

[0399] In various embodiments, one or more polypeptides can be inserted between the IL-15 variant and the antibody of the IL-15 fusion proteins. In various embodiments, the polypeptide can be inserted or conjugated at the N-terminus, at the C-terminus, or both the N-terminus and C- terminus of the antibody. In various embodiments, the polypeptide comprises a polypeptide linker conjugating the IL-15 variant and the antibody.

[0400] In various embodiments, one or more polypeptides can be inserted between the IL-15 variant and the Fc region of the IL-15 fusion proteins. In various embodiments, the polypeptide can be inserted or conjugated at the N-terminus, at the C-terminus, or both the N-terminus and C- terminus of the Fc region. In various embodiments, the polypeptide comprises a polypeptide linker conjugating the IL- 5 variant and the Fc region. In these embodiments, the fusion protein does not include Fab region of an antibody.

[0401] The antibodies that can be fused to IL-15 can include monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, and single chain (ScFv). The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies). Preferably, the antibodies are human or humanized antibodies.

[0402] In various embodiments, the IL-15 fusion proteins are IL-15 fusion proteins listed in Table 1A. That is, an IL-15 fusion protein comprising polypeptide 1, polypeptide 2, and polypeptide 3.

[0403] In various embodiments, the IL-15 fusion protein comprises an IL-15 variant listed in Table 1A or Table 3A.

[0404] In various embodiments, the IL-15 fusion protein comprises polypeptide 1, polypeptide 2, and polypeptide 3 listed in Table 1A, except the linker in polypeptide 2 is a different peptide linker. For example, the linker can be a flexible linker that is generally about 10 to 25 amino acids in length. Additional examples of linkers include but are not limited to a dimer of two amino acids, a tri-mer of three amino acids, or a peptide selected from the group consisting of T, PT, MPT, S, GS, GGS, GGGS (SEQ ID NO:235), (GGGGX_λ (SEQ ID NO:236))n, and (X_λGGGG (SEQ ID NO:317))n wherein X_λ is Q, A, E or S and n=1-5 or an integer larger than 5. In some embodiments, the amino acid linker has the amino acid sequence of (GGGGS (SEQ ID NO:237))n where n is an integer between 1 and 5, thereby an amino acid linker of 25 amino acids or shorter in length.

[0405] Provided herein are methods of producing the IL-15 variants and IL-15 fusion proteins.

[0406] Various embodiments provide for a polynucleotide encoding an IL-15 variant of the present invention described herein. For example, the nucleic acid sequences may encode in a 5' to 3' direction, the IL-15 variant or the IL-15 fusion protein.

[0407] Various embodiments provide for a polynucleotide encoding an IL-15 fusion protein of the present invention described herein. For example, the nucleic acid sequences may encode in a 5' to 3' direction, the IL-15 variant.

[0408] As such, the polynucleotide encoding an IL-15 fusion protein of the present invention comprises polynucleotide 1, polynucleotide 2 and polynucleotide 3 listed in Table 2A. In other embodiments, the polynucleotide encoding an IL-15 fusion protein of the present invention comprises polynucleotide 1, polynucleotide 2 and polynucleotide 3 listed in Table 2A, except the nucleotides encoding the linker encodes a different linker.

[0409] For example, the linker can be a flexible linker that is generally about 10 to 25 amino acids in length. Additional examples of linkers include but are not limited to a dimer of two amino acids, a tri-mer of three amino acids, or a peptide selected from the group consisting of T, PT, MPT, S, GS, GGS, GGGS (SEQ ID NO:235), (GGGGX_λ (SEQ ID NO:236))n, and (X_λGGGG (SEQ ID NO:317))n wherein X_λ is Q, A, E or S and n=1-5 or an integer larger than 5. In some embodiments, the amino acid linker has the amino acid sequence of (GGGGS (SEQ ID NO:237))n

where n is an integer between 1 and 5, thereby an amino acid linker of 25 amino acids or shorter in length. As such, the nucleotides encoding the linker in polynucleotide 2 will encode one of these aforementioned linkers instead.

[0410] Exemplary polynucleotides are in Table 2A.

[0411] Various embodiments also provide for an expression vector comprising any one of the polynucleotides described herein. Thus, the polypeptide encoding the IL-15 variant or IL-15 fusion proteins may also be integrated within a replicable expression vector. Hence, a vector encoding the fusion protein is also provided, which may express the fusion protein in, for example, a bacterial host, an intended recipient, or both.

[0412] Additional embodiments provide cells transformed or transfected with one or more nucleic acid molecules encoding the IL-15 variant or IL-15 fusion protein. The cell can be a prokaryotic cell. Or the cell is a eukaryotic cell, preferably a mammalian cell, and more preferably a human cell.

[0413] In various embodiments, the cell is a mammalian cell. Mammalian cells that are useful include but are not limited to Chinese Hamster Ovary (CHO) cells or HEK-293 cells.

[0414] In various embodiments, the cell is a bacterial cell or a yeast cell.

[0415] Various embodiments provide for a method of producing an IL-15 variant, comprising: culturing any one of the cells described herein, in cell culture medium to allow the Il-15 variant to be produced, and optionally secreted into the cell culture medium.

[0416] In various embodiments, the method further comprises isolating the IL-15 variant from the cell or from the cell culture medium. In various embodiments, the method further comprises purifying the IL-15 variant.

[0417] Various embodiments provide for a method of producing an IL-15 fusion protein, comprising: culturing any one of the cells described herein, in cell culture medium to allow the Il-15 fusion protein to be produced, and optionally secreted into the cell culture medium.

[0418] In various embodiments, the method further comprises isolating the IL-15 fusion protein from the cell or from the cell culture medium. In various embodiments, the method further comprises purifying the IL-15 fusion protein.

[0419] In some embodiments, using a Chinese hamster ovary (CHO) expression system, the IL-15 variant or fusion protein is produced via a process including the steps of: (1) cell recovery, which may be to recover frozen CHO cells via water bath at 37°C; (2) cell subculturing, which may be to sub-culture the cells and adjust the cell density to 6×10^6/ml for transfection; (3) transfection and expression, using a solution 1 (in which a plasmid is diluted with a diluting agent), a solution 2 (in which a transection reagent is diluted with a/the diluting agent), and then mixing the solution 1, the solution 2 and the CHO cells, followed by incubating the mixture at a shaker for expression for 12-14 days at 32°C to collect the supernatant of the culture after centrifuge.

[0420] In some embodiments, a purification process is performed after the expression of the fusion protein. In some embodiments, a purification process includes the steps of: (1) washing a column with a binding buffer (10 times volume) at a flow rate of 1 Ml/min; (2) loading a fusion-protein-containing sample into the column at a flow rate of 1 Ml/min; (3) washing the column with 10x volumes of PBS buffer with a flow rate of 1 Ml/min; (4) eluting the protein from the column with 40 Mm sodium citrate (Ph3.4); optionally the elution sample may be collected into tubes (1ml/min) and measured for optical density (OD) using NanoDrop at 280 nm; and (5) performing dialysis, e.g., against PBS buffer in a dialysis bag overnight.

[0421] Various embodiments of the invention provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering an IL-15 variant of the present invention as described herein, to a subject in need thereof.

[0422] Various embodiments of the invention provide for a method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering an IL-15 fusion protein of the present invention as described herein, to a subject in need thereof.

[0423] In various embodiments, the subject has cancer.

[0424] Various embodiments of the invention provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering an IL-15 variant of the invention as described herein to a subject in need thereof.

[0425] Various embodiments of the invention provide for a method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering an IL-15 fusion protein of the invention as described herein to a subject in need thereof.

[0426] In various embodiments, the disease or condition is cancer.

Pharmaceutical compositions

[0427] In various embodiments, the present invention provides pharmaceutical compositions including a pharmaceutically acceptable excipient along with a therapeutically effective amount of the fusion protein. "Pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

[0428] In certain embodiments, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts, esters, amides, and prodrugs" as used herein refers to those carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the compounds of the present invention

which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use of the compounds of the invention. The term "salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared in situ during the final isolation and purification of the compounds or by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. These may include cations based on the alkali and alkaline earth metals such as sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations including, but not limited to ammonium, tetramethylanunonium, tetraethyl ammonium, methyl amine, dimethyl amine, trimethylamine, triethylamine, ethylamine, and the like (see, e.g., Berge S. M., et al. (1977) J. Pharm. Sci. 66, 1, which is incorporated herein by reference).

[0429] The term "pharmaceutically acceptable esters" refers to the relatively nontoxic, esterified products of the compounds of the present invention. These esters can be prepared in situ during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters via treatment with an alcohol in the presence of a catalyst. The term is further intended to include lower hydrocarbon groups capable of being solvated under physiological conditions, e.g., alkyl esters, methyl, ethyl and propyl esters.

[0430] As used herein, "pharmaceutically acceptable salts or prodrugs" are salts or prodrugs that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subject without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use.

[0431] The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the functionally active one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof. A thorough discussion is provided in T. Higachi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A. C. S. Symposium Series, and in Bioreversible Carriers in: Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference. As used herein, a prodrug is a compound that, upon in vivo administration, is metabolized or otherwise converted to the biologically, pharmaceutically or therapeutically active form of the compound. A prodrug of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof can be designed to alter the metabolic stability or the transport characteristics of one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, to mask side effects or toxicity, to improve the flavor of a compound or to alter other characteristics or properties of a compound. By virtue of knowledge of pharmacodynamic processes and drug metabolism in vivo, once a pharmaceutically active form of the one or more peptides as disclosed herein or a mutant, variant, analog or derivative thereof, those of skill in the pharmaceutical art generally can design prodrugs of the compound (see, e.g., Nogrady (1985) Medicinal Chemistry A Biochemical

Approach, Oxford University Press, N. Y., pages 388-392). Conventional procedures for the selection and preparation of suitable prodrugs are described, for example, in "Design of Prodrugs," ed. H. Bundgaard, Elsevier, 1985. Suitable examples of prodrugs include methyl, ethyl and glycerol esters of the corresponding acid.

[0432] In various embodiments, the pharmaceutical compositions according to the invention may be formulated for delivery via any route of administration. "Route of administration" may refer to any administration pathway known in the art, including but not limited to aerosol, nasal, oral, transmucosal, transdermal or parenteral.

[0433] "Transdermal" administration may be accomplished using a topical cream or ointment or by means of a transdermal patch.

[0434] "Parenteral" refers to a route of administration that is generally associated with injection, including intraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. Via the parenteral route, the compositions may be in the form of solutions or suspensions for infusion or for injection, or as lyophilized powders.

[0435] Via the enteral route, the pharmaceutical compositions can be in the form of tablets, gel capsules, sugar-coated tablets, syrups, suspensions, solutions, powders, granules, emulsions, microspheres or nanospheres or lipid vesicles or polymer vesicles allowing controlled release. Via the parenteral route, the compositions may be in the form of solutions or suspensions for infusion or for injection.

[0436] Via the topical route, the pharmaceutical compositions based on compounds according to the invention may be formulated for treating the skin and mucous membranes and are in the form of ointments, creams, milks, salves, powders, impregnated pads, solutions, gels, sprays, lotions or suspensions. They can also be in the form of microspheres or nanospheres or lipid vesicles or polymer vesicles or polymer patches and hydrogels allowing controlled release. These topical-route compositions can be either in anhydrous form or in aqueous form depending on the clinical indication.

[0437] Via the ocular route, they may be in the form of eye drops.

[0438] The pharmaceutical compositions according to the invention can also contain any pharmaceutically acceptable carrier. "Pharmaceutically acceptable carrier" as used herein refers to a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or a combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It must also be suitable for use in contact with any tissues or organs with which it may come in contact, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

[0439] The pharmaceutical compositions according to the invention can also be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin, saline, alcohols and water. Solid carriers include starch, lactose, calcium sulfate, dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax.

[0440] The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

[0441] The pharmaceutical compositions according to the invention may be delivered in a therapeutically effective amount. The precise therapeutically effective amount is that amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, for instance, by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. For additional guidance, see *Remington: The Science and Practice of Pharmacy* (Gennaro ed. 20th edition, Williams & Wilkins PA, USA) (2000).

Kits

[0442] The present invention is also directed to a kit to treat various diseases. The kit is an assemblage of materials or components, including at least one of the inventive compositions. Thus, in some embodiments the kit contains a composition including fusion proteins, as described above.

[0443] The exact nature of the components configured in the inventive kit depends on its intended purpose. For example, some embodiments are configured for the purposes of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells. Some embodiments are configured for the purpose of treating cancer. In one embodiment, the kit is configured particularly for the purpose of treating mammalian subjects. In another embodiment, the kit is configured particularly for the purpose of treating human subjects. In further embodiments, the kit is configured for veterinary applications, treating subjects such as, but not limited to, farm animals, domestic animals, and laboratory animals.

[0444] Instructions for use may be included in the kit. "Instructions for use" typically include a tangible expression describing the technique to be employed in using the components of the kit to effect a desired outcome, such as to activate, promote differentiation or promote expansion of T cells, B cells or natural killer (NK) cells, to treat diseases, including cancer. Optionally, the kit also contains other useful components, such as, diluents, buffers, pharmaceutically acceptable carriers, syringes, catheters, applicators, pipetting or measuring tools, bandaging materials or other useful paraphernalia as will be readily recognized by those of skill in the art.

[0445] The materials or components assembled in the kit can be provided to the practitioner stored in any convenient and suitable ways that preserve their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures. The components are typically contained in suitable packaging material(s). As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as inventive compositions and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. As used herein, the term "package" refers to a suitable solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding the individual kit components. Thus, for example, a package can be a glass vial used to contain suitable quantities of an inventive composition containing IL-15 variants or fusion proteins. The packaging material generally has an external label which indicates the contents and/or purpose of the kit and/or its components.

EXAMPLES

[0446] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

EXAMPLES

IL15/IL18 VARIANT EXAMPLES

Example 1

[0447] As shown in figure 2, we assessed the impact of an exemplary anti-PD1-pro-IL-18 antibody, one of three exemplary anti-PD1-mutant-IL-15 antibodies or the combination of the anti-PD1-pro-IL-18 antibody and of each of the three mutant IL-15 antibody fusions on IFNγ release mediated by purified T cells exposed to a suboptimal dose of anti-CD3 (Pelicluster CD3, which is capable of crosslinking CD3 and activating T cells independent of FcγR binding) for 72 hours. For anti-PD1-pro-IL-18, the exemplary test article was Fuse694, which incorporates one pro-IL-18 (granzyme B cleavable IL18mutAS) on the C-terminal knob or hole of an IgG1-LALA version of nivolumab (Fuse691). For anti-PD1-mutant-IL-15, the three test articles were Fuse765 (FIG. 2A, open triangle, dashed line), Fuse773 (FIG. 2B, open triangle, dashed line) and Fuse774 (FIG. 2C, open triangle, dashed line), which respectively

incorporated one IL-15m4, IL15m9 or IL15m10 on the C-terminal knob or hole of Fuse691. FIGs 2A-2C are non-linear x-y plots of test article concentration versus IFNγ release.

[0448] In each plot, (1) the Hen Egg Lysosyme (HEL) specific antibody, anti-HEL (gray circle), was used as the human IgG1 isotype control and was associated with background IFNγ release. Fuse691 (open square, dashed line) was observed to induce mild IFNγ release in this system, equating to a maximum value of about 1200 pg/ml. Targeting pro-IL-18 or any of the three IL-15 mutants alone to PD1 using Fuse694, Fuse765, Fuse773 and Fuse774 resulted in only a modest increase in maximum observed IFNγ release relative to Fuse691 with values of approximately 1900 pg/ml, 3000 pg/ml, 2800 pg/ml and 1700 pg/ml, respectively. In contrast, the combination of Fuse765 plus Fuse694 (black triangle) , Fuse773 plus Fuse694 (black triangle) and Fuse774 plus Fuse694 (black triangle) resulted in maximum observed IFNγ release values of approximately 12,000 pg/ml, 11,500 pg/ml and 7,400 pg/ml, respectively. These values correspond to 4-6-fold that of anti-PD1-pro-IL-18 alone (Fuse694) and or any of the anti-PD1-mutant-IL15 variants (Fuse765, Fuse773 and Fuse774) indicating that the combined impact of targeting both IL-15 and IL-18 to PD1 in this system is synergistic. Interestingly, the EC50- IFNγ release, although only semi-quantitative for Fuse691 and the single cytokine fusions to anti-PD1, appeared to cluster within 2-3-fold of each other.

[0449] A summary table of EC50, Emax and AUC (area under the curve) values is shown in FIG. 2D.

Example 2

[0450] As shown in figure 3, we assessed the impact of an exemplary anti-PD1-pro-IL-18 antibody, an exemplary anti-PD1-mutant-IL-15 antibody and an anti-PD1 antibody incorporating both the aforementioned exemplary pro-IL-18 and mutant IL-15, on IFNγ release mediated by purified T cells exposed to a suboptimal dose of anti-CD3 (PeliCluster CD3, which is capable of crosslinking CD3 and activating T cells independent of FcγR binding) for 72 hours. For anti-PD1-pro-IL-18, the exemplary test article was Fuse694 (black square). For anti-PD1-mutant-IL-15, the exemplary test article was Fuse696 (black diamond), which incorporated one IL-15m2 on the C-terminal knob or hole of the anti-PD1 human IgG1 LALA antibody, Fuse691 (open square, dashed line). Finally, Fuse697 (black triangle), incorporated one pro-IL18 on the C-terminal knob and one IL-15m2 on the C-terminal hole of Fuse691. FIG 3 is non-linear x-y plot of test article concentration versus IFNγ release. The human IgG1 antibody, anti-HEL (gray circle), was used as the isotype control and was associated with background IFNγ release. Fuse691 (open square, dashed line) was observed to induce mild IFNγ release in this system, equating to a maximum value of about 830 pg/ml. Targeting pro-IL-18 alone to PD1 using Fuse694 resulted in only a modest increase in maximum observed IFNγ release relative to Fuse691 with a value of approximately 2,000 pg/ml. Targeting IL-15m2 alone to PD1 using Fuse696 resulted in a more pronounced increase in maximum observed IFNγ release relative to Fuse691 with a value of about 9,000 pg/ml. Fuse697 induced a maximum observed IFNγ release values of approximately 21,700 pg/ml, which is about 11-fold greater than that observed for Fuse694 and 2.5-fold greater than that observed for Fuse696. Importantly, maximum observed IFNγ release for Fuse697 equated to more than the sum of the maximum IFNγ release values

associated with Fuse694 plus Fuse696 indicating that IL-15 and IL-18 acted in a synergistic fashion when co-incorporated into anti-PD1. Interestingly, unlike the EC50-IFNγ release for the combination of anti-PD1-pro-IL-18 and anti-PD1-mutant-IL15 relative to Fuse691 and the single cytokine fusions to anti-PD1, the EC50- IFNγ release for Fuse697 was obviously left shifted (more potent) by at least 30-fold relative to Fuse691, Fuse694 or Fuse696. A summary table of EC50, Emax and AUC (area under the curve) values is shown below the x-y plot.

Example 3

[0451] As shown in figure 4, we assessed the capacity of an NKG2D x ROR1 bispecific antibody (bsAb) with or without the incorporation of an exemplary pro-IL18 and IL15 mutant to induce NK cell mediated tumor cell killing and IFNγ release. The NKG2D x ROR1 knobs into holes bsAb was human IgG1 LALA (parent bsAb without cytokines incorporated was Fuse926) incorporated pro-IL18 (granzyme B cleavable IL18mutAV) on the N-terminal hole and IL15m9-1 on the C-terminal hole (Fuse916). Enriched NK cells were expanded from human PBMC (normal donor) and used as the effector cells. The ROR1+ tumor cells were MDA-MB-231 stably expressing firefly luciferase and Egfp (MDA-GL). NK cells were mixed with MDA-GL at an effector to target ratio (E:T) of 5:1 for 48 hours. Tumor cell killing was assessed via luciferase activity and IFNγ release was measured in the culture supernatants via ELISA. FIGs 4A and 4B are non-linear x-y plots of tumor cell killing versus test article concentration and IFNγ release versus test article concentration, respectively. Relative to Fuse926 (black square), Fuse916 (black triangle) induced ~50% more maximum killing and ~7-fold more maximum IFNγ release. The potency (defined as the EC50-killing or EC50- IFNγ release) of Fuse916 was also enhanced relative to Fuse916 in the orders of 5-fold and >10-fold for tumor cell killing and IFNγ release, respectively. The anti-HEL Ab serves as a negative isotype control and Fuse923 (black diamond), a γ9δ2-TCR x ROR1 bsAb incorporating pro-IL18 and IL15m9-1 in the same orientation was used as the non-targeted cytokine incorporating test article. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

Example 4

[0452] As shown in figure 5, we assessed the impact of targeting an exemplary IL-15 variant and an exemplary pro-IL18 to NKG2D on NK cell expansion in PBMC. Both NK cell frequency and numbers were measured after 12 days of incubating PBMC from a healthy human donor with the test articles described below. NK cells were defined as CD56+/CD3- cells. The antibody fusion proteins tested were Fuse 926 (black diamond), which consists of an NKG2D x ROR1 bsAb and Fuse916 (black triangle), which consists of the same bsAb but also incorporates IL-15m9-1 and pro-IL18 (granzyme B cleavable IL18mutAV). Fuse923 (black square), a cytokine bsAb fusion similar to Fuse923 that targets the δ2 region of the γ9δ2 TCR was used as the non targeted control and anti-HEL (gray circle) was used as the isotype matched negative control. Of note, all bsAbs contained the LALA mutation and therefore did not bind nor activate CD16 expressed on NK cells. As such, the expansion of NK cells was limited to targeting of the cytokines to

NKG2D only without co-ligation of NKG2D and CD16, reported to enhance one another's activity www.sciencedirect.com/science/article/pii/S0006497120678016. FIG. 5A and FIG. 5B are x-y plots of NK cell percentages and NK cell numbers measured on day 12, respectively, illustrated as a function of a titration of each test article. Relative to the Fuse926, 100 Nm of Fuse916 induced about a 6-fold expansion of NK cells.

Example 5

[0453] As shown in figure 6, we assessed the impact of targeting an exemplary IL-15 variant and an exemplary pro-IL18 to NKp46 on NK cell expansion in PBMC. Both NK cell frequency and numbers were measured after 12 days of incubating PBMC from a healthy human donor with the test articles described below. NK cells were defined as CD56+/CD3- cells. The antibody fusion proteins tested were Fuse 943 (black triangle), which consists of a monovalent NKp46 Ab which incorporates IL-15m9 and pro-IL18 (granzyme B cleavable IL18mutAV). Fuse944 (black square), was used as the non targeted control in which the NKp46 binder was omitted from the construct. Fuse320 (black diamond) , an NKp46 x EGFR bsAb incorporating the same NKp46 binder but no cytokines served as the targeted/no cytokine control (note that we did not detect expression of EGFR in PBMC; data not shown) and anti-HEL (gray circle) was used as the isotype matched negative control. Of note, all bsAbs contained the LALA mutation and therefore did not bind nor activate CD16 expressed on NK cells. As such, the expansion of NK cells was limited to targeting of the cytokines to NKp46 only without co-ligation of NKp46 and CD16, reported to enhance one another's activity www.sciencedirect.com/science/article/pii/S0006497120678016. FIG. 6A and FIG. 6B are x-y plots of NK cell percentages and NK cell numbers measured on day 12, respectively, illustrated as a function of a titration of each test article. Relative to the Fuse320, 100 Nm of Fuse943 induced about a 5-fold expansion of NK cells.

Example 6

[0454] As shown in figure 7, we assessed the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to γ9δ2 T cells via a Vγ9Vδ2 TCR x ROR1 bsAb cytokine fusion to induce expansion of γ9δ2 T cells in human PBMC.

[0455] Both γ9δ2 cell frequency and numbers were measured after 12 days of incubating PBMC from a healthy human donor with the test articles described below. Given that ROR1 is not expressed on any cells in PBMC (note that ROR1 is expressed on pre-B cells in human bone marrow), expansion observed was considered independent of Vγ9Vδ2 TCR crosslinking via ROR1.

[0456] The antibody fusion proteins tested were cytokine fusions to a Vγ9Vδ2 TCR x ROR1 bsAb (Fuse966; black diamond) incorporating (1) IL-15m9-1 (Fuse968; black square), (2) pro-IL18 (granzyme B cleavable IL18mutAV) (Fuse967; black reverse triangle) and (3) IL-15m9-1 and pro-IL18. Fuse944 (open triangle, dashed line), was used as the non targeted control in which the Vγ9Vδ2 TCR x ROR1 cassette was omitted from the construct. An anti-HEL (gray circle) was used as the isotype matched negative control.

[0457] FIG. 7A and FIG. 7B are x-y plots of γ9δ2 T cell percentages and γ9δ2 T cell numbers measured on day 12, respectively, illustrated as a function of a titration of each test article. Relative to the Fuse966, 100 Nm of Fuse923 induced about a 12-fold expansion of γ9δ2 T cells.

Example 7

[0458] As shown in figure 8, we assessed the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to γ9δ2 T cells via a Vγ9Vδ2 TCR x ROR1 bsAb cytokine fusion to induce γ9δ2 T cell mediated IFNγ release and tumor cell killing. The assay design was as such: γ9δ2 T cells enriched/expanded from the PBMC of a healthy human donor was mixed with the ROR1+ tumor cell, MDA-MB-231 stably expressing Egfp and firefly lucerifase at an E:T ratio of 5:1. After 48 hours, tumor cell cytotoxicity was assessed via luciferase activity (i.e. tumor cell viability) and IFNγ release was measured in the supernatant using a standard ELISA protocol. The antibody fusion proteins tested were cytokine fusions to a Vγ9Vδ2 TCR x ROR1 bsAb (Fuse966; open reverse triangle, dashed line) incorporating (1) IL-15m9-1 (Fuse968 ; open triangle, dashed line), (2) pro-IL18 (granzyme B cleavable IL18mutAV) (Fuse967 ; black reverse triangle) and (3) IL-15m9-1 and pro-IL18. Fuse944 (black diamond), was used as the non targeted control in which the Vγ9Vδ2 TCR x ROR1 cassette was omitted from the construct. An anti-HEL (gray circle) was used as the isotype matched negative control. Shown are non-linear x-y plots of a titration of each test article as a function of tumor cell killing (FIG. 8A) or IFNγ release (FIG. 8B). Relative to Fuse966, the incorporation of cytokine(s) into the bsAb enhanced both tumor cell killing and IFNγ release in the rank order of Fusc923>Fusc967>Fusc968>Fusc966 indicating that the combination of IL-18 and IL-15 was the most potent. The differential readout between test articles was more apparent for IFNγ release compared to tumor cell killing. This is likely due to the high E:T ratio of 5:1 at which maximum tumor cell killing may be reached at a lower γ9δ2 T cell signaling threshold than IFNγ release. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

Example 8

[0459] As shown in figure 9, we assessed the impact of targeting an exemplary IL-15 variant and an exemplary IL18 variant to NKp30 plus or minus NKp46 on NK cell expansion from PBMC. Both NK cell frequency and numbers were measured after 21 days of incubating PBMC from a healthy human donor with 100 Pm of the test articles described below. NK cells were defined as CD56+/TCRαβ- cells. The antibody fusion proteins tested were Fuse 1147 (light grey), which consists of a knobs into holes bispecific antibody with a monovalent arm specific for human NKp30 (Fab) and a monovalent arm specific for human ROR1 (VHH), both located on the N-terminus of the knob (or hole). The ROR1 specific VHH was fused to the C-terminus of the NKp30 specific LC. Fuse 1148 (dark grey) is identical to Fuse1147 expect that it includes an NKp46 specific VHH on the C-terminus of the CH3 domain (knob or hole). Fuse 1145 (black dots) is identical to Fuse1147 except that it includes an exemplary IL-18 variant on the N-

terminus of the hole (or the knob if the NKp30 specific fab is on the hole) and IL-15m10-1 on the C-terminus of the hole (or knob). Fuse 1146 (black stripes) is identical to Fuse1148 except that it includes an exemplary IL-18 variant on the N-terminus of the hole (or the knob if the NKp30 specific fab is on the hole) and IL-15m10-1 on the C-terminus of the hole (or knob if the NKp46 specific VHH is on the hole). Thus, to simplify, Fuse1147, Fuse118, Fuse1145 and Fuse1148 consist of NKp30Xror1, NKp30Xror1xNKp46, NKp30Xror1-IL-18/IL-15 and NKp30Xror1xNKp46-IL-18/IL-15, respectively. Shown in FIG. 9A are flow cytometric dot plots of CD56 versus TCRαβ. A box has been drawn around the NK cells (CD56+/TCRαβ- cells) and the frequency of these cells is shown to the left of the box. The data is also shown graphically as a bar chart in FIG. 9B. The anti-HEL specific antibody (human IgG1 LALA) was used as the negative isotype control. Relative to the negative control and the engagers without the inclusion of cytokines, Fuse1145 and Fuse1146 induced an increased frequency of NK cells by about 8.5 fold and 13.7 fold, respectively. We also measured the total number of NK cells associated with the culture of PBMC with each test article (at 100 Pm). The fold expansion of NK cells derived from this data relative to that observed for anti-HEL is shown in FIG. 9C. No appreciable expansion was observed using engagers without the inclusion of cytokines. In contrast, Fuse1145 and Fuse1146 induced expansion of about 34 fold and 69 fold, respectively. The data indicates that addition NKp46 targeting to NKp30 targeted-IL-18/IL-15 enhances the frequency and expansion of NK cells from human PBMC.

Example 9

[0460] As shown in figure 10, we assessed the agonist activity of γ9δ2 TCR specific VHH fused to the Fc domain of human IgG1 to induce redirected lysis of P815 cells stably transduced with Egfp and firefly luciferase (P815) by expanded γ9δ2 T cells. The assay design was as such: γ9δ2 T cells enriched/expanded from the PBMC of a healthy human donor was mixed with the P815 at an E:T ratio of 3:1. After 24 hours, tumor cell cytotoxicity was assessed via luciferase activity (i.e. tumor cell viability). Non linear x-y plots of killing percentage as a function of test article concentration is shown is FIGs 10A-10B. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

Example 10

[0461] As shown in figure 11, we assessed the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to γ9δ2 T cells via a Vγ9Vδ2 TCR x ROR1 bsAb cytokine fusion to induce γ9δ2 T cell mediated IFNγ release and tumor cell killing. The assay design was as such: γ9δ2 T cells enriched/expanded from the PBMC of a healthy human donor was mixed with the ROR1+ tumor cell, Jurkat stably expressing Egfp and human ROR1 at an E:T ratio of 3:1. After 72 hours, tumor cell cytotoxicity was assessed via GFP fluorescence (i.e. tumor cell viability) and IFNγ release was measured in the supernatant using a standard ELISA protocol. The antibody fusion proteins tested were cytokine fusions to a Vγ9Vδ2 TCR x ROR1 bsAb (Fuse1139; black square) incorporating (1) IL-15m10-1 (Fuse1137 ; black triangle), (2) pro IL18mutA (Fuse1138 ; black reverse triangle) and (3) IL-15m10-1 and pro

IL18mutA (Fuse1136; open circle, dashed line). An anti-HEL (gray circle) was used as the isotype matched negative control. Shown are non-linear x-y plots of a titration of each test article as a function of tumor cell killing (FIG. 11A) or IFNγ release (FIG. 11B). Relative to Fuse1139, the incorporation of cytokine(s) into the bsAb enhanced both tumor cell killing and IFNγ release in the rank order of Fuse1136>Fuse1137>Fusee1138 indicating that the combination of IL-18 and IL-15 was the most potent. The differential readout between test articles was more apparent for IFNγ release compared to tumor cell killing. This is likely due to the high E:T ratio of 3:1 at which maximum tumor cell killing may be reached at a lower γ9δ2 T cell signaling threshold than IFNγ release. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

Example 11

[0462] As shown in figure 12, we assessed the agonist activity of human NKp46 (FIGs 12A-12B) and human NKp30 (FIG. 12C) specific VHH fused to the Fc domain of human IgG1 to induce redirected lysis of P815 cells stably transduced with Egfp and firefly luciferase (P815) by expanded γ9δ2 T cells. The assay design was as such: Expanded NK cells enriched/expanded from the PBMC of a healthy human donor was mixed with the P815 at an E:T ratio of 1:1 (FIG. 12A). 2:1 (FIG. 12B) and 3:1 (FIG. 12C). After 24 hours, tumor cell cytotoxicity was assessed via luciferase activity (i.e. tumor cell viability). Non linear x-y plots of killing percentage as a function of test article concentration is shown is FIGs 12A-12C.

[0463] Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

Example 12

[0464] As shown in figure 13, we assessed the capacity of an exemplary IL-15 variant and/or an exemplary pro-IL18 targeted to human NK cells via a NKp46 x ROR1 bsAb cytokine fusion to induce NK cell mediated IFNγ release and tumor cell killing. The assay design was as such: NK cells enriched/expanded from the PBMC of a healthy human donor were mixed with the ROR1+ tumor cell, Jurkat stably expressing Egfp and human ROR1 at an E:T ratio of 3:1. After 72 hours, tumor cell cytotoxicity was assessed via GFP fluorescence (i.e. tumor cell viability) and IFNγ release was measured in the supernatant using a standard ELISA protocol. The antibody fusion proteins tested were cytokine fusions to a NKp46 x ROR1 bsAb (Fuse1125; black square) incorporating (1) IL-15m10-1 (Fuse1127 ; black triangle), (2) pro IL18mutA (Fuse1126 ; black reverse triangle) and (3) IL-15m10-1 and pro IL18mutA (Fuse1124; open circle, dashed line). An anti-HEL (gray circle) was used as the isotype matched negative control. Shown are non-linear x-y plots of a titration of each test article as a function of tumor cell killing (FIG. 13A) or IFNγ release (FIG. 13B). Relative to Fuse1125, the incorporation of cytokine(s) into the bsAb enhanced both tumor cell killing and IFNγ release in the rank order of Fuse1124>Fuse1127>Fusee1126 indicating that the combination of IL-18 and IL-15 was the most potent. The differential readout between test articles was more apparent for IFNγ release compared to tumor

cell killing. This is likely due to the high E:T ratio of 3:1 at which maximum tumor cell killing may be reached at a lower NK cell signaling threshold than IFNγ release. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

Example 13

[0465] As shown in figure 14, we assessed the capacity of an exemplary IL-15 variant and an exemplary pro-IL18 targeted to human NK cells via a (1) human NKp30 x human ROR1 bsAb cytokine fusion (Fuse1147 and Fuse1145) or (2) NKp30 x NKp46 ROR1 bsAb cytokine fusion (Fuse1148 and Fuse1146), to induce NK cell mediated IFNγ release and tumor cell killing. The assay design was as such: NK cells enriched/expanded from the PBMC of a healthy human donor were mixed with the ROR1+ tumor cell, Jurkat stably expressing Egfp and human ROR1 at an E:T ratio of 3:1. After 72 hours, tumor cell cytotoxicity was assessed via GFP fluorescence (i.e. tumor cell viability) and IFNγ release was measured in the supernatant using a standard ELISA protocol. The antibody fusion proteins tested were a NKp30 x ROR1 bsAb (Fuse1147; black square) a NKp30 x ROR1 bsAb fused to IL-15m10-1 and pro IL18mutA (Fuse1145; open squares, dashed line), a NKp30 x NKp46 x ROR1 bsAb (Fuse1148; black circles) and a NKp30 x NKp46 x ROR1 bsAb fused to IL-15m10-1 and pro IL18mutA (Fuse1146; open circles, dashed line) . An anti-HEL (gray circle) was used as the isotype matched negative control. Shown are non-linear x-y plots of a titration of each test article as a function of tumor cell killing (FIG. 14) or IFNγ release (FIG. 15). Relative to 1148, the incorporation of cytokine(s) into the bsAb enhanced both tumor cell killing and IFNγ release in the rank order of Fuse1145>Fuse1146>Fusee1147 indicating that (1) NKp30 x ROR1 was more potent than NKp30 x NKp46 x ROR1 and the combination of IL-18 and IL-15 enhanced potency. Summary tables of EC50, Emax and AUC (area under the curve) values are shown below each x-y plot.

Example 14

Human peripheral blood mononuclear cell (PBMC) isolation

[0466] PBMCs were isolated from whole blood from healthy donors using Ficoll-Paque Plus medium. In brief, 35 Ml of diluted whole blood (1 volume of whole blood vs 1 volume of PBS) was gently overlayed on top of 15 Ml Ficoll-Paque Plus medium without disturbing the interface in a 50-Ml conical tube. After centrifuging for 40 minutes at 400 × g at room temperature without brake, the buffy coat (interface layer between Ficoll and serum) was collected and diluted in 5 volumes of PBS. After centrifuging for 5 minutes at 500 × g at room temperature, PBMCs were resuspended in PBS and washed once in PBS by centrifuging for 5 minutes at 500 × g at room temperature. PBMCs were then resuspended in 5 Ml of ACK lysis buffer and incubated for 5 minutes at room temperature to remove red blood cell residues. After the 5-minute incubation period, 45 Ml PBS was added to PBMCs and centrifuged for 15 minutes at 100 × g at room temperature. At last, PBMCs were resuspended in culture medium (RPMI1640 with 10% heat-inactivated FBS and 1% penicillin/streptomycin) for cytotoxicity and IFN gamma release assay set-up. To

store PBMCs for further usage, PBMCs were pelleted down by centrifuging for 5 minutes at 500 × g at room temperature and then resuspended in freezing medium (90% heat-inactivated FBS and 10% dimethyl sulfoxide (DMSO)) for storage in liquid nitrogen.

NK cell isolation and expansion

[0467] Fresh PBMCs were washed twice in PBS and resuspended in EasySep Buffer from StemCell at a density of 50 million cells per Ml in 50-Ml conical tube. 50 Ul of isolation cocktail from the NK cell isolation kit from StemCell was added to 1 Ml of cell suspension and incubated at room temperature for 5 minutes. After the 5-minute incubation period, 50 Ul of RapidSpheres from NK cell isolation kit was added to 1 Ml of the cell/antibody cocktail mixture. The total volume of the cell/cocktail/bead mixture was brought up to 25 or 50 Ml with EasySep Buffer and incubated at room temperature for 10 minutes on EASYSEP magnet. After the 10-minute incubation period, the solution was transferred to a new 50-Ml tube by keeping the tube on the magnet and incubated at room temperature for 5 minutes on EASYSEP magnet. The solution with purified NK cell was collected. Freshly purified NK cells were used for expansion or frozen down and stored in liquid nitrogen for further usage.

[0468] To expand NK cells, 1x 500 Ml of ImmunoCult™ NK cell expansion coating material from ImmunoCult NK cell expansion kit from StemCell was added to a non-tissue culture-treated 24-well plate and incubated at room temperature for 2 hours. During the 2-hour incubation, freshly isolated NK cells were resuspended in ImmunoCult NK cell expansion medium from ImmunoCult NK cell expansion kit from StemCell at the density of 1 million per Ml. After the 2-hour incubation, the plate was washed twice with PBS and 500 Ml of NK cell suspension (0.5 million NK cells) was added to one well of the coated 24-well plate. The plate was incubated at 37°C with 5% CO2 for 3 days and 500 Ml of ImmunoCult NK cell expansion medium was added to the well with NK cells. The platE was further incubated for another 4 days. At day 7, NK cells were collecteD by centrifuge at 300 x g for 10 minutes and resuspended in ImmunoCult NK cell expansion medium from ImmunoCult NK cell expansion kit from StemCell at the density of 0.2 million per ML. 1 ML of collected NK cells (0.2 million NK cells) was added to a well of new coated 24-well plate and incubated for 4 days at 37°C with 5% CO2. At day 11, NK cells were collected again by centrifuge at 300 x g for 10 minutes and resuspended in ImmunoCult NK cell expansion medium from ImmunoCult NK cell expansion kit from StemCell at the density of 0.2 million per ML. 1 ML of collected NK cells (0.2 million NK cells) was added to a well of new coated 24-well plate and incubated for another 3 days at 37°C with 5% CO2. NK cells after 14-days' expansion were collected and frozen down and stored in liquid nitrogen for further usage.

T cell isolation

[0469] Fresh PBMCs were washed twice in PBS and resuspended in EasySep Buffer from StemCell at the density of 50 million cells per ML in 50-ML conical tube. 50 UL of isolation cocktail from T cell isolation kit from StemCell was added to 1 ML of cell suspension and incubated at room temperature for 5 minutes. After the 5-minutes' incubation, 50 UL of RapidSpheres from T cell isolation kit was added to 1 ML of the cell/antibody cocktail mixture. The total volume of the cell/cocktail/bead mixture was brought up to 25 or 50 ML with EasySep Buffer and incubated

at room temperature for 10 minutes on EASYSEP magnet. After the 10-minutes' incubation, the solution was transferred to a new 50-ML tube by keeping the tube on the magnet and incubated at room temperature for 5 minutes on EASYSEP magnet. The solution with purified T cell was collected. Freshly purified T cells were frozen down and stored in liquid nitrogen for further usage.

Gamma 9 delta 2 T cell expansion

[0470] PBMCs were recovered from liquid nitrogen, washed twice in PBS and resuspended in culture medium (RPMI1640 with 10% heat-inactivated FBS and 1% penicillin/streptomycin) at the density of 0.5 million cell per ML of medium. 5 UM IPP and 1000 U/ML IL-2 (final concentration) was added to the PBMCs and incubated for 7 days. At the end of 7-day incubation, the percentage of gamma 9 delta 2 T cells was measured by flow cytometry. gamma 9 delta 2 T cells after 7-days' expansion were collected and frozen down and stored in liquid nitrogen for further usage.

P815 redirected lysis assay

On the day of setting up assay plates, collect P815 cell expressing EgfP and firefly luciferase and perform cell count via trypan blue exclusion on cell counter and adjust the density of the viable cell to 2x10^5 cells/ML in complete RPMI1640 medium with 10% heat-inactivated FBS, non-essential amino acid, Sodium pyruvate and 1x pen/strep. Add 50 UL target cell suspension to each well of a black clear flat-bottom 96-well plate using multichannel pipettor to result in a plating density of 10,000 cells/well. Prepare the testing article by serially diluting (3-fold, 5-fold or 8-fold serial dilution) in complete RPMI1640 medium at the concentration of 4x final concentration and add 50 UL of 4x testing article preparation to the designated wells. After incubating the plate for 30 minutes at 37 °C with 5% CO_2, collect the effector cells (expanded NK cell or expanded gamma 9 delta 2 T cell) to perform cell count via trypan blue exclusion on cell counter and adjust effector cells to desired density. Add 100 UL of effector cell suspension to P815 EgfP Flu cell with testing articles to bring the final volume to 200 UL and briefly centrifuge the plate and incubate the plate for desired time length at 37 °C with 5% CO_2. At the end of incubation, remove plates from the incubator, spin for 30s at 300 x g, and remove 150ML of supernatant using a multichannel pipettor. Acclimate ONE-Glo Luciferase Assay solution from Promega to room temperature before use, add 50 UL of One-Glo solution to each well with cells, incubate for 2 min at room temperature and measure bioluminescence intensity (RLU). Killing percentage of target cells in each sample was obtained by using target cell alone group as 0% killing and PBMCs alone group as 100% killing [killing percentage of sample = (RLU of target cell alone-– RLU of sample)/(RLU of target cell alone-– RLU of PBMCs alone)*100]. Killing EC50 and Emax were then obtained by using built-in 4-parameter nonlinear regression cure fit in GRAPHPAD PRISM 8. **Pelicluster CD3 antibody-primed T cell activation assay**

[0471] Isolated T cells were recovered from liquid nitrogen and resuspended in RPMI1640 complete culture medium (RPMI1640 with 10% heat-inactivated FBS and 1% penicillin/streptomycin). The viability of cells was also determined via trypan blue exclusion and the viable T cell density was adjusted to 1.33 million cells/ML in RPMI1640 complete culture medium. 75 UL of 0.67 million cells/ML T cell suspension (100,000 T cells) was added to the designated well in the 96-well plate. Pelicluster CD3 antibody (mouse anti-human CD3 antibody clone CLB-T3/4.E

1XE) was prepared in RPMI1640 complete culture medium at the concentration of 4 times of final concentration ad 75 UL of prepared pelicluster CD3 antibody was added to the cells in the designated well in the 96-well plate. FUSE proteins were prepared and serially diluted (5-fold serial dilution) in RPMI1640 complete medium from 200 NM to 2.56 PM. 150 UL of prepared FUSE proteins at different concentrations were then added to the designated wells in the 96-well plate with T cells and incubated for the dedicated time period (72 hours) at 37 °C with 5% CO2. At the end of the incubation period, the 96-well plates were centrifuged for 1 minute at 500 ×g to transfer the supernatant to V-bottom storage plate using a multichannel pipettor for IFN gamma release assay.

Cytotoxicity assay

[0472] On the day before assay setting up, selective antibiotics were removed from target cell (MDA-MB-231 EgfP FLUC cells). On the day of assay setting up, target cells were collected by brief TrypLE treatment and then washed with culture medium by centrifuging at 500 × g for 5 minutes at room temperature. Target cell lines were then resuspended in culture medium to determine the cell viability by trypan blue exclusion on Cellometer. The viable cell density was adjusted to 50,000 cells/ML in culture media. 100 UL target cell suspension (5,000 target cell) was carefully dispensed to each well of a 96-well black clear flat-bottom tissue culture plate using multichannel pipettor. The plate was then incubated for 4-5 hours in tissue culture incubator to ensure that the target cells have attached to the bottom of the 96-well plate.

[0473] Expanded NK cells or gamma 9 delta 2 T cells were pelleted down by centrifuge for 5 minutes at 500 × g at room temperature and resuspended in culture medium. The viability of cells was also determined via trypan blue exclusion and the viable NK or gamma 9 delta 2 T cell density was adjusted to 0.25 million cells/ML in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin. After 4-5 hours of incubation, culture medium was carefully removed from 96-well plates with target cells. 100 UL of 0.25 million cells/ML NK or gamma 9 delta 2 T cell suspension (25,000 cells) was added to the designated well in the 96-well plate with target cell, which would result in the E:T ratio of 5:1.

[0474] FUSE proteins were prepared and serially diluted (5-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 NM to 2.56 PM. 100 UL of prepared FUSE proteins at different concentrations were then added to the designated wells in the 96-well plate with effector cells and target cells and incubated for the dedicated time period (48 hours) at 37 °C with 5% CO2. At the end of the incubation period, the 96-well plates were centrifuged for 1 minute at 500 ×g to transfer 50ML of supernatant to V-bottom storage plate using a multichannel pipettor for IFN gamma release assay. ONE-Glo luciferase Assay solution was brought to room temperature. 50 UL of One-Glo solution was then added to the designated well and incubated for 2 minutes at room temperature. The bioluminescence (IFU) was measured on a plate reader with preset Bio-luminance protocol. Killing of target cell was calculated by using the formula [Killing percentage of testing sample = (IFU of sample with PBMC and target cell–- IFU of testing sample)/(IFU of sample with PBMC and target cell–- IFU of sample with PBMC alone)*100].

IFN gamma release detection

[0475] IFN gamma release from cytotoxicity assay was measured using human IFN gamma ELISA detection kit. In brief, coating antibody provided in the kit was diluted to suggested concentration by following the protocol provided by the kit manufacturer. 100 ul of diluted coating antibody was added to the Nunc MaxiSorp flat-bottom 96-well plate. Plates were sealed and incubated overnight at 4 °C. On the following day, the plates were then washed 4 times with the Wash Buffer. To block non-specific binding and reduce background, 200 ML 1× Assay Diluent A was added, and the plates were incubated at room temperature for 1 hour on a plate shaker (400 rpm). After blocking, the plates were washed 4 times with Wash Buffer. 100 ML/well of standards (prepared with culture medium) or samples were then added to the appropriate wells and incubated at room temperature for 2 hours on a plate shaker (400 rpm). After the 2-hour incubation with samples or standards, the plates were washed 4 times with Wash Buffer and 100 ML of diluted Detection Antibody solution was added to incubate at room temperature for 1 hour on a plate shaker (400 rpm). After the 1-hour incubation period with detection antibody, the plates were washed 4 times with Wash Buffer and 100 ML of diluted Avidin-HRP solution was added to incubate at room temperature for 30 minutes on a plate shaker (400 rpm). After 30-minute incubation with Avidin-HRP, the plates were washed 5 times with Wash Buffer and 100 ML of freshly mixed TMB Substrate Solution was added to incubate at room temperature for 20 minutes in the dark. 100 ML of Stop Solution was then added to each well to stop the reaction. The absorbance at 450 nm was measured on a plate reader and the concentration of IFN gamma in each sample was back-calculated using the standard curve generated with the absorbance of different concentration of standards.

NK cell or gamma delta T cell expansion assay

[0476] PBMCs were recovered from liquid nitrogen, washed twice in PBS and resuspended in culture medium (RPMI1640 with 10% heat-inactivated FBS and 1% penicillin/streptomycin) at the density of 2 million cell per ML of medium. 500 UL of 2 million cells/ML PBMC suspension was added to the designated well in the 48-well plate, which would result in 1 million cells per well.

[0477] FUSE proteins were prepared and serially diluted (100-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 NM to 0.2 PM. 500 UL of prepared FUSE proteins at different concentrations were then added to the designated wells in the 48-well plate with PBMCs and incubated for the dedicated time period (12 days) at 37 °C with 5% CO_2. At the end of the incubation period, the total cell number was counted in each well and the cells in each well were also collected to check the percentage of NK cells or gamma 9 delta 2 T cells by flow cytometry. The NK cell number or gamma 9 delta 2 T cell number was calculated by multiplying the percentage of NK cells or gamma 9 delta 2 T cells by the total cell number.

IL-15 VARIANT EXAMPLES

Example 15

[0478] As shown in figure 17, we assessed the functional activity of a series of IL-15 variants to activate HEK-Blue IL-2 reporter cells. In this system, engagement of IL-2Rβ and IL-2Rβ by IL-15 induces the expression and secretion of secreted embryonic alkaline phosphatase (SEAP), which is encoded in HEK-Blue IL-2 downstream of a STAT-5 transcriptional promoter element. FIGs 17A-17C are x-y scatterplots of SEAP release (OD at 630 nm) versus the concentration of test article. Unless otherwise noted, all IL-15 variants were fused to the C-term knob of a knobs into holes version of nivolumab. Nivolumab (FUSE691; grey closed circles) served as the negative control while recombinant human IL-15 (black closed squares) served as the positive control. HEK Blue IL-2 does not express PD-1. As such all IL-15 related activity measured was a consequence of non-targeted or "trans" activity. We found that all IL-15 variants were attenuated relative to recombinant human IL-15. The majority of variants formed a cluster with a potency (EC50 SEAP release) between about 40 PM and 300 PM, representing a degree of attenuation between about 11 fold and 80 fold. In FIG. 17A, two IL-15 variants incorporated into FUSE773 (closed triangles) and FUSE774 (closed inverted triangles), respectively, were identified as appreciably more attenuated relative to the aforementioned cluster of variants. Relative to recombinant human IL-15, FUSE773 and FUSE774 were approximately 2000 and 4000 fold less potent, respectively. No appreciable activity was observed from the negative control, FUSE691. In FIG. 17B, an additional four IL-15 variants incorporated into FUSE803 (open hexagons) and FUSE846 (closed triangles) and FUSE847 (closed inverted triangles) were identified as appreciably more attenuated relative to the aforementioned cluster of variants in FIG. 17A. FUSE848 (gray filled triangles) was observed to have no appreciable functional activity. Relative to recombinant human IL-15, FUSE803, FUSE846 and FUSE847 were approximately 1500, 5000, and 52,000 fold less potent, respectively. No appreciable activity was observed from the negative control, FUSE691.

[0479] In FIG. 17C, an additional two IL-15 variants incorporated into FUSE849 (closed stars) and FUSE853 (open squares), were identified as appreciably more attenuated relative to the aforementioned cluster of variants in FIG. 17A. FUSE850 (gray filled triangles) was observed to have little to no functional activity. Relative to recombinant human IL-15, FUSE849 and FUSE853 were approximately 55,000 and 800 fold less potent, respectively. No appreciable activity was observed from the negative control, FUSE691. Summary tables of maximum activity (Emax), EC50 and AUC (area under the curve) are shown beneath each x-y scatterplot. In FIG. 17C, additional two IL-15 variants incorporated into FUSE849 (closed stars) and FUSE853 (open squares), were identified as appreciably more attenuated relative to the aforementioned cluster of variants in FIG. 17A. FUSE850 (gray filled triangles) was observed to have little to no functional activity. Relative to recombinant human IL-15, FUSE849 and FUSE853 were approximately 55,000 and 800 fold less potent, respectively. No appreciable activity was observed from the negative control, FUSE691. Summary tables of maximum activity (Emax), EC50 and AUC (area under the curve) are shown beneath each x-y scatterplot.

Example 16

[0480] As shown in figure 18, we assessed the capacity of a series of IL-15 variants to activate purified human T cells in presence of a low "suboptimal" concentration of the anti-CD3 antibody clone, CLB-T3/4.E, termed Pelicluster CD3. This mouse IgE antibody can crosslink and activate human CD3 in solution (i.e., does not require immobilization). IFNγ release after 72 hours of co-culture was used as the readout of T cell activation. FIG. 18A and FIG. 18B are x-y scatterplots depicting the concentration of IFNγ in the culture supernatant versus the concentration of test article. Unless otherwise noted, all IL-15 variants were fused to the C-term knob of a knobs into holes version of nivolumab. The Hen Egg Lysozyme (HEL) specific antibody served as the negative control (black closed circles). Nivolumab (FUSE691; grey closed circles) served as non-IL-15 incorporating test article for comparison purposes. Recombinant human IL-15 (black closed squares) served as the positive control. The rank order of potency for the IL-15 variants (fused to nivolumab) was observed to be FUSE696 > FUSE765 (open hexagon) > FUSE773 (closed triangle) > FUSE774 (closed inverted triangle) and all induced a greater magnitude of IFNγ release than FUSE691 (nivolumab). This indicates that targeting of IL-15 to PD-1 via attachment of IL-15 to nivolumab enhances the capacity nivolumab to activate T cells. That FUSE773 and FUSE774 were appreciably attenuated relative to recombinant human IL-15 (>30 fold decrease in potency [EC50 IFNγ release] and up to 50% reduced maximum IFNγ release) is consistent with the data obtained using HEK Blue IL-2 in FIG. 18. Summary tables of maximum activity (Emax), EC50 and AUC (area under the curve) is shown beneath each x-y scatterplot.

Example 17

[0481] As shown in figure 19, we assessed the capacity of a series of IL-15 variants to activate purified human T cells in presence of allogeneic dendritic cells (DC). That is, T cells were isolated from a healthy human donor distinct from a second healthy donor from which DC were immobilized from PBMC using GM-CSF and IL-4. IFNγ release after 96 hours of co-culture was used as the readout of T cell activation. FIG. 16A is an x-y scatterplot depicting the concentration of IFNγ in the culture supernatant versus the concentration of test article. Unless otherwise noted, all IL-15 variants were fused to the C-term knob of a knobs into holes version of nivolumab. The Hen Egg Lysozyme (HEL) specific antibody served as the negative control (grey closed circles). Nivolumab (FUSE691; grey closed circles) served as non-IL-15 incorporating test article for comparison purposes. Recombinant human IL-15 (black closed squares) served as the positive control. The rank order of potency for the IL-15 variants (fused to nivolumab) was observed to be FUSE696 (open triangle) > FUSE691 (nivolumab). This indicates that targeting of IL-15 to PD-1 via attachment of IL-15 to nivolumab enhances the capacity nivolumab to activate T cells and is consistent with the data obtained using Pelicluster CD3 FIG. 19A. A summary table of maximum activity (Emax), EC50 and AUC (area under the curve) is shown beneath the x-y scatterplot.

Example 18

Human peripheral blood mononuclear cell (PBMC) isolation

[0482] PBMCs were isolated from whole blood from healthy donors using Ficoll-Paque Plus medium. In brief, 35 ML of diluted whole blood (1 volume of whole blood vs 1 volume of PBS) was gently overlayed on top of 15 ML Ficoll-Paque Plus medium without disturbing the interface in a 50-ML conical tube. After centrifuging for 40 minutes at 400 × g at room temperature without brake, the buffy coat (interface layer between Ficoll and serum) was collected and diluted in 5 volumes of PBS. After centrifuging for 5 minutes at 500 × g at room temperature, PBMCs were resuspended in PBS and washed once in PBS by centrifuging for 5 minutes at 500 × g at room temperature. PBMCs were then resuspended in 5 ML of ACK lysis buffer and incubated for 5 minutes at room temperature to remove red blood cell residues. After the 5-minute incubation period, 45 ML PBS was added to PBMCs and centrifuged for 15 minutes at 100 × g at room temperature. At last, PBMCs were resuspended in culture medium (RPMI1640 with 10% heat-inactivated FBS and 1% penicillin/streptomycin) for cytotoxicity and IFN gamma release assay set-up. To store PBMCs for further usage, PBMCs were pelleted down by centrifuging for 5 minutes at 500 × g at room temperature and then resuspended in freezing medium (90% heat-inactivated FBS and 10% dimethyl sulfoxide (DMSO)) for storage in liquid nitrogen.

T cell isolation

[0483] Fresh PBMCs were washed twice in PBS and resuspended in EasySep Buffer from StemCell at the density of 50 million cells per ML in 50-ML conical tube. 50 UL of isolation cocktail from T cell isolation kit from StemCell was added to 1 ML of cell suspension and incubated at room temperature for 5 minutes. After the 5-minutes' incubation, 50 UL of RapidSpheres from T cell isolation kit was added to 1 ML of the cell/antibody cocktail mixture. The total volume of the cell/cocktail/bead mixture was brought up to 25 or 50 ML with EasySep Buffer and incubated at room temperature for 10 minutes on EASYSEP magnet. After the 10-minutes' incubation, the solution was transferred to a new 50-ML tube by keeping the tube on the magnet and incubated at room temperature for 5 minutes on EASYSEP magnet. The solution with purified T cell was collected. Freshly purified T cells were frozen down and stored in liquid nitrogen for further usage.

Dendritic cell generation

[0484] Fresh PBMCs were resuspended in AIM V medium at the density of 5 million per ml and 40 ML of PBMC suspension was added to a T-174 sterile cell culture flask. The flasks were incubated in a humidified incubator maintained at 37°C and 5% CO_2 for 2 hours. After 2 hours' incubation, the non-adherent cells were removed by firmly tapping the flask and completely aspirating off media in the flask. 40 ML of AIM-V medium was immediately added to the adherent cells with 800 U/ml GM-CSF and 500 U/ml IL-4 to each T-150 cell culture flask containing the adherent monocytes. The flasks were placed in a humidified incubator at 37°C and 5% CO_2 for 7 days. After the 7-day culture period, DCs were harvested, frozen down and stored in liquid nitrogen for further usage.

HEK-Blue-IL-2 cell activation assay with FUSE proteins

[0485] HEK-BLUE-IL-2cells from InvivoGen were maintained in culture medium (DMEM medium with 4.5 g/L glucose, 2 MM L-Glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ML penicillin, 100

μg/ML streptomycin, 100 μg/ML Normocin, 1 μg/ML puromycin and 1× HEK-blue CRLreagent). HEK-BLUE™ IL-2 cells are engineered from the human embryonic kidney 293 (HEK293) cell line to stably express genes encoding the CD25 (aka IL-2Rα), CD122 (IL-2Rβ), and CD132 (IL-2Rγ) and express a Signal transducer and activator of transcription 5 (STAT5)-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene, therefore being useful for detection of bioactive IL-2 or IL-15 by monitoring the activation of the STAT5 pathways via quantification in the supernatant of the SEAP level (which is produced upon activation of STAT5) with a solution such as QUANTI-BLUE™ Solution.

[0486] On the day of experiment setup, HEK-BLUE-IL-2 cells were gently rinsed twice with pre-warmed phosphate buffered saline (PBS) and then detached in PBS by tapping the flask. Detached HEK-Blue-IL-2 cells were resuspended in pre-warmed testing medium (DMEM with 4.5 g/L glucose, 2 MM L-Glutamine, 10% (v/v) heat-inactivated FBS, 100 U/ML penicillin, and 100 μg/ML streptomycin) at the density of 3×10^5 cell per ML. To seed the cells (50,000 cells per well), 180 ML of resuspended HEK-Blue-IL-2 cells were added to designated wells in 96-well plate.

[0487] For FUSE protein activity test, FUSE proteins were diluted in testing medium (DMEM with 4.5 g/L glucose, 2 MM L-Glutamine, 10% (v/v) heat-inactivated FBS, 100 U/ML penicillin, and 100 μg/ML streptomycin) from 1,600 NM to 0.8 PM (10× of final concentration) by 8-fold serial dilution. IL-15 was also diluted in testing medium from 10 NM to 0.3 PM (10× of final concentration) by 8-fold serial dilution. To treat HEK-Blue-IL-2 cells, 20 ML of prepared IL-15 or FUSE proteins were added to the designated wells with 50,000 cells in the 96-well plate and then gently mixed the cells with proteins. The 96-well plate was then incubated at 37°C with 5% CO_2 for 24 hours.

After the 24-hour activation incubation period, 20 ML of HEK-Blue-IL-2 cell culture supernatant containing the secreted alkaline phosphatase was transferred to a new 96-well plate. Meanwhile, QUANTI-Blue solution was prepared by adding 1 ML of QB reagent and 1 ML of QB buffer to 98 ML of sterile water. 80 ML of the prepared QUANTI-Blue solution was then added to the 96-well plate with 20 ML of HEK-Blue-IL-2 cell culture supernatant and incubated at 37°C for 4 hours. The level of secreted alkaline phosphatase related to HEK-Blue-IL-2 cell activation was detected by measuring the absorbance at 630 nm using a spectrophotometer. **T cell and dendritic cell co-culture(MLR) assay**

[0488] Dendritic cells were generated from monocytes in PBMCs with 800 U/ml GM-CSF and 500 U/ml IL-4. Allogeneic T cells from PBMCs were mixed with dendritic cells at the ratio of 10:1 in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin. 100K T cells with 10K dendritic cells in 150 UL of medium were added to the wells in 96-well plate. Fuse proteins were prepared and serially diluted (5-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 NM to 2.56 PM. 150 UL of prepared FUSE proteins at different concentrations were then added to the designated wells in the 96-well plate with T cell and dendritic cells and incubated for 72 hours at 37 °C with 5% CO_2. At the end of the incubation period, the 96-well plates were

centrifuged for 1 minute at 500 ×g to transfer 250ML of supernatant to V-bottom storage plate using a multichannel pipettor for IFN gamma release detection.

Pelicuster-CD3 antibody-primed T cell activation assay

[0489] T cells were resuspended in RPMI1640 medium with 10% FBS, 1% penicillin/streptomycin and 40 ng/ML of mouse anti-human CD3 antibody clone CLB-T3/4.E 1XE (pelicluster CD3) at the density of 0.667 million per ML. 100K T cells in 150 UL of medium were added to the wells in 96-well plate. Fuse proteins or IL-15 were prepared and serially diluted (5-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 NM to 2.56 PM. 150 UL of prepared FUSE proteins at different concentrations were then added to the designated wells in the 96-well plate with T cell and incubated for 72 hours at 37 °C with 5% CO_2. At the end of the incubation period, the 96-well plates were centrifuged for 1 minute at 500 ×g to transfer 250ML of supernatant to V-bottom storage plate using a multichannel pipettor for IFN gamma release detection.

IFN gamma release detection

[0490] IFN gamma release from different assays was measured using human IFN gamma ELISA detection kit. In brief, coating antibody provided in the kit was diluted to suggested concentration by following the protocol provided by the kit manufacturer. 100 ul of diluted coating antibody was added to the Nunc MaxiSorp flat-bottom 96-well plate. Plates were sealed and incubated overnight at 4 °C. On the following day, the plates were then washed 4 times with the Wash Buffer. To block non-specific binding and reduce background, 200 ML 1× Assay Diluent A was added, and the plates were incubated at room temperature for 1 hour on a plate shaker (400 rpm). After blocking, the plates were washed 4 times with Wash Buffer. 100 ML/well of standards (prepared with culture medium) or samples were then added to the appropriate wells and incubated at room temperature for 2 hours on a plate shaker (400 rpm). After the 2-hour incubation with samples or standards, the plates were washed 4 times with Wash Buffer and 100 ML of diluted Detection Antibody solution was added to incubate at room temperature for 1 hour on a plate shaker (400 rpm). After the 1-hour incubation period with detection antibody, the plates were washed 4 times with Wash Buffer and 100 ML of diluted Avidin-HRP solution was added to incubate at room temperature for 30 minutes on a plate shaker (400 rpm). After 30-minute incubation with Avidin-HRP, the plates were washed 5 times with Wash Buffer and 100 ML of freshly mixed TMB Substrate Solution was added to incubate at room temperature for 20 minutes in the dark. 100 ML of Stop Solution was then added to each well to stop the reaction. The absorbance at 450 nm was measured on a plate reader and the concentration of IFN gamma in each sample was back-calculated using the standard curve generated with the absorbance of different concentration of standards.

IL-18 EXAMPLES

Example 29

[0491] Figure 20 shows exemplary illustrations of pro-IL-18 fusion proteins (FIGs 20B-20C, 20E-20F, 20I-20J, 20L-20M) and control fusion proteins (without pro-IL-18; FIGs 20A, 20D, 20G-H, 20K). All proteins incorporate a human IgG scaffold that is either wild type homodimeric IgG1 as in FIGs 20D, 20G and 20J or comprising a knob-in-hole (KIH) heterodimeric IgG1 Fc (FIGs 20A-20C, 20E-20F, 20H-20I, 20J-20M). Unless otherwise noted, all IgG1 Fc domains retain wild type capacity to bind Fc γ Rs and can therefore induce effector mediated Antibody Dependent Cellular Cytotoxicity (ADCC) and Antibody Dependent Cellular Phagocytosis (ADCP). Pro-IL-18, when included in the fusion protein is fused as (a) a singular cassette to either the C-terminal end of the knob (or hole) chain (FIGs 20B, 20E, 20I and 20L) or (b) as singular cassettes to both the C-terminal ends of the knob and hole chains (FIGs 20C, 20F, 20J and 1M). Cleavage sites, when present in the pro-IL-18 cassette, are indicated as a star shape. A tumor associated antigen binding domain (TAA), when present, is fused as a single cassette to the N-terminal end of a knob (or hole) chain (FIGs 20A, 20B, 20C, 20K-20M), or both chains (FIGs 20D-20J) of the KIH. The TAA binding domain may be a VHH (e.g. EGFR specific clone 9G8; FIGs 20A-20F) or a fab (e.g. EGFR specific clone C225 from cetuximab; FIGs 20G-20M).

[0492] In some embodiments, the N-terminal end of the CH1 domain of a knobs into holes (KIH) heterodimeric IgG1 protein was fused to the C-terminal end of an anti-EGFR VHH or fab. The N-terminal of the KIH protein consisted of (a) a single a VHH on the N-terminal HC knob (FIG. 20A), (b) a single fab on the N-terminal HC knob (FIG. 20K) or (c) a fab on the N-terminal HC knob and HC hole (FIG. 20H). In some embodiments, the N-terminal end of the CH1 domain of a homodimeric IgG1 protein was fused to the C-terminal end of a VHH or fab. In some embodiments, the N-terminal of the IgG1 protein consisted of (a) a VHH on both N-terminal HCs (FIG. 20D) and (b) a fab on both N-terminal HCs (FIG. 20G). In some embodiments, the N-terminal of one pro-IL-18mut2AV was fused to the C-terminal of the CH3 domain of the knob-heavy chain (HC) of a knobs into holes (KIH) heterodimeric IgG1 protein. The N-terminal of the Fc fusions consisted of (a) a VHH clone on the N-terminal HC knob (FIG. 20B), (b) a single fab on the N-terminal HC knob (FIG. 20L) or (c) a VHH on the N-terminal HC knob and on the HC hole (FIG. 20E) or (d) a fab on the N-terminal HC knob and HC hole (FIG. 20I). In all cases, a cleavage site, when present (e.g. the Granzyme B cleavage site, IEQD), was inserted within the IL-18 pro-peptide in place of the endogenous Caspase 1 cleavage site. In some embodiments, the N-terminal of pro-IL-18mut2AV was fused to the C-terminal of the CH3 domain of the HC knob and HC hole of a knobs into holes (KIH) heterodimeric IgG1 protein. The N-terminal of the Fc fusions consisted of (a) a single VHH on the N-terminal HC knob (FIG. 20C) and (b) a single fab on the N-terminal HC knob (FIG. 20M). In all cases, a cleavage site, when present, was inserted within the IL-18 pro-peptide. In some embodiments, the N-terminal of pro-IL-18mut2AV was fused to the C-terminal of the CH3 domain of the homodimeric IgG1 protein. The N-terminal of the Fc fusions consisted of (a) a VHH on both N-terminal HCs or (b) a fab on both N-terminal HCs (FIG. 20M). In all cases, a cleavage site, when present, was inserted within the IL-18 pro-peptide.

Example 20

[0493] As shown in figure 21, we assessed the capacity of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), and FUSE686 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18) to induce (1) PBMC mediated Antibody Dependent Cellular Cytotoxicity (ADCC) of the EGFR+ cell line, MDA-MB-231 (FIG. 21A) and (2) PBMC mediated IFNγ release (FIG. 21B). For FUSE686, pro-IL-18 was fused to the C-terminus FUSE556. Pro-IL-18 incorporates a mutant propeptide upstream of IL-18 to mask/attenuate IL-18 activity. We demonstrated herein that such a propeptide variant masking motif vastly reduced the trans activity of the protein, in some cases by >100,000 fold, compared to wild type IL-18.

[0494] To determine the capacity of pro-IL-18 to augment EGFR dependent ADCC/ IFNγ release when delivered to both EGFR (tumor cells) and CD16+ cells (NK cells) as a fusion protein, we mixed freshly isolated PBMC with EGFR+ MDA-MB-231 engineered to express firefly luciferase for 48 hours in the presence of either FUSE556 (black closed circles), FUSE686 (black open circles, dashed line), or the negative control Hen Egg Lysozyme specific antibody (anti-HEL; grey closed circles). The PBMC: tumor target ratio was 10:1. However, since NK cells mediate ADCC and are found at a mean frequency of about 10%, the effective NK: tumor target ratio was approximately 1:1. FUSE686 was more potent (EC50-killing) than FUSE556 (>350 fold) and induced a higher maximum killing (~40% higher). Summary tables of potency and maximum activity for the test articles are shown below each graph. With relation to IFNγ release, FUSE686 was more potent (EC50- IFNγ release) than FUSE556 (>300 fold) and induced a higher maximum IFNγ release (~3.5 fold higher). Given that pro-IL-18 was previously found to have little to no activity when non-targeted (trans-activity), we interpret the results as indicating that pro-IL-18, when targeted to CD16 via wild type IgG1, can bind to the IL-18R complex when bound in close proximity to it (i.e. CD16 on the same cell). Such targeted/proximity related activity has been previously terms as occurring in cis.

Example 21

[0495] As shown in figure 22, we assessed the capacity of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1; targets both EGFR and FcγR), and the combination (1:1, molar equivalence) of FUSE566 and FUSE422 (IgG1-pro-IL-18; only targets FcγR) to induce (1) PBMC mediated Antibody Dependent Cellular Cytotoxicity (ADCC) of the EGFR+ cell line, MDA-MB-231 (FIG. 22A) and (2) PBMC mediated IFNγ release (FIG. 22B). For FUSE422, pro-IL-18 was fused to the C-terminus of the Fc domain of IgG1. FUSE422 is shown herein to be >100,000 fold attenuated in trans compared to wild type IL-18.

[0496] To assess the capacity of pro-IL-18 to augment EGFR dependent ADCC/IFNγ release induced by FUSE566 when co-delivered to CD16+ cells (NK cells) as Fc-pro-IL-18 (FUSE422), we mixed freshly isolated PBMC with EGFR+ MDA-MB-231 engineered to express firefly luciferase for 48 hours in the presence of either FUSE556 (black closed circles), the 1:1 molar combination of FUSE556 and FUSE422 (black open circles, dashed line), FUSE422 alone (black open boxes; dashed line) or the negative control Hen Egg Lysozyme specific antibody (anti-

HEL; grey closed circles). The PBMC: tumor target ratio was 30:1. However, since NK cells mediate ADCC and are found at a mean frequency of about 10%, the effective NK: tumor target ratio was approximately 3:1.

[0497] The combination of FUSE556 and FUSE422 was more potent (EC50-killing) than FUSE556 (>250 fold) and induced a higher maximum killing (~100% higher). Summary tables of potency and maximum activity for the test articles are shown below each graph. Neither FUSE422 nor anti-HEL induced appreciable PBMC (NK) mediated killing of MDA-MB-231. FUSE422 induced a small degree of killing (<5% maximum killing relative to the combination of FUSE556 and FUSE422). This was likely due to targeting of pro-IL-18 to "allo-reactive" NK cells such that the small degree of killing was dependent on "lack of self" and/or expression of ligands for activation receptors such as NKG2D. Indeed, MDA-MB-231 has been reported to express MIC-B, a ligand for NKG2D (see e.g., onlinelibrary.wiley.com/doi/full/10.1002/ijc.28174; www.nature.com/articles/bjc201779; link.springer.com/article/10.1007/s12192-014-0532-5)

[0498] Results related to IFNγ release were similar to cytotoxicity except that FUSE422 induced no cytokine release over background or the negative control anti-HEL antibody. The combination of FUSE556 and FUSE422 was more potent (EC50-killing) than FUSE556 (>150 fold) and induced a higher maximum killing (~700% higher). Summary tables of potency and maximum activity for the test articles are shown below each graph.

[0499] Taken together with the results from FIG.21, our data indicates that pro-IL-18, when targeted to CD16 via wild type IgG1, either as an Fc fusion alone (no intrinsic ADCC capacity) or an EGFR targeted Fc fusion (binds both CD16 and EGFR to induce ADCC), can bind to and activate the IL-18R complex in cis.

Example 22

[0500] As shown in figure 23, we assessed the impact of targeting pro-IL-18 to PD1 on the activation of purified T cells from a healthy human donor when mixed with DC from a second healthy human donor at a T:DC ratio of 10:1. IFNγ release at 72 hours of co-culture was used as the readout of activation. DC were differentiated in-vitro from PBMC using GM-CSF and IL-4 for 7 days. The following proteins were tested: Human recombinant IL-18 (positive control; black closed stars), FUSE691 (nivolumab; black closed circles), FUSE645 (EGFR targeted IgG1-pro-IL-18; non-targeted in this system; gray closed circles-dashed line) and FUSE694 (nivolumab-pro-IL-18; black open circles-dashed line). Consistent with our finding using the HEK-Blue-IL-18 reporter cell line as described herein, trans activating pro-IL-18 used in the non targeted FUSE645 was vastly attenuated relative to human recombinant IL-18 both as a degree of potency (~1000-fold difference in EC50- IFNγ release) and maximum IFNγ release (~10 fold). Targeting pro-IL-18 to PD1 via fusion of pro-IL-18 to the C-terminus of nivolumab (FUSE694) resulted in about a 2-fold and 20-fold increase in potency relative to FUSE645 and FUSE691 (nivolumab), respectively. Importantly, maximum T cell mediated IFNγ release induced by FUSE694 increased by 7-fold and 3.5-fold relative to FUSE645 and FUSE691 (nivolumab), respectively. Summary tables of potency and maximum activity for the test articles are shown below each graph. This data indicates that although pro-IL-18 is largely non-functional when non-targeted (i.e., acting in trans),

when delivered to PD1 and hence placed in close proximity to the IL-18R complex expressed by PD1+ T cells augments IFNγ release, our measure of T cell activation in this system. As described previously for targeting of CD16, the data is supportive of pro-IL-18 acting is cis when targeted to T cells co-expressing PD1 and the IL-18R complex. Given that PD1+ T cells within TIL have been reported to constitute a major fraction of the tumor specific T cells within tumors, PD1 dependent cis activity related to pro-IL-18 may promote the cytotoxic potential, proliferation, survival and memory formation of tumor specific T cells without the indiscriminate activation of other cells that may contribute to systemic toxicity.

Example 23

[0501] As shown in figure 24, we assessed the capacity of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), and FUSE516 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18) to induce PBMC mediated Antibody Dependent Cellular Cytotoxicity (ADCC) of the EGFR+ cell line, MDA-MB-231. For FUSE516, pro-IL-18 included the Granzyme B cleavage site, IEQD, within the pro-peptide such that in addition to the CD16 targeted cis related activity of pro-IL-18, exposure to Granzyme B (released by NK cells mediating ADCC) would release the active mature form (18 kDa) of IL-18. Freshly isolated PBMC were mixed together with MDA-MB-231 engineered to express firefly luciferase for 48 hours in the presence of either FUSE556 (black closed circles), FUSE516 (black open circles, dashed line), FUSE442 (non targeted IgG1-pro-IL-18 control; grey closed circles) or the negative control Hen Egg Lysozyme specific antibody (anti-HEL; grey closed circles). The assay was repeated with PBMC from four different normal human donors as indicated in FIGs 24A-24D. The PBMC: tumor target ratio was 30:1. However, since NK cells mediate ADCC and are found at a mean frequency of about 10%, the effective NK: tumor target ratio was approximately 3:1. For all donors, FUSE516 was more potent (EC50-killing) than FUSE556 (ranging from ~100 fold to >1000 fold) and induced a higher maximum killing (ranging from about 30% to 800% higher). Summary tables of potency and maximum activity for the test articles are shown below each graph.

Example 24

[0502] As shown in figure 25, we assessed the capacity of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1; black closed circles), and FUSE516 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18; black open circles, dashed line) to induce several readouts mediated by freshly isolated PBMC in co-culture with the EGFR+ cell line, MDA-MB-231 after 48 hours of co-culture at an E:T of 30:1. This corresponds to an effective NK: tumor target ratio of approximately 3:1. FUSE442 (non targeted IgG1-pro-IL-18 control; grey closed circles) and the anti-HEL (grey closed circles) were used as negative controls. The functional readouts were as follows: (a) ADCC (FIG. 25A), IFNγ release (FIG. 25B), Granzyme B release (FIG. 25C), and IL-18 release/consumption (FIG. 25D). We observed potent PBMC mediated killing and IFNγ release induced by FUSE516. For ADCC, IFNγ release and Granzyme B release, the potency (EC50-killing, EC50-IFNγ release and EC50-Granzyme B release, respectively) associated with FUSE516 was

>200-fold, >50-fold, ~50-fold greater than FUSE556. Maximum induction for each readout was about 100% (ADCC), 500% (IFNγ) and 250% (Granzyme B) for FUSE516 versus FUSE556. Summary tables of potency and maximum activity for the test articles are shown below each graph. The non-targeted pro-IL-18 control, FUSE442, induced very minimal killing of target cells (EC50-killing >25 NM and max killing <6%), which was independent of ADCC and likely related to the recognition of ligands for NK activation receptor(s) known to be expressed on MDA-MB-231 such as MICA/B (Shen et al., Cell Death and Disease. 2017) or minor degree of T cell mediated allo-reactivity.

[0503] We also measured the magnitude of IL-18 released into the supernatant of the assay described above. Here, the IL-18 reporter cell line, HEK-Blue-IL-18, was exposed to assay supernatants and SEAP activity measured as a surrogate for IL-18 activity. FIG. 25D illustrates the results such that FUSE516 (black open circles, dashed line) that incorporates a Granzyme B cleavage site between its Fc domain and IL-18 but not FUSE556 (black closed circles) released IL-18 is a dose dependent manner in co-culture with PBMC and MDA-MB-231. To estimate the degree of IL-18 consumed by NK cells during the ADCC process, we added rhIL-18 to the PBMC/tumor cell/FUSE556 co-culture (grey closed circles). Here we observed a FUSE556 dose dependent decrease in IL-18 in the assay supernatant. Given that FUSE516 induced strong tumor cell killing and IFNγ release, we interpret that data as indicating that NK cells exposed to FUSE516 consume more IL-18 than those exposed to FUSE556. As such, the magnitude of IL-18 estimated to be released by FUSE516 in FIG. 24D is likely underestimated.

Example 25

[0504] As shown in figure 26, we assessed the capacity of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1), and variants of pro-IL-18 fused to the C-terminus of FUSE566 with different protease cleavage sites between the Fc domain and pro-IL-18 to induce PBMC mediated Antibody Dependent Cellular Cytotoxicity (ADCC) of the EGFR+ cell line, MDA-MB-231. The different protease sites tested in FIG. 26A were Granzyme A (FUSE658), Granzyme B (FUSE516), MMP2/9 (FUSE659), and the combination of Granzyme A, Granzyme B and MMP2/9 (FUSE660). Granzyme A and B would be expected to be released by NK cells mediating ADCC whereas MMP2 has been reported as expressed/secreted by MDA-MB-231 (www.ncbi.nlm.nih.gov/pmc/articles/PMC5661385). Also tested was FUSE627 that contains the PGLALA mutation in the Fc domain to abolish targeting to FcγRs (FIG. 26B) and hence abrogate bridging between FcγR+ NK cells and EGFR+ tumor cells, which is the driver of ADCC in the reported system.

[0505] To assess the impact of the aforementioned protease cleavage sites on the capacity of pro-IL-18 to augment EGFR dependent ADCC, we mixed freshly isolated PBMC with EGFR+ MDA-MB-231 cells engineered to express firefly luciferase for 48 hours in the presence of either FUSE556 (black closed circles; FIGs 26A-26B), FUSE658 (black open diamonds, dashed line; FIG. 26A), FUSE516 (black open boxes; dashed line; FIGs 26A-26B), FUSE659 (black open triangles; dashed lines; FIG. 26A), FUSE660 (black closed stars, dashed lines; FIG. 26A), or the negative control Hen Egg Lysozyme specific antibody (anti-HEL; grey squares). The PBMC: tumor target ratio was

30:1 (FIG. 26A) or 10:1 (FIG. 26B). Since NK cells mediate ADCC and are found at a mean frequency of about 10%, the effective NK: tumor target ratio was approximately 3:1 (FIG. 26A) and 1:1 (FIG. 26B).

[0506] As seen in FIG. 26A, we found that the rank order of killing potency as it relates to cleavage site(s) was (1) the combination of Granzyme A, Granzyme B and MMP2/9 (FUSE660), which was about 2-fold greater than both (2) Granzyme B (FUSE516) and (3) MMP2/9 (FUSE659), while it was about 3-fold greater than (4) Granzyme A (FUSE658). The rank order as it relates to maximum killing was the same except that Granzyme B (FUSE516) was greater than MMP2/9 (FUSE659). In each case, the difference in maximum killing between each test article was about 10%. That the combination of cleavage sites for all three of Granzyme A, Granzyme B and MMP2/9 was superior to any single cleavage site alone is consistent with the inducible trans activity of pro-IL-18. To semi-quantitate the degree of IL-18 dependent tumor cell killing mediated in trans in the absence of effector to tumor bridging, we compared FUSE516 to FUSE627 (FIG. 26B). FUSE627 is identical to FUSE516 except it contains the PGLALA mutation reported to abrogate binding of IgG1 to FcγRs pubmed.ncbi.nlm.nih.gov/27578889. As such, the pro-IL-18 domain in FUSE627 is not targeted to FcγRs expressed on NK cells and does not act in cis. Further, FUSE627 cannot bridge FcγRs expressed on NK cells to EGFR expressed on MDA-MB-231. Any activity associated with FUSE627 (gray open inverted triangles) would be interpreted as stemming from the trans activity of either pro-IL-18 or Granzyme B released mature IL-18. Such release of Granzyme B would likely result from the interaction of "allo-reactive" NK cells with MDA-MB-231 tumor cells that (a) do not express HLA molecules to which the NK cells were educated and/or (b) express MIC-B, a ligand for activation receptor, NKG2D; www.nature.com/articles/bjc201779; link.springer.com/article/10.1007/s12192-014-0532-5). Alloreactivity dependent release of Granzyme B would then be expected to release mature IL-18 and in turn augments NK mediated killing of the allogeneic target, MDA-MB-231

[0507] Unless otherwise specified, in the context of an IL-18 fusion protein disclosed in this invention, any interaction between IL-18 and its ligand, the IL-18 receptor complex, that is not dependent on a targeting moiety to facilitate the interaction between IL-18 and its receptor at close proximity, is considered a trans interaction; and in contrast, any interaction between IL-18 within an IL-18 fusion protein and its receptor that requires the targeting moiety also within the fusion protein to facilitate an interaction between IL-18 and its receptor is considered as occurring in cis. In such a cis based interaction, the targeted molecule (e.g., PD-1) is expressed on the same cell as the IL-18R complex (e.g. an NK cell). Generally speaking, in cellular biology, the term "cis" refers to the interaction between two molecules such as proteins expressed on the same cell. For example the interaction between B7-1 and PD-L1 on T cells is a cis interaction. For multispecific proteins, those that bind two proteins on the same cell simultaneously are also referred to as having a cis interaction. In cellular biology, the term "trans" refers to the interaction between two molecules such as proteins on different cells. For example, the interaction between the prototypical interaction of the T cell Receptor (TCR) and its ligand, an MHC-I/peptide complex, is a trans interaction. For multispecific proteins, those that bind one protein on one cell and a second protein on a separate cell, i.e., forming a "bridge" between the two cells, would be considered as occurring in trans. Further, interaction between a soluble protein and a cell surface protein, although not

forming a cellular bridge, is accepted to fall within the definition of a trans interaction. In the therapeutics landscape, soluble proteins that naturally interact with their ligand in trans are often engineered to be of such low affinity that they no longer bind their ligand unless brought into close proximity of it via a targeting moiety that binds another protein on the same cell. This scenario allows for cell targeted activity of the soluble protein and is categorized as a cis interaction.

[0508] As previously observed, FUSE516 was more potent (EC50-killing) than FUSE556 (~10 fold) and induced a higher maximum killing (~15% higher). In contrast, FUSE627 was observed to induce very weak killing consistent with augmentation of "background" alloreactive cytotoxicity that results in the of small amounts of Granzyme B into the media, which can then release fully active IL-18 from FUSE627. Summary tables of potency and maximum activity for the test articles are shown below each graph.

Example 26

[0509] As shown in figure 27, we assessed the capacity of FUSE556 (single armed anti-EGFR VHH 9G8-IgG1; black closed circles), and FUSE516 (single armed anti-EGFR VHH 9G8-IgG1-pro-IL-18; black open circles, dashed line) to induce two readouts (ADCC and IFNγ release) mediated by expanded NK cells in co-culture with the EGFR+ cell line, MDA-MB-231 after 48 hours of co-culture at E:T ratios of 1:1 and 1:5. An E:T ratio of 5:1 was also tested, and yielded similar results to 1:1 and is not shown. The anti-HEL antibody (grey closed circles) was used as the negative control and no functional activity was observed with this protein. The functional readouts were as follows: (a) ADCC (FIGs 27A and 27B) and IFNγ release (FIGs 27C and 27D). Effector populations of NK cells were expanded from PBMC isolated from two distinct healthy donors. The data shown is calculated based on the average of the two donors.

[0510] At both E:T ratios, FUSE516 was more potent than FUSE556. For ADCC, (a) the difference in potency between FUSE516 and FUSE556 was approximately 60-fold and (b) the difference in maximum killing between FUSE516 and FUSE556 ranged between 30-50%.

[0511] For IFNγ release, the difference in (a) potency between FUSE516 and FUSE556 ranged between 40-fold and >130-fold and may be modesty inversely related to E:T and (b) maximum IFNγ release between FUSE516 and FUSE556 ranged between 250% and 380%. Summary tables of potency and maximum activity for the test articles are shown below each graph. FUSE516 is depicted as open circles and FUSE556 as closed squares. The EC50 values for killing and IFNγ release obtained using an E:T ratio of 5:1 are included in this analysis. This data generated using expanded NK cells of >95% purity (data not shown) is highly similar to that observed with PBMC as effector cells (see FIG. 25), thus providing support that the effector cells within PBMC mediating killing of tumor targets and releasing IFNγ are NK cells. The difference in the magnitude of IFNγ release between FUSE516 and FUSE566 is impressive and suggests that FUSE516 may promote enhanced anti-tumor activity not only via ADCC but also via the promotion of innate and adaptive immunity including tumor antigen presentation and bystander killing.

Example 27

[0512] As shown in figure 28, we assessed the capacity of FUSE555 (dual armed anti-EGFR Fab C225-IgG1-pro-IL-18; black open circles, dashed line), and cetuximab (black closed squares) to induce two readouts (ADCC and IFNγ release) mediated by freshly isolated PBMC in co-culture with the EGFR+ cell line, MDA-MB-231 after 40 hours of co-culture at an E:T ratio of 2:1. The anti-HEL antibody (grey closed circles) was used as the negative control and no functional activity was observed with this protein. The functional readouts were as follows: (a) ADCC (FIG. 28A) and IFNγ release (FIG. 28B). Effector populations of NK cells were expanded from PBMC isolated from two distinct healthy donors. The data shown is calculated based on the average of the two donors.

[0513] For ADCC, (a) the difference in potency between FUSE555 was approximately 3-fold more potent than Cetuximab and (b) maximum killing induced by FUSE555 was about 40% greater than Cetuximab.

[0514] For IFNγ release (see FIG. 28B), the difference in (a) potency between FUSE555 and Cetuximab was about 3-fold and (b) maximum IFNγ release associated with FUSE555 was about 200% higher relative to cetuximab. FUSE555 is depicted as open circles and Cetuximab as closed squares. Summary tables of potency and maximum activity for the test articles are shown below each graph. The difference in the magnitude of IFNγ release between FUSE555 and Cetuximab is highly appreciable. Since IFNγ can impact anti-tumor activity by promoting innate and adaptive immune responses, we potentially consider FUSE555 a far superior therapeutic compared to Cetuximab. FUSE555 may promote enhanced anti-tumor activity not only via ADCC but also via the promotion of innate and adaptive immunity including tumor antigen presentation and bystander killing.

Example 28

[0515] As shown in figure 29, we assessed the capacity of FUSE624 (dual armed anti-HER2 Fab IgG1-pro-IL-18 (black open circles, dashed line), and trastuzumab (black closed squares) to induce two readouts (ADCC and IFNγ release) mediated by freshly isolated PBMC in co-culture with the HER2+ cell line, T47D after 24 hours of co-culture at an E:T ratio of 5:1. The anti-HEL antibody (grey closed circles) was used as the negative control and no functional activity was observed with this protein. The functional readouts were as follows: (a) ADCC (FIG. 29A) and IFNγ release (FIG. 29B).

[0516] For ADCC, FUSE624 was (a) approximately 30-fold more potent than trastuzumab and (b) maximum killing was about 2-fold greater versus trastuzumab.

[0517] For IFNγ release, (a) FUSE624 was about 10-fold more potent than trastuzumab and (b) maximum IFNγ release induced by FUSE624 was approximately 16-fold higher than trastuzumab. FUSE624 is depicted as open circles and trastuzumab as closed squares. Summary tables of potency and maximum activity for the test articles are shown below each graph. The difference in the magnitude of IFNγ release between FUSE624 and trastuzumab is very impressive. Since IFNγ can impact anti-tumor activity by promoting innate and adaptive immune responses, we potentially consider FUSE624 a far superior therapeutic compared to trastuzumab. FUSE624 may promote enhanced

anti-tumor activity not only via ADCC but also via the promotion of innate and adaptive immunity including tumor antigen presentation and bystander killing.

Example 29

[0518] As shown in Figure 30, we assessed the capacity of an anti-PDL1 antibody incorporating a pro-IL-18amut2 variant, designed so that it cannot be cleaved by Granzyme B, to induce T cell mediated IFNγ release. In this context, any enhancement of IFNγ release relative to the impact of anti-PDL1 alone (atezolizumab) is interpreted as the result of (1) a cis based interaction between pro-IL-18amut2 and the IL-18R complex expressed on PD-L1+ activated T cells and/or (2) pro-IL-18amut2 decorated on PD-L1+ activated T cells interacting with IL-18R complexes on neighboring IL-18R+ T cells. The assay system used consisted of human T cells preactivated for 72 hours with CD3/CD28 coated beads to upregulate PDL1 (and PD1) expression mixed with MDA-MB-231 (ROR1+) stably expressing firefly luciferase and EgfP at an E:T ratio of 10:1 and 10 PM of a ROR1 x CD3 bsAb (Fuse608). Fuse608 served to create an artificial synapse between the T cells and ROR1+ tumor cells and deliver a suboptimal signal via the TCR/CD3 complex that resulted in the background IFNγ release. 10 PM was calculated as the concentration of Fuse608 to induce 20% maximal IFNγ release. A titration of the following test articles was added to the aforementioned T cell/tumor cell co-culture system and IFNγ release measured after 48 hours: (1) atezolizumab (black closed circles), (2) Fuse882 (open square, dashed line) consisting of a single non-cleavable pro-IL-18amut2 fused to the C-terminal knob of a knobs into holes human IgG1 version of atezolizumab, (3) Fuse895 consisting of a single non-cleavable pro-IL-18amut2 fused to the N-terminal knob of a knobs into holes human IgG1 version of monovalent atezolizumab whereby the anti-PDL1 fab was fused to the N-terminal hole (open circles, dashed line) or (4) an anti-HEL antibody (grey circles) which served as the isotype matched negative control. As shown in the non-linear x-y plot depicting concentration of test article versus the magnitude of IFNγ release, a highly appreciable enhancement of IFNγ release was observed with Fuse895 relative to atezolizumab at the orders of about 4-fold and 3-fold for maximum IFNγ release and the potency (EC50) of IFNγ release, respectively. A summary table of EC50, Emax and AUC (area under the curve) is shown beneath of x-y plot.

Example 30

[0519] As shown in Figure 31A-31B, we assessed the capacity of an anti-PD1 antibody, incorporating one of two pro-IL-18 variants (pro-IL18amut2 or pro-IL18amut9), designed so that it cannot be cleaved by Granzyme B, to induce T cell mediated tumor cell king (FIG. 31A) and IFNγ release (FIG. 31B). In this context, any enhancement of tumor cell killing and IFNγ release relative to the impact of anti-PD1 alone is interpreted as the result of a cis based interaction between pro-IL-18 and the IL-18R complex expressed on PD-1+ activated T cells. The assay system used consisted of purified human T cells mixed with MDA-MB-231 (ROR1+) stably expressing firefly luciferase and EgfP at an E:T ratio of 10:1 and 10 PM of a ROR1 x CD3 bsAb (Fuse608). As described above, Fuse608 served to create an

artificial synapse between the T cells and ROR1+ tumor cells and deliver a suboptimal signal via the TCR/CD3 complex.

[0520] A titration of test articles was added to the aforementioned T cell/tumor cell co-culture system. Tumor cell killing and IFNγ release were measured after 72 hours. The test articles assessed were: (1) anti-PD-1 with pro-IL-18amut2 incorporated on the C-terminal knob (Fuse783; closed square), (2) anti-PD-1 with pro-IL-18amut2 incorporated on the N-terminal hole (Fuse1042; closed reverse triangle), (3) anti-PD-1 with pro-IL-18amut9 incorporated on the N-terminal hole (Fuse1044; closed triangle), (4) anti-PD1 (Fuse1041; open triangle, dashed line) and (5) Fuse1015 (open reverse triangle, dashed line), which was equivalent to Fuse1042 except specific for mouse PD-1 and hence served as the none-targeted pro-IL-18amut2 control. An anti-HEL antibody (grey closed circle) was used as the isotype matched negative control. FIG. 31A is the non-linear x-y plot depicting concentration of test article versus the magnitude of tumor cell killing. Relative to anti-PD-1 alone, the three test articles incorporating pro-IL-18 were observed to induce between 60-80% more killing (based on Emax), with pro-IL-18amut9 being the most potent. FIG. 31B is a non-linear x-y plot depicting concentration of test article versus the magnitude of IFNγ release. In this setting, the three test articles incorporating pro-IL-18 induced stronger IFNγ release relative to anti-PD-1 alone that ranged in enhanced activity between 8-11 fold (based on Emax) and at least 3-10 fold (based on EC50). As observed for tumor cell killing, pro-IL-18amut9 was the most potent.

Example 31

[0521] As shown in figure 32, we assessed the capacity of several IL-18 variants to induce non-targeted trans based release of SEAP from HEK-Blue-IL-18. As previously indicated, HEK-Blue-IL-18 expresses the human IL-18 receptor complex and responds to exposure to IL-18 via the release of SEAP due to the integration of a transgene encoding SEAP downstream of an AP1/NFκB minimal promoter. HEK-Blue-IL-18 were culture with a titration of test articles for 24 hours. The test articles examined were the following IL-18 variants fused to an IgG1-LALA backbone targeted to PD-1 using the paratope from nivolumab: IL18mut2 (Fuse832; open triangle, dashed line), IL18mut2R (Fuse1116; reverse closed triangle), IL18mut13 (Fuse1109; closed square), IL18mut14 (Fuse1110; open circle, dashed line), IL18mut15 (Fuse1111; open square, dashed line) and IL18mut16 (Fuse1112; closed circle). TNFα (closed gray circle) was used as the negative control and recombinant human IL-18 was used as the reference compound (gray star). Based on the non-linear x-y plot of shown, the rank order of IL-18 induced SEAP release was Fuse832>Fuse1116>Fuse1109>Fuse1111/Fuse1112>>>Fuse1110. The human recombinant IL-18 reference exhibited similar activity to Fuse832. A summary table of EC50s for the test articles are shown below the graph.

Example 32

[0522] As shown in figure 33A and 33B, we assessed the capacity of several IL-18 variants targeted to human PD-1 to induce cis based release of IFNγ from human PBMC derived from a healthy human donor (FIG. 33A)

or human T cells (FIG. 33B). For FIG. 33A, PBMC were treated activated with the CD3 specific antibody, pelicluster CD3, for 48 hours to induce upregulation of PD-1 expression. After washing, these PBMC were culture in a titration of test articles in the presence of a suboptimal concentration of pelicluster CD3 and IFNγ released into the supernatant assessed after 72 hours. The test articles examined were the following IL-18 variants fused to an IgG1-LALA backbone targeted to PD-1 using the paratope from nivolumab: IL18mut2 (Fuse1017; reverse open triangle, dashed line), IL18mut2R (Fuse1116; closed reverse triangle), IL18mut13 (Fuse1109; closed square), IL18mut14 (Fuse1110; open circle, dashed line), IL18mut15 (Fuse1111; open square, dashed line) and IL18mut16 (Fuse1112; closed circle). The anti-HEL specific antibody (gray closed circle) was used as the negative isotype matched control and recombinant human IL-18 was added as a non-targeted reference (gray star). Based on the non-linear x-y plot of shown, the rank order of IL-18 induced IFNγ release was Fuse1116 > Fuse1017 >Fuse1109>Fuse1111/Fuse1112>>>Fuse1110. The PD-1 targeted activity of Fuse1116 and Fuse1017 were both greater than the human recombinant IL-18 reference, whereas Fuse1109, Fuse1112 and Fuse1112 were similar in activity to the human recombinant IL-18 reference and Fuse1110, based on the $EC50_{IFN\gamma\text{-release}}$ was >30x weaker than the human recombinant IL-18 reference. A summary table of EC50s and maximum activity for the test articles are shown below the graph. For FIG. 33B, T cells were mixed with MDA-MB-231 engineered to express high cell surface levels of human ROR1 at an E:T of 5:1 in a titration of test articles in the presence of a suboptimal concentration of a ROR1 x CD3 bispecific antibody equivalent to 20% maximal activity (10 pM of Fuse608) and IFNγ released into the supernatant assessed after 72 hours. The test articles examined were the following IL-18 variants fused to an IgG1-LALA backbone targeted to PD-1 using the paratope from nivolumab: IL18mut2 (Fuse1017; reverse open triangle, dashed line), IL18mut9 containing a 10,000 fold attenuating mutant propeptide on the N-terminal of the mature cytokine (Fuse1044; closed reverse triangle), IL18mut2 containing a 10,000 fold attenuating mutant propeptide on the N-terminal of the mature cytokine (Fuse1042; closed square), IL18mut13 containing a 100 fold attenuating polypeptide on the N-terminal of the mature cytokine (Fuse1177; open circle, dashed line), and Fuse691 (nivolumab biosimilar; black closed circle). The anti-HEL specific antibody (gray closed circle) was used as the negative isotype matched control. Based on the non-linear x-y plot of shown, the rank order of IL-18 induced IFNγ release was Fuse1017 >Fuse1044>Fuse1042/Fuse1177>>Fuse691. A summary table of EC50s and maximum activity for the test articles are shown below the graph.

Example 33

[0523] As shown in figure 34A-34B, we assessed the capacity of an exemplary IL-18 variant targeted to mouse PD-1 to mediate tumor growth inhibition of an aggressive syngeneic melanoma tumor in a fully immunocompetent mouse tumor model. C57BL/6 mice were subcutaneously injected with 1x10^6 B16-F10 tumor cells into which we engineered human ROR1 (B16-F10-R) into the right flank. When the tumors reached 75 mm^3, the mice were randomized into 4 groups of 5 mice each and treatment administered intraperitoneally on days 0, 3 and 6 at a dose of 15 mg/kg. The test articles examined were Fuse952 (open circle; recombinant anti-mouse-PD1 clone RMP1.14

human IgG1 LALA), Fuse1113 (open triangle, dashed line; single arm RMP1.14 Fab x IL-18mut2A human IgG1 LALA in a knobs into holes configuration whereby the mouse PD1 specific Fab and IL-18 variant may be on the N-terminal knob and hole, respectively, or vice versa) and Fuse1176 (closed triangle; recombinant anti-mouse-PD1 clone RMP1.14 human IgG1 LALA in which IL-18mut2A has been fused to the N-terminus of the RMP1.14 light chain). PBS was used as the negative control treatment. Figure 34A is x-y plot of tumor volume in mm^3 versus time in days. Figure 34B is x-y plot of body weight in grams versus time in days. Fuse1113 induced about 100% tumor growth inhibition (TGI) and a complete response rate of 60%. Fuse1176 induced about a 75% TGI and a 40% complete response rate. Transient weight loss was observed between days 10 and 15 in the groups receiving Fuse1113 and 1176. This was attributed to an ADA (anti-drug antibody) response, which is not unexpected for a fully human test article in a fully immunocompetent mouse model, which is xenogeneic relative to the test article.

[0524] On day 5 following the initiation of treatment, tumors were excised (n=5 mice per group), single cell suspensions generated and flow cytometry performed using the protocol described by Xu et al. (pubmed.ncbi.nlm.nih.gov/38091375/). As shown in FIG. 34C, the number of different lymphocyte subsets per gram of tumor was assessed from tumor bearing mice treated with PBS (light grey), anti-PD1 (black), and Fuse1113 (horizontal lines). TIL were defined as live mouse CD45+ cells. Within the TIL subset, T cells were defined as CD3+ cells, CD8+ T cells were defined as CD3+ CD8+ cells, CD8+ T effector memory cells (TEM; and effector CD8+ T cells) were defined as CD3+ CD8+ CD62L- CD44+ cells, CD8+ T central memory cells (TCM) were defined as CD3+ CD8+ CD62L+ CD44+ cells, and anti-PD-1 responsive CD8+ T cells (progenitor exhausted CD8+ T cells) were defined as CD3+ CD8+ PD-1+ TIM3- TCF-1+ cells. FIG. 34D depicts the frequency of total myeloid cells (CD11b+ cells), M2 macrophages (CD11b+ F4-80+ CD11c-CD206+ cells) and the ratios of CD8+ T cells to total Myeloid Derived Suppressor Cells (MDSC; CD11b+ Ly6C+ Ly6G- [M-MDSC] and CD11b+ Ly6C-mid Ly6G+ [G-MDSC]) , CD8+ T cells to M2 macrophages, anti-PD-1 responsive CD8+ T cells to MDSC, and CD8+ T cells to CD4+ T cells. Relative to treatment with anti-PD-1 alone, treatment with Fuse1113 results in about a 10 fold increase in T cells, about a 20 fold increase in CD8+ T cells, about a 12 fold increase in CD8+ TEM cells, about a 13 fold increase in CD8+ TCM cells, and about a 20 fold increase in anti-PD-1 responsive CD8+ T cells. Total myeloid cells were decreased by about 50%, the ratio of CD8+ T cells to MDSC was increased by about 7 fold, CD8+ T cells to M2 macrophages was increased by about 14 fold, CD8+ T cells to CD4+ T cells was increased by about 6 fold and anti-PD-1 responsive CD8+ T cells to MDSC was increased by about 6 fold.

Example 34

[0525] As shown in figure 35, we assessed the capacity of an exemplary IL-18 variant targeted to mouse PD-1 to mediate tumor growth inhibition in a syngeneic colorectal cancer tumor in a fully immunocompetent mouse tumor model. Balb/c mice were subcutaneously injected with 3x10^6 CT26 tumor cells into which human Cadherin-17 was engineered (CT26-C) into the right flank. When the tumors reached 75-100 mm^3, the mice were randomized into 4 groups of 5 mice each and treatment administered intraperitoneally on days 0, 4 and 8 at a dose of 15 mg/kg. The test

articles examined were Fuse952 (open circle, dashed line; recombinant anti-mouse-PD1 clone RMP1.14 human IgG1 LALA), Fuse1113 (open triangle, dashed line; single arm RMP1.14 Fab x IL-18mut2A human IgG1 LALA in a knobs into holes configuration whereby the mouse PD1 specific Fab and IL-18 variant may be on the N-terminal knob and hole, respectively, or vice versa) and a combination of Fuse1114 and Fuse952 (closed triangle). Fuse1114 is identical to Fuse1113 expect that it is targeted to human PD-1, which is not present in Balb/c mice and therefore served as the non-targeted Il-18mut2A control. PBS was used as the negative control treatment. Figure 35A is x-y plot of tumor volume in mm^3 versus time in days. Figure 35B is x-y plot of body weight in grams versus time in days. Fuse1113 induced about a complete response rate of 100%. The combination of Fuse1114 and Fuse952 was not appreciably different from Fuse952 alone. No appreciable weight loss was observed across the four groups of mice.

Example 35

[0526] As shown in figure 36A-36B, we assessed the capacity of an exemplary IL-18 variant targeted to mouse PD-1 to mediate tumor growth inhibition in a syngeneic rapidly growing colorectal cancer tumor in a fully immunocompetent mouse tumor model. C57BL/6 mice were subcutaneously injected into the right flank with 1x10^6 tumor cells of a rapidly growing clone of MC38 termed "MC38i". This growth rate of MC38i is about double that of the widely used MC38 tumor model. It was not engineered. When the tumors reached 75-100 mm^3, the mice were randomized into 4 groups of 5 mice each and treatment administered intraperitoneally on days 0, 4 and 8 at a dose of 15 mg/kg. The test articles examined were Fuse952 (open circle, dashed line; recombinant anti-mouse-PD1 clone RMP1.14 human IgG1 LALA), Fuse1113 (open triangle, dashed line; single arm RMP1.14 Fab x IL-18mut2A human IgG1 LALA in a knobs into holes configuration whereby the mouse PD1 specific Fab and IL-18 variant may be on the N-terminal knob and hole, respectively, or vice versa) and a combination of Fuse1114 and Fuse952 (closed triangle). Fuse1114 is identical to Fuse1113 except that it is targeted to human PD-1, which is not present in Balb/c mice and therefore served as the non-targeted Il-18mut2A control. PBS was used as the negative control treatment. Figure 36A is x-y plot of tumor volume in mm^3 versus time in days. Figure 36B is x-y plot of body weight in grams versus time in days. Fuse1113 induced about a complete response rate of 60%. The combination of Fuse1114 and Fuse952 was not appreciably different from Fuse952 alone. Transient weight loss was observed between days 7 and 15 in the group receiving Fuse1113. This was attributed to an ADA (anti-drug antibody) response, which is not unexpected for a fully human test article in a fully immunocompetent mouse model, which is xenogeneic relative to the test article.

Example 36

[0527] We developed a first-in-class variant of IL-18 termed "HT18" fused to an anti-PD-1 antibody (ApD1-HT18cis). HT18 is 10,000-fold attenuated but retains nearly full activity of native cytokine when delivered to PD-1+ cells.

[0528] HT18 did not bind IL-18BP nor IL- 18Rα in trans but can bind strongly to the IL-18Rα/β complex. Targeting HT18 to PD-1 augmented PD-1 induced T cell mediated proinflammatory cytokine release in-vitro.

[0529] Strong efficacy was observed with ApD1-HT18cis in multiple aggressive mouse tumor models including a complete response (CR) rate ranging from 60%-100% without weight loss or other signs/symptoms of toxicity

[0530] Immune memory and evidence supporting epitope spreading was observed in both mouse tumor models that induce both weak and strong emergency myelopoiesis

[0531] TGI was associated with about a 6- fold increase in TIL numbers relative to the PD-1 antibody benchmark with significant increases in CD8+ T cells, effector and central memory T cells, reduced tumor associated macrophages (TAM) and skewing towards a proinflammatory anti-tumor cellularity.

[0532] Some of these findings are shown in figures 37-41.

Example 37

[0001] As shown in Figure 42A and 42B, we assessed the impact of exemplary anti-PD1-pro-IL-18 antibodies containing different IL-18 variants on IFNγ release mediated by PBMC activated with PeliCluster CD3 for 48 hours, washed and the re-exposed to a 10 fold lower concentration of PeliCluster CD3 for an additional 48 hours. Figure 1A and 1B are non-linear x-y plot of test article concentration versus IFNγ release. The human IgG1 antibody, anti-HEL (gray circle), was used as the isotype control and was associated with background IFNγ release.

[0002] For FIG. 42A and FIG. 42B, PBMC from two healthy human donors were tested. Fuse 599 (IL-18m9A-Fc) was used as the non-targeted benchmark with similar activity to wild type IL-18 (open grey circles, dashed line). Fuse1109 (black squares) and Fuse1177 (black diamonds) consisted of PD-1 targeted IL-18m13A without and with a 100 fold N-terminal attenuator, respectively. Fuse 1229 (black downward facing triangles) and Fuse 1179 (black upward facing triangles) consisted of PD-1 targeted IL-18 m13.1A and IL-18m16A , both containing a 100 fold N-terminal attenuator. Relative to Fuse599, Fuse1109 was about 100 fold attenuated (EC50) whereas Fuse1177, Fuse1229, and Fuse1179 were all greater than 3000 (donor 2) to greater than 1000 fold (donor 1) attenuated (EC50). Relative to Fuse599, the Emax for Fuse1177, Fuse1229, and Fuse1179 was also reduced by >60%. The human IgG1 antibody, anti-HEL (gray circle), was used as the isotype control and was associated with background IFNγ release. A summary table of EC50, Emax and AUC (area under the curve) values is shown below the x-y plots.

Example 38

Human peripheral blood mononuclear cell (PBMC) isolation

[0003] PBMCs were isolated from whole blood from healthy donors using Ficoll-Paque Plus medium. In brief, 35 ML of diluted whole blood (1 volume of whole blood vs 1 volume of PBS) was gently overlayed on top of 15 ML Ficoll-Paque Plus medium without disturbing the interface in a 50-ML conical tube. After centrifuging for 40 minutes at 400 × g at room temperature without brake, the buffy coat (interface layer between Ficoll and serum) was collected and diluted in 5 volumes of PBS. After centrifuging for 5 minutes at 500 × g at room temperature, PBMCs were resuspended in PBS and washed once in PBS by centrifuging for 5 minutes at 500 × g at room temperature. PBMCs were then resuspended in 5 ML of ACK lysis buffer and incubated for 5 minutes at room temperature to remove red blood cell residues. After the 5-minute incubation period, 45 ML PBS was added to PBMCs and centrifuged for 15 minutes at 100 × g at room temperature. At last, PBMCs were resuspended in culture medium (RPMI1640 with 10% heat-inactivated FBS and 1% penicillin/streptomycin) for cytotoxicity and IFN gamma release assay set-up. To store PBMCs for further usage, PBMCs were pelleted down by centrifuging for 5 minutes at 500 × g at room temperature and then resuspended in freezing medium (90% heat-inactivated FBS and 10% dimethyl sulfoxide (DMSO)) for storage in liquid nitrogen.

NK cell isolation and expansion

[0004] Fresh PBMCs were washed twice in PBS and resuspended in EasySep Buffer from StemCell at a density of 50 million cells per ML in 50-ML conical tube. 50 UL of isolation cocktail from the NK cell isolation kit from StemCell was added to 1 ML of cell suspension and incubated at room temperature for 5 minutes. After the 5-minute incubation period, 50 UL of RapidSpheres from NK cell isolation kit was added to 1 ML of the cell/antibody cocktail mixture. The total volume of the cell/cocktail/bead mixture was brought up to 25 or 50 ML with EasySep Buffer and incubated at room temperature for 10 minutes on EASYSEP magnet. After the 10-minute incubation period, the solution was transferred to a new 50-ML tube by keeping the tube on the magnet and incubated at room temperature for 5 minutes on EASYSEP magnet. The solution with purified NK cell was collected. Freshly purified NK cells were used for expansion or frozen down and stored in liquid nitrogen for further usage.

[0005] To expand NK cells, 1x 500 ML of ImmunoCult™ NK cell expansion coating material from ImmunoCult NK cell expansion kit from StemCell was added to a non-tissue culture-treated 24-well plate and incubated at room temperature for 2 hours. During the 2-hour incubation, freshly isolated NK cells were resuspended in ImmunoCult NK cell expansion medium from ImmunoCult NK cell expansion kit from StemCell at the density of 1 million per ML. After the 2-hour incubation, the plate was washed twice with PBS and 500 ML of NK cell suspension (0.5 million NK cells) was added to one well of the coated 24-well plate. The plate was incubated at 37°C with 5% CO2 for 3 days and 500 ML of ImmunoCult NK cell expansion medium was added to the well with NK cells. The plaTE was further incubated for another 4 days. At day 7, NK cells were collecteD by centrifuge at 300 x g for 10 minutes and resuspended in ImmunoCult NK cell expansion medium from ImmunoCult NK cell expansion kit from StemCell at the density of 0.2 million per ML. 1 ML of collected NK cells (0.2 million NK cells) was added to a well of new coated 24-well plate and incubated for 4 days at 37°C with 5% CO2. At day 11, NK cells were collected again by

centrifuge at 300 x g for 10 minutes and resuspended in ImmunoCult NK cell expansion medium from ImmunoCult NK cell expansion kit from StemCell at the density of 0.2 million per ML. 1 ML of collected NK cells (0.2 million NK cells) was added to a well of new coated 24-well plate and incubated for another 3 days at 37°C with 5% CO2. NK cells after 14-days' expansion were collected and frozen down and stored in liquid nitrogen for further usage.

Cytotoxicity assay

[0006] On the day before assay setting up, selective antibiotics were removed from target cell (MDA-MB-231 EgfP FLUC cells or T-47D EgfP FLUC cells). On the day of assay setting up, target cells were collected by brief TrypLE treatment and then washed with culture medium by centrifuging at 500 × g for 5 minutes at room temperature. Target cell lines were then resuspended in culture medium to determine the cell viability by trypan blue exclusion on Cellometer. The viable cell density was adjusted to 50,000 cells/ML in culture media. 100 UL target cell suspension (5,000 target cell) was carefully dispensed to each well of a 96-well black clear flat-bottom tissue culture plate using multichannel pipettor. The plate was then incubated for 4-5 hours in tissue culture incubator to ensure that the target cells have attached to the bottom of the 96-well plate.

[0007] For the cytotoxicity assay using NK cells or PBMCs as effector cells, NK cells or PBMCs were pelleted down by centrifuge for 5 minutes at 500 × g at room temperature and resuspended in culture medium. The viability of cells was also determined via trypan blue exclusion and the viable NK or PBMC density was adjusted to 3 million cells/ML in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin. After 4-5 hours of incubation, culture medium was carefully removed from 96-well plates with target cells. 50 UL of 3 million cells/ML NKs or PBMCs suspension (150,000 cells) was added to the designated well in the 96-well plate with target cell, which would result in the E:T ratio of 30:1. To result in the E:T ratio of 10:1, 5:1, 2:1 or 1:1, 3 million cells/ML of NKs or PBMCs were further diluted with RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin by 3, 6, 15, or 30 times. 50 UL of diluted NK cells or PBMCs suspension was then added to the designated wells in the 96-well plate with target cell.

[0008] For the cytotoxicity assay using activated T cells as effector cells, isolated T cells were pretreated with human CD3/CD28 dynabeads (2 UL per million T cells) for 72 hours. After 72-hour activation, CD3/CD28 dynabeads were removed from activated T cells on magnet. Activated T cells were then pelleted down by centrifuge for 5 minutes at 500 × g at room temperature and resuspended in culture medium. The viability of T cells was also determined via trypan blue exclusion and the viable T cell density was adjusted to 2 million cells/ML in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin. After 4-5 hours of incubation, culture medium was carefully removed from 96-well plates with target cells. 25 UL of 2 million cells/ML activated T cell suspension (50,000 cells) was added to the designated well in the 96-well plate with target cell, which would result in the E:T ratio of 10:1. Meanwhile, Fuse-608 was prepared in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin at the concentration of 40 PM. 25 UL of 40 PM prepared Fuse-608 was added to designated well in the 96-well plate with target cell and activated T cells.

[0009] FUSE proteins were prepared and serially diluted (5-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 NM to 2.56 PM. 50 UL of prepared FUSE proteins at different concentrations were then added to the designated wells in the 96-well plate with PBMC and target cells and incubated for the dedicated time period (24, 48 or 72 hours) at 37 °C with 5% CO2. At the end of the incubation period, the 96-well plates were centrifuged for 1 minute at 500 ×g to transfer 50ML of supernatant to V-bottom storage plate using a multichannel pipettor for IFN gamma release, granzyme B release or HEK-BLUE-IL-18 cell activation assay. ONE-Glo luciferase Assay solution was brought to room temperature. 50 UL of One-Glo solution was then added to the designated well with cells and incubated for 2 minutes at room temperature. The bioluminescence (IFU) was measured on a plate reader with preset Bio-luminance protocol. Killing of target cell was calculated by using the formula [Killing percentage of testing sample = (IFU of sample with PBMC and target cell–- IFU of testing sample)/(IFU of sample with PBMC and target cell–- IFU of sample with PBMC alone)*100].

T cell isolation

[0010] Fresh PBMCs were washed twice in PBS and resuspended in EasySep Buffer from StemCell at the density of 50 million cells per ML in 50-ML conical tube. 50 UL of isolation cocktail from T cell isolation kit from StemCell was added to 1 ML of cell suspension and incubated at room temperature for 5 minutes. After the 5-minutes' incubation, 50 UL of RapidSpheres from T cell isolation kit was added to 1 ML of the cell/antibody cocktail mixture. The total volume of the cell/cocktail/bead mixture was brought up to 25 or 50 ML with EasySep Buffer and incubated at room temperature for 10 minutes on EASYSEP magnet. After the 10-minutes' incubation, the solution was transferred to a new 50-ML tube by keeping the tube on the magnet and incubated at room temperature for 5 minutes on EASYSEP magnet. The solution with purified T cell was collected. Freshly purified T cells were frozen down and stored in liquid nitrogen for further usage.

Dendritic cell generation

[0011] Fresh PBMCs were resuspended in AIM V medium at the density of 5 million per ml and 40 ML of PBMC suspension was added to a T-174 sterile cell culture flask. The flasks were incubated in a humidified incubator maintained at 37°C and 5% CO2 for 2 hours. After 2 hours' incubation, the non-adherent cells were removed by firmly tapping the flask and completely aspirating off media in the flask. 40 ML of AIM-V medium was immediately added to the adherent cells with 800 U/ml GM-CSF and 500 U/ml IL-4 to each T-150 cell culture flask containing the adherent monocytes. The flasks were placed in a humidified incubator at 37°C and 5% CO2 for 7 days. After the 7-day culture period, DCs were harvested, frozen down and stored in liquid nitrogen for further usage.

HEK-Blue-IL-18 cell activation assay with human granzyme B-cleaved FUSE proteins or supernatant from cytotoxicity assay

[0012] HEK-BLUE-IL-18 cells from Invivogen were maintained in culture medium (DMEM medium with 4.5 g/L glucose, 2 MM L-Glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ML penicillin, 100 µg/ML streptomycin, 100 µg/ML Normocin and 1× HEK-blue selection reagent). HEK-BLUE™ IL-18 cells are

engineered from the human embryonic kidney 293 (HEK293) cell line to stably express genes encoding the IL-18 receptor (IL-18R) and IL-18 receptor accessory protein (IL-18RAP) and express an NF- KB /AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene, therefore being useful for detection of bioactive IL-18 by monitoring the activation of the NF-KB and AP-1 pathways via quantification in the supernatant of the SEAP level)which is produced upon activation of NF-KB) with a solution such as QUANTI-BLUE™ Solution. In addition, the responses to human TNF-α and IL-1β have been blocked in HEK-BLUE™ IL-18 cells, and so the HEK-BLUE™ IL-18 cells are responsive specifically to IL-18.

[0013] On the day of experiment setup, HEK-BLUE-IL-18 cells were gently rinsed twice with pre-warmed phosphate buffered saline (PBS) and then detached in PBS by tapping the flask. Detached HEK-Blue-IL-18 cells were resuspended in pre-warmed testing medium (DMEM with 4.5 g/L glucose, 2 MM L-Glutamine, 10% (v/v) heat-inactivated FBS, 100 U/ML penicillin, and 100 µg/ML streptomycin) at the density of 3×10^5 cell per ML. To seed the cells (50,000 cells per well), 180 ML of resuspended HEK-Blue-IL-18 cells were added to designated wells in 96-well plate.

[0014] For FUSE protein cleavage with human granzyme B, human pro-granzyme B was activated with enterokinase (EK) by incubating 2 µg of human pro-granzyme B with 20 ng of EK in 1× PBS for 90 minutes at 37℃. To cleave FUSE protein, 20 µg of FUSE protein was incubated with 2 µg of EK-activated human granzyme B in assay buffer (50 MM HEPES (PH 7.4), 100 MM NaCl, 0.1% CHAPS, 1 MM EDTA, 10% Glycerol) for 10 minutes at 37℃. After the 10-minute incubation period, cleaved FUSE proteins were diluted in testing medium (DMEM with 4.5 g/L glucose, 2 MM L-Glutamine, 10% (v/v) heat-inactivated FBS, 100 U/ML penicillin, and 100 µg/ML streptomycin) from 12,500,000 pg/ML to 160 pg/ML (10× of final concentration) by 5-fold serial dilution. IL-18 was also diluted in testing medium from 4,000 pg/ML to 1.28 pg/ML (10× of final concentration) by 5-fold serial dilution. To treat HEK-Blue-IL-18 cells, 20 ML of prepared IL-18 or cleaved FUSE proteins were added to the designated wells with 50,000 cells in the 96-well plate and then gently mixed the cells with proteins. The 96-well plate was then incubated at 37°C with 5% CO_2 for 24 hours.

[0015] To check the activity of the supernatant from cytotoxicity study in inducing the activation of HEK-Blue-IL-18 cell, 20 ML of supernatant from the cytotoxicity study was added to designated wells containing 50,000 cells in the 96-well plate and then gently mixed the cells with supernatant. The 96-well plate was then incubated at 37°C with 5% CO2 for 24 hours

[0016] After the 24-hour activation incubation period, 20 ML of HEK-Blue-IL-18 cell culture supernatant containing the secreted alkaline phosphatase was transferred to a new 96-well plate. Meanwhile, QUANTI-Blue solution was prepared by adding 1 ML of QB reagent and 1 ML of QB buffer to 98 ML of sterile water. 80 ML of the prepared QUANTI-Blue solution was then added to the 96-well plate with 20 ML of HEK-Blue-IL-18 cell culture supernatant and incubated at 37°C for 4 hours. The level of secreted alkaline phosphatase related to HEK-Blue-IL-18 cell activation was detected by measuring the absorbance at 630 nm using a spectrophotometer.

T cell and dendritic cell co-culture(MLR) assay

[0017] Dendritic cells were generated from monocytes in PBMCs with 800 U/ml GM-CSF and 500 U/ml IL-4. Allogeneic T cells from PBMCs were mixed with dendritic cells at the ratio of 10:1 in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin. 100K T cells with 10K dendritic cells in 150 UL of medium were added to the wells in 96-well plate. Fuse proteins were prepared and serially diluted (5-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 NM to 2.56 PM. 150 UL of prepared FUSE proteins at different concentrations were then added to the designated wells in the 96-well plate with T cell and dendritic cells and incubated for the dedicated time period (48, 72, 96 or 144 hours) at 37 °C with 5% CO2. At the end of the incubation period, the 96-well plates were centrifuged for 1 minute at 500 ×g to transfer 250ML of supernatant to V-bottom storage plate using a multichannel pipettor for IFN gamma release detection.

IFN gamma release detection

[0018] IFN gamma release from cytotoxicity assay was measured using human IFN gamma ELISA detection kit. In brief, coating antibody provided in the kit was diluted to suggested concentration by following the protocol provided by the kit manufacturer. 100 ul of diluted coating antibody was added to the Nunc MaxiSorp flat-bottom 96-well plate. Plates were sealed and incubated overnight at 4 °C. On the following day, the plates were then washed 4 times with the Wash Buffer. To block non-specific binding and reduce background, 200 ML 1× Assay Diluent A was added, and the plates were incubated at room temperature for 1 hour on a plate shaker (400 rpm). After blocking, the plates were washed 4 times with Wash Buffer. 100 ML/well of standards (prepared with culture medium) or samples were then added to the appropriate wells and incubated at room temperature for 2 hours on a plate shaker (400 rpm). After the 2-hour incubation with samples or standards, the plates were washed 4 times with Wash Buffer and 100 ML of diluted Detection Antibody solution was added to incubate at room temperature for 1 hour on a plate shaker (400 rpm). After the 1-hour incubation period with detection antibody, the plates were washed 4 times with Wash Buffer and 100 ML of diluted Avidin-HRP solution was added to incubate at room temperature for 30 minutes on a plate shaker (400 rpm). After 30-minute incubation with Avidin-HRP, the plates were washed 5 times with Wash Buffer and 100 ML of freshly mixed TMB Substrate Solution was added to incubate at room temperature for 20 minutes in the dark. 100 ML of Stop Solution was then added to each well to stop the reaction. The absorbance at 450 nm was measured on a plate reader and the concentration of IFN gamma in each sample was back-calculated using the standard curve generated with the absorbance of different concentration of standards.

Granzyme B release detection

[0019] Granzyme B release from cytotoxicity assay was measured using human granzyme B ELISA detection kit. In brief, coating antibody provided in the kit was diluted to suggested concentration by following the protocol provided by the kit manufacturer. 100 ul of diluted coating antibody was added to the Nunc MaxiSorp flat-bottom 96-well plate. The plates were sealed and incubated overnight at 4 °C. The plates were then washed 3 times with the Wash Buffer. To block non-specific binding and reduce background, 200 ML 1× Assay Diluent A was added, and

the plates were incubated at room temperature for 1 hour on a plate shaker (400 rpm). After blocking, the plates were washed 3 times with the Wash Buffer. 100 ML/well of standards (prepared with culture medium) or samples were then added to the appropriate wells and incubated at room temperature for 2 hours on a plate shaker (400 rpm). After the 2-hour incubation period with samples or standards, the plates were washed 3 times with Wash Buffer and 100 ML of diluted Detection Antibody solution was added to incubate at room temperature for 1 hour on a plate shaker (400 rpm). After the 1-hour incubation period with detection antibody, the plates were washed 3 times with Wash Buffer and 100 ML of diluted Avidin-HRP solution was added to incubate at room temperature for 30 minutes on a plate shaker (400 rpm). After 30-minute incubation with Avidin-HRP, the plates were washed 4 times with Wash Buffer and 100 ML of freshly mixed TMB Substrate Solution was added to incubate at room temperature for 20 minutes in the dark. 100 ML of Stop Solution was then added to each well to stop the reaction. The absorbance at 450 nm was measured on a plate reader and the concentration of granzyme B in each sample was back-calculated by using the standard curve generated with the absorbance of different concentration of standards.

Example 39

[0020] The N-terminal of pro-IL-18 was fused to the C-terminal of an IgG1 CH3 domain so as to engineer a variant of IL-18 expressible in mammalian cells. Pro -IL-18 was fused to the knob of a knob into hole heterodimeric IgG1 protein. Further modification was made to pro-IL-18 to reduce aggregation of the molecule such that each cysteine residue in both the pro-peptide and mature IL-18 was substituted with serine ("IL-18AS", as in FUSE-480), alanine ("IL-18AA", as in FUSE-481), or valine ("IL-18AV", as in FUSE-442). Illustrations of the three variants are shown in panel A of figure 43.

[0021] Transient transfection in the ExpiCHO system resulted in titers of 191, 214, and 198 mg/L, for FUSE-480, FUSE-481, and FUSE-442, respectively. These Fc-pro-IL-18 fusion proteins were hypothesized as harboring a "masked" version of IL-18, where the biological activity of the fused IL-18 would be reduced until the propeptide is cleaved off. To evaluate cleavage of the propeptide, a cleavage site specific for the enterokinase (EK) was inserted within the propeptide ("pp") upstream of mature IL-18 sequence in the location of the endogenous Caspase 1 site. EK was chosen because of its robust protease activity and activity in phosphate buffered saline. To evaluate the biological activity of the Fc-pro-IL-18 fusion proteins before and after treatment with EK, a reporter system was used. Here, HEK-Blue-IL-18 cells were used to quantify IL-18 activity. HEK-Blue IL-18 cells are made from HEK-293 engineered to express the human IL-18 receptor complex (IL-18Rα/β) and an NF-κb/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. The cells are also engineered not to respond to human TNF-α and IL-1β. Upon exposure to IL-18, HEK-Blue IL-18 produce SEAP in a dose dependent manner, which can be quantified via a colorimetric assay. We observed that compared to recombinant human mature IL-18, all three mutants induced lower biological activity greater than 1000 fold and up to 100,000 fold. Upon demasking (enzymatic cleavage) by treatment with EK, the biological activity of the released protein from the alanine-substituted mutant (FUSE-480) and the valine-substituted mutant (FUSE-442) was not substantially different from recombinant human mature IL-18. Following

release of the serine mutant with EK (Fig. 43B), however, although biological activity was restored by >30,000 fold, it was ~50 fold less potent than recombinant human mature IL-18. Potency was measured as the concentration of test article that induced have maximal SEAP production (EC50-SEAP).

[0022] As shown in figure 44, pro-IL-18mut2 was fused to the knob of a knob into hole heterodimeric IgG1 protein. IL-18mut2 incorporates four amino acid substitutions hypothesized to reduce binding to IL-18BP while maintaining wild type binding to the IL-18 receptor complex. Further modification was made to pro-IL-18mut2 to reduce aggregation of the molecule such that each cysteine residue in both the pro-peptide and mature IL-18mut2 was substituted with serine (IL-18mut2AS), alanine (IL-18mut2AA), or valine (IL-18mut2AV). Illustrations of the three variants are shown in panel A of figure 44. As in figure 43, the biological activity was assessed using HEK-Blue. Compared to recombinant human mature IL-18, all three mutants induced lower biological activity of at least 100,000 fold. Upon demasking (enzymatic cleavage) by treatment with EK, the biological activity of the released protein from the alanine-substituted mutant (FUSE-423) and the valine-substituted mutant (FUSE-424) was not substantially different from recombinant human mature IL-18. Following release of the serine mutant with (FUSE422), however, although biological activity was restored by about 100,000 fold, it was about 100 fold less potent than recombinant human mature IL-18. Potency was measured as the concentration of test article that induced have maximal SEAP production (EC50-SEAP).

[0023] As shown in figure 45, we examined the impact of a propeptide on masking of IL-18AV biological activity in the context of Fc fusions variants incorporating IL-18AV on the C-terminal of the Fc with (panel A, FUSE-442; Fc-eKpp-IL-18AV) or without (panel B, FUSE-505; Fc-EK-IL-18AV) the propeptide. For the fusion in which the propeptide was removed, the EK cleavage site that replaced the Caspase 1 site was moved to a position directly in between the CH2 domain of the knob and mature IL-18AV without the addition of a flexible linker. Using the HEK-Blue IL-18AV reporter cell assay as a readout, we exposed these cells to a titration of FUSE-442 or FUSE-505 with or without treatment with EK. The biological activity, measured as the EC50-SEAP, of FUSE-442 and -505 were attenuated compared to human recombinant IL-18AV at the orders of ~150 fold and ~15,000 fold, respectively (panel C) such that incorporation of the propeptide contributed to ~100-fold additional attenuation compared to the Fc fusion alone. Treatment with EK, designed to cleave off the IL-18AV fragment from FUSE-505 and FUSE-442 led to restoration of biological activity. In the case of FUSE-442, treatment with EK resulted in biological activity that was not substantially different from recombinant human mature IL-18AV. For FUSE-505, however, although biological activity was restored following treatment with EK, potency remained reduced by ~30 fold compared to recombinant human mature IL-18AV. The EC50-SEAP for each compound is shown in panel D. Given that FUSE-505 and FUSE-442 both harbor the same IL-18AV, the difference in activity after demasking with EK could not be explained by any difference in IL-18AV variant. Rather, we observed that EK was far less efficient at releasing IL-18AV from FUSE-505 (<10% cleavage efficiency) than FUSE-4442 (>95% cleavage efficiency). Hence, the reduced biological activity observed from EK cleaved FUSE-505 versus EK cleaved 442 was the result of less IL-18AV being released by the former compared to the latter. We hypothesize that the lack of a flexible linker between the CH3 domain of the knob

and IL-18AV in FUSE-505 resulted in a mostly inaccessible EK cleavage site. In contrast, the propeptide incorporated into FUSE-442 allowed for sufficient access of EK to its cleavage site for efficient release of IL-18AV. The data indicates strongly that the in the context of an IL-18AV Fc fusion protein, the propeptide is not required but contributes to masking of IL-18AV biological activity. In the absence of the propeptide, masking is likely the consequence of steric hindrance mediated by the protein fused to the N-terminal of IL-18AV

[0024] As shown in figure 46, as in figure 45 with IL-18AV, we examined the impact of a propeptide on masking of IL-18mut2AV biological activity in the context of Fc fusions variants incorporating IL-18aVmut2 on the C-terminal of the Fc with (panel A of figure 46, FUSE-424; Fc-eKpp-IL-18mut2AV) or without (panel B, FUSE-441; Fc-EK-IL-18mut2AV) the propeptide. The biological activity of FUSE-441 and FUSE424 were highly attenuated compared to human recombinant IL-18 at the orders of >100,000 fold (panel C). Treatment with EK led to restoration of biological activity. In the case of FUSE-424, treatment with EK resulted in biological activity that was not substantially different from recombinant human mature IL-18. For FUSE-441, however, although the vast majority of biological activity was restored following treatment with EK, potency remained reduced by ~10 fold compared to recombinant human mature IL-18. The EC50-SEAP for each compound is shown in panel D. Given that FUSE-441 and FUSE-442 both harbor the same IL-18mut2AV, the difference in activity after demasking with EK could not be explained by any difference in IL-18 variant. Rather, we observed that EK was far less efficient at releasing IL-18mut2AV from FUSE-441 (<20% cleavage efficiency) than FUSE-424 (>95% cleavage efficiency). Hence, the reduced biological activity observed from EK cleaved FUSE-441 versus EK cleaved FUSE-424 was likely the result of less IL-18mut2AV being released by the former compared to the latter. We hypothesize that the lack of a flexible linker between the CH3 domain of the knob and IL-18mut2AV in FUSE-441 resulted in a mostly inaccessible EK cleavage site. For example, a flexible linker can be the propeptide; or the propeptide behaves as a flexible linker. In contrast, the propeptide incorporated into FUSE-424 allowed for sufficient access of EK to its cleavage site for efficient release of IL-18mut2AV. The data indicates strongly that the in the context of an IL-18mut2AV Fc fusion protein, the propeptide is not required for masking of IL-18mut2AV biological activity but is rather the consequence of steric hindrance mediated by the protein fused to the N-terminal of IL-18mut2AV. As such, we hypothesize that any N-terminal protein of sufficient size (e.g., about 4 kDa or larger, e.g., the propeptide is about 4 kDa, Fc is about 28 kDa as a monomer,HSA is about 66 kDa, VHH is about 14 kDa) would be capable of masking the biological activity of IL-18mut2AV; and that an EK cleavage site containing linker of sufficient size (e.g., 25 amino acid or longer) to allow access to EK, when incorporated between the Fc or other N-terminal protein mask and IL-18mut2AV, would substitute for the EK containing propeptide. Further, should such N-terminal masking protein or fragment thereof be engineered or naturally be transported through the Endoplasmic Reticulum (ER), this would result in expression yields from transient transfection of mammalian cells such as Expi-CHO acceptable for therapeutic development.

[0025] As shown in figure 1547 we examined the impact of fusing the propetide-IL-18AV fusion to the C-terminus of wild type IgG1 (FUSE-507, panel A) and wild type IgG4 (FUSE-509, panel B). In both formats, one molecule of the propetide-IL-18AV fusion are linked to each Fc-CH3 domain, resulting in two molecules of the

propetide-IL-18AV fusion per IgG1 or IgG4 homodimer. As previously described, a cleavage site specific for EK was inserted between the propeptide and mature IL-18 sequence in the location of the endogenous Caspase-I site. To evaluate the biological activity of the Fc-pro-IL-18 fusion proteins before and after treatment with EK, the HEK-Blue-IL-18 cell reporter system was used. Using this systems as a readout, we exposed HEK-Blue-IL-18 cells to a titration of FUSE-507 (IgG1fc-EKpp-IL-18AV) or FUSE-509 (IgG4fc-EKpp-IL-18AV) with or without treatment with EK. The biological activity, measured as the EC50-SEAP, of FUSE-507 and -509 were highly attenuated compared to human recombinant IL-18 at the orders of >10,000 fold (panel C). Treatment with EK, designed to cleave off the IL-18AV fragment from FUSE-507 and FUSE-509 led to restoration of biological activity that was approximately 2 fold greater than hrIL-18. This difference was likely the result of two molecules of IL-18AV being release per IgG1 or IgG4 fusion resulting in about a 2:1 molar ratio of IL-18AV to hrIL-18 when the IgG1 or IgG4 fusion proteins were fully cleaved by EK. The EC50-SEAP for each compound is shown in panel D. We also generated versions of FUSE-507 and FUSE-509 without the propeptide; IgG1Fc-EK-IL-18AV and IgG4Fc-EK-IL-18AV, respectively. These did not express well, likely due to the propensity of IL-18 to form homodimers and the absence of a flexible linker between the CH3 domain of IgG1 or IgG4 and mature IL-18AV (data not shown). The data indicate that the in the context of a propeptide-IL-182AV fusion, it is possible to obtain both expression exceeding 135 mg/L and masking/attenuation of IL-18AV biological activity as high or greater than 100,000 fold (compare Figure 47 to Figure 43 and 45) whether IL-18AV is fused to the C-terminus of wild type IgG1 or IgG4 (two molecules of IL-18AV) or an IgG1 knob into hole heterodimer (one molecule of IL-18AV). The formats utilizing wild type IgG1 or IgG4 indicate allowance for straightforward plug and play fusion of propeptide-IL-18 fusions, their variants and fragments thereof to an array of monoclonal antibodies including commercialized ones such as Avelumab (anti-PDL1), Cetuximab (anti-EGFR), Trastuzumab (anti-HER2/neu), etc.

[0026] As shown in figure 48, to address the universality of masking of IL-18 and its variants by a polypeptide fused to the N-terminus of IL-18's mature form, we examined the capacity of an N-terminal protein (mask) distinct from the Fc domain of IgG to attenuate the biological activity of IL-18-AV with or without the propeptide. The N-terminus of IL-18AV with (panel A, FUSE-501) or without (panel B, FUSE-503) the propeptide was fused to the C-terminus of Human Serum Albumin (HSA). For the fusion in which the propeptide was removed, the EK cleavage site that replaced the Caspase 1 site was moved to a position directly in between the C-terminus of HSA and mature IL-18AV without the addition of a flexible linker. Using the HEK-Blue IL-18 reporter cell assay as a readout, we exposed these cells to a titration of FUSE-501 (HSA-EKpp-IL-18AV) or FUSE-503 (HSA-EK-IL-18AV) with or without treatment with EK. The biological activity, measured as the EC50-SEAP of FUSE-501 (panel C) was highly attenuated compared to human recombinant IL-18 at the order of ~35,000 fold. FUSE-503 (panel D), which does not contain the propeptide, was also attenuated at the order of ~3,500 fold. Treatment with EK, designed to cleave off the IL-18AV fragment from FUSE-501 and FUSE-503 led to restoration of biological activity. In the case of FUSE-501, treatment with EK resulted in biological activity that was not substantially different from recombinant human mature IL-18. For FUSE-503, however, although biological activity was increased by treatment with EK, it remained ~40 fold weaker

than human recombinant IL-18. The EC50-SEAP for each compound is shown in panel E. Given that FUSE-501 and FUSE-503 both harbor the same IL-18AV, the difference in activity after demasking with EK could not be explained by any difference in IL-18 variant. Rather, we observed that EK was far less efficient at releasing IL-18AV from FUSE-503 (<20% cleavage efficiency) than FUSE-501 (>95% cleavage efficiency). Hence, the reduced biological activity observed from EK cleaved FUSE-503 versus EK cleaved -501 was the result of less IL-18AV being released by the former compared to the latter. We hypothesize that the lack of a flexible linker between the C-terminus of HSA and IL-18AV in FUSE-503 resulted in a mostly inaccessible EK cleavage site. In contrast, the propeptide incorporated into FUSE-501 allowed for sufficient access of EK to its cleavage site for efficient release of IL-18AV. Taken together with data obtained from the Fc fusions, the results indicate strongly that the in the context of an HSA-IL-18AV fusion protein, the propeptide is not required for masking of IL-18AV biological activity but may contribute to greater attenuation when present in HSA-IL-18 fusion proteins. For both FUSE-501 and -503, masking appears to be the consequence of steric hindrance mediated by the protein fused to the N-terminal of IL-18AV. As such, this data provides further support that any N-terminal protein of sufficient size would be capable of masking the biological activity of IL-18AV and that an EK cleavage site containing linker of sufficient size to allow access to EK, when incorporated between the Fc, HSA or other N-terminal protein mask and IL-18AV, would substitute for the EK containing propeptide. Further, should such N-terminal masking protein or fragment thereof be engineered or naturally be transported through the Endoplasmic Reticulum (ER), this would result in expression yields from transient transfection of mammalian cells such as Expi-CHO acceptable for therapeutic development.

[0027] As shown in figure 49, to address the universality of masking of IL-18 and its variants by a polypeptide fused to the N-terminus of IL-18's mature form, we examined the capacity of an N-terminal protein (mask) distinct from the Fc domain of IgG to attenuate the biological activity of IL-18-mut2AV with or without the propeptide. The N-terminus of IL-18mut2AV with (panel A, FUSE-502) or without (panel B, FUSE-504) the propeptide was fused to the C-terminus of Human Serum Albumin (HSA). For the fusion in which the propeptide was removed, the EK cleavage site that replaced the Caspase 1 site was moved to a position directly in between the C-terminus of HSA and mature IL-18AV without the addition of a flexible linker. Using the HEK-Blue IL-18 reporter cell assay as a readout, we exposed these cells to a titration of FUSE-502 (HSA-EKpp-IL-18mu2AV) or FUSE-504 (HSA-EK-IL-18mut2AV) with or without treatment with EK. The biological activity, measured as the EC50-SEAP, of FUSE-502 (panel C) and FUSE-504 (panel D) were highly attenuated compared to human recombinant IL-18 at the orders of at least ~30,000 fold and ~50,000 fold, respectively. Treatment with EK, designed to cleave off the IL-18mut2AV fragment from FUSE-504 and FUSE-502 led to restoration of biological activity. In the case of FUSE-502, treatment with EK resulted in biological activity that was not substantially different from recombinant human mature IL-18 (panel C). For FUSE-504, although the vast majority of biological activity was restored following treatment with EK, potency remained reduced by ~3 fold compared to recombinant human mature IL-18 (panel D). The EC50-SEAP for each compound is shown in panel E. Given that FUSE-504 and FUSE-502 both harbor the same IL-18mut2AV, the difference in activity after demasking with EK could not be explained by any difference in IL-18 variant. Rather, we observed that EK was

far less efficient at releasing IL-18mut2AV from FUSE-504 (<20% cleavage efficiency) than FUSE-502 (>95% cleavage efficiency). Hence, the reduced biological activity observed from EK cleaved FUSE-504 versus EK cleaved -502 was the result of less IL-18mut2AV being released by the former compared to the latter. We hypothesize that the lack of a flexible linker between the C-terminus of HAS and IL-18mut2AV in FUSE-504 resulted in a mostly inaccessible EK cleavage site. In contrast, the propeptide incorporated into FUSE-502 allowed for sufficient access of EK to its cleavage site for efficient release of IL-18mut2AV. Taken together with data obtained from the Fc fusions, the results indicate strongly that in the context of an HSA-IL-18mut2AV fusion protein, the propeptide is not required for masking of IL-18mut2AV biological activity but is rather the consequence of steric hindrance mediated by the protein fused to the N-terminal of IL-18mut2AV. As such, this data provides further support that any N-terminal protein of sufficient size would be capable of masking the biological activity of IL-18mut2AV and that an EK cleavage site containing linker of sufficient size to allow access to EK, when incorporated between the Fc, HAS or other N-terminal protein mask and IL-18mut2AV, would substitute for the EK containing propeptide. Further, should such N-terminal masking protein or fragment thereof be engineered or naturally be transported through the Endoplasmic Reticulum (ER), this would result in expression yields from transient transfection of mammalian cells such as Expi-CHO acceptable for therapeutic development.

[0028] As shown in figure 50, we examined the impact of the propeptide on the biological activity of a single IL-18AV fused to the N-terminus of IgG1 Fc. Panels A and B are exemplary illustrations of IL-18AV fused on the knob of a knob into hole IgG1-Fc domain with the propeptide (FUSE-499; Fc-pp-IL-18AV; panel A) or without the propeptide (FUSE-500; Fc-IL-18AV; panel B). In this case, we used a wild type propeptide in which all cysteine residues were substituted for valine but the Caspase 1 site was maintained. To allow for translocation of both constructs into the Endoplasmic Reticulum (ER) of mammalian cells and hence expression/secretion, the signal peptide from the Ig Kappa chain (IgK leader) was encoded upstream of either the propeptide of FUSE-499 or the IL-18AV of FUSE-500. Using the HEK-Blue IL-18 reporter cell assay as a readout, we examined the capacity of our C→V (AV) propeptide to mask IL-18AV (FUSE-499) and the ability of Caspase 1 to demask/restore biological activity. The biological activity, measured as the EC50-SEAP, of FUSE499 was highly attenuated compared to human recombinant IL-18 at the order of >50,000 fold (panel C). In contrast, in the absence of the propeptide in FUSE-500, there was no reduction in biological activity compared to human recombinant IL-18, indicating that in the absence of a the propeptide (or another polypeptide) attached to the N-terminus of mature IL-18, the IL-18-Fc fusion protein is fully functional. Importantly, de-masking of FUSE-499 with Caspase 1 resulted in restoration of biological activity that was not appreciably different from to human recombinant IL-18. Treatment of FUSE-500, which did not contain a masking domain nor Caspase 1 cleavage site, with Caspase 1 served as a negative control and indeed had no effect on biological activity. This data indicates that in the configuration whereby IL-18AV is linked to the N-terminus of an IgG, the propeptide is required for attenuation. Taken together with our previous data that a CH3 domain or HSA attenuated the biological activity of IL-18AV and IL-18mut2AV when fused to the N-terminus of each IL-18 variant without the presence of a propeptide, we hypothesize that any polypeptide of sufficient size fused to the N-terminal of mature IL-18

and/or its variants and fragments thereof would be capable of masking the biological activity of IL-18. While the smallest polypeptide tested was the propeptide (~6 kDa), polypeptides as small as 2 kDa would also be of sufficient size. In various embodiments, the short polypeptide or protein being about 2 kDa or no greater than 250 kDa. As additional examples, the short polypeptide or protein is about 2-5 kDa, about 6-10kDa, about 11-20 kDa, about 21-30 kDa, about 31-40 kDa, about 41-50 kDa, about 51-75 kDa, about 76-100 kDa, about 101-125 kDa, about 126-150 kDa, about 151-175 kDa, about 176-200 kDa, about 201-225 kDa, or about 256-250 kDa. As further examples, the short polypeptide is 1, 2, 3, or 4 amino acids, or flexible linkers of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, or 35 amino acids. In various embodiments, the short polypeptide is 1, 2, 3, or 4 amino acids, or flexible linkers or amino acid chain of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, or 35 amino acids. Additional examples small polypeptides capable of masking the biological activity of IL-18 and IL-18 variants include but are not limited to a dimer of two amino acids, a tri-mer of three amino acids, a 4-mer of four amino acids, a 5-mer of five amino acids, or a peptide selected from the group consisting of T, PT, MPT, S, GS, GGS, GGGS (SEQ ID NO:235), and $(GGGGX_\lambda$ (SEQ ID NO:236)$)_n$ wherein X_λ is Q, A, E or S and n=1-5 or an integer larger than 5. In some embodiments, the amino acid linker has the amino acid sequence of (GGGGS (SEQ ID NO:237))n where n is an integer between 1 and 5, thereby an amino acid linker of 25 amino acids or shorter in length. Additional examples include $(X_\lambda GGGG$ (SEQ ID NO:317)$)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GX_\lambda GGG$ (SEQ ID NO:318)$)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GGX_\lambda GG$ (SEQ ID NO:319)$)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GGGX_\lambda G$ (SEQ ID NO:320)$)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5. Still additional examples include $(X_\lambda GGG)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GX_\lambda GG)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GGX_\lambda G)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GGGX_\lambda)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5. Still additional examples include $(X_\lambda GG)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GX_\lambda G)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GGX_\lambda)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments. Still additional examples include $(X_\lambda G)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5, $(GX_\lambda)_n$ wherein X_λ is Q, A, E or S and n=1-5 or in some embodiments, an integer larger than 5.

[0029] Further, the propeptide in FUSE-499 is not naturally transported through the ER but was engineered to do so via addition of an IgK leader sequence upstream to it. This indicates that any polypeptide of sufficient size fused to the N-terminus of mature IL-18 and/or its variants and fragments thereof would be expected to attenuate the biological activity of IL-18. That is, the N-terminal polypeptide might naturally translocate into the Endoplasmic Reticulum (ER) or it may be engineered to do so. In both cases, transport through the ER is important for obtaining expression yields from transient transfection of mammalian cells such as Expi-CHO acceptable for therapeutic development.

[0030] As shown in figures 51A-51I, we examined whether proteases other than the proof of concept EK could be used to demask/activate pro-IL-18. As such, we chose (a) the matrix metalloproteinase (MMP), MMP2, reported to be preferentially over-expressed in the tumor microenvironment and (b) Granzyme B, which is released by cytotoxic lymphocytes including NK cells and CD8+ T cells and may therefore accumulate in inflamed tumors. For MMP2, we replaced the EK cleavage site within the propeptide (pp) of FUSE-442 (Fc-EKpp-IL-18AV) with the (a) the MMP2/9 cleavage sequence (GPLGVR (SEQ ID NO:89)) to generate FUSE-486 (Fc-MMP2pp-IL-18AV) and (b) the MMP9/2 cleavage sequence (VHMPLGFLGP (SEQ ID NO:90)) to generate FUSE-487 (Fc-MMP2/9pp-IL-18AV). (Desnoyers et al., *Sci Transl Med.* 2013 Oct 16;5(207):207ra144.) In each case, the MMP to the left of the forward slash preferentially cleaves the aforementioned peptide sequence. For Granzyme B, we substituted the EK cleavage site within the propeptide (pp) of both FUSE-442 (Fc-EKpp-IL-18AV) and FUSE-424 (Fc-EKpp-IL-18mut2AV) with a prototypical cleavage site for Granzyme B (IEQD (SEQ ID NO:88)); thus, generating FUSE-485 (Fc-GzmBpp-IL-18AV) and FUSE-462 (Fc-GzmBpp-IL-18mut2AV). FUSE-486, FUSE-487, FUSE-485 and FUSE-462 are illustrated in FIGs. 51A and 51D. Using the HEK-Blue IL-18 reporter cell assay as a readout, we exposed these cells to a titration of (a) FUSE-486 or FUSE-487 (19B) with or without treatment with recombinant human MMP2 or (b) FUSE-485 or FUSE-462 with or without recombinant human Granzyme B (FUSE-485 and FUSE-462) (see FIG. 51D). The biological activity, measured as the EC50-SEAP, of the MMP prodrug fusions, FUSE486 and FUSE487, were greatly attenuated at the orders of up to approximately 5000-fold compared to rhIL8. Following treatment of FUSE-486 with MMP2, the biological activity of the demasked IL-18AV fragment was restored to within 3-fold that of rhIL-18. Next, we added a Granzyme B cleavage site immediately C-terminal to the MMP site in FUSE486 to create FUSE587 (black triangle). As seen in FIG. 51C, FUSE587 was about 3,000-fold attenuated relative to recombinant human IL-18.

[0031] Interestingly, we observed that cleavage of FUSE587 with MMP2 released and IL-18AV variant that was still about 100-fold attenuated relative to recombinant IL-18. In contrast, cleavage with Granzyme B released an IL-18AV variant with activity similar activity as recombinant IL-18. Cleavage with Granzyme B results in release of mature IL-18AV without any N-terminal residues constituting an overhang, whereas 11 and 15 amino acid N-terminal polypeptide overhangs remain after cleavage of FUSE486 and FUSE587, respectively, with MMP2. We speculated that these overhangs might be attenuating IL-18AV activity, albeit to a lesser degree than the full size variant propeptide. This phenomenon was further investigated in FIG. 43 and FIG. 45.

[0032] FUSE-485 and FUSE-462 were also highly attenuated compared to human recombinant IL-18 at the orders of >15,000-fold (19E and 19F). Treatment with recombinant human Granzyme B (rhGb), designed to cleave off the IL-18AV fragment and the IL-18mut2AV fragment from FUSE-485 and FUSE-462, respectively, led to restoration of biological activity. In both cases, the masked IL-18 variants displayed nearly identical attenuation and the restored biological activity was about 2-fold weaker than recombinant human mature IL-18. Further, we did not observe an appreciable difference between the activity of the demasked IL-18AV and IL-18mut2AV.

[0033] Summary tables of the potencies (EC50-SEAP) for each of the test articles is shown below FIGs 51A-51B and in FIG. 51F.

[0034] We next examined whether our finding could form the basis for a plug and play masked IL-18 platform for targeting pro-IL-18 variants to cell surface proteins including but not limited to tumor associated antigens (TAA). As such, we used Cetuximab as our proof-of-concept TAA targeting protein for pro-IL-18 fusion. Cetuximab is an EGFR targeting monoclonal antibody that is commercially used for the treatment of multiple cancer indications including colorectal and head and neck cancer. First, we fused a Granzyme B cleavable pp-IL-18AV onto the C-terminus of Cetuximab's Fc domain. We found that fusion of pp-Gb-IL-18AV onto the CH3 domain of Cetuximab, that forms a natural homodimer, expressed poorly. This was presumably due to the presence of two fabs because a knob into hole IgG1 format in which ppGb-IL-18AV was fused to the C-terminus knob (or hole) and the EGR specific fab was fused to the N-terminal knob (or hole) expressed well. FUSE-517 (Cetuximab_ KiH_ppGb-IL-18-AV), as illustrated in panel G consists of the fab from Cetuximab fused to the N-terminus of the Fc-knob and ppGb-IL-18AV fused to the C-terminus of the Fc-knob. Using the HEK-Blue IL-18 reporter cell assay as a readout, we exposed these cells to a titration of FUSE-517 (19H) with or without treatment with rhGb. Relative to rhIL-18, FUSE-517 was highly attenuated at the order of >250,000-fold. Treatment of FUSE-517 with rhGb, designed to cleave off IL-18AV, led to the restoration of biological activity within 2-fold that of rhIL-18.

[0035] FIG. 51I is summary tables of potencies (EC50-SEAP) related to the data shown in FIG. 51H. Overall, the data indicates strongly that both MMP2 and granzyme B are capable of demasking/activating IL-18AV (and IL-18mut2AV with Granzyme B) in the context of a pp mask fused in-between an IgG CH3 domain and the mature IL-18AV fragment. Given our ability to successfully use other polypeptides that translocate through the ER as masks when fused to the N-terminus of multiple variants of IL-18, our observation suggests strongly that masked fusion proteins of IL-18, its variants or fragments thereof can be engineered to be selectively activated by proteases found in tumors/inflamed tumors including MMPs and Granzymes. As shown in figure 52, we examined the susceptibility of select IL-18 variants to attenuation of biological activity by its natural antagonist, IL-18 BP. Panel A of figure 52 is an illustration of the IL-18 fusion proteins examined. That is, FUSE-442 (Fc-EKpp-IL-18AV) and FUSE-424 (Fc-EKpp-IL-18mut2AV). Both variants contain the same cysteine to valine substitutions, however, FUSE-424 harbors a mature IL-18AV (termed IL-18mut2AV) downstream of the polypeptide (pp) that includes the following mutations: M87K, K89G, M696L and M149V. These four residues within the mature 18 kDa fragment of IL-18 were previously described as important to binding/masking of wild type IL-18. We therefore hypothesized that substitution of these residues would reduce binding of IL-18 BP such that IL-18AVmut2 would maintain biological activity in the presence of IL-18BP, which is often over-represented relative to IL-18 in the tumor microenvironment (TME). As in previous examples, HEK Blue IL-18 was used to assess biological activity via MYD88 driven SEAP and potency reported as the EC50-SEAP. FUSE-442 (Fc-EKpp-IL-18AV) and FUSE-424 (Fc-EKpp-IL-18mut2AV) were treated with EK to release mature IL-18AV or IL-18mut2AV, respectively. Each cleavage product was then titrated from 1 pg/ml to 1

μg/ml in media alone or media containing 1.25 μg/ml human recombinant IL-18-BP (hrIL-18-BP). Human recombinant IL-18 (hrIL-18) was used as the reference molecule for wild type inhibition of HEK Blue IL-18 reporter activity mediated by rhIL-18BP. Panels B (rhIL-18), D (FUSE-442), and F (FUSE-424) are non-linear x-y plots of SEAP release (IL-18R reporter activity) on the y-axis versus the concentration of test article on the x-axis in the presence or absence of IL-18BP. A summary of biological potencies (EC50-SEAP) relevant to each graph is shown as panels C, E, and G (beneath each x-y plot). As is well documented, we observed strong attenuation of rhIL-18 by rhIL-18BP in the order of at least 300-1000-fold. For IL-18AV released from FUSE442, we observed about 100 to 150-fold attenuation of biological activity (i.e., ~3-6 fold less than rhIL-18) suggesting that apparent affinity of IL-18AV for IL-18BP is weaker than that of rhIL-18 for IL-18BP. Importantly, the cleavage product of FUSE424, IL-18mut2AV, appeared resistant to biological attenuation by rhIL-18BP. As seen in panel F, we observed no appreciable difference in biological activity between IL-18mut2AV released from FUSE424 and the same molecule exposed to rhIL-18BP. This data suggests strongly that IL-18mut2AV binds with far weaker apparent affinity to rhIL-18BP compared to IL-18AV or rhIL-18. As such, a masked version of IL-18mut2AV designed to be released by proteases present in the TME including but not limited to MMP2, 9 and 14 and/or proteases released in inflamed tumors including but not limited to Granzymes A, B and M may be predicted to function in the presence of IL-18BP to promote anti-tumor activity via multiple pathways including but not limited to IFNγ mediated Th1 and Tc1 activity.

[0036] As shown in figure 53, we examined the impact of the size of polypeptides fused to the N-terminus of mature IL-18 on the biological activity of a single IL-18AV fused to the N-terminus of IgG1 Fc. Panels A are exemplary illustrations of IL-18AV fused on the knob of a knob into hole IgG1-Fc domain with different size polypeptides ranging from a propeptide variant (FUSE-499; described in FIG. 50) to 35 amino acids (FUSE756) to 15 amino acids (FUSE757 and FUSE758). To allow for translocation of all constructs into the Endoplasmic Reticulum (ER) of mammalian cells and hence expression/secretion, the signal peptide from the Ig Kappa chain (IgK leader) was encoded upstream of the polypeptides (and cleaved off the signal peptidase in the ER). The polypeptides of FUSE756 and FUSE757 are made up of a series of glycine and serine residues whereas that of FUSE758 is the sequence of the overhang generated by MMP2 mediated cleavage of the MMP cleavage site, KPLGLQARVVGGGG (SEQ ID NO:568). In these three cases, the C-terminus of the N-terminal polypeptides also incorporated the Granzyme B site, IEQD (SEQ ID NO:88). Using the HEK-Blue IL-18 reporter cell assay as a readout, we examined the capacity of the different size polypeptides to attenuate/mask IL-18AV-Fc (FUSE500; closed squares) or rhIL-18 (black cross hatches "X"). All polypeptides fused to the N-terminus of mature IL-18AV reduced biological activity, measured as EC50-SEAP, by at least 100-fold. FUSE499 (closed triangles) was the most attenuated (>10,000-fold). FUSE757 (closed diamonds) and 758 (open reverse triangle), both of which included 11 amino acid polypeptides fused to the N-terminus of mature IL-18AV, were about 250-fold less biologically active relative to FUSE500 or rhIL-18. FUSE756 (open triangles), which contained a polypeptide of 31 amino acids polypeptides fused to the N-terminus of mature IL-18AV was approximately 1000-fold less biologically active relative to FUSE500 or rhIL-18. Thus, all polypeptides tested and larger proteins including HSA and fragments of IgG from previous examples, fused to the N-terminus of mature IL-

18AV, regardless of size or amino acid constitution, attenuated the biological activity of mature IL-18. The degree of attenuation appears to correlate positively with the size of the polypeptide/protein fused to the N-terminus of IL-18.

Example 40

Techniques and Procedures

HEK-Blue-IL-18 cell activation assay with IL-18 or FUSE proteins

[0037] HEK-BLUE™-IL-18 cells from Invivogen were maintained in culture medium (DMEM medium with 4.5 g/L glucose, 2 mM L-Glutamine, 10% (v/v) heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 100 μg/mL Normocin and 1× HEK-blue selection reagent). HEK-BLUE™ IL-18 cells are engineered from the human embryonic kidney 293 (HEK293) cell line to stably express genes encoding the IL-18 receptor (IL-18R) and IL-18 receptor accessory protein (IL-18RAP) and express an NF-Kb/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene, therefore being useful for detection of bioactive IL-18 by monitoring the activation of the NF-Kb and AP-1 pathways via quantification in the supernatant of the SEAP level (which is produced upon activation of NF-Kb) with a solution such as QUANTI-BLUE™ Solution. In addition, the responses to human TNF-α and IL-1β have been blocked in HEK-BLUE™ IL-18 cells, and so the HEK-BLUE™ IL-18 cells are responsive specifically to IL-18.

[0038] On the day of experiment setup, HEK-BLUE-IL-18 cells were gently rinsed twice with pre-warmed phosphate buffered saline (PBS) and then detached in PBS by tapping the flask. Detached HEK-Blue-IL-18 cells were resuspended in pre-warmed testing medium (DMEM with 4.5 g/L glucose, 2 mM L-Glutamine, 10% (v/v) heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin) at the density of 5×10^5 cell per mL. To seed the cells (50000 cells per well), 100 μL of resuspended HEK-Blue-IL-18 cells were added to designated wells in 96-well plate.

[0039] For protein preparation, FUSE proteins were diluted in testing medium from 500000 pg/mL to 6.4 pg/mL (2× of final concentration) by 5-fold serial dilution. IL-18 was also diluted in testing medium from 800 pg/mL to 1.28 pg/mL (2× of final concentration) by 5-fold serial dilution. To treat HEK-Blue-IL-18 cells, 100 μL of prepared IL-18 or FUSE proteins were added to the designated wells with 50000 cells in the 96-well plate, and then the proteins were gently mixed the cells. The 96-well plate was then incubated at 37°C with 5% CO_2 for 24 hours.

[0040] After 24-hour activation, HEK-Blue-IL-18 cells released secreted alkaline phosphatase in the supernatant. 20 μL of HEK-Blue-IL-18 cell culture supernatant was transferred to a new 96-well plate. Meanwhile, QUANTI-Blue solution from Invivogen was prepared by adding 1 mL of QB reagent and 1 mL of QB buffer to 98 mL of sterile water. 180 μL of prepared QUANTI-Blue solution was then added to the 96-well plate with 20 μL of HEK-Blue-IL-18 cell culture supernatant and incubated at 37°C for 4 hours. The level of secreted alkaline phosphatase related to HEK-Blue-IL-18 cell activation was detected by measuring the OD at 630 nm using a spectrophotometer.

HEK-Blue-IL-18 cell activation assay with enterokinase (EK)-cleaved FUSE proteins

[0041] HEK-Blue-IL-18 cells from Invivogen were maintained in culture medium. On the day of experiment setup, HEK-Blue-IL-18 cells were gently rinsed twice with pre-warmed phosphate buffered saline (PBS) and then detached in PBS by tapping the flask. Detached HEK-Blue-IL-18 cells were resuspended in pre-warmed testing medium at the density of 5×10^6 cell per mL. To seed the cells (50000 cells per well), 100 µL of resuspended HEK-Blue-IL-18 cells were added to designated wells in 96-well plate.

[0042] For FUSE protein cleavage, 4 µg of FUSE protein was mixed with 80 ng of EK enzyme and 2 µL of 10× PBS in a tube. Sterile water was added to the tube to bring the total reaction volume to 20 µL. The tube was then incubated at 25 °C for 40 minutes. After 40-minute incubation, cleaved FUSE proteins were diluted in testing medium from 500000 pg/mL to 6.4 pg/mL (2× of final concentration) by 5-fold serial dilution. IL-18 was also diluted in testing medium from 800 pg/mL to 1.28 pg/mL (2× of final concentration) by 5-fold serial dilution. To treat HEK-Blue-IL-18 cells, 100 µL of prepared IL-18 or cleaved FUSE proteins were added to the designated wells with 50000 cells in the 96-well plate and then gently mixed the cells with proteins. The 96-well plate was then incubated at 37°C with 5% CO2 for 24 hours.

[0043] After 24-hour activation, 20 µL of HEK-Blue-IL-18 cell culture supernatant with secreted alkaline phosphatase was transferred to a new 96-well plate. Meanwhile, QUANTI-Blue solution was prepared by adding 1 mL of QB reagent and 1 mL of QB buffer to 98 mL of sterile water. 180 µL of prepared QUANTI-Blue solution was then added to the 96-well plate with 20 uL of HEK-Blue-IL-18 cell culture supernatant and incubated at 37°C for 4 hours. The level of secreted alkaline phosphatase related to HEK-Blue-IL-18 cell activation was detected by measuring the OD at 630 nm using a spectrophotometer.

HEK-Blue-IL-18 cell activation assay with matrix metalloproteinase (MMP)-cleaved FUSE proteins

[0044] HEK-Blue-IL-18 cells from Invivogen were maintained in culture medium. On the day of experiment setup, HEK-Blue-IL-18 cells were gently rinsed twice with pre-warmed phosphate buffered saline (PBS) and then detached in PBS by tapping the flask. Detached HEK-Blue-IL-18 cells were resuspended in pre-warmed testing medium at the density of 5×10^6 cell per mL. To seed the cells (50000 cells per well), 100 µL of resuspended HEK-Blue-IL-18 cells were added to designated wells in 96-well plate.

[0045] For FUSE protein cleavage, 1 µg of FUSE protein was mixed with 280 ng of MMP2 or MMP9 enzyme and 2.8 µL of 10× assay buffer (500 mM Tris, 100 mM CaCl2, 1500 mM NaCl, 0.5% (w/v) Brij-35, pH 7.5),) in a tube. Sterile water was added to the tube to bring the total reaction volume to 28 µL. The tube was then incubated at 37 °C for 2 hours. After 2-hour incubation, cleaved FUSE proteins were diluted in testing medium from 500000 pg/mL to 6.4 pg/mL (2× of final concentration) by 5-fold serial dilution. IL-18 was also diluted in testing medium from 800 pg/mL to 1.28 pg/mL (2× of final concentration) by 5-fold serial dilution. To treat HEK-Blue-IL-18 cells, 100 µL of prepared IL-18 or cleaved FUSE proteins were added to the designated wells with 50000 cells in the 96-well plate and then gently mixed the cells with proteins. The 96-well plate was then incubated at 37°C with 5% CO2 for 24 hours.

[0046] After 24-hour activation, 20 μL of HEK-Blue-IL-18 cell culture supernatant with secreted alkaline phosphatase was transferred to a new 96-well plate. Meanwhile, QUANTI-Blue solution was prepared by adding 1 mL of QB reagent and 1 mL of QB buffer to 98 mL of sterile water. 180 μL of prepared QUANTI-Blue solution was then added to the 96-well plate with 20 μL of HEK-Blue-IL-18 cell culture supernatant and incubated at 37°C for 4 hours. The level of secreted alkaline phosphatase related to HEK-Blue-IL-18 cell activation was detected by measuring the OD at 630 nm using a spectrophotometer.

HEK-Blue-IL-18 cell activation assay with Caspase I-cleaved FUSE proteins

[0047] HEK-Blue-IL-18 cells from Invivogen were maintained in culture medium. On the day of experiment setup, HEK-Blue-IL-18 cells were gently rinsed twice with pre-warmed phosphate buffered saline (PBS) and then detached in PBS by tapping the flask. Detached HEK-Blue-IL-18 cells were resuspended in pre-warmed testing medium at the density of 5×10^5 cell per mL. To seed the cells (50000 cells per well), 100 μL of resuspended HEK-Blue-IL-18 cells were added to designated wells in 96-well plate.

[0048] For FUSE protein cleavage, 14 μg of FUSE protein was mixed with 0.5 unit of Caspase I enzyme and 2.8 μL of 10× assay buffer (500 mM Hepes, pH 7.2, 500 mM NaCl, 1% Chaps, 100 mM EDTA, 50% Glycerol, and 100 mM DTT) in a tube. Sterile water was added to the tube to bring the total reaction volume to 28 μL. The tube was then incubated at 37 °C for 2 hours. After 2-hour incubation, cleaved FUSE proteins were diluted in testing medium from 500000 pg/mL to 6.4 pg/mL (2× of final concentration) by 5-fold serial dilution. IL-18 was also diluted in testing medium from 800 pg/mL to 1.28 pg/mL (2× of final concentration) by 5-fold serial dilution. To treat HEK-Blue-IL-18 cells, 100 μL of prepared IL-18 or cleaved FUSE proteins were added to the designated wells with 50000 cells in the 96-well plate and then gently mixed the cells with proteins. The 96-well plate was then incubated at 37°C with 5% CO_2 for 24 hours.

[0049] After 24-hour activation, 20 μL of HEK-Blue-IL-18 cell culture supernatant with secreted alkaline phosphatase was transferred to a new 96-well plate. Meanwhile, QUANTI-Blue solution was prepared by adding 1 mL of QB reagent and 1 mL of QB buffer to 98 mL of sterile water. 180 μL of prepared QUANTI-Blue solution was then added to the 96-well plate with 20 μL of HEK-Blue-IL-18 cell culture supernatant and incubated at 37°C for 4 hours. The level of secreted alkaline phosphatase related to HEK-Blue-IL-18 cell activation was detected by measuring the OD at 630 nm using a spectrophotometer.

HEK-Blue-IL-18 cell activation assay with granzyme B-cleaved FUSE proteins

[0050] HEK-Blue-IL-18 cells from Invivogen were maintained in culture medium. On the day of experiment setup, HEK-Blue-IL-18 cells were gently rinsed twice with pre-warmed phosphate buffered saline (PBS) and then detached in PBS by tapping the flask. Detached HEK-Blue-IL-18 cells were resuspended in pre-warmed testing medium at the density of 5×10^5 cell per mL. To seed the cells (50000 cells per well), 100 μL of resuspended HEK-Blue-IL-18 cells were added to designated wells in 96-well plate.

[0051] Mature active human granzyme B was generated by cleaving human pro-granzyme B using EK enzyme. In brief, 4 μg of human pro-granzyme B was mixed with 40 ng of EK enzyme and 2 μL of 10× PBS in a tube. Sterile water was added to the tube to bring the total reaction volume to 20 μL. The tube was then incubated at 25 °C for 40 minutes. After 40-minute incubation, activated human granzyme B was used to cleave FUSE proteins. In brief, 10 μg of fuse proteins were mixed with 1 ug of activated human granzyme B in assay buffer (50 mM HEPES (pH 7.4), 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 10% Glycerol) and incubated for certain time indicated in each experiment at 37℃.

[0052] Granzyme B-cleaved fuse proteins were then diluted in testing medium from 500000 pg/mL to 6.4 pg/mL (2× of final concentration) by 5-fold serial dilution. IL-18 was also diluted in testing medium from 800 pg/mL to 1.28 pg/mL (2× of final concentration) by 5-fold serial dilution. To treat HEK-Blue-IL-18 cells, 100 μL of prepared IL-18 or cleaved FUSE proteins were added to the designated wells with 50000 cells in the 96-well plate and then gently mixed the cells with proteins. The 96-well plate was then incubated at 37°C with 5% CO_2 for 24 hours.

[0053] Non-cleaved or cleaved FUSE proteins were prepared in testing medium from 1000000 pg/ml to 12.8 pg/ml (4× of final concentration) by 5-fold serial dilution. IL-18BP was diluted in testing medium at the concentration of 5,000,000 pg/ml (4× of final concentration) by. To treat HEK-Blue-IL-18 cells, 50 μL of prepared non-cleaved or cleaved FUSE proteins and 50 μL of prepared IL-18BP were mixed, incubated for 1 hour, and then added to the designated wells with 50000 cells in the 96-well plate and then gently mixed the cells with proteins. The 96-well plate was then incubated at 37°C with 5% CO2 for 24 hours. Since our fusion proteins have attenuated binding to the IL-18R complex, we conceive that they will also have reduced binding to IL-18BP, compared to cleaved FUSE proteins, because both IL-18R and IL-18BP compete for binding to IL-18. The recent crystal structures of binary and ternary complexes of hIL-18 with its receptors have shown that IL-18BP competes directly with the hIL-18Rα D3 domain for binding hIL-18, overlapping the previously identified hIL-18 binding site II (Krumm, et al., *Acta Crystallogr F Struct Biol Commun.* 2015 Jun 1; 71(Pt 6): 710–717).

[0054] After 24-hour activation, 20 uL of HEK-Blue-IL-18 cell culture supernatant with secreted alkaline phosphatase was transferred to a new 96-well plate. Meanwhile, QUANTI-Blue solution was prepared by adding 1 mL of QB reagent and 1 mL of QB buffer to 98 mL of sterile water. 180 μL of prepared QUANTI-Blue solution was then added to the 96-well plate with 20 μL of HEK-Blue-IL-18 cell culture supernatant and incubated at 37°C for 4 hours. The level of secreted alkaline phosphatase related to HEK-Blue-IL-18 cell activation was detected by measuring the OD at 630 nm using a spectrophotometer.

Binding assay by Biolayer Interferometry

[0055] Coating proteins were prepared in 1×PBS with 0.02% Tween-20 with the final concentration of 15 ug/ml. Capturing protein were also prepared, and serial diluted (4-fold dilution ranging from 400 nM to 1.6 nM) in 1×PBS with 0.02% Tween-20. The biosensors were pre-moistened in 200 uL of 1×PBS with 0.02% Tween-20 for 10 minutes. Meanwhile, Octet BLI system (ForteBio) was prewarmed for 30 minutes and the flow rate was set to 1000

rpm. The biosensors (Capture biosensor) were soaked in 250 uL of 1×PBS with 0.02% Tween-20 for 60 seconds at 30℃ to get an initial baseline reading. After the 60-second baseline reading, the biosensors were exposed to coating proteins for 300 seconds at 30℃ for the association between antibody and the biosensors (coupling coating proteins with biosensor). The biosensors with coating proteins were then exposed to capturing proteins in 250 uL of 1×PBS with 0.02% Tween-20 at 30℃ for 300 seconds for the association reaction between the coating proteins and capturing proteins (association curve). After 300-second association reaction between coating proteins and capturing proteins, the biosensors with coating proteins and capturing proteins were then exposed to 250 uL of 1×PBS with 0.02% Tween-20 at 30℃ for 300 seconds for the dissociation reaction between coating proteins and capturing proteins (dissociation curve). For binding to IL18BP, each IL18 mutant was coated onto AHC biosensor tips and probed with recombinant His tagged IL-18BP at concentrations ranging from 400 nM to 1.6 nM. For binding to IL18Rα, His tagged IL18Rα was coated into nickel biosensor tips and probed with recombinant each IL18 mutant at concentrations ranging from 400 nM to 1.6 nM. The binding affinity was calculated by the built-in data fitting algorithm.

Example 41

[0056] As shown in figure 54, we engineered eleven mutants of human IL-18 (IL-18AV shown) and measured their ability to bind recombinant human IL-18BP and recombinant human IL-18RA (also termed IL-18Rα). All test articles were generated as Fc fusion proteins with the IL-18 variant fused to the N-terminus of the Fc (see FIG. 43A). The locations and amino acid substations associated with each variant are depicted in FIG. 54.

[0057] To assess binding to human IL18BP or human IL18Rα, kinetic binding graphs were generated via Bio-Layer Interferometry (BLI) using an Octet system (ForteBio). For binding to IL18BP, each IL18 mutant was coated onto AHC biosensor tips and probed with recombinant His tagged IL-18BP at concentrations ranging from 400 nM to 1.6 nM (FIG. 54B). For binding to IL18Rα, His tagged IL18Rα was coated into nickel biosensor tips and probed with recombinant each IL18 mutant at concentrations ranging from 400 nM to 1.6 nM (FIG. 54D). Summary tables for binding to IL-18BP and IL18-RA (binding affinity (KD), on rate (k-on) and off rate (k-dis)) are shown in FIGs 54C and 54E, respectively.

[0058] All eleven IL-18 mutants bound to IL18BP with weaker affinity than FUSE500 (wild type human IL-18-AV-Fc). Five mutants were associated with no appreciable binding. These are FUSE545, FUSE599, FUSE600, FUSE601 and FUSE602.

[0059] In contrast, all eleven mutants maintained appreciable binding to IL18RA. The affinities ranged from 11 nM to 30 nM, which was only between 1.5-fold and 4-fold weaker than the affinity of FUSE500 for IL-18RA (7.4 nM).

Example 42

[0060] As shown in the table below, we tabulated the binding affinities of eleven mutant variants of human IL-18 (IL-18AV shown) to human IL18BP and human IL18Rα. Each IL-18 protein was generated as an Fc fusion proteins whereby the IL-18 variant was fused to the N-terminus of the Fc (see FIG. 53A).

Protein	Mutant	IL-BP Kd (nM)	IL-18Rα Kd (nM)
IL18		4E-10***	2E-08****
FUSE500	Wild type AV	7.28E-10*	7.4E-09**
FUSE 545	mut2	-	1.9E-08
FUSE 546	mut3	1.4E-07	1.8E-08
FUSE 547	mut4	4.0E-08	2.6E-08
FUSE 583	mut5	3.2E-09	1.2E-08
FUSE 584	mut6	4.0E-08	2.5E-08
FUSE 585	mut7	1.5E-07	2.0E-08
FUSE 586	mut8	2.6E-09	1.6E-08
FUSE 599	mut9	-	3.0E-08
FUSE 600	mut10	-	1.8E-08
FUSE 601	mut11	-	2.1E-08
FUSE 602	mut12	-	1.1E-08

*mean of 11 measurements, ** mean of 9 measurements

***www.pnas.org/doi/10.1073/pnas.97.3.1190

****arthritis-research.biomedcentral.com/articles/10.1186/ar3295

Example 43

[0061] As shown in figure 55, we examined the impact of the size of polypeptides fused to the N-terminus of mature IL-18 on the biological activity of a single IL-18AV fused to the N-terminus of IgG1 Fc.

[0062] A single N-terminal amino acid (FUSE874; downward open triangles) and different size polypeptides ranging from five amino acids (FUSE875) to the propeptide variant of FUSE-499 (upward closed triangles; see FIG. 53 for description) were investigated. FUSE500 was used as the fully active control without a N-terminal polypeptide.

[0063] To allow for translocation of all constructs into the Endoplasmic Reticulum (ER) of mammalian cells and hence expression/secretion, the signal peptide from the Ig Kappa chain (IgK leader) was encoded upstream of the polypeptides (and cleaved off the signal peptidase in the ER).

[0064] The polypeptides of FUSE875 (5 residues; closed stars), FUSE876 (10 residues; open diamonds), FUSE757 (15 residues; open circles), FUSE756 (35 residues; downward closed triangles) are made up of a series of glycine and serine residues. The N-terminal polypeptide associated with FUSE758 (open upward triangles) is the sequence of the overhang generated by MMP2 cleavage of FUSE486 (see FIG. 51). For FUSE756, FUSE757 and FUSE758, the final four amino acids of the N-terminal peptide consisted of the Granzyme B cleavage site, IEQD (SEQ ID NO:88) (see FIG. 53).

[0065] Using the HEK-Blue IL-18 reporter cell assay as a readout, we examined the capacity of the different size polypeptides to attenuate/mask IL-18AV-Fc (FUSE500; closed squares) or rhIL-18 (black crosses "X"). All

polypeptides and the single amino acid (serine) fused to the N-terminus of mature IL-18AV reduced biological activity, measured as EC50-SEAP, by at least 10-fold.

[0066] The degree of attenuation increased as the size of the polypeptide increased, such that the rank order of attenuation was observed as FUSE499>FUSE756, FUSE757, FUSE758>FUSE876, FUSE875>FUSE874. A summary table of potencies (EC50-SEAP) is shown beneath the non-linear x-y graph.

Example 44

[0067] As shown in figure 56A-56H, we examined the impact of the substituting the cysteine residue in the pro-peptide and cysteine residues in the mature IL18, which were fused together to form the pro-IL-18 variant cassette, on the biological activity of each variant using the HEK Blue IL18 assay system. Unless otherwise stated, the proteins were generated such that the propeptide-IL-18 variant was fused to the C-terminal knob or hole of a knobs into holes human IgG1 Fc as previously depicted in FIG. 43A with a Granzyme B cleavage site added between the pro-peptide variant and the mature IL18 variant. The three variants assessed in FIG. 43 contained serine substitutions (FUSE480), alanine substitutions (FUSE481) or valine substitutions (FUSE442) at all the cysteine residues in pro-IL-18. Mature IL-18 variants released from FUSE442 and FUSE481 were about as active as recombinant human IL-18 whereas mature IL18 released from FUSE480 (serine substituted) was approximately 100-fold attenuated versus recombinant human IL-18 (see FIGs 43B-43D).

[0068] For this example, the variant of pro-IL-18 in which all the cysteines were substituted for valine included an N-terminus EGFR specific VHH (FUSE516; closed triangle) and the variant of pro-IL-18 in which all the cysteines were substituted for serine included an N-terminus PD-1 specific Fab (FUSE694; closed diamond). As in FIG. 43, biological activity of the pro-IL18 test articles were assessed using HEK Blue IL18. Each was tested as an intact untreated protein or following exposure to recombinant human Granzyme B.

[0069] FIG 56A shows the results with FUSE516 (closed triangle), in which the cysteine residue in its pro-peptide variant is replaced with valine and all the cysteines in its mature IL18 variant are replaced by valine. Intact FUSE516 is about 1000-fold attenuated relative to recombinant human IL18 and that the mature IL18 variant released from FUSE516 (open triangle) by Granzyme B is approximately as active as recombinant human IL18.

[0070] FIG 56B shows the results with FUSE694 (closed diamond), in which the cysteine residue in its pro-peptide variant is replaced with serine and all of the cysteines in its mature IL18 variant are replaced by serines. Intact FUSE694 is greater than 10,000-fold attenuated relative to recombinant human IL18 and that the mature IL18 variant released from FUSE694 (open diamond) by Granzyme B is approximately 60-fold less active than recombinant human IL18.

[0071] FIG 56C shows the results with FUSE887 (closed reverse triangle), in which the cysteine residue in its pro-peptide variant is replaced with threonine and all the cysteines in its mature IL18 variant are replaced by serines. Intact FUSE887 is greater than 10,000-fold attenuated relative to recombinant human IL18 and that the mature IL18

variant released from FUSE887 by Granzyme B (open reverse triangle) is approximately 100-fold less active than recombinant human IL18.

[0072] FIG 56D shows the results with FUSE888 (closed triangle), in which the cysteine residue in its pro-peptide variant is replaced with glutamine and all the cysteines in its mature IL18 variant are replaced by serines. Intact FUSE888 is greater than 10,000-fold attenuated relative to recombinant human IL18 and that the mature IL18 variant released from FUSE888 (open triangle) by Granzyme B is approximately 100-fold less active than recombinant human IL18.

[0073] FIG 56E shows the results with FUSE889 (closed square), in which the cysteine residue in its pro-peptide variant is replaced with aspartic acid and all the cysteines in its mature IL18 variant are replaced by alanines. Intact FUSE889 is greater about 10,000-fold attenuated relative to recombinant human IL18 and that the mature IL18 variant released from FUSE889 by Granzyme B (open square) is approximately 100-fold less active than recombinant human IL18.

[0074] FIG 56F shows the results with FUSE890 (closed reverse triangle), in which the cysteine residue in its pro-peptide variant is replaced with phenylalanine and all of the cysteines in its mature IL18 variant are replaced by alanines. Intact FUSE890 is about than 10,000-fold attenuated relative to recombinant human IL18 and that the mature IL18 variant released from FUSE890 by Granzyme B (open reverse triangle) is approximately 100-fold less active than recombinant human IL18.

[0075] FIG 56G shows the results with FUSE891 (closed triangle), in which the cysteine residue in its pro-peptide variant is replaced with isoleucine and all the cysteines in its mature IL18 variant are replaced by valines. Intact FUSE891 is about 3,000-fold attenuated relative to recombinant human IL18 and that the mature IL18 variant released from FUSE891 (open triangle) by Granzyme B is approximately 100-fold less active than recombinant human IL18.

[0076] FIG 56H shows the results with FUSE892 (closed reverse triangle), in which the cysteine residue in its pro-peptide variant is replaced with histidine and all the cysteines in its mature IL18 variant are replaced by valines. Intact FUSE892 is greater about 3,000-fold attenuated relative to recombinant human IL18 and that the mature IL18 variant released from FUSE892 (open reverse triangle) by Granzyme B is approximately 100-fold less active than recombinant human IL18.

[0077] Summary tables of potency (EC50-SEAP) are show to the right of each non-linear x-y plot.

Example 45

[0078] As shown in figure 57A-57B, we examined the impact of targeting pro-IL18 to within close proximity of its receptor complex (i.e., "cis activity"). Pro-IL-18 variants tested were (a) that in which all the cysteine residues were substituted with serines (pro-IL18AS, FUSE782 and FUSE827) and (b) that in which all the cysteines were substituted with valines (pro-IL18AV, FUSE783 and FUSE785). Pro-IL18AS or pro-IL18AV were fused to the N-terminal knob or hole of a PD1 specific knobs into holes antibody (FUSE782 and FUSE783). The fab for these

fusion proteins was derived from nivolumab. To test whether targeting the pro-IL18 variants to PD1 decorated on the same cell that expressed the IL-18R complex (cis effect) enhanced biological activity compared to cells that were not decorated with PD-1 (trans effect), (1) we used HEK Blue IL18 as our IL18R complex positive reporter system, and (2) either used the cell without modification or decorated the cell line with the ectodomain of PD-1 using a bispecific antibody that contains a CD46 specific fab (clone YS5) on the N-terminal hole and the PD-1 ectodomain on the N-terminal knob (FUSE986). CD46 was chosen because of its reported expression the parental HEK 293 cell line (jitc.bmj.com/content/6/1/55), which we confirmed on HEK Blue IL18 (not shown). We also generated pro-IL18AS (FUSE827) and pro-IL18AV (FUSE775) that were functional non targeted in this and could only function in trans. FUSE827 contained no targeting domain (i.e., Fc only) and FUSE775 replaced the PD-1 specific fab with an EGFR specific VHH, 9G8, the ligand for which (EGFR) was not expressed on HEK Blue IL18 (data not shown). FIG. 57A and FIG. 57B are nonlinear x-y plots of IL18 biological activity versus the concentration of each test article using HEK Blue IL18 (FIG. 57A) and PD-1 decorated HEK Blue IL18 (FIG. 57B). As previously observed for pro-IL18AS and pro-IL18AV, serine substitutions results in greater attenuation than valine substitutions. As such, when HEK Blue IL18 (FIG. 57A; trans activity only), was treated with (a) the valine mutants FUSE783 (open reverse triangle) or FUSE775 (closed triangle), we observed about a 1000-fold attenuation relative to recombinant human IL18 and (b) the serine mutants FUSE782 (open triangle) and FUSE827 (closed reverse triangle), we observed about greater than a 100,000-fold attenuation relative to recombinant human IL18.

[0079] With regards to cis activity (FIG. 57B using PD-1 decorated HEK Blue IL18), we observed increased biological activity from the PD-1 targeted versions of pro-IL18AS (FUSE782; about 100-fold relative to non targeted FUSE827) and pro-IL18AV (FUSE783; approximately 30-fold relative to non targeted FUSE775).

[0080] FUSE691 (nivolumab) was used as the negative control antibody in both FIG. 57A and FIG. 57B. No biological activity was observed from this test article. No appreciable difference was observed in the biological activities of recombinant human IL18 or the non targeted test articles (FUSE775 and FUSE827), allowing for comparison across FIG. 57A and FIG. 57B. In this context, the PD-1 targeted versions of pro-IL18AS (FUSE782) and pro-IL18AV (FUSE783) were about 100 and 30-fold more active when exposed to PD-1 decorated HEK Blue IL-18 than non-decorated HEK Blue L18.

[0081] Summary tables of the potency of each pro-IL18 variant are shown below each graph.

Example 46

[0082] As shown in figure 58, panel A depicts an exemplary bispecific TCE in format 11A whereby a CD3-specific Fab is fused to the N-terminus of a knob chain of a knob-in-hole (KIH) heterodimeric IgG1 Fc scaffold and two anti-TAA VHH are fused in tandem to the N-terminus of a hole chain of the KIH scaffold. Panel B is an exemplary bispecific TCE in format 13cA whereby a CD3-specific Fab is fused in one of its two polypeptide chains to the N-terminus of the knob chain of a KIH scaffold, a first TAA-specific VHH is fused to the N-terminus of a hole chain, and

a second TAA-specific VHH is fused to the C-terminus of the light chain of the CD3-specific Fab. Panel C is an exemplary bispecific TCE in format Ac13 whereby a CD3-specific Fab is fused in one of its polypeptide chains to the N-terminus of a hole chain of a KIH scaffold, a first TAA-specific VHH is fused to the N-terminus of a knob chain, and a second TAA-specific VHH is fused to the C-terminus of the light chain of the CD3-specific Fab. Format Ac13 in panel B is a mirror image of 13cA depicted in panel B. Panels D and E represent control bispecific TCEs used for the examples below in formats 12A (panel D) and 1A (panel E), respectively. In both cases, the CD3-specific Fab is fused in one of its two polypeptide chains to the N-terminus of the knob chain of a KIH scaffold and a well described TAA specific scFV is fused to the N-terminus of the hole chain (formats 1A and 12A) or to both the N-terminus and C-terminus of the hole chain (format 12A). In some embodiments, the CD3-specific Fab can be a variant of clone of the 1F3 antibody, hu-1F3.

[0083] As shown in figure 59, tables are presented of the protein yields from the transient CHO cell transfections of exemplary TCEs (FIG. 59A) and their melting temperatures (FIG. 59B).

[0084] As shown in figure 60, the apparent binding affinity (EC_{50}) of TCE for human and non-human primate (NHP, Cynomolgus) CD3-expressing T cells were studied. We examined the magnitude by which ROR1-specific TCEs bound to cell surface expressed CD3 (selected TCEs, FUSE-393 and FUSE-394, are shown). Graphs in panels A to D are non-linear x-y plots illustrating the apparent binding affinities (EC50-binding) of FUSE-393 (format 11A incorporating the 5A1 ROR1-specific VHH) and FUSE-394 (format 13cA incorporating the 5A1 ROR1-specific VHH), for human CD3 on Jurkat T cells (panel A), human CD3 on CD3 knockout Jurkat T cells (panel B), NHP CD3 on cynomolgus pan-T cells (panel D), and human CD3 on T cells isolated from human PBMC (panel C). The CD3-specific Fab binding arm is presumed responsible for the observed binding. A Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. Binding was measured via flow cytometry at various concentrations of test articles using a fluorescently labeled secondary antibody specific for the Fc domain of IgG1. The apparent affinity calculated for each test subject based on the data is shown under each graph. A less than 2-fold difference in the apparent binding affinity for human and for cynomolgus CD3 was observed for FUSE-393 and FUSE-394; and no appreciable non-specific binding was observed. Binding to mouse CD3 was not tested because the anti-CD3 clone, 1F3-3, incorporated into the TCE is a humanized variant of clone SP34, which was derived in mouse and is well described as not binding mouse CD3.

[0085] As shown in figure 61, the apparent binding affinity (EC_{50}) of TCEs for ROR1 at different cell surface densities of the target tumor cell was studied. Panels A-D are non-linear x-y plots illustrating the apparent binding affinities (EC_{50}-binding) of FUSE-394 (i.e., the apparent affinity derived from the ROR1-specific VHH in format 13cA incorporating the ROR1-specific VHH having the 5A1 sequence) and FUSE-393 (format 11A incorporating the ROR1-specific specific VHH, 5A1) for human ROR1 expressed at different densities on the surface of engineered MDA-MB-231 cell variants. The cell surface densities are as follows: approximately 2,000,000 molecules per cell (panel A), approximately 500,000 molecules per cell (panel B), approximately 35,000 molecules per cell (panel C), and

approximately 17,000 molecules per cell (panel D). In addition, a ROR1-knock out MDA-MB-231 cell line was also generated using CRISPR/Cas9, which served as a control for non-specific binding (panel E). A Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. Binding was measured via flow cytometry at various concentrations of test articles using a fluorescently labeled secondary antibody specific for the Fc domain of IgG1. The apparent affinity calculated based on the data for each test article is shown under each graph. FUSE-394 was found to bind to ROR1 with a higher apparent binding affinity than FUSE-393, which ranged from approximately 4-fold to less than 2-fold greater depending on the cell surface density of ROR1. In general, the apparent affinity for both FUSE-393 and FUSE-394 correlated positively with cell surface density of ROR1. For FUSE-394, the apparent affinity ranged from approximately 100 nM at a low cell surface density to approximately 5 nM at a high cell surface density. For FUSE-393, the apparent affinity ranged from approximately 300 nM at a low cell surface density to approximately 15 nM at a high cell surface density. No appreciable non-specific binding was observed with either for the HEL-specific control with ROR1+ MDA-MB-231 cells or for the TCEs with MDA-MB-231 ROR1-knockout cells. No binding to mouse ROR1 was observed using mouse 4T1 cell engineered to stably express mouse ROR1 (data not shown). Qi et al. (see www.pubmed.ncbi.nlm.nih.gov/29844189/) previously reported that the ROR1 specific domain targeted by a CD3 x ROR1 bispecific antibody or TCE can impact its potency. This includes the EC50 of pro-inflammatory cytokine release and EC50 of killing of ROR1 expressing tumor cells (but not necessarily maximum killing). The authors demonstrated that the closer the TCE's ROR1 binder's epitope is to the cell membrane, the more potent the TCE. The ROR1 ectodomain consists of three domains. The membrane proximal Kringle domain, the membrane distal Ig domain and the Frizzled domain in-between. Hence, the authors demonstrated that the Kringle domain specific binder, R11, when formatted as a CD3 bispecific antibody is more potent than a binder specific for the region between the Ig and Frizzled domain. Importantly, Gohil demonstrated that the same ROR1 binder (hF(1x1)) when formatted as a (scFV)2 or BiTE (short synapse) but not a symmetric bispecific antibody (larger synapse), mediated killing of ROR1+ tumor cells (see www.discovery.ucl.ac.uk/id/eprint/10042372/1/GOHIL_SH_DEPOSIT.pdf). This relationship between synaptic size and potency is thought to be the result of the magnitude of the signal transmitted via the TCR/CD3 complex, whereby the shorter the bridge or synapse between the T cell and targeted tumor, the stronger the activation signal. This is mediated, at least in part, by greater engagement of adhesion molecules exclusion of large inhibitory molecules such as CD45 from the smaller synapse. The strong activation signal, however, is associated with an important caveat. That is, that although the signal strength required to induce killing is lower than that required for cytokine release (see www.pnas.org/doi/10.1073/pnas.2334336100), very short synapses are unable to take advantage of this phenomenon to induce strong tumor cell killing with modulated or tuned cytokine release to reduce the risk of cytokine mediated toxicities such as Cytokine Release Syndrome (CRS) and neurotoxicity. While not wishing to be bound by any particular theory, we believe that although binding to the Kringle or Frizzled domain may maximize killing potency, it may be possible to dose higher into similar maximal killing by targeting the membrane distal Ig domain. FIGs 61F-61H are overlayed flow cytometric histograms of binding of exemplary test articles to HEK-293 cells (negative control; FIG. 61F), HEK-293 cells transfected with full length human ROR1 (FIG. 61G) and HEK-293

cells transfected with the ROR1 Ig domain linked to the cell surface via the GPI linker from CD59 (FIG. 61H). Binding of 2A11 (black line), 5A1 (dark grey line) and VHH8 (medium grey line) were tested in TCE format 12A. The commercial ROR1 specific Ab reported to bind the ROR1-Ig domain 2A2 (light grey line), served as the positive control and anti-HEL (dotted black line) served as the negative control. To better visualize binding of each test article to the three cells lines, we plotted MFI on the y-axis for each test article for HEK-293 cells (negative control; FIG. 61I), HEK-293 cells transfected with full length human ROR1 (FIG. 61J) and HEK-293 cells transfected with the ROR1 Ig domain linked to the cell surface via the GPI linker from CD59 (FIG. 61K). We found that the ROR1 specific 2A11 and 5A1 VHHs bound to the ROR1 Ig domain but ROR1 specific VHH8 did not.

[0086] As shown in figures 62A-62D, the binding affinities (KD) of TCEs for ROR1 were assessed. We examined the magnitude by which several TCEs and mutants thereof bind to recombinant ROR1. Kinetic binding graphs were generated via Bio-Layer Interferometry (BLI) using an Octet system. For each TCE, the biosensor tips were coated with FUSE proteins at 15 μg/ml and probed with either recombinant His tagged ROR1 at concentrations ranging from 100 pM to 10 μM. The parental TCEs tested were FUSE-211 (format 11A incorporating 2A11, a ROR1-specific VHH), FUSE-393 (format 11A incorporating 5A1, a ROR1-specific VHH), and FUSE-394 (format 13cA incorporating 5A1, a ROR1-specific VHH), shown in figures 62A-62C, respectively. The binding affinity (KD), on rate (k-on) and off rate (k-dis), are summarized for ROR1 in FIG. 62D.

[0087] We further examined the impact of mutating the cysteine residues located in CDR1 and CDR3 of 2A11 VHH and 5A1 VHH. As such the parental 2A11 VHH and 5A1 VHH were expressed as VHH-human-Fc fusion proteins, FUSE-112 and FUSE-179, respectively. For the mutant 2A11 (FUSE-453) and mutant 5A1 (FUSE-454) the cysteine residues in both CDR1 and CDR3 were mutated to valines such that 2A11-CDR1 was changed from GSTYSANC (SEQ ID NO:325) (FUSE-112) to GSTYSANV (SEQ ID NO:569) (FUSE-453), 2A11-CDR3 was changed from AYGGSRCVYNY (SEQ ID NO:327) (FUSE-112) to AYGGSRVVYNY (SEQ ID NO:570) (FUSE-453), 5A1-CDR1 was changed from GYTNRLKC (SEQ ID NO:328) (FUSE-179) to GYTNRLKV (SEQ ID NO:571) (FUSE-454) and 5A1-CDR3 was changed from DVRPDGTTCHYN (SEQ ID NO:572) (FUSE-179) to DVRPDGTTVHYN (SEQ ID NO:573) (FUSE-454). The binding affinity (KD), on rate (k-on) and off rate (k-dis) are summarized for ROR1 in FIG. 62G and 62J. The binding curves for FUSE-112, FUSE-453, FUSE-179 and FUSE-454 are shown in FIGs 62E, 62F, 62H, and 62I, respectively where FIG. 62E versus FIG. 62F compares 2A11 parental to the 2A11 mutant and FIG. 62H versus 62I compares 5A1 parental to the 5A1 mutant. Both FUSE-112 and FUSE-179 bound to ROR1 with KDs of about 4 nM, whereas binding of FUSE-453 and FUSE-454 to ROR1 was so weak that a reliable KD could not be calculated or was below the limit of calculation (BLC). We estimate the affinity of FUSE-453 and FUSE-454 for ROR1 is >100 fold lower than FUSE-112 and FUSE-454, respectively. As such, the cysteine residues within CDR1 and CDR3 of 2A11 VHH and 5A1 VHH are critical to the ROR1 specific paratopes.

[0088] As shown in figures 63A-6H, non-linear x-y plots illustrate the induction ROR1-dependent, human peripheral blood mononuclear cells (PBMC) release of IFNγ and cytotoxic activity (i.e. killing of ROR1+ MDA-MB-

231 tumor cells) induced by fusion proteins FUSE-211 (format 11A incorporating 2A11, a ROR1-specific VHH), FUSE-394 (format 13cA incorporating 5A1, a ROR1-specific VHH), and FUSE-393 (format 11A incorporating 5A1, a ROR1-specific VHH). In FIGs 63I and 63J, IFNγ release and cytotoxicity of the aforementioned TCE are compared to a highly potent TCE, FUSE-277 (format 12A incorporating R11, a ROR1 specific scFV) and the ratio of cytotoxicity to cytokine (IFNγ) release measured as the "decoupling ratio". We examined the capacity of the aforementioned TCEs to induce killing of ROR1$^+$ target cells, MDA-MB-231. Results from three exemplary PBMC donors are shown in FIGs 63A, 63B and 63C. FIG. 63D shows the potency of killing defined as the EC_{50}-killing. We further examined the capacity of several TCEs to induce PBMC mediated IFNγ release in the presence of ROR1$^+$ target cells, MDA-MB-231. Results from three exemplary PBMC donors are shown in FIGs 63E, 63F and 63G. FIG. 63H shows the potency of cytokine release defined as the EC_{50}- IFNγ. For both readouts, a Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. To measure cytotoxic potency and IFNγ release, freshly isolated human PBMCs were co-cultured for 48 hours with human ROR1$^-$ MDA-MB-231 tumor cells engineered to stably express firefly luciferase and GFP. Supernatant was collected and IFNγ measured via ELISA. Luminescent activity induced by firefly luciferase in the presence of its substrate, D-Luciferin, was used as a measure of ROR1$^+$ MDA-MB-231 cell viability. We observed ROR1-dependent cytotoxic potency in the pM range for FUSE-211, FUSE-393 and FUSE-394 across three sets of PBMCs isolated from different healthy control donor subjects. FUSE-394 was the most potent followed by FUSE-393 and FUSE-211, the latter two of which were not appreciably different. We also observed ROR1-dependent IFNγ release. Again, FUSE-394 was the most potent followed by FUSE-393 and FUSE-211. The values for IFNγ release for FUSE-211 at 100 nM were calculated based on the curve. For all the aforementioned TCEs, IFNγ release was weaker, both in EC50 and max release, than what would be expected from a traditional TCE such as a Bi-specific T-cell engager (BiTE) or Dual-Affinity Re-Targeting (DART) molecule. Both BiTES and DARTs contain small linkers between their CD3 binder and target antigen binder. As such, the synapse or bridge formed between T cells and tumor cells is generally short unless in the very rare circumstance that the target antigen is very large and the epitope is very membrane distal. This results in a stronger activation signal via the TCR/CD3 complex, which has been hypothesized to occur because more adhesion molecules are engaged, and CD45, a key inhibitory phosphatase, is excluded from the T cell/tumor synapse. In contrast, FUSE-211, FUSE-393 and FUSE-394 incorporate ROR1 specific VHH that bind the membrane distal ROR1 Ig domain and geometries selected for high killing efficacy (e.g., maximum killing) but reduced pro-inflammatory cytokine release (IFNγ used as example since early neutralization of IFNγ can reduce symptoms of CRS (see e.g., www.pubmed.ncbi.nlm.nih.gov/8370401/)) to decrease the risk of toxicity. Of note, the expression of IFNγ following T cell activation correlates with that of IL-2, TNFα and IL-6 is well accepted. TNFα and IL-6 are also tightly linked to CRS. IL-2 has also been linked toxicity and could have implications to Treg activity, which constitutively express the high affinity IL-2 receptor, CD25. We therefore compared ROR1 dependent PBMC mediated IFNγ release and cytotoxicity of MDA-MB-231 associated with FUSE-211, FUSE-393 and FUSE-394 with a TCE that bridges CD3 to the membrane proximal Kringle domain of ROR1. The latter TCE, FUSE-277, binds to

ROR1 via the well described ROR1 specific clone, R11. The non-linear x-y plot illustrating the killing of MDA-MB-231 by the average of three donor PBMCs is shown in FIG. 63I. The non-linear x-y plot illustrating MDA-MB-231 ROR1 dependent PBMC mediated IFNγ release (mean of three donors) is shown in FIG. 63J. For PBMC mediated cytotoxicity (FIG. 63I), we observed that the potency ($EC50_{KILLING}$) of FUSE-277 was approximately 2 pM, or 40-50 fold greater than FUSE-211, FUSE-393 and FUSE-394. Importantly, killing efficacy for FUSE-277 was only 6-10% greater than those observed for FUSE-211, FUSE-393 and FUSE-394 indicating that the latter TCE could be dosed to a nearly equivalent efficacy. Further, the extremely potent nature of FUSE-277 may result in difficulty identifying a sufficiently low dose in the clinic to prevent tumor lysis syndrome when tumor burden is high. For PBMC mediated IFNγ release (FIG. 63J), we observed that the potency ($EC50_{IFNγ}$) of FUSE-277 was approximately 5 pM, on average about 1000 fold greater than FUSE-211, FUSE-393 and FUSE-394. Perhaps more critical, the maximum release of IFNγ induced by FUSE-277 was on average approximately 3 fold greater than those observed for FUSE-211, FUSE-393 and FUSE-394. This data suggests one could dose FUSE-211, FUSE-393 and FUSE-394 into similar killing efficacy as FUSE-277 with a reduced risk of CRS. Of interest, although FUSE-211, FUSE-393 and FUSE-394 were observed to reach maximum killing efficacy at about 1 nM, IFNγ levels slowly increased from close to baseline at 1 nM to between 2.5-5 ng/ml at 20 nM. This gap represents about a 20,000 pM range, or "in-vitro therapeutic window", in which the dose of FUSE-211, FUSE-393 and FUSE-394 can be titrated to achieve a dose that incorporates both maximizing killing and a safe/effective dose of pro-inflammatory cytokine, important for inflaming the tumor and facilitating bystander killing (see FIG. 70). Thus, the data suggests that it may be possible to dose FUSE-211, FUSE-393 and FUSE-394 to maximize efficacy but titrate the cytokine release to achieve tumor inflammation with a reduced risk of CRS. In contrast, no such "in-vitro therapeutic window" was observed with FUSE-277. To further quantitate the degree by which each TCE decoupled cytotoxicity from cytokine (IFNγ) release, we measured killing magnitude as the area under the curve (AUC) for cytotoxicity from FIG. 63I and IFNγ release magnitude as the area under the curve (AUC) for IFNγ from FIG. 63J. A "decoupling ratio", defined as the magnitude of killing divided by the magnitude of IFNγ release (normalized to FUSE-277, for which an "in-vitro therapeutic window" was not observed) was then assigned to each TCE (FIG. 63K). The decoupling ratios for FUSE-277, FUSE-211, FUSE-393 and FUSE-394 were 1 (no decoupling), 4.4, 5.4 and 4.3, respectively. We wished to confirm that TCE induced tumor cell cytotoxicity and IFNγ release observed using PBMC as effectors was mediated by T cells. As such, we performed the same assay shown in FIGs 63I and 63J but substituted purified Pan-T cells for PBMC. FIG. 63L and 63M are non-linear x-y plots of specific killing or IFNγ release versus test article concentration, respectively. Both assays were run for 24 hours and unlike PBMC that were freshly isolated, Pan-T cells were cryo-preserved. In both cases, FUSE-394 was compared to the non-decoupling control TCE, FUSE-277. Anti-HEL was used as the negative control, for which non-specific activity was absent. A summary of potencies (EC50s) for ROR1 dependent cytotoxicity and IFNγ release is shown in FIG. 63O. Similar to that observed with PBMC, FUSE-277 was about 15 fold more potent ($EC50_{KILLING}$) than FUSE-394 but both reached similar efficacy readouts (maximum killing) and the potency of FUSE-394 remained in the low

triple digit pM range (FIG. 63L). We consider such a potency more conducive for managing tumor lysis syndrome. In contract to ROR1 dependent tumor cell cytotoxicity, we observed about a 200 fold reduced potency related to T cell mediated IFNγ release induced by FUSE-394 compared to FUSE-277. Importantly, maximum IFNγ release associated with FUSE-394 plateaued at approximately 30% that of FUSE-277 (FIG. 63M) and the "in-vitro therapeutic window" calculated for FUSE-394 was about 20,000 pM. This resulted in a decoupling ratio for FUSE-394 that was between 4-5x greater than that for FUSE-277 (FIG. 63N). Although, the comparison was between PBMC and Pan-T, it is interesting to note that reducing the assay time period from 48 hours (PBMC) to 24 hours (T cells) led to only about a 25% drop maximum killing but 4-5 fold drop in maximum IFNγ release. To obtain further insight into the kinetics of cytotoxicity and cytokine release induced by (a) a non-decoupling TCE (FUSE-277) and (b) exemplary TCEs that decouple cytotoxicity from cytokine release (FUSE-211, FUSE-393 and FUSE-394), we performed target dependent cytotoxicity (TDCC) assays and target dependent cytokine release (TDCR) assays for 24 hours, 48 hours and 72 hours. MDA-MB-231 was used as the ROR1 expressing tumor cell, PBMC as effector cells and IFNγ as the exemplary proinflammatory cytokine. As seen in FIGs 63P, 63R and 63T, FUSE-277 was about 50-100 fold more potent (EC50-killing) than FUSE-211, FUSE-393 and FUSE-394 but the maximum killing (efficacy) induced by the decoupled TCEs was able to reach values not appreciably different from FUSE-277. Killing potency induced by FUSE-211, FUSE-393 and FUSE-394 was within 2-3 fold of one another. As would be expected, maximum killing mediated by all test articles increased with time with values of approximately 70%, 85% and 95% at 24, 48 and 72 hours, respectively. Unlike ROR1 dependent killing, maximum cytokine release induced by FUSE-211, FUSE-393 and FUSE-394 was consistently about 3 fold less than FUSE-277 at each timepoint (FIGs 63Q, 63S and 63U). Potency (EC50-IFNγ) associated with the three decoupled TCEs was as much as 10,000 fold weaker than FUSE-277, the "in-vitro therapeutic window" ranged from 10,000 to 20,000 pM and decoupling ratios (FIG. 63V) ranged from values of about 4 (FUSE-211 and 394) to between 4.5-5 (FUSE-393). Although there was no significant difference in decoupling ratios at different timepoints, a trend developed such that the decoupling ratio at 24 hours (the key safety timepoint) appeared to be the highest. As observed with cytotoxicity, potency associated with cytokine release varied only between 2-3 fold between FUSE-211, FUSE-393 and FUSE-394. Most importantly, the absolute values for maximum IFNγ release at 24 hours for the three decoupled TCEs did not exceed 1.5 ng/ml and could be titrated between about 200 pg/ml and 1.5 ng/ml while maintaining maximum killing. Matthys and colleagues (see www.pubmed.ncbi.nlm.nih.gov/8370401/) reported that early but not late neutralization of IFNγ was capable of reducing the symptoms of CRS. In fact, rebound levels of IFNγ at later timepoints that exceeded treatment with saline did not contribute to such symptoms. This suggests that initiating treatment of a patient with a dose of FUSE-211, FUSE-393 or FUSE-394 that induces maximum killing but minimal IFNγ should reduce the risk of CRS and the need for expensive interventions such neutralization of IFNγ, TNFα and/or IL-6 thus vastly improving patient compliance. Importantly, at later timepoints the dose of FUSE-211, FUSE-393 or FUSE-394 can be safely increased such that IFNγ levels accumulate to the point that secondary

mechanisms of tumor cell killing that involve tumor inflammation and bystander killing mediated by death receptors are supported. The latter is explored in FIG. 70.

[0089] As shown in figures 64A-64C, none-linear x-y plots illustrate the induction of human PBMC-mediated, ROR1-independent cytotoxic activity by FUSE-211 (format 11A incorporating the ROR1 specific VHH, 2A11), FUSE-394 (format 13cA incorporating the ROR1 specific VHH, 5A1), and FUSE-393 (format 11A incorporating the ROR1 specific VHH, 5A1). We examined the ROR1 specificity of several TCEs with reference to their induction of killing of target cells that do not express ROR1, i.e., T47-D cell line. Results from three exemplary PBMC donors are shown in panels A-C, respectively. A Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. To measure non-specific cytotoxic potency, freshly isolated human PBMC were co-cultured for 48 hours with human ROR1-negative T-47D tumor cells engineered to stably express firefly luciferase and GFP. Luminescent activity induced by firefly luciferase in the presence of its substrate, D-Luciferin, was used as a measure of $ROR1^+$ target viability. We observed very little to no appreciable cytotoxicity towards T-47D target cells. At the highest and supraphysiological concentration of 100 nM, non-specific cytotoxicity was observed towards <10% of the T-47D cells in donors 2 and 3 but not in donor 1. Further, such non-specific killing was highly variable between test articles including the anti-HEL isotype control, which was indicative of irrelevant artifacts likely resulting from the IgG1 backbone or perhaps NK cell-mediated antibody dependent cellular cytotoxicity.

[0090] As shown in figures 65A-65E, and 65G-65K, we provide non-linear x-y plots of dose contingent TCE induced human PBMC-mediated ROR1-dependent (a) cytotoxic activity, and (b) IFNγ release, respectively, when admixed with one of a series of tumor cells. The TCEs tested were the non-decoupling control TCE, FUSE-277, and the following TCEs that decouple cytotoxicity from cytokine release: FUSE-211, FUSE-393 and FUSE-394. From highest to lowest cell surface density of ROR1, the cell lines tested were NCCIT (FIGs 65A, 65G), MDA-MB-231 (FIGs 65B, 65H), NCI-H1975 (FIGs 65C, 65I), DU-145 (FIGs 65D, 65J) and ROR1-negative T-47D (FIGs 65E and 65K). A Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. We observed PBMC mediated killing of all $ROR1^+$ cell lines and very little to no appreciable non-specific cytotoxicity towards the ROR1-negative target cells, i.e., T-47D cells. For each TCE, the potency of killing (EC_{50}-killing) was observed to weaken as the density of cell surface ROR1 decreased. As expected, the potency of FUSE-277 ranged between 70-100 fold greater than the 3 decoupled TCEs, which demonstrated very similar killing potencies across ROR1 cell surface density whereby the fold difference between any two was less than 2 fold. Further, we observed that maximum killing trended positively with ROR1 cell surface density on the target cells and importantly, with the exception of killing of NCI-1975, FUSE-211, FUSE-393 and FUSE-394 reached maximum killing values that were not appreciably different from FUSE-277. A summary table of EC_{50}-killing for each test article is shown in FIG. 65F. As previously observed when MDA-MB-231 tumor cells were employed as ROR1+ targets, we observed that compared to FUSE-277, for which no "in-vitro therapeutic window" was observed, the three decoupled TCEs induced PBMC mediated cytokine release that was associated with (a) 1000 to >10,000 fold reduced potency (EC_{50}-IFNγ), (b) maximum IFNγ values that were at least

3 fold lower and (c) "in-vitro therapeutic windows" of approximately 20,000 pM, (d) decoupling ratios (FIG. 65M) between 4-5 (NCCIT, MDA-MB-231 and NCI-H1975) and 6-7 (DU-145), and (e) potencies and maximum levels within 2 fold of each other across the entire cell line array. A summary table of EC_{50}-IFNγ for each test article is shown in FIG. 65L that includes the lack of non-specific activity observed with T-47D (FIG. 65K). The values for IFNγ release for FUSE-211 at 100 nM were calculated based on the curve. Like ROR1 dependent cytotoxicity, ROR1 dependent cytokine release trended positively with ROR1 cell surface density, which was measured as between 5,000-7,000 molecules per cell on DU-145 (prostate cancer cell line). This level is consistent with that thought to be expressed on most normal tissues that express ROR1, including gut epithelial cells and pancreatic islet cells (see www.pubmed.ncbi.nlm.nih.gov/27852699/) Importantly, unlike FUSE-277 that induced appreciable PBMC mediated IFNγ release, that induced by the three decoupled TCEs was negligible (≤250 pg/ml). This suggests that FUSE-211, FUSE-393 and FUSE-394 are able to "distinguish" between "normal" and malignant levels of ROR1 such that these TCE do not induce appreciable cytokine production when ROR1 density reaches levels at or below about 7,000 cell surface molecules per cell.

[0091] As shown in figures 66A-66E, and 66G-66K, we provide non-linear x-y plots of dose contingent TCE induced human PBMC-mediated ROR1-dependent (a) cytotoxic activity, and (b) IFNγ release, respectively, when admixed with one of a series of tumor cells as described below. The TCEs tested were the non-decoupling control TCE, FUSE-277, and the following TCEs that decouple cytotoxicity from cytokine release: FUSE-211, FUSE-393 and FUSE-394. The target cells consisted of MDA-MB-231 cells engineered to stably express different cell surface densities of ROR1. The cell surface densities were as follows: ~2,000,000 molecules per cell (MDA++; FIGs 66A and 66G), ~500,000 molecules per cell (MDA+; FIGs 66B and 66H), ~35,000 molecules per cell (MDA; FIGs 66C and 66I), and 17,000 molecules per cell (MDA-low; FIGs 66D and 66J). In addition, a ROR1-knock out, MDA-MB-231 cell line (MDA-KO; FIGs 66F and 66K) was generated using CRISPR/Cas9, which served as a control and reflected ROR1-independent, non-specific cytotoxicity. A Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. To measure cytotoxic potency and IFNγ release, freshly isolated human PBMCs were co-cultured for 48 hours with each of the MDA-MB-231 ROR1 density variants. We observed very little to no appreciable non-specific IFNγ release from PBMC nor cytotoxicity of the MDA-MB-231 ROR1-knockout target cells. For each TCE, the potency of killing (EC_{50}-killing) and IFNγ release (EC_{50}-IFNγ) were observed to weaken as the density of cell surface ROR1 decreased. As anticipated, the potency of FUSE-277 ranged between 70-100 fold greater than the 3 decoupled TCEs, which demonstrated very similar killing potencies across ROR1 cell surface density whereby the fold difference between any two was less than 2 fold. Further, we observed that maximum killing trended positively with ROR1 cell surface density on the target cells and importantly, FUSE-211, FUSE-393 and FUSE-394 reached maximum killing values that were not appreciably different from FUSE-277. A summary table of EC_{50}-killing for each test article is shown in FIG. 66F. No "in-vitro therapeutic window" was observed with FUSE-277. In contrast, the observed "in-vitro therapeutic window" for the three decoupled TCEs was consistently about 20,000 pM. Further, PBMC mediated

cytokine release induced by FUSE-211. FUSE-393 and FUSE-393 was associated with (a) 1000 to >10,000 fold reduced potency (EC_{50}-IFNγ), (b) maximum IFNγ values that were at least 3 fold lower and (c) decoupling ratios (FIG. 66M) between 4-5.5. Of note, a trend appeared such that decoupling ratios increased in value as the density of ROR1 on the cell surface of the MDA-MB-231 variants decreased. A summary table of EC_{50}-IFNγ for each test article is shown in FIG. 66L. The values for IFNγ release for FUSE-211 at 100 nM were calculated based on the curve. We calculated the area under the curve for IFNγ release termed the magnitude of IFNγ release and performed a regression analysis of such versus ROR1 cell surface density (FIG. 66N). Each dot in the regression analysis represents a different variant of MDA-MB-231 whereby the density of ROR1 increases from left to right. FUSE-277, the non-decoupling TCE control, was associated with an appreciably steeper slope than FUSE-394, the exemplary non-decoupling TCE shown. FUSE-211 and FUSE-393 were associated with similar results (data not shown). Importantly, FUSE-277 but not FUSE-394 was observed to be associated with IFNγ release at ROR1 densities below 10,000 cell surface molecules, discussed above as the likely demarcation between the expression of ROR1 on most normal versus malignant cells. Taken together with the data from FIG. 66F

[0092] As shown in figures 67A-67C, we examined the capacity of anti-ROR1 TCEs to induce activation of T cells when ligated to ROR1 on target cells (MDA-MB-231). Exemplary TCEs that decouple cytotoxicity from cytokine release, FUSE-211, FUSE-393 and FUSE-394 are shown relative to the non-decoupling control TCE, FUSE-399 (format 1A incorporating the R11 scFV specific for the ROR1 Kringle domain). PBMCs were mixed with MDA-MB-231 for 24 hours after which flow cytometry was used to assess the early and late activation markers, CD69 (Fig. 67A) and CD25 (Fig. 67B), respectively, on gated CD3+ T cells. Mean Fluorescence Intensity (MFI) for each of CD69 and CD25 is shown on the y-axis of each pot. Each x-axis is representative of a titration of test article ranging from 5 pM to 100 nM. A Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control (filled closed circles). We observed ROR1 dependent induction of CD69 and CD25 expression. For both activation markers, the potency by which expression was induced (EC50-CD69 and EC50-CD25) by FUSE-399 (filled triangles) was between 200-600 fold greater than that of FUSE-211 (inverted filled triangles), FUSE-394 (open circles) or FUSE-393 (filled diamonds). In all cases, the difference in EC50-CD69 and EC50-CD25 between each of the three decoupled TCEs was no greater than 2 fold and averaged about 10 nM. Non specific activation was not observed with the anti-HEL negative control. A summary table of potencies for induction of CD69 and CD25 by each TCE is shown in FIG. 67C. We had previously observed an average potency of ROR1 dependent cytotoxicity (EC50-killing) of between 75-150 pM for FUSE-211, FUSE-393 and FUSE-394. Thus, this data suggested that in a similar fashion to cytokine release, these three TCEs may also decouple cytotoxicity from induction of early and late activation markers. To address this possibility, we used FUSE-394 as the exemplary TCE that decouples cytotoxicity from cytokine release to calculate the area under the curve (AUC) for induction of CD69 (FIG. 67A) and induction of CD25 (FIG. 67B) and used these values for calculating the divisor of AUC-killing divided by AUC-69 (Cytotoxicity/CD69 decoupling ratio; FIG. 67D) or AUC-killing divided by AUC-CD25 (Cytotoxicity/CD25 decoupling ratio; FIG. 67F), normalized to FUSE-399 (set to a

value of 1). We calculated that FUSE-394 decoupled cytotoxicity from CD69 and CD25 induction at the orders of 2 fold and 2.4 fold, respectively. Given that CD25 and IFNγ both reach peak expression between 24-48 hours after T cell activation, the decoupling of cytotoxicity from CD25 induction was not surprising. However, CD69, in part due to the protein being preformed in T cells, is rapidly expressed on the surface of activated T cells such that it can be observed in under 4 hours and peaks roughly 24 hours after TCR/CD3 stimulation. Thus, it was surprising that relative to FUSE-399, FUSE-394 decoupled cytotoxicity from CD69. Given that expression of CD69 has been linked to an exhaustion-like phenotype of tumor infiltrating T cells and reduced anti-tumor activity (see www.academic.oup.com/intimm/article/30/12/559/5067178), our data suggest that FUSE-394 (as well as FUSE-393 and FUSE-211) may be associated with a reduced risk of inducing T cell exhaustion and more improved anti-tumor activity compared to non-decoupled TCEs such as FUSE-399 (and FUSE-277). To our knowledge, we are the first to observe decoupling of cytotoxicity from induction of CD69 expression suggesting that release of perforin and cytotoxic proteases including Granzyme B may not always be associated with expression of CD69. Given that the ratio for decoupling cytotoxicity from induction of CD69 and CD25 was measured as between 2-2.4 for FUSE-394, that is about 2 fold lower than its capacity to decouple cytotoxicity from cytokine (IFNγ) release (average ratio for FUSE-394 was about 4; see above), while not wishing to be bound by any particular theory, we believe that FUSE-394 (as well as FUSE-393 and FUSE-211) likely decouple the induction of CD69 and CD25 expression from cytokine (IFNγ) release. This would be especially interesting for CD25 since, as stated above, the kinetics of CD25 and IFNγ expression after T cell activation are very similar. As shown in FIG. 67E and FIG. 67G, compared to FUSE-399, FUSE-394 decoupled the induction of CD69 and CD25 expression from cytokine (IFNγ) release by 2 fold and 1.8 fold, respectively. Overall, the data indicate that cytotoxicity associated with FUSE-394 is decoupled from both cytokine release and the induction of activation markers but that the latter is intermediate compared to the former such that induction of activation markers is further decoupled from cytokine release. FUSE-211 and FUSE-394 were not appreciably different whereas FUSE-393 was approximately 2-fold less potent. No non-specific activation was observed with the anti-HEL antibody.

[0093] As shown in figures 68A-68C, we examined the single dose PK in wild type C57BL/6 mice of two TCEs, FUSE-211 (format 11A incorporating the ROR1 specific VHH, 2A11) and FUSE-394 (format 13cA incorporating the ROR1 specific VHH, 5A1). A recombinant version of the clinical stage ROR1-specific monoclonal antibody, Cirmzuzimab (also denoted as FUSE-366), generated from the published sequence, was used as the IgG antibody control described to have pharmacokinetics consistent with a wild type antibody. Figure 68A and 68B are x-y plots of the concentration of test article identified in mouse serum (FUSE-211, FUSE-394, or FUSE-366) on the y-axis versus time on the x-axis. In each case, mice received a single dose of a test article at either 5 mg/kg (Fig 68A) or 0.5 mg/kg (Fig 68B). Cirmzuzimab (also denoted as FUSE-366) was only included in the 5 mg/kg cohort. In all cases, mice were injected IV with one of the 3 test articles at time zero and serum collected at the several time points (in hours): 0, 1, 6, 24, 48, 72, 168, and 240. Three mice were sampled at each time point. After collection of serum, the concentration of each test article was quantitated using an ELISA specific for the Fc domain of human IgG. No appreciable difference

was observed in the PK of FUSE-211 versus FUSE-366, and both exhibited very similar alpha distribution and beta elimination phases. Importantly, although the slope of the late beta elimination phase (>150 hours) at 5 mg/kg for the control antibody, FUSE-366, was gentler than that of FUSE-211 and FUSE-394 (Fig. 68A), the PK for both TCEs was not appreciably different from the control antibody between 0-150 hours. This indicates that both FUSE-211 and FUSE-394 have PK properties similar to a standard monoclonal antibody.

[0094] As shown in figures 69A-69E, we examined the capacity of FUSE-394 and the non-decoupled control TCE, FUSE399, to mediate tumor growth inhibition in two humanized mouse xenograft models. In both cases, the highly aggressive human ROR1$^+$ teratoma, NCCIT was used and injected subcutaneously. In the first model, NSG mice were injected with a mixture of NCCIT and expanded T cells such that the ratio of tumor to CD8+ T cells constituted 1:4. In an attempt to mimic the phenotype of human TIL that would reflect the "human condition" and not necessarily be expected to fully control tumor growth in the absence of circulating T cells, T cells were expanded for 12 days and recovered for an additional 3 days in IL-2 to mimic a progenitor exhausted phenotype, which would not be expected to be fully functional nor proliferate in-vivo in a hypoxic tumor mass. As such, these T cells did not display alloreactivity towards NCCIT in-vitro. A treatment regimen of four intravenous (IV) injections was initiated on Day 1 and thereafter the mice were monitored for "breakthrough" tumor growth. Due to concerns that matrigel may be toxic to TIL, the former was omitted from this study making tumors more difficult to measure, especially in the treated groups in which tumors growth, if present, was flat in nature and therefore subjective. On Day 21, we observed what appeared to be possible tumor formation in 2 mice that received the low dose of FUSE-394 (0.15 mg/kg), 2 mice that received the higher dose of FUSE-394 (1.5 mg/kg), 1 mouse that received the low dose of FUSE-399 (0.15 mg/kg) and 1 mouse that received the higher dose of FUSE-399 (1.5 mg/kg). We interpreted this finding as possible "breakthrough" tumors and therefore injected all the mice with 3.5 million expanded T cells and initiated a second round of treatment (4 IV injections) on Day 24. Such a dosing regimen resembled the "several weeks on, several weeks off" nature of TCE dosing often used in the clinic. At this point, circulating T cells could be recruited into any tumors that evaded TIL. By day 28, with the exception of one mouse in the group receiving the low dose of FUSE-394, all tumors shrunk to below 10 mm cubed. Thereafter, with the exception of the same mouse from the group receiving the low dose of FUSE-394 and one mouse receiving the low dose of FUSE-399, no tumors were visible in any of the treatment groups. In contrast, tumors grew in mice that were treated with PBS, all of which were visibly raised. In the second model, we investigated the capacity of FUSE-394 to control tumor growth of established NCCIT tumors growing rapidly. In this case, NSG mice in which both MHC-I and MHC-II were knocked out (NSG-MHC-I/II dKO; n=25) were implanted subcutaneously with 5 million ROR1$^+$ NCCIT cells. When the tumor volume reached ~100 mm^3, mice were injected intravenously with 30 million freshly isolated PBMC from a healthy human donor. T cell frequency within the PBMC was ~50%, of which a further 60% were CD4+ T cells and ~3% were regulatory T cells. T cells did not expand nor reconstitute in our hands. This is because there are no major xeno-antigens present in these mice. As such, PBMC reconstitution in NSG-MHC-I/II dKO is highly donor dependent and is often not-successful. As such, this system best reflects the capacity of naïve circulating T cells to infiltrate a non-

immunogenic highly aggressive tumor absent of TIL ("desert tumor") in which the only agent inducing tumor reactivity is the TCE. Once the tumor volume reached ~100 mm^3, the mice were randomly divided into three groups; two groups of ten that would receive bolus IV injections of FUSE-394 at either 5 mg/kg or 0.5 mg/kg. A third group of 5 mice were designated to receive the control treatment, Cirmtuzumab, a ROR1 targeting antibody that blocks Wnt5-alpha signaling but has little to no effect in mouse xenograft tumor models. This allowed for the additional study of the impact of CD3/ROR1 bridging with FUSE-394 versus ROR1 binding alone with a well characterized clinical stage antibody. Mice received treatment (repeated IV bolus injection of a test article at either 5 mg/kg or 0.5 mg/kg) on days 0, 2, and 7. FIG. 69D is an x-y plot of tumor volume (y-axis) versus time in days (x-axis). Tumor growth inhibition (TGI) mediated by FUSE-394 was observed as early as 6 days after treatment and achieved a magnitude of >45% versus Cirmtuzumab after ten days (FIG. 69E). No appreciable difference between 0.5 mg/kg and 5 mg/kg was observed at day 10.

[0095] As shown in figures 70A-70D, we investigated the kinetics by which FUSE-394 (format 13cA incorporating the ROR1 specific VHH, 5A1) induces T cell (PBMC derived T cells expanded for 10 days) mediated killing of ROR1-positive MDA-MB-231 tumor cells (FIG. 70A), ROR1-negative T-47D tumor cells (FIG. 70B) or the mixture of ROR1-positive MDA-MB-231 and ROR1-negative T-47D tumor cells (FIG. 70C and 70D). Shown are non-linear x-y plots of tumor viability on the y-axis and time on the x-axis. To distinguish between ROR1-positive MDA-MB-231 tumor cells and ROR1-negative T-47D tumor cells, the former was engineered to express RFP and the latter to express GFP. As seen in FIG. 70A, in the presence of 800 pM FUSE-394 but not the anti-HEL negative control, T cells killed between ~85% of ROR1-positive MDA-MB-231 with an $IC50_{VIABILITY}$ of 14.4 hours. A full titration of TCE from 100 nM to 1.28 pM was performed. Similar maximum killing was observed at concentrations of FUSE-394 between 800 pM and 100 nM (data not shown). At 160 pM, 32 pM, 6.4 pM, and 1.28 pM of FUSE-395, maximum killing dropped to ~58%, 47%, 25% and 16%, respectively (data not shown). When ROR1-negative T47-D tumor cells were co-cultured with T cells, FUSE-394 or anti-HEL, no appreciable loss in tumor cell viability was observed at 800 pM of test article (FIG. 70B) nor any concentration between 1.28 pM and 100 nM (data not shown). This indicates that FUSE-394 is specific for ROR1 and in the absence of ROR1-positive cells, T cells do not mediate killing of T47-D. Importantly and specific to FUSE-394 and not anti-HEL, when T cells were used as effectors in co-culture with a mixture of ROR1-positive MDA-MB-231 and ROR1-negative T-47D tumor cells, we observed killing of both types of tumor cells. The $IC50_{VIABILITY}$ for ROR1-positive MDA-MB-231 was 14.3 hours and that for ROR1-negative T-47D tumor cells was 32.6 hours, which equates to an 18.3 hour delay between ROR1-positive and ROR1-negative tumor cells. This timing is consistent with the phenomenon referred to direct (early) and bystander (late) killing whereby MDA-MB-231 is killed primarily via Perforin/Granzyme B and T-47D is killed via several death receptor mediated pathways. These include Fas that are upregulated by IFNγ, which influences not only the expression of death receptors (e.g. TRAIL-R, TNFR, FAS) but also adhesion molecules such as ICAM-1 that help strengthen T cell-tumor synaptic junctions. The counter-receptors (e.g. FasL, TRAIL, TNFα, LFA-1) are induced by T cell activation, in this

case mediated by cross-linking of the TCR/CD3 complex by the bridge formed by FUSE-394 between ROR1 and CD3ε. TCE mediated bystander killing was previously described for EGFR specific BiTES (see www.pubmed.ncbi.nlm.nih.gov/28837681/), which was observed to induce cytokine release and killing in a coupled fashion. The data suggests that the amount of IFNγ induced by FUSE-394, although safer than that associated non-decoupled TCEs, is still sufficient to mediate similar killing efficacy of tumors expressing heterogeneous levels of ROR1. Indeed, as seen in FIG. 70D, we observed no appreciable difference in maximum bystander killing (killing efficacy) induced by FUSE-394 and FUSE-399, that latter being a non decoupled TCE in format 1A incorporating an R11 scFV specific for the membrane proximal Kringle domain. As shown in FIG. 70E, after a 24 hour co-culture of T cells and MDA-MB-231, the potency and maximum IFNγ release induced by FUSE-394 is 100-300 fold lower and less than 50% that of FUSE-399. Importantly, at 800 pM of test article, while FUSE-399 was within 10% of its maximum cytokine release (~$EC90_{IFN\gamma}$), FUSE-394 had reached only about its $EC30_{IFN\gamma}$. This provides further support for our view that compared to a standard non-decoupled TCE, the size of the window to safely tune cytokine production to maximize both efficacy and safety would be far larger with FUSE-394.

[0096] As shown in FIGs 71A and 71B, we assessed the capacity of exemplary TCEs to induce T cell mediated killing of a constant number of ROR1+ tumor cells (MDA-MB-231) across a range of T cell numbers. This allows for the calculation of (a) the number of T cells needed by different TCEs to kill a given percentage of ROR1+ tumor cells (FIG. 71A) and (b) the average number of tumor cells killed per T cell, also termed "serial killing" (FIG. 71B). To generate the data, different numbers of activated CD8+ T cells ranging from 300 to 40,000 cells were mixed with 5000 MDA-MB-231 (ROR1+) tumor cells. Tumor cell viability was measured after 24 hours. The range of CD8+ T cell numbers added to the assay equated to effector to target ratios of 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1. FUSE-399 (format 1A incorporating R11 scFV specific for the ROR1 Kringle domain) was used as the ROR1 specific non-decoupling TCE control, and was compared to FUSE-211 (format 11A incorporating 2A11, ROR1-Ig domain-specific VHH), FUSE-393 (format 11A incorporating 5A1, a ROR1-Ig domain-specific VHH) and FUSE-394 (format 13cA incorporating 5A1, a ROR1-Ig domain-specific VHH). At E:T ratios of 1:2 to 8:1 (FIG. 71A, right segment of split x-axis), we observed no appreciable difference in tumor cell killing between any of the test articles indicating that at E:T ratios consistent with hematological tumors, the decoupled TCEs (FUSE-211, FUSE-393 and FUSE-394) were not outperformed in terms of killing efficacy (maximum killing) by FUSE-399. At lower T cell numbers corresponding to between 300 and 1250 T cells (E:T of 1:16, 1:8 and 1:4), the decoupled TCEs were observed to induce greater T cell mediated ROR1 dependent tumor cell killing than FUSE-399, both in terms of potency and efficacy. FUSE-393 and FUSE-211 were associated with nearly identical killing curves with maximum killing about 0.3 fold weaker than FUSE-394. Compared to FUSE-399, FUSE-211, FUSE-393 and FUSE-394 were between 2.5-3 fold more potent and 2-2.5 fold more efficacious. For example, to kill approximately 25% of the tumor cells, FUSE-211, FUSE-393 and FUSE-394 required about 450, 450 and 300 T cells, respectively, whereas FUSE-399 required about 1100 T cells. At very low E:T ratios (e.g. 1:16 and 1:8), each T cell (on average) likely killed more than one tumor cell. For example, at

an E:T of 1:16 (312 T cells added to the assay), FUSE-394 was associated with 28-29% killing, which equates to about 1450 tumor cells. As such, on average each of the 312 T cells killed about 4 tumor cells. The phenomenon of a single T cell killing more than one tumor cell is termed serial killing and is especially important in solid tumors where the number of T cells may be sparse with E:T ratios ranging from 1:2 to <1:8. FIG 71B is a bar chart illustrating serial killing for each TCE, which is calculated as the mean number of tumor cells killed per T cell at an E:T of 1:16 and 1:8. In alignment with the overall cytotoxicity observed in FIG. 71A, FUSE-394 was observed to support the highest degree of serial killing at approximately 4.1 tumor cells/T cell. FUSE-211 and FUSE-393 supported slightly less serial killing between 3-3.2 tumor cells/T cell. Importantly, the non-decoupling TCE control, FUSE-399, only supported serial killing at the order of about 1.4 tumor cells/T cell. The mechanism of action for the observed difference likely stems from multiple factors that lead to a longer T cell/tumor residency time and/or T cell "over-activation" with FUSE-399 compared to FUSE-211, FUSE-393 and FUSE-394. For example, the shorter T cell/tumor synapse mediated by FUSE-399 versus FUSE-211, FUSE-393 and FUSE-394, will (a) result in a stronger activation signal that could result in more release and hence less availability of Granzyme B and perforin for multiple kills and (b) likely lead to a tighter bridge between T cell and tumor due to engagement of a greater number and higher affinity adhesion molecules. All data reported are derived from the average of two donor T cells. We wished to confirm that TCE induced tumor cell cytotoxicity and IFNγ release observed using PBMC as effectors was mediated by T cells. As such, we performed the same assay shown in FIGs 63I and 63J but substituted purified Pan-T cells for PBMC.

Example 47

Techniques and Procedures

[0097] The following techniques and procedures are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Human peripheral blood mononuclear cell (PBMC) isolation

[0098] PBMCs were isolated from whole blood from healthy donors using Ficoll-Paque Plus medium. In brief, 35 mL of diluted whole blood (1 volume of whole vs 1 volume of PBS) was gently overlayed on the top of 15 mL Ficoll-Paque Plus medium without disturbing the interface in a 50-mL conical tube. After centrifuge for 40 min at 400 × g at room temperature without brake, the buffy coat (interface layer between Ficoll and serum) was collected and diluted in 5 volumes of PBS. After centrifuge for 5 min at 500 × g at room temperature, PBMCs were resuspended in PBS and washed once in PBS by centrifuging for 5 min at 500 × g at room temperature. PBMCs were then resuspended in 5 mL of ACK lysis buffer and incubate for 5 minutes at room temperature to remove red blood cell residues. After 5-minute's incubation, 45 mL PBS was added to PBMCs and centrifuge for 15 min at 100 × g at room

temperature. At last, PBMCs were resuspended in culture medium (RPMI1640 with 10% FBS and 1% penicillin/streptomycin for cytotoxicity and IFN gamma release assay setting up.

Human T cell isolation, activation, and recovery

[0099] PBMCs were isolated from whole blood from healthy donors following the procedure described above. T cell were isolated from fresh human PBMCs using EASAYSEP human T cell isolation kit from STEMCELL. In Brief, PBMCs were mixed with isolation antibody cocktail (provided with the kit) in Ca^{2+}- and Mg^{2+}-free PBS containing 2% FBS and 1 mM EDTA. After 5-minute incubation at room temperature, RapidSpheres™ bead (provided with the kit) was added to the mixture and then immediately assembled on EASY50 EASY SEP magnet from Stemcell. After 10-minute incubation at room temperature, cells in mixture solution were collected and assembled on EASY50 EASY SEP magnet from Stemcell again. After 5-minute incubation at room temperature, purified T cells in mixture solution were collected and pelleted by centrifugation. Purified T cells were frozen down and kept in liquid nitrogen for further usage.

[0100] To activate purified T cell, isolated T cells were resuspended in ImmunoCult™-XF T cell expansion medium from Stemcell. ImmunoCult™ human CD3/CD28 T cell activator from Stemcell and 100 U/mL human IL-2 from R&D System were added to the T cells to activate for 12 days. During the 12-days activation, fresh ImmunoCult™-XF T cell expansion medium with 100 U/mL human IL-2 was added to the cells whenever the cell density was above 1 million cells per milliliter. At the end of 12-day activation, activated T cells were frozen down and kept in liquid nitrogen.

[0101] To recover T cells, frozen activated T cells or T cells without activation were recovered from liquid nitrogen and thawed in 37℃ water bath. The recovered T cells were resuspended in RMPI1640 medium with 10% heat-inactivated FBS with 250 U/mL human IL-2. After 48-hour incubation at 37℃, T cells were ready for further usage.

Human CD8+ T cell isolation, activation, and recovery

[0102] PBMCs were isolated from whole blood from healthy donors following the procedure described above. CD8+ T cell were isolated from fresh human PBMCs using EASAYSEP human CD8+ T cell isolation kit from STEMCELL. In Brief, PBMCs were mixed with isolation antibody cocktail (provided with the kit) in Ca^{2+}- and Mg^{2+}-free PBS containing 2% FBS and 1 mM EDTA. After 5-minute incubation at room temperature, RapidSpheres™ bead (provided with the kit) was added to the mixture and then immediately assembled on EASY50 EASY SEP magnet from Stemcell. After 10-minute incubation at room temperature, cells in mixture solution were collected and assembled on EASY50 EASY SEP magnet from Stemcell again. After 5-minute incubation at room temperature, purified CD8+ T cells in mixture solution were collected and pelleted by centrifugation. Purified CD8+ T cells were frozen down and kept in liquid nitrogen for further usage.

[0103] To activate CD8+ T cell, purified human CD8+ T cells were resuspended in ImmunoCult™-XF T cell expansion medium from Stemcell. ImmunoCult™ human CD3/CD28 T cell activator from Stemcell and 100

U/mL human IL-2 from R&D System were added to the T cells to activate for 12 days. During the 12-days activation, fresh ImmunoCult™-XF T cell expansion medium with 100 U/mL human IL-2 was added to the cells whenever the cell density was above 1 million cells per milliliter. At the end of 12-day activation, activated T cells were frozen down and kept in liquid nitrogen.

To recover CD8+ T cells, activated CD8+ T cells or CD8+ t cells were recovered from liquid nitrogen and thawed in 37℃ water bath. CD8+ T cells were resuspended in RMPI1640 medium with 10% heat-inactivated FBS with 250 U/mL human IL-2. After 48-hour incubation at 37℃, T cells were ready for further usage.

Cytotoxicity assay

[0104] On the day before assay setting up, selective antibiotics was removed from target cell lines. On the day of assay setting up, target cell lines were collected by brief TrypLE treatment and then washed with culture medium by centrifuge at 500 ×g for 5 min at room temperature. Target cell lines were then resuspended in culture medium to determine the viability by trypan blue exclusion on Cellometer. The viable cell density was adjusted to 50,000 cells/mL in culture media. 100 uL target cell suspension (5000 target cell) was carefully dispensed to each well of a 96-well black clear flat-bottom tissue culture plate using multichannel pipettor. The plate was then incubated for 4-5 hours in tissue culture incubator to make sure that the target cells have attached to the bottom of the 96-well plate.

[0105] Fresh PBMCs or recovered T cells were pelleted down by centrifuge for 5 min at 500 × g at room temperature and resuspended in culture medium. The viability of cells was also determined via trypan blue exclusion. The viable PBMC density was adjusted to 3 million cells/mL in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin while the viable T cell density was adjusted to 0.5 million cells/mL in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin. After 4-5 hours' incubation, culture medium was carefully removed from the 96-well plates with target cells. 50 uL of 3 million cells/mL PBMCs suspension (150,000 PBMCs) or 50 uL of 0.5 million cells/mL PBMCs suspension (25,000 T cells) was added to the designated well in the 96-well plate with target cell, which would result in the E:T ratio of 30:1 for PBMC and 5:1 for T cells.

[0106] CD3xROR1 T-cell-engagers (TCEs) were prepared and serially diluted (5-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 nM to 2.56 pM. 50 uL of prepared CD3xROR1 TCEs at different concentrations was then added to the designated wells in the 96-well plate with PBMC and target cells and incubated for one, two or three days at 37 °C with 5% CO_2. At the end of incubation, the 96-well plates were centrifuged for 1 minute at 500 ×g to transfer 50μL of supernatant to V-bottom storage plate using a multichannel pipettor for IFN gamma release assay. ONE-Glo Luciferase Assay solution was brought to room temperature. 50 uL of One-Glo solution was then added to the designated well and incubate for 2 min at room temperature. The bioluminescence was measured on a plate reader with preset Bio-luminance protocol. The killing percentage was calculated using the formula: killing percentage = 100* (bioluminescence intensity of target cell alone - bioluminescence intensity of sample)/(bioluminescence intensity of target cell alone - bioluminescence intensity of PBMC alone).

IFN gamma release detection

[0107] IFN gamma release from cytotoxicity assay was measured using human IFN gamma ELISA detection kit. In brief, coating antibody provided with the kit was diluted to suggested concentration by following the protocol provided by the kit manufacturer. 100 ul of diluted coating antibody was added to the Nunc MaxiSorp flat-bottom 96-well plate, sealed plate and incubated at 4 °C overnight. The plates were then washed 4 times with Wash Buffer. To block non-specific binding and reduce background, 200 µL Assay Diluent A was added and incubated the plate at room temperature for 1 on a plate shaker (400 rpm). After blocking, the plates were washed 4 times with Wash Buffer. 100 µL/well of standards (prepared with culture medium) or samples were then added to the designated wells and incubated at room temperature for 2 hours on a plate shaker (400 rpm). After 2-hour incubation with samples or standards, the plates were washed 4 times with Wash Buffer and 100 µL of diluted Detection Antibody solution was added to incubate at room temperature for 1 hour on a plate shaker (400 rpm). After 1-hour incubation with detection antibody, the plates were washed 4 times with Wash Buffer and 100 µL of diluted Avidin-HRP solution was added to incubate at room temperature for 30 minutes on a plate shaker (400 rpm). After 30-minute incubation with Avidin-HRP, the plates were washed 5 times with Wash Buffer and 100 µL of freshly mixed TMB Substrate Solution was added to incubate at room temperature for 20 minutes in the dark. 100 µL of Stop Solution was then added to the well to stop the reaction. The absorbance at 450 nm was measured on a plate reader and the concentration of IFN gamma in each sample was backcalculated by using the standard curve generated with the absorbance of different concentration of standards.

Decoupling ratio calculation

Cytotoxicity and IFN gamma release were plotted against the concentration of testing articles in GraphPad Prism. Area under the curve (AUC) for cytotoxicity and IFN gamma release was calculated using the AUC algorithm in Prism. The decoupling ratio was generated by dividing the AUC of cytotoxicity with the AUC of IFN gamma release for the same testing article. All decoupling ratios were normalized to certain non-decoupling testing article to compare the variation among different experiments.

Binding assay by flow cytometry

[0108] Adherent target cells were collected by brief TrypLE treatment while suspension cells were directly collected. Collected cells were washed once with PBS by centrifugation at 500 ×g for 5 minutes at room temperature and added to V-bottom 96-well plate (200,000 – 300,000 cells per well). To prepared Live/Dead fixable red, one vial of powder was dissolved into 50 uL of DMSO to make stock solution and then diluted 1 uL of stock Live/Dead fixable red in 1 mL of PBS to prepare the working solution. 100 uL of Live/Dead fixable red working buffer was then added to the cell in the V-bottom 96-well plate (200,000 – 300,000 cells per well) and incubated for 15 minutes at room temperature. After the 15-minute incubation, the cells were washed twice with PBS by centrifugation at 500 g for 5 minutes at room temperature. The cells were then cultured with antibodies at different concentrations in 100 uL of Biolegend staining buffer at 4 °C for 45 minutes. After the 45-minute incubation with primary antibody, the cells were washed with PBS by centrifugation at 500 g for 5 minutes at room temperature. The cells were then cultured with PE-Cy7-conjugated

mouse-anti-human IgG secondary antibody in Biolegend staining buffer at 4 °C for 45 minutes. After the 45-minute incubation with secondary antibody, the cells were washed with PBS by centrifugation at 500 g for 5 minutes at room temperature and resuspended in 100 uL of BioLegend fixation buffer. The samples were then analyzed on Cytek flow cytometer. Data were analyzed with FlowJo and the gating strategy was followed: In brief, target cell population was gated by FSC and SSC. Single cells were selected from target cell population by FSC-A and FSA-H. Living single cells were gated from single cells by live/dead red dye low. The binding to target was determined by specific antibody staining and the gate was decided by the staining of isotype control of antibody on the same cell.

HEK293 cell transfection and binding assay

[0109] HEK293 cells from ATCC were maintained in Eagle's Minimum Essential Medium with 10% heat-inactivated FBS. On one day before transfection, HEK293 cells were harvested and resuspended in Eagle's Minimum Essential Medium with 10% heat-inactivated FBS at the density of 0.4 million cells per milliliter. HEK293 cells were then added to T-75 flask by 20 mL of cell suspension per T-75 flask and incubated at 37°C for 24 hours. On the day of transfection, 19 ug of DAN plasmid was mixed with 1.9 mL of Opti-MEM® I Reduced-Serum Medium from ThermoFisher and 57 uL of TransIT-293 Reagent from Mirus and incubated for 30 minutes at room temperature. After 30-minute incubation, DNA plasmid/ TransIT-293 Reagent mixture was added to the T-75 flask with HEK293 cells drop-by-drop at different area of the flask. The T-75 flasks were gently rocked back-and-forth and from side-to-side to evenly distribute the TransIT-293 Reagent:DNA complexes.

[0110] After 48-hour incubation, cells were collected to check the expression of transfected target and the binding of testing antibodies to the transfected target by flow cytometry as described herein under *Binding assay by flow cytometry*. In brief, collected cells were washed once with PBS in V-bottom 96-well plate (200,000 – 300,000 cells per well) and resuspended in 100 uL of Live/Dead fixable red working buffer. After the 15-minute incubation, the cells were washed twice with PBS and cultured with antibodies at different concentrations in 100 uL of Biolegend staining buffer at 4 °C for 45 minutes. After the 45-minute incubation with primary antibody, the cells were washed with PBS and cultured with PE-Cy7-conjugated mouse-anti-human IgG secondary antibody in Biolegend staining buffer at 4 °C for 45 minutes. After the 45-minute incubation with secondary antibody, the cells were washed with PBS and resuspended in 100 uL of BioLegend fixation buffer. The samples were then analyzed on Cytek flow cytometer. Data were analyzed with FlowJo and the gating strategy was followed: In brief, target cell population was gated by FSC and SSC. Single cells were selected from target cell population by FSC-A and FSA-H. Living single cells were gated from single cells by live/dead red dye low. The binding to target was determined by specific antibody staining and the gate was decided by the staining of isotype control of antibody on the same cell.

Binding assay by Biolayer Interferometry

[0111] Antibodies were prepared in 1×PBS with 0.02% Tween-20 with the final concentration of 15 ug/ml. Human ROR1-His or CD3e-His protein were also prepared, and serial diluted (4-fold dilution ranging from 400 nM to 0.098 nM) in 1×PBS with 0.02% Tween-20. The biosensors were pre-moistened in 200 uL of 1×PBS with 0.02%

Tween-20 for 10 minutes. Meanwhile, Octet BLI system (ForteBio) was prewarmed for 30 minutes and the flow rate was set to 1000 rpm. The biosensors (Anti-hIgG Fc Capture biosensor) were soaked in 250 uL of 1×PBS with 0.02% Tween-20 for 60 seconds at 30℃ to get an initial baseline reading. After the 60-second baseline reading, the biosensors were exposed to testing antibody for 300 seconds at 30℃ for the association between antibody and the biosensors (coupling antibody with biosensor). The biosensors with antibody were then exposed to human ROR1 or CD3e in 250 uL of 1×PBS with 0.02% Tween-20 at 30℃ for 300 seconds for the association reaction between antibody and human ROR1 or CD3e (association curve). After 300-second association reaction between antibody and antigen, the biosensors with antibody and antigen were then exposed to 250 uL of 1×PBS with 0.02% Tween-20 at 30℃ for 300 seconds for the dissociation reaction between antibody and antibody (dissociation curve). The binding affinity was calculated by the built-in data fitting algorithm.

CD69 and CD25 detection on T cells

[0112] Following the experiment setting up for cytotoxicity assay, target cells were added to the wells (5,000 target cells per well) in 96-wells plate and incubated for 5 hours. After 5-hour incubation, cell culture medium was removed from the plate without touching the target cells and 50 uL of fresh PBMCs (150,000 PBMCs per well) were added to the wells with target cells (E:T ratio 0f 30:1). 50 uL of CD3xROR1 TCEs were then added to the designated wells with 150,000 PBMCs and 5,000 target cells. After incubating at 37℃ overnight, PBMCs were collected and washed once with PBS by centrifugation at 500 ×g for 5 minutes at room temperature and added to V-bottom 96-well plate. To prepared Live/Dead fixable red, one vial of powder was dissolved into 50 uL of DMSO to make stock solution and then diluted 1 uL of stock Live/Dead fixable red in 1 mL of PBS to prepare the working solution. 100 uL of Live/Dead fixable red working buffer was then added to the cell in the V-bottom 96-well plate and incubated for 15 minutes at room temperature. After the 15-minute incubation, the cells were washed twice with PBS by centrifugation at 500 g for 5 minutes at room temperature. The cells were then cultured with antibodies (PE-Cy5-conjugated mouse anti-human TCRa/b antibody, PE-conjugated mouse anti-human CD19 antibody, BB515-conjugated mouse anti-human CD56 antibody, BB700-conjugated mouse anti-human CD14 antibody, PE-Cy7-conjugated mouse anti-human CD69 or CD25 antibody) in 100 uL of Biolegend staining buffer at 4 °C for 45 minutes. After the 45-minute incubation with antibody, the cells were washed with PBS by centrifugation at 500 g for 5 minutes at room temperature and resuspended in 100 uL of BioLegend fixation buffer. The samples were then analyzed on Cytek flow cytometer. Data were analyzed with FlowJo and the gating strategy was followed: In brief, target cell population was gated by FSC and SSC. Single cells were selected from target cell population by FSC-A and FSA-H. Living single cells were gated from single cells by live/dead red dye low. T cells were gated from living single cells by TCRa/b high. CD69 and CD25 expression was then gated on T cells with CD69 or CD25 high.

CD107a detection on T cells

[0113] Following the experiment setting up for cytotoxicity assay, target cells were added to the wells (5,000 target cells per well) in 96-wells plate and incubated for 5 hours. After 5-hour incubation, cell culture medium was removed from the plate without touching the target cells and 50 uL of fresh PBMCs (150,000 PBMCs per well) were

added to the wells with target cells (E:T ratio 0f 30:1). 50 uL of CD3xROR1 TCEs were then added to the designated wells with 150,000 PBMCs and 5,000 target cells. After incubating at 37℃ for 5 hours, PE-Cy7-conjugated mouse anti-human CD107a antibody (40:1) and Monensin (1500:1) was added to the culture medium and incubated for 8 hours. PBMCs were then collected and washed once with PBS by centrifugation at 500 ×g for 5 minutes at room temperature and added to V-bottom 96-well plate. To prepared Live/Dead fixable red, one vial of powder was dissolved into 50 uL of DMSO to make stock solution and then diluted 1 uL of stock Live/Dead fixable red in 1 mL of PBS to prepare the working solution. 100 uL of Live/Dead fixable red working buffer was then added to the cell in the V-bottom 96-well plate and incubated for 15 minutes at room temperature. After the 15-minute incubation, the cells were washed twice with PBS by centrifugation at 500 g for 5 minutes at room temperature. The cells were then cultured with antibodies (PE-Cy5-conjugated mouse anti-human TCRa/b antibody, PE-conjugated mouse anti-human CD19 antibody, BB515-conjugated mouse anti-human CD56 antibody, BB700-conjugated mouse anti-human CD14 antibody) in 100 uL of Biolegend staining buffer at 4 °C for 45 minutes. After the 45-minute incubation with antibody, the cells were washed with PBS by centrifugation at 500 g for 5 minutes at room temperature and resuspended in 100 uL of BioLegend fixation buffer. The samples were then analyzed on Cytek flow cytometer. Data were analyzed with FlowJo and the gating strategy was followed: In brief, target cell population was gated by FSC and SSC. Single cells were selected from target cell population by FSC-A and FSA-H. Living single cells were gated from single cells by live/dead red dye low. T cells were gated from living single cells by TCRa/b high. CD107a was then gated on T cells with CD107a high.

Bystander killing assay

[0114] On the day before assay setting up, selective antibiotics was removed from target cell lines (ROR1+ MDA-MB-231 RFP cell or ROR1- T-47D eGFP FLUC cell). On the day of assay setting up, target cell lines were collected by brief TrypLE treatment and then washed with culture medium by centrifuge at 500 ×g for 5 min at room temperature. Target cell lines were then resuspended in culture medium to determine the viability by trypan blue exclusion on Cellometer. The viable cell density was adjusted to 50,000 cells/mL in culture media. 100 uL ROR1- target cell suspension (5000 cell) was carefully dispensed to the designated well of a 96-well black clear flat-bottom tissue culture plate using multichannel pipettor for the group with ROR1- target cell only. 100 uL ROR1+ target cell suspension (5000 cell) was dispensed to the designated well of the 96-well black clear flat-bottom tissue culture plate for the group with ROR1+ target cell only. For the group with both ROR1+ and ROR1- target cells, 100 uL ROR1- target cell suspension (5000 cell) and 100 uL ROR1+ target cell suspension (5000 cell) was added to the designated well of the 96-well black clear flat-bottom tissue culture plate. The plate was then incubated for 4-5 hours in tissue culture incubator to make sure that the target cells have attached to the bottom of the 96-well plate.

[0115] Recovered T cells were pelleted down by centrifuge for 5 min at 500 × g at room temperature and resuspended in culture medium. The viable T cell density was adjusted to 0.5 million cells/mL in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin. After 4-5 hours' incubation, culture medium was carefully removed from 96-well plates with target cells. 50 uL of 0.5 million cells/mL T cell suspension (25,000 T cells) was added to the designated well in the 96-well plate with target cell, which would result in the E:T ratio of 5:1 for T cells.

[0116] CD3xROR1 T-cell-engagers (TCEs) were prepared and serially diluted (5-fold serial dilution) in RPMI1640 medium with 10% FBS and 1% penicillin/streptomycin ranging from 200 nM to 2.56 pM. 50 uL of prepared CD3xROR1 TCEs at different concentrations was then added to the designated wells in the 96-well plate with T cells and target cells and incubated in CELLINK CELLCYTE X at 37 °C with 5% CO_2. Cell number of ROR1+ MD-MB-231 RFP cell and ROR1- T-47D eGFP FLUC cell in each well was monitored every three hours for 72 hours. The change of target cell number in each well was plotted against time and the killing percentage of target cell was calculated by using target cell number at the time zero as the 100% base.

Serial killing assay

[0117] On the day before assay setting up, selective antibiotics was removed from target cell lines. On the day of assay setting up, target cell lines were collected by brief TrypLE treatment and then washed with culture medium by centrifuge at 500 ×g for 5 min at room temperature. Target cell lines were then resuspended in culture medium to determine the viability by trypan blue exclusion on Cellometer. The viable cell density was adjusted to 200,000 cells/mL in culture media. A serial dilution of target cell (factor 2) was made from 200,000 cells/mL to 3,125 cells/mL (200,000 cells/mL, 100,000 cells/mL, 50,000 cells/mL, 25,000 cells/mL, 12,500 cells/mL, 6,250 cells/mL, 3,125 cells/mL, 0 cell/ mL) with culture medium. 50 uL target cell suspension at different densities was carefully dispensed to designated standard control wells of a 96-well black clear flat-bottom tissue culture plate using multichannel pipettor, which yielded 10,000 cells, 5,000 cells, 2,500 cells, 1,250 cells, 625 cells, 313 cells, 156 cells, 0 cell per well as standard controls. For all testing article wells, 100 uL target cell suspension at the density of 50,000 cell/mL (5,000 cell per well) was carefully dispensed to designated wells. The plate with target cells were incubated at 37℃ for 4 hours.

[0118] After 48-hour recovery in 250 U/mL IL-2, activated CD8+ T cells were pelleted down by centrifuge for 5 min at 500 × g at room temperature and resuspended in culture medium. The viability of cells was also determined via trypan blue exclusion. The viable recovered CD8+ T cells was adjusted to 800,000 cells/mL in culture medium. A serial dilution of activated CD8+ T cell (factor 2) was made from 800,000 cells/mL to 6,250 cells/mL (800,000 cells/mL, 400,000 cells/mL, 200,000 cells/mL, 100,000 cells/mL, 50,000 cells/mL, 25,000 cells/mL, 12,500 cells/mL, 6,250 cells/ mL) with culture medium. 50 uL recovered activated CD8+ T cell suspension at different densities was carefully dispensed to designated wells with 5,000 target cells for testing articles or controls in a 96-well black clear flat-bottom tissue culture plate using multichannel pipettor, which yielded 40,000 cells (E:T ratio of 8:1), 20,000 cells (E:T ratio of 4:1), 10,000 cells (E:T ratio of 2:1), 5,000 cells (E:T ratio of 1:1), 2,500 cells (E:T ratio of 1:2), 1,250 cells (E:T ratio of 1:4), 625 cells (E:T ratio of 1:8), 313 cells (E:T ratio of 1:16) per well for all testing articles or controls.

[0119] Testing articles and proper negative controls were prepared in culture medium at the concentration of 1 nM. 100 uL of prepared testing articles of negative controls was added to the designated wells with 5,000 target cells and different number of activated CD8+ T cells. For the wells with different number of target cells as standard controls, 150 uL of culture medium was added to bring the total volume to 200 uL. The cells were incubated for one day at 37 °C with 5% CO_2. At the end of incubation, the 96-well plates were centrifuged for 1 minute at 500 ×g to transfer 150μL of

supernatant to V-bottom storage plate using a multichannel pipettor for further usage. ONE-Glo Luciferase Assay solution was brought to room temperature. 50 uL of One-Glo solution was then added to the designated well and incubate for 2 min at room temperature. The bioluminescence was measured on a plate reader with preset Bio-luminance protocol.To calculate the killing frequency, the bioluminescence intensity of target cell at different seeding numbers as standard controls were plotted against the cell numbers to generate a standard curve. The number of living target cell number in each testing sample wells were calculated with the standard curve by using the bioluminescence intensity of each testing sample well. The killed target cell number was calculated by subtracting the calculated living target cell number for each testing sample from 5000 (plating number of target cells). The killing frequency of target cells in each well was generated by dividing killed target cell number with the input CD8+ T cell number for the same well.

Mouse pharmacokinetics (PK) study

[0120] Single dose pharmacokinetics (PK) for antibody-like protein was performed in 8-10-week-old wild type C57BL/6j mice obtained from the Jackson Laboratory. Each protein test article was prepared in sterile phosphate buffered saline (PBS) pH 7.4 at a concentration of 100 ug/50 uL (2 mg/ml) for a dose of 5 mg/kg or 10 ug/50 uL (0.2 mg/ml) for a dose of 0.5 mg/kg. At time "0", 50 uL of protein was injected as an intravenous (IV) bolus into 9 mice per test article dose level such that 3 mice could be sampled per time points of 0, 1, 6, 24, 48, 72, 168, 240 hours and no single mouse was sampled more than twice. Peripheral blood was collected (approximately 60 μL) into pre-chilled (0-4°C) lithium heparin tubes via the sublingual, retrobulbar or via tail vein route (tail snip) under inhalation anesthesia (isoflurane) prior to IV injection and at 1, 6, 24, 48, 72, 168, 240 hours. After blood collection, blood samples were centrifuged (2500 g/10 minutes at 4°C) and plasma immediately stored at -80°C.

[0121] The concentration of each test article (FUSE protein with human IgG1 backbone) in mouse serum was quantified using human IgG SimpleStep ELISA Kit from Abcam. In brief, all reagents, working standards, and samples were brought to room temperature before the assay. All samples and standards were prepared in manufacturer-provided *Sample Diluent NS.* When the assay began, 50 μL of all samples or standard were added to the designated wells in the 96-well plate provided by the manufacturer. 50 μL of Antibody Cocktail were then added to the wells. After sealing with film, the plate was incubated for 40 minutes at room temperature on a plate shaker (400 rpm). After the 40-minutes incubation, the plate was washed three time with manufacturer-provided 1X Wash Buffer PT buffer and 100 μL of manufacturer-provided *TMB Development Solution* was then added to each well. After incubating for 5 minutes in the dark on a plate shaker (400 rpm), 100 μL of manufacturer-provided *Stop Solution* was added to the well. The plate was shaken on a plate shaker for 1 minute to measure the OD at 450 nm for each well on a plate reader. The concentration of testing article in each sample was backcalculated by using the standard curve generated with the absorbance of different concentration of standard human IgG.

Mouse xenograft tumor models in immunocompromised mice

[0122] In-vivo PK/PD (Pharmacodynamics) and efficacy were assessed using two human ROR1+ xenograft models in humanized immunocompromised mice. In the first model, NSG mice were injected with a mixture of 5 million NCCIT and expanded T cells such that the ratio of tumor to CD8+ T cells constituted 1:4 without matrigel. Such T cells were expanded for 12 days and recovered for an additional 3 days in IL-2 to mimic a progenitor exhausted phenotype. T cells from 2 healthy donors were assessed for alloreactivity via an in-vitro bioluminescent cytotoxicity and IFNγ release (as described above) assay using NCCIT engineered to constitutively express firefly luciferase. The donor observed to display minimal alloreactivity (defined as <15% maximum killing and <150 pg/ml maximum IFNγ release at an E:T of 1:3) towards NCCIT in-vitro (donor 2) was used in-vivo. Mice were either treated with PBS (n=4), FUSE-394 (0.15 mg/kg; n=6), FUSE-394 (1.5 mg/kg; n=6), FUSE-399 (0.15 mg/kg; n=6) or FUSE-399 (1.5 mg/kg; n=6) on day 1, 2, 4 and 7. In all cases the volume of treatment was 50 μL in pBS pH 7.4. Next, we examined mice for tumor growth. If tumor breakthrough was suspected, all mice were intravenously injected with 3.5 million expanded T cells and a second round of treatment initiated such that intravenous injections of test articles were given of days 0, 2, 4 and 6 following the adoptive transfer of T cells. Tumor volumes were measured every 3-5 days. Mice were sacrificed at the end of the study. In the second model, NSG mice in which both MHC-I and MHC-II were knocked out (NSG-MHC-I/II dKO; n=25) were implanted subcutaneously with 5 million ROR1+ NCCIT teratoma cells mixed with matrigel. When the tumor volume reached 50 mm cubed, mice were injected intravenously with 30 million freshly isolated PBMC from a healthy human donor. Once the tumor volume reached 100 mm cubed ("day 0"), the mice were randomly divided into three groups. Group 1 (n=5) received 5 mg/kg of the negative control treatment, Cirmtuzumab (FUSE-366), a ROR1 targeting antibody that blocks Wnt5-alpha signaling but has little to no effect in mouse xenograft tumor models. Groups 2 and 3 (n=10 each) received a selected TCE at either 5 mg/kg or 2 mg/kg, respectively. Treatment consisted of bolus IV injections of test article prepared in PBS pH 7.4 such that 50 μL was injected IV via the tail vein. For 5 mg/kg, 100 μg of test article was injected in 50 μL. For 0.5 mg/kg, 10 μg of test article was injected in 50 μL. Mice received treatment on days 0, 2, and 7. Tumor volume was measured every 4 days starting on day 0. Mice were sacrificed at the end of the study.

Mouse xenograft tumor model in humanized immunocompromised mice

[0123] The capacity of the ROR1 x CD3 bsAbs to mediate in-vivo tumor growth inhibition (TGI) was assessed using the human ROR1+ xenograft TNBC mouse tumor model, MDA-MB-231. Briefly, NSG (NOD-*scid* IL2Rgamma^{null}; Jackson Laboratory) mice were injected subcutaneously with 5 million MDA-MB-231 admixed in Matrigel Matrix (Corning) into the right dorsal flanks of the animals. On the same day, the mice were also injected intravenously with 5 million expanded human T cells. Expanded T cells for injection were selected from several healthy donors based on minimal alloreactivity towards MDA-MB-231. In short, T cells from two healthy donors were expanded using ImmunoCult™ human CD3/CD28 T cell activator reagent (Stemcell) as per the manufacturer's recommended protocol. Alloreactivity was examined using our in-vitro bioluminescent cytotoxicity and IFNγ release assays (as described above) and was defined as maximum killing equal to 15% or less and maximum IFNγ release

equal to 300 pg/ml or less at an E:T of 5:1. When tumor volumes reached between 75-100 cubic millimeters, the mice were randomized into 5 groups. Group 1, consisting of 5 mice, received the mock treatment, PBS twice weekly. Groups 2-4 consisted of 10 mice each and received two weekly doses of FUSE608 at 0.03 mg/kg, 0.3 mg/kg or 3 mg/kg. Group 5 consisted of 7 mice and received two weekly doses of FUSE399 at 3 mg/kg. Tumor volumes were measured every 3-5 days and the survival of mice was also recorded. Mice were sacrificed at the end of the study.

Example 48

A ROR1 specific CD3 bispecific T-cell Engager engineered for solid tumors with an expanded therapeutic window

[0124] We engineered two ROR1 specific TCEs with properties to increase the size of their therapeutic windows using a unique ROR1 specific VHH from Camelus bactrianus (clone 5A1) that contains a non-canonical disulfide-bond between CDR1 and CDR3 and ROR1 specific valency that (1) decouples cytotoxicity from cytokine release and (2) facilitates the discrimination between ROR1 cell surface density on normal versus tumor cells.

[0125] When 5A1 was paired with an anti-CD3 Fab in two orientations that include a half-life extending Fc domain, we were able to establish a T cell/tumor synapse that resulted in sufficient activation to induce degranulation/tumor-killing at TCE concentrations of 10-30 pM (EC50 ~ 150-200 nM). In contrast, expression of CD69, CD25 and IFNγ, the latter of which is directly upstream of CRS, was not observed until the concentration of TCEs reached ~1 nM (EC50 ~ 5-10 nM). Compared to the non-decoupling control TCE, 5A1 TCEs demonstrated 3.5-4.5 fold greater decoupling. In the context of JeKo1 CD19+/ROR1+ tumor targets, the 5A1 TCEs were 6-7.5 fold better decoupled compared to blinatumomab. We also observed an EC50-cytotoxicity for blinatumomab of below 100 fM, suggesting that the risk of both CRS and TLS are multiple folds higher than for 5A1 TCEs. Compared to the non-decoupling control TCE, the 5A1 TCEs mediated superior cytotoxicity/serial killing at an effector to target ratio (E:T) as low as 1:16, more consistent with solid-tumors. The 5A1 TCEs were also utilized ROR1 specific binding valency to discriminate between cell surface densities of ROR1 typical to tumor cells (>15k molecules/cell) versus normal cells (generally <10,000 molecules/cell). The concentration of TCEs required for maximum IFNγ release and killing of tumor cells registered in the pM range, as opposed to the supraphysiologic nM/uM range that was insufficient to induce more than 25% and 35% of IFNγ release and cytotoxicity, respectively, on cells expressing <10,000 molecules of ROR1.

Example 49

[0126] As shown in figure 58C, the format(s) used for the CD3 x ROR1 bispecific antibodies (bsAbs) are that of knobs into holes and contain modular subunits of the humanized CD3 specific fab 1F3-3 (or variants thereof) and the ROR1 specific VHHs, 5A1 or 2A11 or variants thereof. The ROR1 specific scFV, R11, which binds to the membrane proximal domain of ROR1 (termed the Kringle domain) was used to generate the non-decoupled benchmark bsAb, FUSE399. As an example, Format Ac13 consists of a 1F3-3 fab genetically fused to the N-terminus

of the heavy chain hole and ROR1 specific binders genetically fused to the N-terminus of the heavy chain knob and C-terminus of the light chain.

Example 50

[0127] As shown in the tables below, mouse B16-F10 melanoma (Tables 19A-19F) or LS174T human colorectal adenocarcinoma (Tables 19G-19H) cells genetically engineered to express human ROR1 at high and low densities, respectively, were stained for flow cytometric analysis with 100 nM of humanized variants of the ROR1 specific VHHs, 5A1 or 2A11 in either a VHH-Fc format or the Ac13 format shown in FIG. 58. Cells were analyzed on a flow cytometer after the primary test article was fluorescently tagged with a goat anti human IgG-Fc specific PE secondary antibody. The positive control used was PE conjugated anti-human ROR1 clone 2A2. Negative controls included a PE labeled mouse IgG Ab, the secondary antibody only, the combination of a Hen Egg Lysozyme(HEL) specific antibody plus goat anti human IgG-Fc specific PE and Fluorescence Minus One (FMO). Other controls included the non-humanized version of 5A1-Fc (FUSE179), 2A11-Fc (FUSE112) and 5A1-Ac13 (FUSE488). Humanized variants were generated using computational design (FUSE489, FUSE497-498, FUSE563, FUSE569-570, and BI-HU9-VHH11-33), a yeast display library in which mutations were inserted into the Vernier zone residues of human framework indicated in GenBank accession number M99660 (FUSE524-525, FUSE589, FUSE597-598, FUSE537, FUSE661-662, BI-HU9-HK and BI-HU9-HK-1), and a second yeast display library in which mutations were inserted into the Vernier zone residues of human framework indicated in GenBank accession number AWH66715 and amino acid position 103 within CDR3 (FUSE559, FUSE590-596, BI-HU9-VH9-1, FUSE654-655 and FUSE661-662. Affinity matured variants of FUSE559 were also generated using computational design (F22158101-21; Table 19H).

Table 19A. Single concentration FACS screen

B16-F10-ROR1-H	MFI
FMO	5.96
mIgG-PE	6.1
anti-human ROR1(clone 2A2)-PE	35240
2nd Anti-human IgG-PE antibody	5.67
100 nM anti-HEL4-hIgG1	9.86
100 nM FUSE-179-2 (5A1 Fc)	4220
100 nM FUSE-488 (5A1-Ac13)	3909
100 nM FUSE-489 (h5A1-Ac13)	1055
100 nM FUSE-497 (h5A1-Ac13)	811
100 nM FUSE-498 (h5A1-Ac13)	1363
100 nM FUSE-524 (h5A1-HK1_Fc)	**6473**
100 nM FUSE 525 (h5A1-HK1v Fc)	5.84

Table 19B. Single concentration FACS screen

B16-F10-ROR1-H	MFI
FMO	4.07
mIgG-PE	4.36
anti-human ROR1(clone 2A2)-PE	19948
2nd Anti-human IgG-PE antibody	15.5
100 nM anti-HEL4-hIgG1	12.1
100 nM FUSE-179-2-AKTA	3732
100 nM FUSE-559 (h5A1-FA9-HK1 Fc)	6082
100 nM FUSE-563 (h5A1-FA11 Fc)	388
100 nM FUSE-569 (h5A1-FA17 Fc)	590
100 nM FUSE-570 (h5A1-FA18 Fc)	583

Table 19C. Single concentration FACS screen

B16-F10-ROR1-H	MFI
FMO	4.32
mIgG-PE	4.31
anti-human ROR1(clone 2A2)-PE	38704
2nd Anti-human IgG-PE antibody	4.45
100 nM anti-HEL4-hIgG1	6.85
100 nM FUSE-179-2 (5A1 Fc)	8479
100 nM FUSE-112-2 (2A11 Fc)	11934
100 nM FUSE-597 (h5A1-HK2 Fc)	10088
100 nM FUSE-598 (h5A1-HK6 Fc)	9555

Table 19D. Single concentration FACS screen

B16-F10-ROR1-H	MFI
FMO	2.14
mIgG-PE	2.08
anti-human ROR1(clone 2A2)-PE	21570
2nd Anti-human IgG-PE antibody	3.01
100 nM anti-HEL4-hIgG1	3.86
100 nM FUSE537 (h2A11-HK1_Fc)	862

Table1 9E. Single concentration FACS screen

B16-F10-ROR1-H	MFI
FMO	6.48
mIgG-PE	6.53
anti-human ROR1(clone 2A2)-PE	27104
2nd Anti-human IgG-PE antibody	9.92
100 nM anti-hCD47	9.77

100 nM anti-HEL4-hIgG1	13.3
100 nM FUSE-179-2 (5A1 Fc)	4475
100 nM FUSE-112-2 (2A11 Fc)	7840
100 nM FUSE-589 (h2A11-HK5 Fc)	6951
100 nM FUSE-590 (h5A1-FA9-HK2_Fc)	7329
100 nM FUSE-591 (h5A1-FA9-HK3_Fc)	7768
100 nM FUSE-592 (h5A1-FA9-HK4_Fc)	7539
100 nM FUSE-593 (h5A1-FA9-HK5_Fc)	7525
100 nM FUSE-594 (h5A1-FA9-HK6_Fc)	7911
100 nM FUSE-595 (h5A1-FA9-HK7_Fc)	7180
100 nM FUSE-596 (h5A1-FA9-HK8 Fc)	6256

Table 19F. Single concentration FACS screen

B16-F10-ROR1-H	MFI
FMO	356
mIgG-PE	94.6
anti-human ROR1(clone 2A2)-PE	416000
2nd Anti-human IgG-PE antibody	125
100 nM anti-HEL4-hIgG1	1135
100 nM FUSE-179-2 (5A1 Fc)	93806
100 nM FUSE-112-2 (2A11 Fc)	207782
100 nM FUSE-559 (h5A1-FA9-HK1 Fc)	198683
100 nM FUSE-560 (h2A11-HK2 Fc)	159403
100 nM FUSE-561(h2A11-HK3 Fc)	157168
100 nM FUSE-562 (h2A11-HK4 Fc)	155016
100 nM (BI-HU9-VHH11 h5A1 Fc)	14127
100 nM (BI-HU9-VHH12 h5A1 Fc)	21000
100 nM (BI-HU9-VHH13 h5A1 Fc)	21114
100 nM (BI-HU9-VHH21 h5A1 Fc)	15601
100 nM (BI-HU9-VHH22 h5A1 Fc)	22260
100 nM (BI-HU9-VHH23 h5A1 Fc)	23588
100 nM (BI-HU9-VHH31 h5A1 Fc)	17939
100 nM (BI-HU9-VHH32 h5A1 Fc)	20060
100 nM (BI-HU9-VHH33 h5A1 Fc)	21956
100 nM (BI-HU9-VH9 h5A1 Fc)	14450
100 nM (BI-HU9-VH9-1 h5A1 Fc)	16607
100 nM (BI-HU9-HK h5A1 Fc)	133
100 nM (BI-HU9-HK-1 h5A1 Fc)	135858

Table 19G. Single concentration FACS screen

LS174T-ROR1-L	MFI
FMO	22.3

2nd Anti-human IgG-PE antibody	22.6
mIgG-PE	23.2
anti-human ROR1(clone 2A2)-PE	7956
100nM Human IgG1	30.8
100nM FUSE-179-2 (5A1 Fc)	**836**
100 nM FUSE-559 (h5A1-FA9-HK1 Fc)	3120
100nM FUSE-654 - h5A1-FA9-HK6-R19K Fc	4558
100nM FUSE-655 - h5A1-FA9-HK6-N84S Fc	4010
100nM FUSE-112-2 (2A11)	**4336**
100nM FUSE-661- h2A11-HK2-3-N84S Fc	4881
100nM FUSE-662 – h2A11-HK2-16-N84S Fc	4351

Table 19H. Single concentration FACS screen

LS174T-ROR1-L	MFI
FMO	58.4
mIgG-PE	54
anti-human ROR1(clone 2A2)-PE	36206
2nd Anti-human IgG-PE antibody	45.3
100 nM Human IgG1	316
100 nM FUSE-179-2 (5A1 Fc)	3058
100 nM FUSE-559 (h5A1-FA9-HK1 Fc)	24426
100 nM F22158101 (h5A1-BI-001 Fc)	19719
100 nM F22158102 (h5A1-BI-002 Fc)	20481
100 nM F22158103 (h5A1-BI-003 Fc)	15800
100 nM F22158104 (h5A1-BI-004 Fc)	28869
100 nM F22158105 (h5A1-BI-005 Fc)	22268
100 nM F22158106 (h5A1-BI-006 Fc)	17400
100 nM F22158107 (h5A1-BI-007 Fc)	11471
100 nM F22158108 (h5A1-BI-008 Fc)	25086
100 nM F22158109 (h5A1-BI-009 Fc)	34983
100 nM F221581010 (h5A1-BI-010 Fc)	18659
100 nM F221581011 (h5A1-BI-011 Fc)	19368
100 nM F221581012 (h5A1-BI-012 Fc)	14200
100 nM F221581013 (h5A1-BI-013 Fc)	23838
100 nM F221581014 (h5A1-BI-014 Fc)	28696
100 nM F221581015 (h5A1-BI-015 Fc)	29280
100 nM F221581016 (h5A1-BI-016 Fc)	14976
100 nM F221581017 (h5A1-BI-017 Fc)	28569
100 nM F221581018 (h5A1-BI-018 Fc)	17492
100 nM F221581019 (h5A1-BI-019 Fc)	33815
100 nM F221581020 (h5A1-BI-020 Fc)	26535
100 nM F221581021 (h5A1-BI-021 Fc)	35788

Example 51

[0128] As shown in figure 72 the apparent binding affinity (EC50-binding) of the ROR1 specific VHH-Fc incorporating either the non humanized VHH clone 5A1 (FUSE179; black circle), the humanized clone 5A1-FA9-HK1 (FUSE559; black square) or the humanized clone 5A1-HK1 (FUSE524; black triangle) was calculated. Shown is a non-linear x-y plot of binding to B16-F10 cell engineered to express human ROR1 relative to the concentration of test article used for staining. A Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. Binding was measured via flow cytometry using a fluorescently labeled secondary antibody specific for the Fc domain of IgG1. A summary table of EC50s and maximum MFI is shown beneath under the graph.

Binding assay by Surface Plasmon Resonance (SPR)

[0129] Protein test article binding to recombinant human ROR1 (Acro Biosystems, RO1-H522y) was detected on a BIAcore 8K (Cytiva) biosensor system using an SPR assay. Briefly, a carboxymethylated CM5 sensor chip was activated with 1:1 mixture of 0.4 M N-ethyl-N -(3-dimethylaminopropyl) carbodiimide and 0.1 M′ N-hydroxysuccinimide. Recombinant human ROR1 in 10 mM NaOAc (pH5) was then immobilized on the sensor chip by amine-coupling according to the manufacturer's instructions to yield the stable RU of 4400. Unreacted sites were blocked with 1M ethanolamine/HCl (pH 8). Control flow cells (blank) were activated and blocked in the absence of ROR1. Flow cells were routinely equilibrated with running buffer (PBS, 0.005 % surfactant P20). Test article proteins used for assessment of binding to recombinant human ROR1 binding were diluted in HBS-EP running buffer and injected into the sensor chip at different concentrations. Kinetic and affinity data of ROR1/test article binding were obtained at a flow rate of 30 µL/min, with a set association time of 100 sec and a dissociation time of 200 sec. Data from duplicate assays were modeled for binding equilibrium and dot plots for 200 nM of protein test article was calculated. Association constant (ka), dissociation constant (kd) and binding affinity (KD = kd / ka) were generated from integrated software in the BIAcore 8K system.

PD-1, TIGIT, and CD69 detection on T cells

[0130] Following the experiment setting up for cytotoxicity assay, target cells were added to the wells (5,000 target cells per well) in 96-wells plate and incubated for 5 hours. After 5-hour incubation, cell culture medium was removed from the plate without touching the target cells and 50 uL of fresh PBMCs (150,000 PBMCs per well) were added to the wells with target cells (E:T ratio 0f 30:1). 50 uL of CD3xROR1 TCEs were then added to the designated wells with 150,000 PBMCs and 5,000 target cells. After incubating at 37℃ for 24, 48 or 72 hours, PBMCs were collected and washed once with PBS by centrifugation at 500 ×g for 5 minutes at room temperature and added to V-bottom 96-well plate. To prepare Live/Dead fixable red, one vial of powder was dissolved into 50 uL of DMSO to make stock solution and then diluted 1 uL of stock Live/Dead fixable red in 1 mL of PBS to prepare the working solution. 100 uL of Live/Dead fixable red working buffer was then added to the cell in the V-bottom 96-well plate and incubated for 15 minutes at room temperature. After the 15-minute incubation, the cells were washed twice with PBS by centrifugation at 500 g for 5 minutes at room temperature. The cells were then cultured with antibodies (PE-Cy5-

conjugated mouse anti-human TCRa/b antibody, PE-conjugated mouse anti-human CD19 antibody, BB515-conjugated mouse anti-human CD56 antibody, BB700-conjugated mouse anti-human CD14 antibody, PE-Cy7-conjugated mouse anti-human PD-1, TIGIT or CD69 antibody) in 100 uL of Biolegend staining buffer at 4 °C for 45 minutes. After the 45-minute incubation with antibody, the cells were washed with PBS by centrifugation at 500 g for 5 minutes at room temperature and resuspended in 100 uL of BioLegend fixation buffer. The samples were then analyzed on Cytek flow cytometer. Data were analyzed with FlowJo and the gating strategy was followed: In brief, target cell population was gated by FSC and SSC. Single cells were selected from target cell population by FSC-A and FSA-H. Living single cells were gated from single cells by live/dead red dye low. T cells were gated from living single cells by TCRa/b high. PD-1, TIGIT and CD69 expression was then gated on T cells with PD-1, TIGIT or CD69 high.

Example 52

[0131] As shown in figure 73, the affinity (KD) of the ROR1 specific VHH-Fc incorporating either the non humanized VHH clone 5A1 (FUSE179; FIG. 73A) or the humanized clone 5A1-FA9-HK1 (FUSE559; FIG. 73B) was calculated. Shown are sensorgrams of surface plasmon resonance (SPR) data generated via Biacore analysis. A summary table of binding affinity (KD), on rate (k-a) and off rate (kd) is shown below FIG. 73B.

Example 53

[0132] As shown in figure 74, we assessed the apparent binding affinity (EC50-binding) of the two bispecific antibodies (bsAbs), FUSE488 (humanized CD3 clone 1F3.3 x ROR1 VHH 5A1) and FUSE608 (humanized CD3 clone 1F3.3 x humanized clone 5A1-FA9-HK1) for human ROR1 (FIG. 74A) and human CD3 (FIG. 74B).

[0133] FIG. 74A is a non-linear x-y plot depicting binding of FUSE488 (black triangle) or FUSE608 (black circle) to the human TNBC ROR1+ MDA-MB-231 cell line at various concentrations of test articles. FUSE608 demonstrated appreciably greater apparent affinity for ROR1 than FUSE608. This may be attributed to the "optimization" of the Vernier zone residues as well as the amino acid substitution in CDR3 of 5A1-5A9-HK1, D103H.

[0134] FIG. 74B is a non-linear x-y plot depicting binding of FUSE488 (black triangle) or FUSE608 (black circle) to the human lymphoma CD3+ Jurkat cell line (which has been engineered to express Lucia luciferase downstream of an NFAT promoter) at various concentrations of test articles. Given that both FUSE488 and FUSE608 use the same CD3 binder and incorporate the format, it is not surprising that we observed no appreciable difference in apparent binding for CD3 between the two bsAbs.

[0135] An Hen Egg Lysozyme (HEL)-specific antibody was used as the negative isotype control. Binding was measured via flow cytometry using a fluorescently labeled secondary antibody specific for the Fc domain of IgG1.

[0136] A summary table of EC50s and maximum MFI is shown beneath each graph.

Example 54

[0137] As shown in figure 75, we examined the capacity of several ROR1 x CD3 specific bsAbs to induce (1; FIG. 75A) T cell mediated killing of ROR1+ MDA-MB-231 tumor cells and (2; FIG. 75B) T cell mediated release of IFNγ when co-mixed with ROR1+ MDA-MB-231 tumor cells. Both assays were read out at 24 hours. The test articles examined were (a) the non decoupled ROR1 x 1F3-3 benchmark bsAb, FUSE399 (non humanized Kringle domain specific scFV, R11, x humanized 1F3-3 bsAb), (b) FUSE488 (non humanized 5A1 x 1F3-3 bsAb in format Ac13) and (c) FUSE608 (humanized CD3 clone 1F3-3 x humanized clone 5A1-FA9-HK1). The negative controls tested were non targeted 1F3-3 control, FUSE397 and the human IgG1 anti-HEL isotype control antibody. The assay system utilized expanded T cells purified from healthy donors.

[0138] Shown are exemplary non-linear x-y plots of tumor cell killing (FIG. 75A) and IFNγ release (FIG. 75B) whereby the rank order of both potency (EC50) and efficacy (Emax) were observed to as FUSE399 (black reverse triangle) > FUSE608 (black circle) > FUSE488 (black triangle). Importantly, the difference in the potency/efficacy of tumor cell killing between FUSE608 and FUSE399 was not appreciable. In contrast, FUSE608 was about 25x less potent at IFNγ release than FUSE399 indicating that a concentration range of FUSE608 was observed in which maximum tumor cell killing can be achieved without concomitant maximum release of IFNγ. This property associated with FUSE608, which may be termed "decoupling of cytotoxicity from cytokine release" might reduce the risk of toxic side effects, such as Cytokine Release Syndrome (CRS), associated with CD3 bsAbs, without sacrificing tumor cell killing. An appreciable concentration range with similar properties was not observed for FUSE399, which was specifically chosen as the "non-decoupled benchmark".

[0139] Little to no activity was observed in either assay with FUSE397 (black square) nor the anti-HEL (gray diamond) negative control test article. Summary tables of EC50, Emax and AUC for each test article are shown below each graph.

Example 55

[0140] As shown in figure 76, we examined the capacity of several ROR1 x CD3 specific bsAbs to induce (1; FIGs 19A-19D) PBMC mediated killing of four different tumor cell lines and (2; FIGs 76E-76H), PBMC mediated release of IFNγ when co-mixed with the aforementioned tumor cells. Both assays were read out at 24 hours. The test articles examined were (a) the non decoupled ROR1 x 1F3-3 benchmark bsAb, FUSE399 (non humanized Kringle domain specific scFV, R11, x humanized 1F3-3 bsAb) and (b) FUSE608 (humanized CD3 clone 1F3-3 x humanized clone 5A1-FA9-HK1). The human IgG1 anti-HEL antibody served as the negative isotype control. The assay system utilized PBMC purified from healthy donors and the following different tumor cell lines: (1) the ROR1+ triple negative breast cancer (TNBC), MDA-MB-231 (FIG. 76A, 76E), the ROR1+ non-small cell lung cancer (NSCLC) NCI-H1975 (FIG. 76B, 76F), ROR1+ mantle cell lymphoma, JeKo-1 (FIG. 76C, 76G), and ROR1 negative breast cancer, T-47D (FIG. 76D, 76H). The rank order cell surface expression of ROR1 was as follows: JeKo-1>MDA-MB-231>NCI-H1973>T-47D. As shown in the exemplary non-linear x-y plots labelled as FIGs 76A-76D, we observed that FUSE608

(black circle) induced potent (EC50) and efficacious (Emax) PBMC mediated ROR1 dependent tumor cell killing within 3 fold the EC50 and within 5% the Emax attributed to the benchmark FUSE399 (black reverse triangle). No non-specific killing was associated with the anti-HEL control nor was any observed with FUSE399 or FUSE608 in the context of the ROR1 negative target, T-47D. In contrast, as shown in FIGs 76E-76H, FUSE608 was between 25-30x less potent at IFNγ release than FUSE399 across the three ROR1 positive tumor cell lines examined. This result indicated that a concentration range of FUSE608 in which maximum tumor cell killing can be achieved without concomitant maximum release of IFNγ is not a singular observation related to MDA-MB-231but can rather be extended to all the ROR1+ tumor cell lines tested. No appreciable concentration range with similar properties was observed for FUSE399. Further, No non-specific IFNγ release was associated with the anti-HEL control nor was any observed with FUSE399 or FUSE608 in the context of the ROR1 negative target, T-47D.

[0141] Summary tables of EC50, Emax and AUC for each test article are shown below FIGs 76A-D and FIGs 76E-H (FIGs 76I and 76J).

Example 56

[0142] As shown in figure 77, we examined the capacity of several ROR1 x CD3 specific bsAbs to induce (1; FIG. 77A) PBMC mediated killing of ROR1+ MDA-MB-231 tumor cells and (2; FIG. 77B), PBMC mediated release of IFNγ when co-mixed with ROR1+ MDA-MB-231 tumor cells, (3. FIG. 77C) upregulation of cell surface CD69 expression on T cells within PBMC when co-mixed with ROR1+ MDA-MB-231 tumor cells, (4. FIG. 77D) upregulation of cell surface PD-1 expression on T cells within PBMC when co-mixed with ROR1+ MDA-MB-231 tumor cells, and (5. FIG. 77E) upregulation of cell surface TIGIT expression on T cells within PBMC when co-mixed with ROR1+ MDA-MB-231 tumor cells. All readouts were assessed at 72 hours. The test articles examined were (a) the non decoupled ROR1 x 1F3-3 benchmark bsAb, FUSE399 (non humanized Kringle domain specific scFV, R11, x humanized 1F3-3 bsAb) and (b) FUSE608 (humanized CD3 clone 1F3-3 x humanized clone 5A1-FA9-HK1). The human IgG1 anti-HEL antibody served as the negative isotype control. We observed that the potency (EC50) and efficacy (Emax) of tumor cell killing was not appreciably different between FUSE608 (black circle) and FUSE399 (black reverse triangle). Importantly, in a similar fashion to the "decoupling of cytotoxicity from cytokine (IFNγ) release" observed for FUSE608 but not the non-decoupled benchmark CD3 bsAb, FUSE399, (see FIG. 77B) we observed that upregulation of cell surface CD69, PD-1 and TIGIT on T cells within the PBMC co-mixed with MDA-MB-231 and test article was also decoupled from tumor mediated killing. That is, FUSE608 was about 10x, 30x and 27x less potent at inducing the upregulation of CD69, PD-1 and TIGIT, respectively, compared to FUSE399. These results indicate that a concentration range of FUSE608 was observed in which maximum tumor cell killing can be achieved without concomitant maximum upregulation of CD69, PD-1 and TIGIT. An appreciable concentration range with similar properties was not observed for FUSE399. This may be of physiological significance because CD69 (pubmed.ncbi.nlm.nih.gov/30085193/), PD-1 (www.ncbi.nlm.nih.gov/pmc/articles/PMC5341794/) and TIGIT

(jitc.bmj.com/content/8/2/e000957) have all been reported to be markers of T cell (and NK cell) "exhaustion" and the treatment of tumor bearing mice with antagonist antibodies specific for each protein has been reported to mediate tumor growth inhibition.

[0143] No non-specific tumor cell killing, IFNγ release nor induction of CD69, PD-1 or TIGIT was associated with the anti-HEL negative control isotype control antibidy.

[0144] Summary tables of EC50, Emax and AUC for each test article are shown following FIGs 77A-77E (FIG. 77F)

Example 57

[0145] As shown in figure 78, we examined the capacity of FUSE399 (black reverse triangle) and FUSE608 (black circle) to induce CD8+ T cell mediated killing of a constant number (5000 cells) of ROR1+ MDA-MB-231 cells across a range of CD8+ T cell numbers from 313 to 40,000. This allows for the calculation of (a) the number of T cells needed for a given ROR1 x CD3 bsAb to induce T cell mediating killing of a given amount of ROR1+ tumor cells (FIG. 78A and 78B) and (b) the average number of tumor cells killed per T cell, also termed "serial killing" (FIG. 78C). Tumor cell viability was measured after 24 hours of co-culture. Effector to target ratios of 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1 were analyzed. FUSE397 (black square) and anti-HEL (gray diamond) were used as the negative control test articles. Two distinct healthy donor derived expanded CD8+ T cells were tested. Donor 1 and 2 are shown in FIGs. 78A and 21B, respectfully. At E:T ratios of 1:2 to 8:1 no significant difference in tumor cell killing efficacy (Emax) was observed. At lower E:T ratios, FUSE608 was observed to induce greater T cell mediated ROR1 dependent tumor cell killing than FUSE399. For example, to kill approximately 30% of the tumor cells, FUSE399 required about 1100 T cells, whereas FUSE608 required about 450 T cells. At very low E:T ratios (e.g. 1:16 and 1:8), each T cell, on average, killed more than one tumor cell. For example, at an E:T ratio of 1:16 (312 T cells added to the assay), FUSE608 was associated with 18% killing, which equates to approximately 900 tumor cells or about 3 tumor cells killed per T cell per 24 hours.

[0146] Figure 78C is a bar chart illustrating the average serial killing associated with FUSE399 and FUSE608 derived from the T cells from each individual donor shown in FIG. 78A and FIG. 78B. The value is reported as the number of tumor cells killed per T cell over a 24 hour period. It was calculated as the average value from the E:T ratios of 1:16 and 1:8. FUSE608 supports serial killing at approximately 3 tumor cells/T cell, whereas this number is less than 1.5 tumor cells/T cell for FUSE399. The phenomenon whereby a single T cell kills more than one tumor cell is especially important in solid tumors, where the number of T cells may be sparse with E:T ratios ranging from 1:2 to < 1:8.

Example 58

[0147] As shown in figure 79, we addressed whether FUSE608 induced bystander killing of ROR1 negative tumors co-mixed with ROR1 positive tumors. Bystander killing has been reported to be induced by death receptors, impacted by adhesion molecules and that the upregulation of both is dependent on IFNγ (journals.plos.org/plosone/article?id=10.1371/journal.pone.0183390). Therefore, assessment of this parameter is critical for a CD3 bsAb such as FUSE608 that has been engineered for decoupling of cytotoxicity from cytokine release. A high-level indirect questions is "does FUSE608 induce sufficient IFNγ release to support bystander killing?"

[0148] As such, we examined the kinetics by which FUSE608 (black circle) and FUSE399 (black reverse triangle; the non-decoupled benchmark ROR1 x CD3 bsAb) induced expanded T cell mediated killing of ROR1+ MDA-MB-231 tumor cells (FIG. 79A), ROR1-negative T-47D tumor cells (FIG. 79B) or a 1:1 mixture of both cells (FIGs 79C-79D). Shown are x-y plots of tumor viability on the y-axis and time on the x-axis. To distinguish between ROR1 positive MDA-MB-231 and ROR1 negative T-47D cells, the former were engineered to express RFP and the latter to express eGFP. FUSE397 (black square) was used as the negative control.

[0149] As seen in FIG. 79A and consistent with the results from our previous killing assays, in the presence of expanded T cells and MDA-MB-231 RFP alone, both FUSE399 and FUSE608 (but not the control test article, FUSE397) induced strong T cell mediated killing of ROR1+ MDA-MB-231 RFP with potencies ($IC50_{VIABILITY}$) and Imax values that were not appreciably different from one another. Also consistent with our previous observations, when ROR1-negative T-47D eGFP cells were co-cultured with expanded T cells, neither FUSE608 nor FUSE399 (or the control test article, FUSE397) induced loss in T-47D eGFP viability (FIG. 79B), indicating that the killing observed with MDA-MB-231 RFP was ROR1 dependent.

[0150] To examine bystander killing, expanded T cells were used as effector cells in co-culture with a 1:1 mixture of ROR1-positive MDA-MB-231 RFP and ROR1-negative T-47D eGFP. Test articles were tested at multiple concentrations. Shown is the 1 nM concentration because this level was previously observed to be the most consistent concentration at which both FUSE608 and FUSE399 induced similar maximum killing of MDA-MB-231 but dramatically differed in IFNγ release. That is, at 1 nM, FUSE399 reached maximum IFNγ release but the release of IFNγ by FUSE608 was below its EC50 value and therefore well within the decoupled portion of its curve.

[0151] Here, we observed that FUSE608 was as efficient at inducing direct killing of ROR1-positive MDA-MB-231 RFP (FIG. 79C) and bystander killing of ROR1-negative T-47D eGFP (FIG. 79D) as FUSE399. This indicates that although FUSE608 can be dosed for lower IFNγ release than FUSE399 while maintaining maximum killing of ROR1+ tumors, FUSE608 still induces sufficient IFNγ to mediate bystander killing. No loss in either tumor cell viability was observed with the negative control test article, FUSE397.

[0152] Killing of ROR1-positive MDA-MB-231 RFP (FIG. 79D) when admixed with T-47D eGFP occurred with similar kinetics as that observed without co-mixture of T-47D eGFP cells (see FIG. 79A). Such "direct killing" has been previously shown to be perforin/granzyme mediated

(journals.plos.org/plosone/article?id=10.1371/journal.pone.0183390). In contrast, bystander killing of ROR1-negative T-47D eGFP only occured when co-mixed with MDA-MB-231 and is delayed with an

[0153] $IC50_{VIABILITY}$ of about 36 hours (i.e., approximately 22 hours after perforin/granzyme mediated killing of MDA-MB-231 RFP). This timing is consistent with a previous report describing CD3 bsAb induced IFNγ dependent bystander killing (journals.plos.org/plosone/article?id=10.1371/journal.pone.0183390).

Example 59

[0154] As shown in figure 80, we examined the capacity of FUSE608 and the non-decoupled benchmark ROR1 x CD3 bsAb, FUSE399, to mediate tumor growth inhibition (TGI) in a mouse tumor xenograft model. NSG mice were subcutaneously implanted with the human ROR1+ TNBC cell line, MDA-MB-231 and intravenously reconstituted with expanded human T cells from a healthy human donor. T cells from several human donors were first screened to identity those with minimal "background" alloreactivity against MDA-MB-231 but which induced potent ROR1 dependent tumor cell killing when co-mixed with FUSE399 or FUSE608 in a similar fashion to that observed in figure 75.

[0155] When the tumor volume reached ~100 mm3, mice were treated as such: group 1 (gray reverse open triangle) received PBS, group 2 (open circle) received 3 mg/kg of FUSE608, group 3 (black closed circle) received 0.3 mg/kg of FUSE608, group 4 (gray closed triangle) received 0.03 mg/kg of FUSE608 and group 5 (black reverse triangle) received 3 mg/kg of FUSE399. Tumor volume and survival as a function of time are shown in FIGs. 80A and 80B, respectively. Test articles were administered intraperitoneally twice a week.

[0156] We observed highly appreciable TGI from FUSE608 with dose levels of 0.3 mg/kg and 3 mg/kg associated with slightly better efficacy than 0.03 mg/kg.

[0157] Importantly, all mice treated with FUSE608 survived whereas all mice treated with FUSE399 died within the first 5 days after the first treatment from toxicity consistent with CRS.

Example 60

Humanization libraries of V_HH clones 5A1 and 2A11

[0158] Many tumors, such as lung or breast cancer, show increased expression of ROR1. ROR1, which is normally only expressed during embryogenesis except for hematogones, plays a crucial role in cell proliferation, migration, and survival. Two ROR1 targeting V_HH variants (2A11 and 5A1) were obtained from a *Camelus dromedarius* immunization campaign and contained a putative non-canonical disulfide bridge between CDR1 and CDR3, which is present in about 30% of all V_HHs of HCAbs from *Camelus dromedarius*. The position of an additional disulfide bridge depends on the camelid species. In *Camelus dromedarius*, for example, it is located between the CDR1 and CDR3 loops, whereas in llama V_HHs, the CDR3 loop is linked to the CDR2 loop. The two V_HH clones have a 71.3 % sequence identity . Despite their many desirable properties, the non-human origin of V_HH could lead to the

development of undesirable immune responses upon therapeutic administration. Thus, to broaden the range of therapeutic applications, humanization is a promising method. The first comprehensive report describing the humanization of V_HHs was published by Vincke *et al.* in 2009. In their study, they proposed two different general methods for the humanization of V_HHs: CDR grafting and resurfacing (Veneering). In the grafting approach, the CDRs of a V_HH are grafted onto another human or humanized framework (16A). In the Veneering strategy, residues in the framework regions of the camelid V_HH are substituted to mimic the sequence of human V_HS.

[0159] The putative CDR loops were determined based on a statistical comparison of V_HHs by Melarkode Vattekatte et al. In the context of humanization, undesirable effects such as affinity attenuation or impaired protein stability may occur due to the modulation of framework regions. Therefore, so-called Vernier residues are of great importance for specificity and affinity. They underlie the CDRs and can potentially impact the conformation of the CDR loops. It has been shown that back-mutations of Vernier residues to the non-human amino acid can restore the original conformation of CDR loops.

[0160] For each clone, eight putative Vernier positions underlying the CDR loops were proposed based on the work of Vincke et al., Soler et al., and Sang et al., as well as on the comparison with the crystal structure of a similar V_HH. The nearest human framework (FR), which is IGHV3-23*01 in both cases, was identified by utilizing Ig-blast (www.ncbi.nlm.nih.gov/igblast/; accessed 01.06.2022). For clone 2A11, the sequence identity is 71.1%, and for 5A1, it is 64.9%. To identify which human amino acids should be allowed besides the camelid one at each Vernier position, the five human frameworks with the highest sequence identity were chosen as templates. A characteristic difference between human V_H and camelid V_HH is the replacement of conserved hydrophobic amino acids in the FR2 (V42F; G49E; L50R; W52G). Conserved key residues in FR2 were not altered, except for glutamate to leucine at position 52, as this increased V_HH stability in the past.

[0161] Further substitutions with potential beneficial effects for the V_HH properties were made. For example, a reduced aggregation could be observed for the Q1E mutation in framework region 1 (FR1) and the exchange to a hydrophobic residue at position 5 residue has been shown to improve stability. In FR4, the exchange of tryptophan to arginine can improve solubility and reduce aggregation by masking hydrophobic residues. Thus, 16 residues outside from the Vernier residues were replaced in the 5A1 humanization library. In the 2A11 humanization library, seven such residues were altered. The minimal guaranteed identity to the human germline gene IGHV3-23 with this library approach is 75.3% with consideration of the CDR regions and 87.6% without these regions.

[0162] For the generation of yeast surface display libraries, the protocol of Benatuil et al. was utilized. Inserts were obtained via a PCR assembly reaction with primers containing degenerated codons. They were designed to allow the selected human amino acids and the camelid residue at the Vernier positions (Figure 72). Yeast Surface Display takes advantage of eukaryotic post-translational modification machinery. Folding in the oxidizing environment of the endoplasmatic reticulum (ER) and glycosylation enhances the presentation of natively proteins. Furthermore, chaperones and foldases assist in the presentation of correctly folded, more complex proteins. This also allows the

surface presentation of multidomain proteins containing multiple disulfide bridges. This makes YSD an efficient method for screening antibody libraries. The function of displayed proteins can be rapidly and quantitatively determined by using fluorescence activated cell sorting (FACS). FACS-based screening over several rounds was used to find functional variants.

Simultaneous Vernier-optimization and affinity maturation based on FUS497

[0163] The computational humanized clone FUSE497, showed improved expression properties compared to the parental 5A1 clone. Therefore, this clone was used as template for an additional humanization campaign. The FUSE497-based library (Figure 73) allows for the optimization of Vernier residues, the potential increasement of the proportion of human residues and affinity maturation by randomization of a position in CDR3 with a degenerated NNS codon. New putative Vernier residues were introduced in the positions marked in orange compared to the previous libraries. On the one hand, this allows back mutations at potential Vernier positions and on the other hand, it enables an increase in sequence homology with the human germline gene IGHV-3-23*01 compared to the template FUSE497. They were identified as described above.

[0164] The insert for the library was generated with degenerated primers. Twelve different PCRs with varying NNS codons in the CDR3 region were performed and mixed in a stochiometric ratio.

Yeast library generation

[0165] Yeast library generation was performed according to the protocol published by Benatuli et al. in 2010. 50 mL of YPD medium were inoculated with *S. cerevisiae* EBY100 and incubated overnight at 30°C and 200 rpm. The next day, 1 L YPD Medium was inoculated with the pre-culture to a starting OD_{600} of 0.3 and incubated at 30°C and 200 rpm until an OD_{600} of 1.6 was reached. The cells were sedimented by centrifugation at 4000 rpm for 3 min. After washing twice with ice-cold water and once with ice-cold electroporation buffer (1 M sorbitol; 1 mM $CaCl_2$), cells were collected in 250 mL resuspension buffer (0.1 M LiAc; 10 mM DTT) and incubated at 30°C and 200 rpm for 30 min. Finally, the cells were washed four times with ice-cold electroporation buffer, collected in 10 mL electroporation buffer, and kept on ice until electroporation. For each electroporation, to 400 µL of the electrocompetent EBY100 solution, 12 µg insert and 4 µg restricted vector (pCT digested by Bamh I and Nhe I) was added. After transfer to an ice-cold electroporation cuvette, the mixture was incubated for 5 min on ice. Afterward, cells were electroporated at 200 Ω, 2.5 kV, 25 µF, transferred to 8 mL of a 1:1 mixture of 1 M Sorbitol:YPD, and incubated at 30°C and 200 rpm for 1 h. To determine the library size, a dilution series was prepared up to a dilution of 10-9. Dilution 10^{-4} to 10^{-9} were plated on selective SD agar plates. After an incubation of 3 days at 30°C, the colonies were calculated to determine the number of transformed cells. The rest of the regenerated cells were transferred to 1 L of selective SD medium (5.4 g/L Na_2HPO_4; 8.6 g/L NaH_2PO_4; 20 g/L glucose; 5 g/L ammonium sulfate; 1.7 g/L yeast nitrogen base; 5 g/L Bacto Casamino Acids (-Trp)) and incubated for 2 d at 30°C and 200 rpm.

Induction of gene expression for YSD

[0166] Surface presentation of V_HHs was mediated via Aga2p (Figure 75). Gene expression of those linked proteins was controlled by the Gal1 promoter. Therefore, expression of the Aga2p fusion protein is induced by galactose in the culture medium. In order to start to display gene expression, cells were grown in SG medium (5.4 g/L Na_2HPO_4; 8.6 g/L NaH_2PO_4; 20 g/L galactose; 5 g/L ammonium sulfate; 1.7 g/L yeast nitrogen base; 5 g/L Bacto Casamino Acids (-Trp)) or a mixture of 4:1 SG:SD medium at 30°C and 200 rpm for 12 to 48 h.

[0167] While not wishing to be bound by any particular theory, we believe that our ROR1 TCEs provide a superior therapeutic index and safety profile associated with reduced risks of CRS and TLS, the capacity to dose higher, avoid continuous infusion and potentially mediate better efficacy in solid tumors.

[0168] Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

[0169] The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

[0170] While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are useful to an embodiment, yet open to the inclusion of unspecified elements, whether useful or not. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). Although the open-ended term "comprising," as a synonym of terms such as including, containing, or having, is used herein to describe and claim the invention, the present invention, or embodiments thereof, may alternatively be described using alternative terms such as "consisting of" or "consisting essentially of."

[0171] Unless stated otherwise, the terms "a" and "an" and "the" and similar references used in the context of describing a particular embodiment of the application (especially in the context of claims) may be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein may be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (for example, "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the application and does not pose a limitation on the scope of the application otherwise claimed. The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example." No language in the specification should be construed as indicating any non-claimed element essential to the practice of the application.

[0172] "Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

[0173] Groupings of alternative elements or embodiments of the present disclosure disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

WHAT IS CLAIMED IS:

1. A fusion protein comprising a scaffold polypeptide and at least two of (a)-(c):
    a. one or more of an interleukin 15 (IL-15) variant,
    b. one or more of an interleukin 18 (IL-18), a fragment of the IL-18, an IL-18 variant, or a fragment of the IL-18 variant, and
    c. one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA).
2. The fusion protein of claim 1, wherein the one or more polypeptides capable of binding the TAA comprise a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH.
3. The fusion protein of claim 1, wherein the TAA comprises EGFR, HER2, or DLL.
4. The fusion protein of claim 1, wherein the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, or wherein the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40.
5. The fusion protein of claim 1, comprising one or more of an interleukin 15 (IL-15) variant and one or more of an interleukin 18 (IL-18), a fragment of the IL-18, an IL-18 variant.
6. The fusion protein of any one of claims 1-5, further comprising a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell,

    wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, and

    wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40.
7. The fusion protein of claim 1, wherein the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, are each independently fused to the C-terminus of the scaffold polypeptide.
8. The fusion protein of claim 1, wherein the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, are each independently fused to the N-terminus of the scaffold polypeptide.
9. The fusion protein of claim 1, wherein the scaffold polypeptide is an antibody, the IL-15 variant, the interleukin 18 (IL-18), the fragment of the IL-18, the IL-18 variant, or the fragment of the IL-18 variant, or the receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, are each independently fused to any one of one of a C-terminus or an N-terminus of a heavy chain or a light chain, or fused to any one of a C_H2 domain, or hinge region of the antibody.

10. The fusion protein of claim 1, wherein the scaffold polypeptide is an antibody or a fragment thereof.
11. The fusion protein of claim 10, wherein the antibody is an IgA, IgM, IgG, or IgE antibody.
12. The fusion protein of claim 10, wherein the antibody is an anti-PD-1 antibody or anti-PD-L1 antibody.
13. The fusion protein of claim 1, wherein the scaffold polypeptide is a fragment crystallizable (Fc) region or a fragment thereof.
14. The fusion protein of claim 13, wherein the fragment crystallizable (Fc) region is an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG1.
15. The fusion protein of claim 13, wherein the fragment crystallizable (Fc) region is a knobs-in-hole (KiH) Fc.
16. The fusion protein of claim 1, wherein the scaffold polypeptide is a polypeptide or protein capable of translocating into an endoplasmic reticulum (ER), or a fragment thereof.
17. The fusion protein of claim 1, wherein the scaffold polypeptide is selected from fragment crystallizable (Fc) region, human serum albumin (HSA), beta2microglobulin, transferrin, fragment antigen-binding region (Fab region), VHH antibody, single-chain variable fragment (scFv), anticalin, designed ankyrin repeat protein (DARPin), a binding domain thereof, and a fragment thereof.
18. The fusion protein of any one of claims 1-17, wherein IL-15 variant comprises the sequence of Formula I: X_1WVX_4VISDLKKIEDLIQSMHIX_{22}ATLYTESX_{30}VHPSCKVTAMX_{41}CFLX_{45}ELQX_{49}ISLX_{53}SGDASIH DTVX_{64}NL$X_{67}X_{68}$LANNSLSSNGX_{79}VTESGCKECEELEX_{93}KNIKEFLQSX_{103}VHIVX_{108}MFIX_{112}TS, wherein
    a. X_1 is any amino acid,
    b. X_4 is any amino acid,
    c. X_{22} is any amino acid,
    d. X_{30} is any amino acid,
    e. X_{41} is any amino acid,
    f. X_{45} is any amino acid,
    g. X_{49} is any amino acid except V,
    h. X_{53} is any amino acid except E,
    i. X_{64} is any amino acid,
    j. X_{67} is any amino acid,
    k. X_{68} is any amino acid,
    l. X_{79} is any amino acid,
    m. X_{93} is any amino acid,
    n. X_{103} is any amino acid,
    o. X_{108} is any amino acid, and
    p. X_{112} is any amino acid; (SEQ ID NO:533).
19. The fusion protein of any one of claims 1-18, wherein the IL-18 variant comprises

a1. amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQG NRPLFEDMTDSDVRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMN PPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO:250), with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of SEQ ID NO:250, OR

a2. amino acid positions 37-193 of MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENIEQDYFGKLESKLSVIRNLNDQVLFIDQG NRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMN PPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO:251) with one to five amino acid substitutions at positions E42, M87, K89, M96, and M149 of SEQ ID NO:251, optionally further comprising amino acid substitutions at one or more of C74, C104, C112, and C164, each independently substituted to valine, alanine or serine, OR

a3. an IL-18 variant selected from Table 6B; and

b1. optionally, wherein the one to five amino acid substitutions are one or more of: E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; or M149V or M149I; or

b2. optionally, wherein the one to five amino acid substitutions are E42K, E42R, E42A, E42H, or E42Q; M87K, or M87H; K89G, K89A, or K89E; M96L, or M96I; and M149V or M149I.

20. The fusion protein of any one of claims 1-19, wherein the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant further comprises its propeptide (PP) or a PP variant, and optionally, wherein the PP or the PP variant is on the N-terminus end relative to the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant.

21. The fusion protein of any one of claims 1-19, wherein the IL-18, the fragment of IL-18, the IL-18 variant, or the fragment of the IL-18 variant further comprises a short polypeptide or protein.

22. The fusion protein of any one of claims 1-21, wherein the fusion protein further comprises one or more cleavage sites and the fusion protein is cleaved at the one or more cleavage sites by one or more proteases.

23. The fusion protein of claim 22, wherein the one or more cleavage sites is

between the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant and the scaffold polypeptide, or

within the PP, between PP or the PP variant and the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, or

within the PP, between the PP or the PP variant and the scaffold polypeptide, or

within the IL-18, a fragment of IL-18, an IL-18 variant, or a fragment of the IL-18 variant, or within the PP, or

a combination thereof.

24. The fusion protein of any one of claims 2-23, wherein the ROR1 VHH comprises:

a polypeptide having SEQ ID NO:325 (complementarity-determining region (CDR) 1 of 2A11), a polypeptide having SEQ ID NO:326 (CDR2 of 2A11), a polypeptide having SEQ ID NO:327 (CDR3 of 2A11), or a combination thereof; OR

a variant of the polypeptide having SEQ ID NO:325 (CDR1 of 2A11), a variant of the polypeptide having SEQ ID NO:326 (CDR2 of 2A11), a variant of the polypeptide having SEQ ID NO:327 (CDR3 of 2A11), or a combination thereof, wherein the variant of the polypeptide having SEQ ID NO:325 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:325, wherein the variant of the polypeptide having SEQ ID NO:326 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:326, wherein the variant of the polypeptide having SEQ ID NO:327 comprises one or more deletions, additions or a substitutions of an amino acid residue in the polypeptide having SEQ ID NO:327, and

wherein:

the variant of the polypeptide having SEQ ID NO:325 and the variant of the polypeptide having SEQ ID NO:327 do **not** replace cysteine residues in the polypeptide having SEQ ID NO:325 and the polypeptide having SEQ ID NO:327, or

the variant of the polypeptide having SEQ ID NO:325 and the variant of the polypeptide having SEQ ID NO:327 **replaces** one or both of the cysteine residues in the polypeptide having SEQ ID NO:325 and/or one or both of the cysteine residues in the polypeptide having SEQ ID NO:327 **with** an amino acid that contains a cross-linking functional group.

25. A polynucleotide encoding a fusion protein of any one of claims 1-24.

26. An expression vector comprising the polynucleotide of claim 25.

27. A cell transfected with the expression vector of claim 26.

28. The cell of claim 27, wherein the cell is a mammalian cell.

29. The cell of claim 28, wherein the mammalian cell is a CHO cell or a HEK-293 cell.

30. The cell of claim 27, wherein the cell is a bacterial cell or yeast cell.

31. A method of producing a fusion protein, comprising:

culturing the cell of any one of claims 27-30, in cell culture medium to allow the fusion protein to be produced, and optionally secreted into the cell culture medium.

32. The method of claim 31, further comprising isolating the fusion protein.

33. The method of claim 32, further comprising purifying the fusion protein.

34. A method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising

administering a fusion protein of any one of claims 1-24, to a subject in need thereof.

35. The method of claim 34, wherein the subject has cancer.

36. A method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising

administering a fusion protein of any one of claims 1-24, to a subject in need thereof.

37. The method of claim 36, wherein the disease or condition is cancer.

38. A method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising administering at least two of the following to a subject in need thereof:

a. interleukin 15 (IL-15) variant or IL-15 fusion protein,

b. interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein,

c. a multispecific antibody construct comprising

i. a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and

ii. a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell,

wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, and

wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40, and

d. one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA)

39. The method of claim 38, wherein the subject has cancer.

40. A method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising administering at least two of the following to a subject in need thereof:

a. interleukin 15 (IL-15) variant or IL-15 fusion protein,

b. interleukin 18 (IL-18), a fragment of the IL-18, IL-18 variant, a fragment of the IL-18 variant, or an IL-18 fusion protein,

c. a multispecific antibody construct comprising

i. a receptor tyrosine kinase-like orphan receptor 1 (ROR1) VHH, and

ii. a receptor binding polypeptide capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell,

wherein optionally the activation receptor comprises cluster of differentiation (CD) 3, CD16, γ9 TCR, δ2 TCR or δ1 TCR, and

wherein optionally the costimulatory receptor comprises cluster of differentiation (CD) 137, CD28, DNAM-1, NKp46, NKG2D, NKp30, CD2, ICOS, OX40, CD40L, or CD40, and

d. one or more receptor binding polypeptides capable of binding an activation receptor and/or a costimulatory receptor expressed on an immune cell, or one or more polypeptides capable of binding a tumor associated antigen (TAA).

41. The method of claim 40, wherein the disease or condition is cancer.

42. An interleukin 15 (IL-15) variant comprising the sequence of Formula I: X_1WVX$_4$VISDLKKIEDLIQSMHIX$_{22}$ATLYTESX$_{30}$VHPSCKVTAMX$_{41}$CFLX$_{45}$ELQX$_{49}$ISLX$_{53}$SGDASIH DTVX$_{64}$NLX$_{67}$X$_{68}$LANNSLSSNGX$_{79}$VTESGCKECEELEX$_{93}$KNIKEFLQSX$_{103}$VHIVX$_{108}$MFIX$_{112}$TS, wherein

a. X_1 is any amino acid,
b. X$_4$ is any amino acid,
c. X$_{22}$ is any amino acid,
d. X$_{30}$ is any amino acid,
e. X$_{41}$ is any amino acid,
f. X$_{45}$ is any amino acid,
g. X$_{49}$ is any amino acid except V,
h. X$_{53}$ is any amino acid except E,
i. X$_{64}$ is any amino acid,
j. X$_{67}$ is any amino acid,
k. X$_{68}$ is any amino acid,
l. X$_{79}$ is any amino acid,
m. X$_{93}$ is any amino acid,
n. X$_{103}$ is any amino acid,
o. X$_{108}$ is any amino acid, and
p. X$_{112}$ is any amino acid; (SEQ ID NO:533).

43. The IL-15 variant of claim 42, wherein

a. X_1 is N (SEQ ID NO:534),
b. X$_4$ is N (SEQ ID NO:535),
c. X$_{22}$ is D (SEQ ID NO:536),
d. X$_{30}$ is D (SEQ ID NO:537),
e. X$_{41}$ is K (SEQ ID NO:538),

f. X_{45} is L (SEQ ID NO:539),
g. X_{64} is E (SEQ ID NO:540),
h. X_{67} is I (SEQ ID NO:541),
i. X_{68} is I (SEQ ID NO:542),
j. X_{79} is N (SEQ ID NO:543),
k. X_{93} is E (SEQ ID NO:544),
l. X_{103} is F (SEQ ID NO:545),
m. X_{108} is Q (SEQ ID NO:546), or
n. X_{112} is N (SEQ ID NO:547), or
o. any two or more of a-o.

44. The IL-15 variant of claim 42, wherein
a. X_1 is N (SEQ ID NO:534),
b. X_4 is N (SEQ ID NO:535),
c. X_{22} is D (SEQ ID NO:536),
d. X_{30} is D (SEQ ID NO:537),
e. X_{41} is K (SEQ ID NO:538),
f. X_{45} is L (SEQ ID NO:539),
g. X_{49} is R or K (SEQ ID NO:548),
h. X_{53} is G, K, I or A (SEQ ID NO:549),
i. X_{64} is E (SEQ ID NO:540),
j. X_{67} is I (SEQ ID NO:541),
k. X_{68} is I (SEQ ID NO:542),
l. X_{79} is N (SEQ ID NO:543),
m. X_{93} is E (SEQ ID NO:544),
n. X_{103} is F (SEQ ID NO:545),
o. X_{108} is Q (SEQ ID NO:546), or
p. X_{112} is N (SEQ ID NO:547), or
q. any two or more of a-p.

45. The IL-15 variant of claim 42, wherein
a. X_1 is N or G (SEQ ID NO:550),
b. X_4 is N, K or L (SEQ ID NO:551),
c. X_{22} is D or A (SEQ ID NO:552),
d. X_{30} is D or N (SEQ ID NO:553),
e. X_{41} is K or Q (SEQ ID NO:554),
f. X_{45} is L or S (SEQ ID NO:555),

g. X_{49} is R or K (SEQ ID NO:548),
h. X_{53} is G, K, I, or A (SEQ ID NO:549),
i. X_{64} is E or Q (SEQ ID NO:556),
j. X_{67} is I or T (SEQ ID NO:557),
k. X_{68} is I or S (SEQ ID NO:558),
l. X_{79} is N or Y (SEQ ID NO:559),
m. X_{93} is E or A (SEQ ID NO:560),
n. X_{103} is F or L (SEQ ID NO:561),
o. X_{108} is Q or T (SEQ ID NO:562), or
p. X_{112} is N or R (SEQ ID NO:563), or
q. any two or more of a-p.

46. The IL-15 variant of claim 42, wherein
X_{49} is R or K, and
X_{53} is G, K, I or A; (SEQ ID NO:564).

47. The IL-15 variant of claim 42,
X_{30} is N (SEQ ID NO:565).

48. The IL-15 variant of claim 42, wherein
X_{64} is Q (SEQ ID NO:566).

49. The IL-15 variant claim 42, wherein
X_1 is G (SEQ ID NO:567).

50. The IL-15 variant of claim 42, wherein the IL-15 variant comprises an IL-15 variant listed in Table 1A or Table 3A.

51. A fusion protein, comprising
the IL-15 variant of any one of claims 42-50; and
a scaffold polypeptide.

52. The fusion protein of claim 51, wherein the IL-15 variant is fused to the C-terminus of the scaffold polypeptide.

53. The fusion protein of claim 51 or claim 52, wherein the scaffold polypeptide is an antibody or a fragment thereof.

54. The fusion protein of claim 53, wherein the antibody is an IgA, IgM, IgG, or IgE antibody.

55. The fusion protein of claim 53, wherein the antibody is an anti-PD-1 antibody or anti-PD-L1 antibody.

56. The fusion protein of claim 51, wherein the scaffold polypeptide is a Fc region or a fragment thereof.

57. The fusion protein of claim 51, wherein the scaffold polypeptide is a Fc region or a fragment thereof and the scaffold polypeptide does not comprise an Fab.

58. The fusion protein of claim 56, wherein the Fc region is an Fc region from an IgG4, knobs-in-hole (KiH) Fc, or IgG1.
59. The fusion protein of claim 56, wherein the Fc region is a knobs-in-hole (KiH) Fc.
60. The fusion protein of claim 51, wherein the IL-15 variant is fused to the knob of the KiH Fc.
61. The fusion protein of claim 51, wherein the IL-15 variant is fused to the hole of the KiH Fc.
62. A polynucleotide encoding an IL-15 variant of any one of claims 42-50, or a fusion protein of any one of claims 51-61.
63. An expression vector comprising the polynucleotide of claim 62.
64. A cell transfected with the expression vector of claim 63.
65. The cell of claim 64, wherein the cell is a mammalian cell.
66. The cell of claim 65, wherein the mammalian cell is a CHO cell or a HEK-293 cell.
67. The cell of claim 64, wherein the cell is a bacterial cell or yeast cell.
68. A method of producing an IL-15 variant or a fusion protein, comprising:

culturing the cell of any one of claims 64-67, in cell culture medium to allow the IL-15 variant or the fusion protein to be produced, and optionally secreted into the cell culture medium.

69. The method of claim 68, further comprising isolating the IL-15 variant or the fusion protein, or purifying the IL-15 variant or the fusion protein.
70. A method of activating, promoting differentiation or promoting expansion of T cells, B cells or natural killer (NK) cells, comprising

administering an IL-15 variant of any one of claims 42-50, or an IL-15 fusion protein of any one of claims 51-61, to a subject in need thereof.

71. The method of claim 70, wherein the subject has cancer.
72. A method of ameliorating, inhibiting or treating a disease or condition in a subject in need thereof, comprising

administering an IL-15 variant of any one of claims 42-50, or an IL-15 fusion protein of any one of claims 51-61, to a subject in need thereof.

73. The method of claim 72, wherein the disease or condition is cancer.