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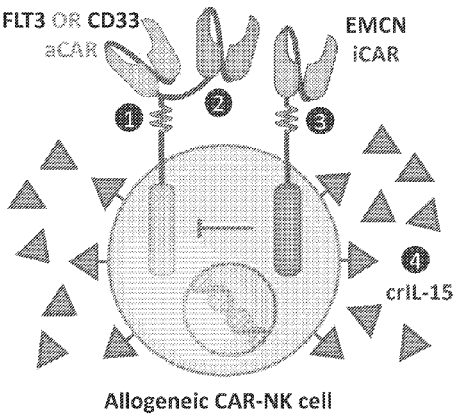
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(54) Title: MULTICISTRONIC CHIMERIC PROTEIN EXPRESSION SYSTEMS



Gene Circuit	Logic Gating: FLT3 OR CD33 NOT EMCN
1	FLT3-targeting scFv; part of bivalent *activating* CAR (aCAR)
2	CD33-targeting scFv; part of bivalent *activating* CAR (aCAR)
3	EMCN *inhibitory* CAR (iCAR)
4	crIL-15 (novel IL-15 molecule)

FIG. 1

(57) Abstract: Described herein are multicistronic expression systems that encode chimeric proteins, specifically membrane-cleavable chimeric systems and chimeric antigen receptors. Also described herein are nucleic acids, cells, and methods directed to the same.

WO 2023/205614 A3

[Continued on next page]

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MULTICISTRONIC CHIMERIC PROTEIN EXPRESSION SYSTEMS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of each of U.S. Provisional Application Nos. 63/333,483 filed on April 21, 2022; 63/370,219 filed on August 2, 2022; and 63/440,668 filed January 23, 2023, each of which is hereby incorporated in its entirety by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on XXXX, 20XX, is named XXX-XXX_sequencelisting.txt and is ###,### bytes in size.

BACKGROUND

Cell-based therapy platforms provide promising avenues for treating a variety of diseases. One such promising platform is CAR-T based therapies in the treatment of cancer. Given their promise, improvements in cell-based therapies are needed. An active area of exploration is engineering cell-based therapies to produce and/or secrete effector molecules such as cytokines, a process referred to as armoring, that enhance the cell-based therapy. For example, unarmored CAR-T therapies have poor efficacy in solid tumors and armoring can impact the entire cancer immunity cycle and boost the activity of CAR-T. However, uncontrolled or unregulated armoring strategies can have negative impacts on treatment, such as off-target effects and toxicity in subjects. Thus, additional methods of controlling and regulating the armoring of cell-based therapies, such as regulating production and/or secretion of payload effector molecules, are required.

SUMMARY

Provided herein, in some embodiments, is a cell-based therapy platform involving regulated armoring of the cell-based therapy, including chimeric antigen receptor (CAR)-based therapies (*e.g.* NK CARs), such as regulated secretion of payload effector molecules. Also provided herein, in some embodiments, is a combinatorial cell-based immunotherapy involving regulated armoring for the targeted treatment of cancer, such as ovarian cancer, breast cancer, colon cancer, lung cancer, and pancreatic cancer.

The therapy provided herein, however, can limit systemic toxicity of armoring. For example, the immunotherapy provided herein can be tumor-specific and effective while limiting systemic toxicity and/or other off-target effects due to armoring. These therapies deliver proteins of interest, such as immunomodulatory effector molecules, in particular IL-15, in a regulated manner, including regulation of secretion kinetics, cell state specificity, and cell or tissue specificity. The design of the delivery vehicle is optimized to improve overall function in cell-based therapies, such as cancer therapy, including, but not limited to, optimization of the membrane-cleavage sites, promoters, linkers, signal peptides, delivery methods, combination, regulation, and order of the immunomodulatory effector molecules.

The design of the delivery vehicle is also optimized for expression of multiple components for CAR-based therapies in a multicistronic system, including chimeric antigen receptors (both activating aCARs and inhibitory iCARs) and membrane-cleavable chimeric protein.

Provided for herein is a multicistronic expression system comprising an engineered nucleic acid comprising: (A) an exogenous polynucleotide encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula: S – C – MT or MT – C – S, wherein S comprises a secretable effector molecule, wherein the secretable effector molecule comprises IL-15, C comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein S – C – MT or MT – C – S is configured to be expressed as a single polypeptide; (B) an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response, and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and (C) an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: (i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response, and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

In some aspects, the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are linked together by a polynucleotide linker encoding a 2A ribosome skipping element. In some aspects, the 2A ribosome skipping element is selected from the group consisting of: a T2A ribosome skipping element, a E2A ribosome skipping element, a P2A ribosome skipping element, a F2A ribosome

skipping element, ribosome skipping element fusions thereof, and combinations thereof. In some aspects, the ribosome skipping element fusion comprises an E2A/T2A ribosome skipping element. In some aspects, the E2A/T2A ribosome skipping element comprises the amino acid sequence QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221). In some aspects, the E2A/T2A ribosome skipping element is encoded by a polynucleotide sequence comprising the sequence CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATCCTGG ACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACGTGGAGGAA AACCCTGGACCT (SEQ ID NO: 222) or the sequence CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTCGAGAGTAACCCAGG ACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGTAGAAGAAA ATCCTGGACCT (SEQ ID NO: 223).

In some aspects, the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR. In some aspects, the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) iCAR; and (iii) bivalent aCAR.

In some aspects, the secretable effector molecule comprises a signal peptide or a signal-anchor sequence. In some aspects, the signal peptide comprises a native signal peptide native to the secretable effector molecule. In some aspects, the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule. In some aspects, the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-CSFRa. In some aspects, the non-native signal peptide comprises an IgE signal peptide. In some aspects, the IgE signal peptide comprises the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228). In some aspects, the IgE signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229).

In some aspects, the IL-15 comprises the amino acid sequence NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID

NO: 224). In some aspects, the IL-15 is encoded by a polynucleotide sequence comprising the sequence AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCAT GCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGA CCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGAC GCCAGCATCCACGATACCGTGGAAAATCTGATCATCCTGGCCAACAACAGCCTGTC CAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAG AAGAACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAA CACCTCA (SEQ ID NO: 225).

In some aspects, the protease cleavage site further comprises the N-terminal peptide linker, optionally selected from the group consisting of: SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; and EAAAKEAAAKEAAAKEAAAK. In some aspects, the protease cleavage site further comprises the N-terminal peptide linker SGGGGSGGGGSG (SEQ ID NO: 230).

In some aspects, the protease cleavage site further comprises a C-terminal peptide linker, optionally selected from the group consisting of: SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; and EAAAKEAAAKEAAAKEAAAK. In some aspects, the protease cleavage site further comprises the C-terminal linker GGGSGGGGSGGGSLQ (SEQ ID NO: 231).

In some aspects, the protease cleavage site comprises a Tumor Necrosis Factor-α Converting Enzyme (TACE)-specific cleavage site. In some aspects, the TACE-specific cleavage site comprises the amino acid sequence VTPEPIFSLI (SEQ ID NO: 191). In some aspects, the TACE-specific cleavage site comprises the amino acid sequence SGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQ (SEQ ID NO: 250). In some aspects, the TACE-specific cleavage site is encoded by a polynucleotide sequence comprising the sequence TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTT

CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAA (SEQ ID NO: 251).

In some aspects, the cell membrane tethering domain comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, LIR1, B7-1, and BTLA. In some aspects, the cell membrane tethering domain comprises a B7-1 transmembrane domain. In some aspects, B7-1 transmembrane domain comprises the amino acid sequence LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204). In some aspects, B7-1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252).

In some aspects, the membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula S – C – MT. In some aspects, the membrane-cleavable chimeric protein comprises the amino acid sequence MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM KCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQS FVHIVQMFINTSSGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQLLPSWAITLISV NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 226). In some aspects, the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence comprising the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAAT TGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCA CATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCG CCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGACGCC AGCATCCACGATACCGTGGAAAATCTGATCATCCTGGCCAACAACAGCCTGTCCAG CAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG AACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAACACC TCATCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTAT CTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTC AATTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCT GCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGG CTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

In some aspects, the antigen-binding domain specific for EMCN comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the EMCN-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 296), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and the EMCN-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 299), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 300), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 301), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme. In some aspects, the EMCN-VH comprises the amino acid sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNT HYHSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS (SEQ ID NO: 302); and the EMCN-VL comprises the amino acid sequence DVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKL DSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQGTKLEIK (SEQ ID NO: 310).

In some aspects, the EMCN-VH and the EMCN-VL are separated by a peptide linker. In some aspects, the antigen-binding domain specific for EMCN comprises the structure VH-L-VL or VL-L-VH, wherein L is the peptide linker. In some aspects, the peptide linker comprises the amino acid sequence SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; or EAAAKEAAAKEAAAKEAAAK. In some aspects, the peptide linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 244). In some aspects, the peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253).

In some aspects, the antigen-binding domain specific for EMCN comprises the structure VH-L-VL and comprises the amino acid sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNT HYHSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSSGGG GSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPG QSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQ GTKLEIK (SEQ ID NO: 311). In some aspects, the antigen-binding domain specific for EMCN is encoded by a polynucleotide sequence comprising the sequence GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAG ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTGGGTCCG ACAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACGGCAACA CACACTACCACAGCGCCCTGAAGTCCCGGTTCACCATCTCCAGAGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTA CTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTG GAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCAT GACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCT GCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTC CAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGGA TAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAA GATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGGCATCC ATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAG (SEQ ID NO: 312).

In some aspects, the intracellular inhibitory domain comprises a LIR1 intracellular inhibitory domain. In some aspects, the LIR1 intracellular inhibitory domain comprises the amino acid sequence LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKH TQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEED RQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 285). In some aspects, the LIR1 intracellular inhibitory domain is encoded by a polynucleotide sequence comprising the sequence CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACT TTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGG CGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCA CACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACC CTCAGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGC AAGCCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCG

AAGAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGATGT GACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCAC CTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTC AC (SEQ ID NO: 286).

In some aspects, the signal peptide is present in the iCAR. In some aspects, the signal peptide of the iCAR comprises a native signal peptide native or a non-native signal peptide, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF. In some aspects, the signal peptide of the iCAR comprises a CD8 signal peptide. In some aspects, the CD8 signal peptide comprises the amino acid sequence MALPVTALLLPLALLLHAARP (SEQ ID NO: 137). In some aspects, the CD8 signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGCTGCT AGACCA (SEQ ID NO: 416).

In some aspects, the hinge domain is present in the iCAR comprises. In some aspects, the hinge domain of the iCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof. In some aspects, the hinge domain of the iCAR comprises a LIR1 hinge. In some aspects, the LIR1 hinge comprises the amino acid sequence HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 417). In some aspects, the LIR1 hinge comprises is encoded by a polynucleotide sequence comprising the sequence CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAGCAGC CCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAAC AGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418). In some aspects, the hinge domain of the iCAR comprises a CD8 hinge. In some aspects, the CD8 hinge comprises the amino acid sequence TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271). In some aspects, the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGCAACC

CCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCACACTA GAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283).

In some aspects, the transmembrane domain of the iCAR is present. In some aspects, the transmembrane domain of the iCAR comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, LIR1, and BTLA. In some aspects, the transmembrane domain of the iCAR comprises a LIR1 transmembrane domain. In some aspects, the LIR1 transmembrane domain comprises the amino acid sequence VIGILVAVILLLLLLLLLFLI (SEQ ID NO: 259). In some aspects, the LIR1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCC TGATC (SEQ ID NO: 260).

In some aspects, the iCAR comprises the amino acid sequence MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYD MHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNSLRAEDT AVYYCTLRIKDWGQGTMVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASI SCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCLQGIHLPWTFGQGTKLEIKHPSDPLELVVSGPSGGPSSPTTGPTSTSG PEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILLLLLLLLLFLILRHRRQGKHWTSTQRK ADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDE DPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVT YAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 430); or

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYD MHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNSLRAEDT AVYYCTLRIKDWGQGTMVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASI SCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCLQGIHLPWTFGQGTKLEIKngaaHPSDPLELVVSGPSGGPSSPTTGPTS TSGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILLLLLLLLLFLILRHRRQGKHWTSTQ RKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPH DEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQD VTYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 419).

In some aspects, the iCAR is encoded by a polynucleotide sequence comprising the sequence ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGCTGCT AGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATC

TCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTG
GGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACG
GCAACACACACTACCACAGCGCCCTGAAGTCCCGGTTCACCATCTCCAGAGACAAC
AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGT
GTACTACTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTT
CTTCTGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTG
GTCATGACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCAT
CAGCTGCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACT
GGTTCCAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAG
CTGGATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACC
CTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGG
CATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAGAATGGCG
CTGCACACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTA
GCAGCCCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACA
CCAACAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTTGTGATCGG
CATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCCTGATCCTG
CGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACTTTC
AGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGGCGG
TCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCACAC
CCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACCCTC
AGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGCAAG
CCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCGAAG
AGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGATGTGACA
TATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTC
TCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTCAC
(SEQ ID NO: 420).

In some aspects, the iCAR comprises the amino acid sequence

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYD
MHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNSLRAEDT
AVYYCTLRIKDWGQGTMVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASI
SCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKIS
RVEAEDVGVYYCLQGIHLPWTFGQGTKLEIKTTTPAPRPPTPAPTIALQPLSLRPEACRP
AAGGAVHTRGLDFACDVIGILVAVILLLLLLLLLFLILRHRRQGKHWTSTQRKADFQHP
AGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVT

YAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHS
LTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 431); or

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYD
MHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNSLRAEDT
AVYYCTLRIKDWGQGTMVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASI
SCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKIS
RVEAEDVGVYYCLQGIHLPWTFGQGTKLEIKngaaTTTPAPRPPTPAPTIALQPLSLRPEA
CRPAAGGAVHTRGLDFACDVIGILVAVILLLLLLLLLFLILRHRRQGKHWTSTQRKADF
QHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQ
AVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQ
LHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 421).

In some aspects, the iCAR is encoded by a polynucleotide sequence comprising the sequence

ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGCTGCT
AGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATC
TCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTG
GGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACG
GCAACACACACTACCACAGCGCCCTGAAGTCCCGGTTCACCATCTCCAGAGACAAC
AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGT
GTACTACTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTT
CTTCTGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTG
GTCATGACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCAT
CAGCTGCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACT
GGTTCCAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAG
CTGGATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACC
CTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGG
CATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAGAATGGCG
CTGCAACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTG
CAACCCCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCA
CACTAGAGGCCTGGACTTTGCCTGTGACGTGATCGGCATTCTGGTCGCCGTGATCCT
GCTCCTGTTGCTCCTGCTGCTTCTGTTCCTGATCCTGCGGCACAGAAGGCAGGGCAA
GCACTGGACAAGCACCCAGAGAAAGGCCGACTTTCAGCATCCTGCTGGCGCCGTTG
GACCTGAGCCTACAGATAGAGGACTGCAGTGGCGGTCTAGCCCTGCCGCTGATGCC
CAAGAGGAAAATCTTTACGCCGCCGTGAAGCACACCCAGCCTGAGGATGGCGTGGA
AATGGACACCAGATCTCCCCACGATGAGGACCCTCAGGCCGTGACATACGCAGAAG

TGAAGCACTCCAGACCTCGGAGAGAGATGGCAAGCCCTCCATCTCCTCTGAGCGGC
GAGTTCCTGGACACCAAAGACAGACAGGCCGAAGAGGACAGACAGATGGATACCG
AAGCCGCCGCTTCTGAAGCCCCACAGGATGTGACATATGCCCAGCTGCATAGCCTG
ACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAAGGCCCATCTCCTGC
CGTGCCTTCCATCTATGCCACTCTGGCCATTCAC (SEQ ID NO: 422).

In some aspects, the antigen-binding domain specific for FLT3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the FLT3-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GGTFSSYAIS (SEQ ID NO: 360), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIIPIFGTANYAQKFQG (SEQ ID NO: 361), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQAFDI (SEQ ID NO: 362), and the FLT3-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASQSISSYLN (SEQ ID NO: 363), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme. In some aspects, the FLT3-VH comprises the amino acid sequence
EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTAN
YAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTV
TVSS (SEQ ID NO: 315); and the FLT3-VL comprises the amino acid sequence
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIK (SEQ ID NO: 316).

In some aspects, the antigen-binding domain specific for CD33 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the CD33-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and the CD33-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and a light chain

complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme. In some aspects: the CD33-VH comprises the amino acid sequence QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGG TGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTV SS (SEQ ID NO: 329); and the CD33-VL comprises the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQG SGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIK (SEQ ID NO: 330).

In some aspects, the FLT3-VH, the FLT3-VL, the CD33-VH, and the CD33-VL are separated by peptide linkers. In some aspects, the aCAR antigen binding domains comprises the structure (FLT3-VH) – L1 – (CD33-VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a third peptide linker, respectively. In some aspects, the L1, L2, and/or L3 are each independently selected from the group consisting of: SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; and EAAAKEAAAKEAAAKEAAAK. In some aspects, the L1 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or GGGGSGGGGS (SEQ ID NO: 243). In some aspects, the L1 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGCGGCGGTGGCTCT (SEQ ID NO: 254) or the L1 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256). In some aspects, the L2 peptide linker is the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247). In some aspects, the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGGGC (SEQ ID NO: 249). In some aspects, the L2 peptide linker is the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243). In some aspects, the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257). In some aspects, the L3

peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242)or GGGGSGGGGS (SEQ ID NO: 243). In some aspects, the L3 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGCGGATCC (SEQ ID NO: 255) or the L3 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258).

In some aspects, the aCAR intracellular signaling domains that stimulate an immune response is selected from the group consisting of: CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278, FcεRI, DAP10, DAP12, CD66d, CD97, CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, LFA-1, CD7, LIGHT, NKG2C, B7-H3, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a SLAM protein, an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLAl, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and combinations thereof. In some aspects, the aCAR intracellular signaling domains that stimulate an immune response comprise a CD28 co-stimulatory domain and a CD3ζ signaling domain. In some aspects, the CD28 co-stimulatory domain comprises the amino acid sequence RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 287). In some aspects, the CD28 co-stimulatory domain is encoded by a polynucleotide sequence comprising the sequence AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCTAGAC GGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCC GCCTACCGGTCC (SEQ ID NO: 288). In some aspects, the CD3ζ signaling domain comprises the amino acid sequence RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 289). In some aspects, the CD3ζ signaling domain is encoded by a polynucleotide sequence comprising the sequence

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACC
AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG
AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT
TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC
TCGC (SEQ ID NO: 290).

In some aspects, the hinge domain of the aCAR is present. In some aspects, the hinge domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof. In some aspects, the hinge domain of the aCAR comprises a CD8 hinge. In some aspects, the CD8 hinge comprises the amino acid sequence ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACD (SEQ ID NO: 272). In some aspects, the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCCCGCC AAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAG CCAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAGCTGCTGGCGGAGCCGTGC ATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284).

In some aspects, the transmembrane domain of the aCAR is present. In some aspects, the transmembrane domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof. In some aspects, the transmembrane domain of the aCAR comprises a CD8 hinge. In some aspects, the CD8 transmembrane comprises the amino acid sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206). In some aspects, the CD8 transmembrane comprises is encoded by a polynucleotide sequence comprising the sequence ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCTGGTC ATCACCCTGTACTGCAACCACAGA (SEQ ID NO: 261).

In some aspects, the aCAR signal peptide of the aCAR is present. In some aspects, the signal peptide of the aCAR is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8,

NKG2D, TNFR2, GMCSF, and GM-CSFRa. In some aspects, the signal peptide of the aCAR comprises a GM-CSFRa signal peptide. In some aspects, the GM-CSFRa signal peptide comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIP (SEQ ID NO: 423). In some aspects, the GM-CSFRa signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTTTCTGCTTATTCCT (SEQ ID NO: 424).

In some aspects, the aCAR comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 414).

In some aspects, the aCAR comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK

DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 415).

Also provided herein is an expression vector comprising any of the multicistronic expression systems provided herein. In some aspects, the expression vector comprises the polynucleotide of SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 433, SEQ ID NO: 432, or SEQ ID NO: 429.

Also provided herein is an isolated cell comprising any of the multicistronic expression systems provided herein or any of the expression vectors provided herein. In some aspects, the cell comprises an immune cell. In some aspects, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell. In some aspects, the cell is an NK cell.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 433, SEQ ID NO: 432, or SEQ ID NO: 429.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 425.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 426.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 427.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 428.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 429.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 432.

Also provided herein is a polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 433.

Also provided herein is a method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells provided herein or any of the polynucleotides provided herein.

Also provided herein is a method of treating a subject with cancer, the method comprising administering to the subject an immunotherapy comprising of any of the isolated cells provided herein or any of the polynucleotides provided herein.

A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells of any of the isolated cells provided herein or any of the polynucleotides provided herein. In some aspects, the isolated cell is derived from the subject. In some aspects, the isolated cell is allogeneic with reference to the subject.

Also provided herein is a method of making an engineered cell, comprising transducing an isolated cell with any of the multicistronic expression systems provided herein, any of the expression vectors provided herein, or any of the polynucleotides provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 provides a schematic illustrating components and organization of the overall FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell system.

FIG. 2 provides a schematic illustrating components assessed in the optimization of the FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell system.

FIG. 3 shows the gating strategy for assessing iCAR and aCAR expression by flow cytometry.

FIG. 4 shows aCAR and iCAR expression at Day 15 for the indicated series of RetroVec constructs.

FIG. 5 shows aCAR and iCAR expression at Day 15 for the indicated series of self-inactivated (SIN) γ-retroviral SINvec constructs.

FIG. 6 shows representative flow cytometry plots for membrane-bound IL-15 expression at Day 15 for the indicated series of RetroVec constructs.

FIG. 7 shows representative flow cytometry plots for membrane-bound IL-15 expression at Day 15 for the indicated series of SINvec constructs.

FIG. 8 shows a first series of comparisons for aCAR, iCAR expression, and membrane-bound IL-15 for the indicated series of RetroVec and SINvec constructs.

FIG. 9 shows a second series of comparisons for aCAR, iCAR expression, and membrane-bound IL-15 for the indicated series of RetroVec and SINvec constructs.

FIG. 10 shows a third series of comparisons for aCAR, iCAR expression, and membrane-bound IL-15 for the indicated series of RetroVec and SINvec constructs.

FIG. 11 shows a summary of aCAR and iCAR expression at Day 15 as assessed by MFI for the indicated series of RetroVec and SINvec constructs.

FIG. 12 shows a general summary of expression considerations for the various components and organizations assessed.

FIG. 13 shows total cytotoxicity against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) for constructs using a LIR1 ICD, both for constructs ordered in an aCAR to iCAR organization (top panel) and an iCAR to aCAR organization (bottom panel).

FIG. 14 shows normalized cytotoxicity against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) for constructs using a LIR1 ICD.

FIG. 15 shows total cytotoxicity at Day 17 against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) at the indicated effector:target ratios for constructs using a LIR1 ICD for constructs ordered in an aCAR to iCAR organization.

FIG. 16 shows normalized cytotoxicity at Day 10 against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) at the indicated effector:target ratios for constructs using a LIR1 ICD, both for constructs ordered in an aCAR to iCAR organization (top panel) and an iCAR to aCAR organization (bottom panel).

FIG. 17 illustrates a schematic of the Membrane-Cleavable system described herein in which a desired payload is expressed as a chimeric protein in which a protease cleavage site is inserted between the payload and membrane-tethering domain. The left panel illustrates a schematic of the Membrane-Cleavable system using a transmembrane spanning architecture (*e.g.*, the chimeric protein contains a transmembrane domain). The right panel illustrates a schematic of the Membrane-Cleavable system using a membrane-associated architecture (*e.g.*, the chimeric protein contains a post-translational modification tag allowing association with a cell membrane).

FIG. 18 illustrates a representative Membrane-Cleavable system for the regulated secretion of IL-15 in which IL-15 is expressed as a chimeric protein having a cleavage site capable of cleavage by TACE inserted between the IL-15 payload and membrane-tethering domain. The left panel illustrates a Type I transmembrane architecture (*e.g.*, S – C – MT) through use of Type I transmembrane domains, such as PDGFR-beta and CD8. The right panel a Type II transmembrane architecture (*e.g.*, MT – C – S) through use of Type II transmembrane domains, such as NKG2D and TNFR2.

FIG. 19 shows components and their order of the indicated constructs encoding either bivalent FLT3 OR CD33 aCARs or monospecific aCARs.

FIG. 20 shows survival curves in MV4-11 murine tumor models for bivalent FLT3 OR CD33 CAR-NK cells and monospecific CAR-NK cells.

FIG. 21 shows representative tumor imaging in MV4-11 murine tumor models for bivalent FLT3 OR CD33 CAR-NK cells and monospecific CAR-NK cells.

FIG. 22 illustrates various tagged and tagless combinations of aCARs and iCARS.

FIG. 23 shows representative flow cytometry plots for a "no virus" control used for assessing tagged and tagless CARs.

FIG. 24 shows representative flow cytometry plots for tagless constructs SB07401 and SB07405 at Day 8.

FIG. 25 shows representative flow cytometry plots for iCAR tagged only construct SB06467 at Day 8.

FIG. 26 shows a summary of CD33/EMCN double positive cell percentage for the indicated constructs having either Loop 6 (top panel) or Loop 4 (bottom panel) linkers.

FIG. 27 shows a summary of expression for each of the CD33, FLT3, and EMCN CARs for the indicated constructs having Loop 4 linkers.

FIG. 28 shows a summary of expression for each of the CD33, FLT3, and EMCN CARs for the indicated constructs having Loop 6 linkers.

FIG. 29 shows total cytotoxicity against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) at a 1:2 effector:target ratio for constructs using a LIR1 ICD.

FIG. 30 shows normalized cytotoxicity (normalized to no-virus control) against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) at a 1:2 effector:target ratio for constructs using a LIR1 ICD.

FIG. 31 shows normalized cytotoxicity (normalized to no-virus control) against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) at a 1:4 effector:target ratio for constructs using a LIR1 ICD.

FIG. 32 shows specific cytotoxicity of target cell lines expressing CD33 (SEM) and target cell lines also expressing the NOT gate target antigen (SEM + EMCN) at a 1:2 effector:target ratio. The figure provides SEM EMCN- in the left columns, and EMCN+ in the right columns, respectively.

FIG. 33A shows specific cytotoxicity of target cell lines expressing CD33 and target cell lines also expressing the NOT gate target antigen (SEM + EMCN) at a 1:2 effector:target ratio in a serial killing assay at 24 hours. **FIG. 33B** shows specific cytotoxicity of target cell lines

expressing CD33 and target cell lines also expressing the NOT gate target antigen (SEM + EMCN) at a 1:2 effector:target ratio in a serial killing assay at 48 hours. **FIG. 33C** shows specific cytotoxicity of target cell lines expressing CD33 and target cell lines also expressing the NOT gate target antigen (SEM + EMCN) at a 1:2 effector:target ratio in a serial killing assay at 96 hours. EMCN+ right columns, respectively.

FIG. 34A shows a general schematic of the *in vivo* experiment to assess the NOT-logic gated CAR-NK cells. **FIG. 34B** shows the characterization the 50:50 mixture of SEM CD33+ and SEM CD33+/EMCN+ cells shown in **FIG. 34A.**

FIG. 35 shows the results of an *in vivo* experiment where a 1:1 mixture of SEM +/- EMCN was injected into mice and treated with NK cells transduced with the respective vector. The top row shows the BLI image at day 11, whereas the bottom panel shows the BLI image at 19 days after tumor injection.

FIG. 36 shows the results of **FIG. 35**, where the region of interest (ROI) is quantified at days 11 and 19.

FIG. 37A shows the proportion of healthy cells in mice on day 14 of the in vivo study where mice were injected with a 1:1 mixture of SEM +/- EMCN and treated with NK cells transduced with the respective constructs. The circled portion compares the percentage of EMCN+ SEM cells between OR/NOT- and OR- GATED CAR-NK cell treatments showing a significantly higher percentage of EMCN+ SEM cells in the animal treated with OR/NOT GATED CAR-NK cells, demonstrating NOT GATE protection of EMCN+ cells. **FIG. 37B** shows the proportion of EMCN+ SEM cells on day 21 of the study shown in **FIG. 37A**. **FIG. 37C** shows the proportion of EMCN+ SEM cells on day 27 of the study shown in **FIG. 37A**.

FIG. 38A shows the average BLI measurement of mice on the respective days of the in vivo study where mice were injected with MV4-11 cells and treated with NK cells transduced with the respective constructs. **FIG. 38B** shows the quantified BLI signal of the mice shown in **FIG. 38A** over the course of the *in vivo* study shown in **FIG. 38A**.

FIG. 39A shows the BLI measurement of mice on the respective days of the in vivo study where mice were injected with MV4-11 cells and treated with NK cells transduced with the respective constructs.

FIG. 39B shows a representative image on day 55 post tumor transplantation of mice treated with PBS, untransduced NK cells, or NK cells transduced with SB07412 or SB07418.

FIG. 39C shows a survival curve and the media survival (days) of the tested treated groups.

FIG. 39D shows a continuation of the study shown in **FIG. 39C**, where data has been collected until day 120.

FIG. 40 shows testing of NK cells expressing an aCAR and different iCAR intracellular domains in a cytotoxicity assay.

FIG. 41A shows a comparison of cell-mediated cytotoxicity against MOLM-13 using non-engineered or engineered NK cells.

FIG. 41B shows a comparison of cell-mediated cytotoxicity against MV4-11 using non-engineered or engineered NK cells.

FIG. 41C shows a comparison of cell-mediated cytotoxicity against SEM-CD33 using non-engineered or engineered NK cells.

FIG. 41D shows NK cytotoxicity of MOLM-13 cells at multiple E:T ratios.

FIG. 41E shows NK cytotoxicity of MV4-11 at multiple E:T ratios.

FIG. 41F shows NK cytotoxicity of engineered NK cells and non-engineered NK cells from multiple experiments plotted against CD33 CAR expression.

FIG. 42 shows a heat map to visualize fold expression analysis of cytokine production by NK cells following co-culture with MV4-11 or MOLM-13.

FIG. 43A shows NK-cell cytotolxicity of engineered and non engineered NK cells derived from donor A that are co-cultured with primary AML samples.

FIG. 43B shows NK-cell cytotolxicity of engineered and non engineered NK cells derived from donor B that are co-cultured with primary AML samples.

FIG. 44 shows a heat map analysis of NK activation markers as analyzed by flow cytometry.

FIG. 45A shows expression of surface associated IL15 in transduced NK cells derived from different donors.

FIG. 45B shows expression of secreted IL15 in transduced NK cells derived from different donors.

FIG. 45C shows expression of the CD33/FLT3 bivalent aCAR in transduced NK cells derived from different donors.

FIG. 45D shows expression of the EMCN iCAR in engineered NK cells derived from different donors.

FIG. 46A shows results from a cytotoxicity assay of engineered NK cells incubated with human stem cells expressing EMCN.

FIG. 46B shows results from a cytoxicity assay of engineered NK cells incubated with AML (Leukemia cells).

FIG. 46C shows results from a cytoxicity assay of engineerered NK cells incubated with human stem progenitor cells.

FIG. 46D shows results from a cytoxicity assay of engineered NK cells incubated with primary healthy MPPs. Shown are results for the whole healthy MPP population.

FIG. 46E shows results from a cytoxicity assay of engineered NK cells incubated with primary healthy MPPs. Shown are results for the healthy EMCN+ MPPs population.

FIG. 47 shows EMCN expression on various hematopoietic stem and progenitor cell (HSPC) subpopulations in CD34-enriched primary human bone marrow cells, including hematopoietic progenitor cells (HPC), lympho-myeloid primed progenitor cells (LMPPs), multipotent progenitor cells (MPPs), in addition to HSCs, across two representative donors.

FIG. 48 shows a flow cytometry gating strategy to count the number of live remaining EMCN+ HSCs after killing assay. From left to right and top to bottom, progressive gates on (1) cell size parameters, (2) single cells, (3) CD56-negative cells (i.e., not NK cells), (4) live CD34+ undifferentiated cells, (5) CD45RA–CD38– cells (i.e., HSCs and MPPs), (6) CD90+ cells (i.e., HSCs), and (7) EMCN+ HSCs. Gates for CD90 and EMCN were set using FMO controls.

DETAILED DESCRIPTION

The present disclosure provides multicistronic expression systems. The multicistronic systems encode (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR. The multicistronic systems include FLT3 OR CD33 NOT EMCN logic-gated CARs and a membrane-cleavable chimeric protein encoding IL-15.

Provided are multicistronic expression systems that include an engineered nucleic acid encoding:

(A) an exogenous polynucleotide encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula: S – C – MT or MT – C – S wherein S includes a secretable effector molecule, wherein the secretable effector molecule includes IL-15, C includes a protease cleavage site , and MT includes a cell membrane tethering domain. S – C – MT or MT – C – S is configured to be expressed as a single polypeptide;

(B) an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) including: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response, and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and

(C) an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) including: (i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling

domains that stimulate an immune response, and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

The expression systems described herein are multicistronic, *i.e.*, more than one separate polypeptide (*e.g.*, multiple chimeric proteins) can be produced from a single mRNA transcript. Engineered nucleic acids can be multicistronic through the use of various linkers, *e.g.*, a polynucleotide sequence encoding a first chimeric proteins can be linked to a nucleotide sequence encoding a second chimeric protein, such as in a first gene:linker:second gene 5' to 3' orientation. A linker can encode a 2A ribosome skipping element, such as T2A. Other 2A ribosome skipping elements include, but are not limited to, E2A, P2A, and F2A. 2A ribosome skipping elements allow production of separate polypeptides encoded by the first and second genes are produced during translation. 2A ribosome skipping elements can include fusion peptides of 2A ribosome skipping elements, including, but not limited to, an E2A/T2A ribosome skipping element. In certain embodiments, the E2A/T2A ribosome skipping element comprises the amino acid sequence of GSGQCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 219). One exemplary nucleic acid encoding the E2A/T2A ribosome skipping element is GGTAGCGGCCAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATC TAATCCTGGACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACG TGGAGGAAAACCCTGGACCT (SEQ ID NO: 220). In certain embodiments, a nucleic acid encoding the E2A/T2A ribosome skipping element includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 220. In some embodiments, an engineered nucleic acid disclosed herein comprises an E2A/T2A ribosome skipping element. In certain embodiments, the E2A/T2A ribosome skipping element comprises the amino acid sequence of QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221). One exemplary nucleic acid encoding the E2A/T2A ribosome skipping element is CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATCCTGG ACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACGTGGAGGAA AACCCTGGACCT (SEQ ID NO: 222). Another exemplary nucleic acid encoding the E2A/T2A ribosome skipping element is CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTCGAGAGTAACCCAGG ACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGTAGAAGAAA ATCCTGGACCT (SEQ ID NO: 223). In certain embodiments, a nucleic acid encoding the

E2A/T2A ribosome skipping element includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 222 or SEQ ID NO: 223.

A linker can encode a cleavable linker polypeptide sequence, such as a Furin cleavage site or a TEV cleavage site, wherein following expression the cleavable linker polypeptide is cleaved such that separate polypeptides encoded by the first and second genes are produced. A cleavable linker can include a polypeptide sequence, such as such a flexible linker (*e.g.*, a Gly-Ser-Gly sequence), that further promotes cleavage.

A linker can encode an Internal Ribosome Entry Site (IRES), such that separate polypeptides encoded by the first and second genes are produced during translation. A linker can encode a splice acceptor, such as a viral splice acceptor.

A linker can be a combination of linkers, such as a Furin-2A linker that can produce separate polypeptides through 2A ribosome skipping followed by further cleavage of the Furin site to allow for complete removal of 2A residues. In some embodiments, a combination of linkers can include a Furin sequence, a flexible linker, and 2A linker. Accordingly, in some embodiments, the linker is a Furin-Gly-Ser-Gly-2A fusion polypeptide. In some embodiments, a linker of the present disclosure is a Furin-Gly-Ser-Gly-T2A fusion polypeptide.

In general, a multicistronic system can use any number or combination of linkers, to express any number of genes or portions thereof (*e.g.*, an engineered nucleic acid can encode a first, a second, and a third chimeric protein, each separated by linkers such that separate polypeptides encoded by the first, second, and third chimeric proteins are produced).

In general, the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR can be encoded in any order. For example, the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR can be encoded by the multicistronic system in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR. In another example, the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR can be encoded by the multicistronic system in order from 5' to 3': i) membrane-cleavable chimeric protein; (ii) iCAR; and (iii) bivalent aCAR.

Membrane Cleavable Chimeric Proteins

The multicistronic systems herein encode membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S**, oriented from N-terminal to C-terminal and expressed as a single polypeptide. S refers to a secretable effector molecule. C refers to a protease cleavage site. MT refers to a cell membrane tethering domain. The membrane-cleavable chimeric protein is engineered such that secretion of the effector molecule can be regulated in a protease-

dependent manner. Specifically, the membrane-cleavable chimeric protein is engineered such that secretion of the effector molecule can be regulated as part of a "Membrane-Cleavable" system, where incorporation of a protease cleavage site ("C") and a cell membrane tethering domain ("MT") allow for regulated secretion of an effector molecule in a protease-dependent manner. Without wishing to be bound by theory, the components of the Membrane-Cleavable system present in the membrane-cleavable chimeric protein generally regulate secretion through the below cellular processes:

- MT: The cell membrane tethering domain contains a transmembrane domain (or a transmembrane-intracellular domain) that directs cellular-trafficking of the chimeric protein such that the protein is inserted into, or otherwise associated with, a cell membrane ("tethered")
- C: Following expression and localization of the chimeric protein into the cell membrane, the protease cleavage site directs cleavage of the chimeric protein such that the effector molecule is released ("secreted") into the extracellular space. Generally, the protease cleavage site is protease-specific, including sites engineered to be protease-specific. The protease cleavage site can be selected or engineered to achieve optimal protein expression, cell-type specific cleavage, cell-state specific cleavage, and/or cleavage and release of the payload at desired kinetics (*e.g.*, ratio of membrane-bound to secreted chimeric protein levels)

FIG. 17 illustrates a schematic of the Membrane-Cleavable system described herein in which a desired payload is expressed as a chimeric protein in which a protease cleavage site is inserted between the payload and membrane-tethering domain. The left panel illustrates a schematic of the Membrane-Cleavable system using a transmembrane spanning architecture (*e.g.*, the chimeric protein contains a transmembrane domain). The right panel illustrates a schematic of the Membrane-Cleavable system using a membrane-associated architecture (*e.g.*, the chimeric protein contains a post-translational modification tag allowing association with a cell membrane). **FIG. 18** illustrates a representative Membrane-Cleavable system for the regulated secretion of IL-15 in which IL-15 is expressed as a chimeric protein having a cleavage site capable of cleavage by TACE inserted between the IL-15 payload and membrane-tethering domain. The left panel illustrates a Type I transmembrane architecture (*e.g.*, S – C – MT) through use of Type I transmembrane domains, such as PDGFR-beta and CD8. The right panel a Type II transmembrane architecture (*e.g.*, MT – C – S) through use of Type II transmembrane domains, such as NKG2D and TNFR2.

In some aspects, membrane-cleavable chimeric proteins (or engineered nucleic acids encoding the membrane-cleavable chimeric proteins) are provided for herein having a protein of

interest (*e.g.*, any of the effector molecules described herein), a protease cleavage site, and a cell membrane tethering domain.

An "effector molecule," refers to a molecule (*e.g.*, a nucleic acid such as DNA or RNA, or a protein (polypeptide) or peptide) that binds to another molecule and modulates the biological activity of that molecule to which it binds. For example, an effector molecule may act as a ligand to increase or decrease enzymatic activity, gene expression, or cell signaling. Thus, in some embodiments, an effector molecule modulates (activates or inhibits) different immunomodulatory mechanisms. By directly binding to and modulating a molecule, an effector molecule may also indirectly modulate a second, downstream molecule.

In certain embodiments described herein (*e.g.*, in general, for all membrane-cleavable chimeric proteins described herein), an effector molecule is a secretable effector molecule (*e.g.*, referred to as "S" in the formula **S – C – MT** or **MT – C – S** for membrane-cleavable chimeric proteins described herein). Non-limiting examples of effector molecules include cytokines, chemokines, enzymes that modulate metabolite levels, growth factors, co-activation molecules, tumor microenvironment modifiers, ligands, peptides, enzymes, antibodies, antibodies or decoy molecules that modulate cytokines, homing molecules, and/or integrins.

The term "modulate" encompasses maintenance of a biological activity, inhibition (partial or complete) of a biological activity, and stimulation/activation (partial or complete) of a biological activity. The term also encompasses decreasing or increasing (*e.g.*, enhancing) a biological activity. Two different effector molecules are considered to "modulate different tumor-mediated immunosuppressive mechanisms" when one effector molecule modulates a tumor-mediated immunosuppressive mechanism (*e.g.*, stimulates T cell signaling) that is different from the tumor-mediated immunosuppressive mechanism modulated by the other effector molecule (*e.g.*, stimulates antigen presentation and/or processing).

Modulation by an effector molecule may be direct or indirect. Direct modulation occurs when an effector molecule binds to another molecule and modulates activity of that molecule. Indirect modulation occurs when an effector molecule binds to another molecule, modulates activity of that molecule, and as a result of that modulation, the activity of yet another molecule (to which the effector molecule is not bound) is modulated.

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in an increase in an immunostimulatory and/or anti-tumor immune response (*e.g.*, systemically or in the tumor microenvironment) by at least 10% (*e.g.*, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 200%). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in an increase in an immunostimulatory and/or anti-tumor immune response by at least 20%, at least 30%, at least

40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in an increase in an immunostimulatory and/or anti-tumor immune response 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%. It should be understood that "an increase" in an immunostimulatory and/or anti-tumor immune response, for example, systemically or in a tumor microenvironment, is relative to the immunostimulatory and/or anti-tumor immune response that would otherwise occur, in the absence of the effector molecule(s).

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in an increase in an immunostimulatory and/or anti-tumor immune response (*e.g.*, systemically or in the tumor microenvironment) by at least 2 fold (*e.g.*, 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in an increase in an immunostimulatory and/or anti-tumor immune response by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in an increase in an immunostimulatory and/or anti-tumor immune response by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold.

Non-limiting examples of immunostimulatory and/or anti-tumor immune mechanisms include T cell signaling, activity and/or recruitment, antigen presentation and/or processing, natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, dendritic cell differentiation and/or maturation, immune cell recruitment, pro-inflammatory macrophage signaling, activity and/or recruitment, stroma degradation, immunostimulatory metabolite production, stimulator of interferon genes (STING) signaling (which increases the secretion of IFN and Th1 polarization, promoting an anti-tumor immune response), and/or Type I interferon signaling. An effector molecule may stimulate at least one (one or more) of the foregoing immunostimulatory mechanisms, thus resulting in an increase in an immunostimulatory response. Changes in the foregoing immunostimulatory and/or anti-tumor immune mechanisms may be assessed, for example, using *in vitro* assays for T cell proliferation or cytotoxicity, *in vitro* antigen presentation assays, expression assays (*e.g.*, of particular markers), and/or cell secretion assays (*e.g.*, of cytokines).

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in a decrease in an immunosuppressive response (*e.g.*, systemically or in the tumor microenvironment) by at least 10% (*e.g.*, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 200%). For example, modulation of a tumor-mediated

immunosuppressive mechanism may result in a decrease in an immunosuppressive response by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in a decrease in an immunosuppressive response 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%. It should be understood that "a decrease" in an immunosuppressive response, for example, systemically or in a tumor microenvironment, is relative to the immunosuppressive response that would otherwise occur, in the absence of the effector molecule(s).

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in a decrease in an immunosuppressive response (*e.g.*, systemically or in the tumor microenvironment) by at least 2 fold (*e.g.*, 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in a decrease in an immunosuppressive response by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in a decrease in an immunosuppressive response by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold.

Non-limiting examples of immunosuppressive mechanisms include negative costimulatory signaling, pro-apoptotic signaling of cytotoxic cells (*e.g.*, T cells and/or NK cells), T regulatory (Treg) cell signaling, tumor checkpoint molecule production/maintenance, myeloid-derived suppressor cell signaling, activity and/or recruitment, immunosuppressive factor/metabolite production, and/or vascular endothelial growth factor signaling. An effector molecule may inhibit at least one (one or more) of the foregoing immunosuppressive mechanisms, thus resulting in a decrease in an immunosuppressive response. Changes in the foregoing immunosuppressive mechanisms may be assessed, for example, by assaying for an increase in T cell proliferation and/or an increase in IFNγ production (negative co-stimulatory signaling, T_{reg} cell signaling and/or MDSC); Annexin V/PI flow staining (pro-apoptotic signaling); flow staining for expression, *e.g.*, PDL1 expression (tumor checkpoint molecule production/maintenance); ELISA, LUMINEX®, RNA via qPCR, enzymatic assays, *e.g.*, IDO tryptophan catabolism (immunosuppressive factor/metabolite production); and phosphorylation of PI3K, Akt, p38 (VEGF signaling).

In some embodiments, effector molecules function additively: the effect of two effector molecules, for example, may be equal to the sum of the effect of the two effector molecules

functioning separately. In other embodiments, effector molecules function synergistically: the effect of two effector molecules, for example, may be greater than the combined function of the two effector molecules.

Effector molecules that modulate tumor-mediated immunosuppressive mechanisms and/or modify tumor microenvironments may be, for example, secreted factors (*e.g.*, cytokines, chemokines, antibodies, and/or decoy receptors that modulate extracellular mechanisms involved in the immune system), inhibitors (*e.g.*, antibodies, antibody fragments, ligand TRAP and/or small blocking peptides), intracellular factors that control cell state (*e.g.*, microRNAs and/or transcription factors that modulate the state of cells to enhance pro-inflammatory properties), factors packaged into exosomes (*e.g.*, microRNAs, cytosolic factors, and/or extracellular factors), surface displayed factors (*e.g.*, checkpoint inhibitors, TRAIL), and and/or metabolic genes (*e.g.*, enzymes that produce/modulate or degrade metabolites or amino acids).

In some embodiments, at least one of the effector molecules stimulates an immunostimulatory mechanism in the tumor microenvironment and/or inhibits an immunosuppressive mechanism in the tumor microenvironment.

In some embodiments, at least one of the effector molecules (a) stimulates T cell signaling, activity and/or recruitment, (b) stimulates antigen presentation and/or processing, (c) stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, (d) stimulates dendritic cell differentiation and/or maturation, (e) stimulates immune cell recruitment, (f) stimulates pro-inflammatory macrophage signaling, activity and/or recruitment or inhibits anti-inflammatory macrophage signaling, activity and/or recruitment, (g) stimulates stroma degradation, (h) stimulates immunostimulatory metabolite production, (i) stimulates Type I interferon signaling, (j) inhibits negative costimulatory signaling, (k) inhibits pro-apoptotic signaling of anti-tumor immune cells, (l) inhibits T regulatory (T_{reg}) cell signaling, activity and/or recruitment, (m) inhibits tumor checkpoint molecules, (n) stimulates stimulator of interferon genes (STING) signaling, (o) inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment, (p) degrades immunosuppressive factors/metabolites, (q) inhibits vascular endothelial growth factor signaling, and/or (r) directly kills tumor cells.

In some embodiments, effector molecules may be selected from the following non-limiting classes of molecules: cytokines, antibodies, chemokines, nucleotides, peptides, and enzymes. Non-limiting examples of the foregoing classes of effector molecules are listed in **Table 1** and specific sequences encoding exemplary effector molecules are listed in **Table 2**. Effector molecules can be human, such as those listed in **Table 1** or **Table 2** or human equivalents of murine effector molecules listed in **Table 1** or **Table 2**. Effector molecules can be human-derived, such as the endogenous human effector molecule or an effector molecule

modified and/or optimized for function, *e.g.*, codon optimized to improve expression, modified to improve stability, or modified at its signal sequence (*see* below). Various programs and algorithms for optimizing function are known to those skilled in the art and can be selected based on the improvement desired, such as codon optimization for a specific species (*e.g.*, human, mouse, bacteria, etc.).

In some embodiments, the effector molecule comprises interleukin 12 (IL-12), for example, p35 and p40 as a dimer that is generally referred to in the art as IL12p70. In some embodiments, the first effector molecule comprises an IL12p70 fusion protein. In some embodiments, the IL12p70 fusion protein is a human IL12p70 fusion protein. In some embodiments, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 203.

In some embodiments, the effector molecule comprises interleukin 15 (IL-15). In some embodiments, the effector molecule consists of IL-15 (see, e.g., SEQ ID NO: 199). In some embodiments, the effector molecule comprises a fusion protein including IL-15 and an extracellular portion of IL-15 Receptor α (IL-15Rα), such as the sushi domain as shown in SEQ ID NO: 201. An exemplary IL-15/IL-15Rα sushi domain fusion is provided as SEQ ID NO: 202. In some embodiments, the effector molecule includes IL-15 having the amino acid sequence NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 224). In some embodiments, IL-15 is encoded by a polynucleotide sequence comprising the sequence AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCAT GCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGA CCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGAC GCCAGCATCCACGATACCGTGGAAAATCTGATCATCCTGGCCAACAACAGCCTGTC CAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAG AAGAACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAA CACCTCA (SEQ ID NO: 225). In some embodiments, IL-15 is encoded by a polynucleotide sequence that includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCAT GCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGA CCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGAC GCCAGCATCCACGATACCGTGGAAAATCTGATCATCCTGGCCAACAACAGCCTGTC CAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAG

AAGAACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAA CACCTCA (SEQ ID NO: 225).

In some embodiments, the membrane-cleavable chimeric protein includes the amino acid sequence

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM KCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQS FVHIVQMFINTSSGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQLLPSWAITLISV NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 226). In some embodiments, the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence comprising the sequence

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAAT TGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCA CATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCG CCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGACGCC AGCATCCACGATACCGTGGAAAATCTGATCATCCTGGCCAACAACAGCCTGTCCAG CAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG AACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAACACC TCATCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTAT CTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTC AATTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCT GCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGG CTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227). In some embodiments, the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence that includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAAT TGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCA CATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCG CCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGACGCC AGCATCCACGATACCGTGGAAAATCTGATCATCCTGGCCAACAACAGCCTGTCCAG CAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG AACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAACACC TCATCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTAT CTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTC AATTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCT

GCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGG CTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

Table 1. Exemplary Effector Molecules

Effector name	Category	Function
anti-CD40 or CD40 Ligand	Agonist antibody	Stimulates B-cells and antigen presenting cells.
Flt3L	Ligand agonist	Stimulates myeloid cells and antigen presenting cells
CXCL10-11 fusion	Chemokine	Attracts T-cells
TGFb blocking peptides	Antagonist peptides	Inhibit TGFb pathway, TME modifier
Adenosine deaminase (ADA)	TME modifier	Degradation of suppressive adenosine in the TME
Kyneurinase	TME modifier	Degradation of kyneurine
HPGE2	TME modifier	Degradation of PGE2
CXCL13	Chemokine	Attracts B-cells
anti PD-1/PD-L1	Agonist antibody	Remove checkpoint
anti-CTLA-4	Agonist antibody	Remove checkpoint
anti-VEGF	Antagonist antibody	Neutralizes an immunosuppressive/angiogenesis factor
anti-TNFa	Antagonist antibody	Neutralizes cytokine/pro-tumor factor
anti-IL-10	Antagonist antibody	Neutralizes immunosuppressive cytokine
anti-SDF1/CXCL12	Antagonist antibody	Neutralizes pro-tumor chemokine
(TβRII)2 trap	Capture trap	Neutralizes an immunosuppressive cytokine
CCL21	Chemokine	Attracts leukocytes/NK
CCL1	Chemokine	Attracts leukocytes/NK
CCL17	Chemokine	Attracts leukocytes/NK
CCL19	Chemokine	Attracts leukocytes/NK
CCL21	Chemokine	Attracts leukocytes/NK
CCL20	Chemokine	Attracts leukocytes/NK
CCL21a	Chemokine	Attracts leukocytes/NK
MIP1b (CCL5)	Chemokine	Attracts leukocytes/NK
CXCL10	Chemokine	Attracts leukocytes/NK
CXCL11	Chemokine	Attracts leukocytes/NK
CCL2	Chemokine	Attracts monocytes
MIP-1alpha (CCL3)	Chemokine	Attracts leukocytes/NK
XCL1	Chemokine	Attracts leukocytes/NK
IFNbeta	Cytokine	T cell response, tumor cell killing
IFNgamma	Cytokine	T cell response, tumor cell killing
IL-12	Cytokine	T cells, NK cells
IL-1beta	Cytokine	T cells, NK cells

Effector name	Category	Function
IL-15	Cytokine	Stimulates T-cells and NK
IL-2	Cytokine	Stimulates T-cells and NK
IL-21	Cytokine	Stimulates T-cells
IL-24	Cytokine	Stimulates T-cells
IL36-gamma	Cytokine	Stimulates T-cells
IL-7	Cytokine	Stimulates T-cells
IL-22	Cytokine	Stimulates T-cells
IL-18	Cytokine	Stimulates T-cells
Granzymes/Perforin	Enzyme	Direct tumor cell killing
OX86 (anti-OX40)	ligand	Stimulates T-cells
anti-TGFbeta	Neutralizing antibody	Neutralizes an Immunosuppressive cytokine
TRAIL	Receptor/ligand	Direct tumor cell killing
FASL (CD49L)	Receptor/ligand	Direct tumor cell killing
OX40-L	Receptor/Ligand	Stimulates T-cells
cGAS	secreted molecule	Stimulates antigen-presenting cells
41BBL	secreted molecule	Co-activation of T-cells
CD40L	secreted molecule	Stimulates T-cells
GM-CSF	secreted molecule	Growth factor for monocytes
STING	secreted molecule	Stimulates antigen-presenting cells
HAC-V 'microbody'_PD1	Antagonist antibody	inhibits checkpoint
yCD	Pro-drug	Converts to cytotoxic molecule upon activation
CpG/Nucleotides	Nucleotides	STING agonist

Table 2: Exemplary effector molecule sequences

IL-12 (Human) (SEQ ID NO: 56)
ATGTGCCATCAGCAGCTTGTCATATCTTGGTTTTCACTTGTATTCCTGGCCAGCCCTTTGGTTGCG ATCTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGTG AGATGGTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACCAGTC CTCCGAAGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGGAC AATACACATGCCACAAGGGAGGCGAGGTGTTGTCTCATAGTTTGCTGCTTCTCCACAAGAAAGA GGATGGAATCTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAAATAAGACATTTCTG CGATGTGAGGCTAAGAACTATAGTGGCCGCTTCACTTGTTGGTGGCTGACTACCATCAGCACAG ATCTCACGTTTTCAGTAAAAAGTAGTAGAGGTTCAAGTGATCCTCAAGGGGTAACGTGCGGTGC TGCAACACTGTCTGCTGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCGTAGAATGC CAAGAGGACAGTGCTTGTCCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGACGCGG TGCATAAACTGAAATATGAGAACTACACAAGCAGTTTTTTTATAAGAGATATCATCAAGCCCGA TCCGCCGAAGAATTTGCAACTTAAACCGCTTAAAAACTCACGCCAGGTTGAAGTATCCTGGGAG TATCCGGATACATGGTCAACACCACACAGCTATTTTTCCCTTACCTTCTGTGTGCAGGTCCAAGG GAAGAGCAAAAGGGAGAAGAAGGACAGGGTATTCACTGATAAAACTTCCGCGACGGTCATCTG CCGAAAAAACGCTAGTATATCTGTACGGGCGCAGGATAGGTACTATAGTTCTTCTTGGTCTGAGT GGGCCTCAGTTCCGTGCTCTGGGGGAGGAAGTGGAGGAGGGTCCGGCGGTGGAAGCGGGGGAG GGAGTCGCAACTTGCCAGTGGCTACACCAGATCCAGGCATGTTTCCATGTCTGCATCATTCCCAG AATCTCCTGAGAGCGGTGTCAAATATGCTCCAAAAAGCGAGACAAACACTGGAATTTTACCCGT GTACCAGTGAGGAGATTGATCACGAGGACATAACCAAGGACAAGACCTCAACTGTAGAAGCGT GTTTGCCGCTGGAGTTGACTAAGAATGAGTCCTGCCTCAATTCCAGAGAAACTTCATTCATTACT AACGGCAGTTGTCTTGCATCCCGGAAAACGTCCTTTATGATGGCCCTTTGCCTTAGTTCAATTTAC

GAGGATCTTAAAATGTATCAAGTGGAGTTTAAAACCATGAATGCTAAACTTCTTATGGACCCCA
AACGACAAATTTTTCTGGATCAGAATATGCTTGCCGTGATAGACGAACTCATGCAGGCGCTTAAT
TTTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAGAACCTGATTTTTATAAAACGAAGAT
TAAACTGTGTATCCTGCTGCATGCCTTTCGCATCCGAGCTGTCACAATCGATAGGGTTATGTCCT
ACCTTAACGCGAGCtaG

IL-12p70 (Human; codon optimized; bold denotes signal sequence) (SEQ ID NO: 57)

ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCCTCGCTTCCCCTCTGGT
CGCCATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCT
GGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCTGGATC
AGAGCTCCGAGGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCGGCGACGC
GGGCCAGTACACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCTGCTGCACAAG
AAAGAGGATGGAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACAAGACC
TTCCTCCGCTGCGAAGCCAAGAACTACAGCGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTC
CACCGACCTGACTTTCTCCGTGAAGTCGTCACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTG
GAGCCGCCACTCTGTCCGCCGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTGG
AATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTCATGGTCGA
TGCCGTGCATAAGCTGAAATACGAGAACTACACTTCCTCCTTCTTTATCCGCGACATCATCAAGC
CTGACCCCCCCCAAGAACTTGCAGCTGAAGCCACTCAAGAACTCCCGCCAAGTGGAAGTGTCTTG
GGAATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGC
AGGGAAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCGCCACTGTGA
TTTGTCGGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTCCTCCTGGAG
CGAATGGGCCAGCGTGCCTTGTTCCGGTGGCGGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGA
GGAGGTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGGAATGTTCCCGTGCCTACACCACTC
CCAAAACCTCCTGAGGGCTGTGTCGAACATGTTGCAGAAGGCCCGCCAGACCCTTGAGTTCTAC
CCCTGCACCTCGGAAGAAATTGATCACGAGGACATCACCAAGGACAAGACCTCGACCGTGGAAG
CCTGCCTGCCGCTGGAACTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAGACAAGCTTTAT
CACTAACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTCATGATGGCGCTCTGTCTTTCCTCGA
TCTACGAAGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGAACGCCAAGCTGCTCATGGA
CCCGAAGCGGCAGATCTTCCTGGACCAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCC
CTGAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCGGACTTCTACAAGA
CCAAGATCAAGCTGTGCATCCTGTTGCACGCTTTCCGCATTCGAGCCGTGACCATTGACCGCGTG
ATGTCCTACCTGAACGCCAGT

IL-12 (Mouse) (SEQ ID NO: 58)

ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCCTCCTGGTGAGCCCACTCATGGC
AATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAGGG
GAGACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACCAAC
GCCATGGCGTAATAGGGAGTGGGAAAACACTCACGATCACAGTTAAAGAGTTCTTGGATGCTGG
TCAATATACTTGCCATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCATAAAAAAG
AGAATGGCATTTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAGACATTTCTCAAGTGTGA
GGCCCCTAATTACAGCGGCAGGTTCACGTGCTCATGGCTGGTCCAGCGCAACATGGACCTCAAG
TTTAACATAAAATCTTCTTCCTCTTCACCTGACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTG
AGCGCAGAAAAAGTAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGTCAAGAGG
ATGTTACGTGCCCGACGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACA
AAACAAGTATGAAAACTACAGTACAAGCTTCTTTATACGGGATATAATTAAACCCGATCCCCCC
AAGAACTTGCAAATGAAACCACTTAAGAACAGCCAGGTGGAAGTTTCCTGGGAGTATCCAGACT
CATGGAGTACTCCTCACAGCTATTTTTCTCTGAAATTCTTTGTAAGGATACAACGGAAGAAAGAG
AAGATGAAAGAGACCGAGGAGGGTTGTAATCAGAAGGGAGCGTTTCTCGTGGAGAAAACGTCT
ACCGAAGTCCAATGTAAAGGTGGCAATGTGTGCGTCCAAGCTCAGGATAGATACTATAATTCAA
GTTGCTCCAAGTGGGCCTGTGTTCCATGCCGCGTTCGGAGCGGGGGAGGTAGCGGAGGAGGTAG
TGGGGGTGGGTCAGGAGGAGGGAGTCGAGTTATCCCGGTGTCAGGCCCCGCACGCTGCTTGAGC
CAGAGTCGCAACCTCCTTAAGACAACAGATGACATGGTGAAAACAGCACGCGAAAAGCTTAAA
CACTACTCTTGTACGGCGGAGGATATTGATCACGAGGATATTACCCGAGACCAAACTAGCACTTT
GAAAACCTGTCTGCCCCTTGAACTTCATAAAAATGAGAGCTGTCTGGCTACACGAGAGACGTCA
AGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAAGACAAGCCTCATGATGACGCTCTGTTTGG
GTTCCATTTACGAGGACTTGAAAATGTATCAAACGGAGTTCCAGGCTATAAATGCGGCGTTGCA
GAACCATAACCATCAACAAATTATACTTGATAAAGGCATGTTGGTGGCGATTGATGAACTCATG
CAGAGTCTCAATCACAACGGGGAAACGTTGAGACAGAAACCCCCAGTCGGTGAAGCGGACCCA
TATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACGCATTCAGCACTAGAGTTGTTACCATCAA
CCGGGTAATGGGATATCTCTCCAGTGCGtaG

IL21 (Human; codon optimized; bold denotes signal sequence) (SEQ ID NO: 59)

ATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGTCGA
GCAGCCAGGGACAGGACAGGCACATGATTAGAATGCGCCAGCTCATCGATATCGTGGACCAGT
TGAAGAACTACGTGAACGACCTGGTGCCCGAGTTCCTGCCGGCCCCCGAAGATGTGGAAACCAA
TTGCGAATGGTCGGCATTTTCCTGCTTTCAAAAGGCACAGCTCAAGTCCGCTAACACCGGGAACA
ACGAACGGATCATCAACGTGTCCATCAAAAAGCTGAAGCGGAAGCCTCCCTCCACCAACGCCGG
ACGGAGGCAGAAGCATAGGCTGACTTGCCCGTCATGCGACTCCTACGAGAAGAAGCCGCCGAA
GGAGTTCCTGGAGCGGTTCAAGTCGCTCCTGCAAAAGATGATTCATCAGCACCTGTCCTCCCGGA
CTCATGGGTCTGAGGATTCA

IL-12p70_T2A_IL21 (Human; codon optimized; bold denotes signal sequences) (SEQ ID NO: 60)

ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCCTCGCTTCCCCTCTGGT
CGCCATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCT
GGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCTGGATC
AGAGCTCCGAGGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCGGCGACGC
GGGCCAGTACACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCTGCTGCACAAG
AAAGAGGATGGAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACAAGACC
TTCCTCCGCTGCGAAGCCAAGAACTACAGCGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTC
CACCGACCTGACTTTCTCCGTGAAGTCGTCACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTG
GAGCCGCCACTCTGTCCGCCGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTGG
AATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTCATGGTCGA
TGCCGTGCATAAGCTGAAATACGAGAACTACACTTCCTCCTTCTTTATCCGCGACATCATCAAGC
CTGACCCCCCCCAAGAACTTGCAGCTGAAGCCACTCAAGAACTCCCGCCAAGTGGAAGTGTCTTG
GGAATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGC
AGGGAAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCGCCACTGTGA
TTTGTCGGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTCCTCCTGGAG
CGAATGGGCCAGCGTGCCTTGTTCCGGTGGCGGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGA
GGAGGTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGGAATGTTCCCGTGCCTACACCACTC
CCAAAACCTCCTGAGGGCTGTGTCGAACATGTTGCAGAAGGCCCGCCAGACCCTTGAGTTCTAC
CCCTGCACCTCGGAAGAAATTGATCACGAGGACATCACCAAGGACAAGACCTCGACCGTGGAAG
CCTGCCTGCCGCTGGAACTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAGACAAGCTTTAT
CACTAACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTCATGATGGCGCTCTGTCTTTCCTCGA
TCTACGAAGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGAACGCCAAGCTGCTCATGGA
CCCGAAGCGGCAGATCTTCCTGGACCAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCC
CTGAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCGGACTTCTACAAGA
CCAAGATCAAGCTGTGCATCCTGTTGCACGCTTTCCGCATTCGAGCCGTGACCATTGACCGCGTG
ATGTCCTACCTGAACGCCAGTAGACGGAAACGCGGAAGCGGAGAGGGCAGAGGCTCGCTGCTT
ACATGCGGGGACGTGGAAGAGAACCCCGGTCCG**ATGGAACGCATTGTGATCTGCCTGATGGT**
CATCTTCCTGGGCACCTTAGTGCACAAGTCGAGCAGCCAGGGACAGGACAGGCACATGATTA
GAATGCGCCAGCTCATCGATATCGTGGACCAGTTGAAGAACTACGTGAACGACCTGGTGCCCGA
GTTCCTGCCGGCCCCCGAAGATGTGGAAACCAATTGCGAATGGTCGGCATTTTCCTGCTTTCAAA
AGGCACAGCTCAAGTCCGCTAACACCGGGAACAACGAACGGATCATCAACGTGTCCATCAAAAA
GCTGAAGCGGAAGCCTCCCTCCACCAACGCCGGACGGAGGCAGAAGCATAGGCTGACTTGCCCG
TCATGCGACTCCTACGAGAAGAAGCCGCCGAAGGAGTTCCTGGAGCGGTTCAAGTCGCTCCTGC
AAAAGATGATTCATCAGCACCTGTCCTCCCGGACTCATGGGTCTGAGGATTCA

IL-12_2A_CCL21a (Human) (SEQ ID NO: 61)

ATGTGCCATCAGCAGCTTGTCATATCTTGGTTTTCACTTGTATTCCTGGCCAGCCCTTTGGTTGCG
ATCTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGTG
AGATGGTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACCAGTC
CTCCGAAGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGGAC
AATACACATGCCACAAGGGAGGCGAGGTGTTGTCTCATAGTTTGCTGCTTCTCCACAAGAAAGA
GGATGGAATCTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAAATAAGACATTTCTG
CGATGTGAGGCTAAGAACTATAGTGGCCGCTTCACTTGTTGGTGGCTGACTACCATCAGCACAG
ATCTCACGTTTTCAGTAAAAAGTAGTAGAGGTTCAAGTGATCCTCAAGGGGTAACGTGCGGTGC
TGCAACACTGTCTGCTGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCGTAGAATGC
CAAGAGGACAGTGCTTGTCCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGACGCGG
TGCATAAACTGAAATATGAGAACTACACAAGCAGTTTTTTTATAAGAGATATCATCAAGCCCGA
TCCGCCGAAGAATTTGCAACTTAAACCGCTTAAAAACTCACGCCAGGTTGAAGTATCCTGGGAG
TATCCGGATACATGGTCAACACCACACAGCTATTTTTCCCTTACCTTCTGTGTGCAGGTCCAAGG

GAAGAGCAAAAGGGAGAAGAAGGACAGGGTATTCACTGATAAAACTTCCGCGACGGTCATCTG
CCGAAAAAACGCTAGTATATCTGTACGGGCGCAGGATAGGTACTATAGTTCTTCTTGGTCTGAGT
GGGCCTCAGTTCCGTGCTCTGGGGGAGGAAGTGGAGGAGGGTCCGGCGGTGGAAGCGGGGGAG
GGAGTCGCAACTTGCCAGTGGCTACACCAGATCCAGGCATGTTTCCATGTCTGCATCATTCCCAG
AATCTCCTGAGAGCGGTGTCAAATATGCTCCAAAAAGCGAGACAAACACTGGAATTTTACCCGT
GTACCAGTGAGGAGATTGATCACGAGGACATAACCAAGGACAAGACCTCAACTGTAGAAGCGT
GTTTGCCGCTGGAGTTGACTAAGAATGAGTCCTGCCTCAATTCCAGAGAAACTTCATTCATTACT
AACGGCAGTTGTCTTGCATCCCGGAAAACGTCCTTTATGATGGCCCTTTGCCTTAGTTCAATTTAC
GAGGATCTTAAAATGTATCAAGTGGAGTTTAAAACCATGAATGCTAAACTTCTTATGGACCCCA
AACGACAAATTTTTCTGGATCAGAATATGCTTGCCGTGATAGACGAACTCATGCAGGCGCTTAAT
TTTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAGAACCTGATTTTTATAAAACGAAGAT
TAAACTGTGTATCCTGCTGCATGCCTTTCGCATCCGAGCTGTCACAATCGATAGGGTTATGTCCT
ACCTTAACGCGAGCCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGCG
GCGATGTTGAAGAAAATCCTGGTCCCATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTC
TTGGCCTTCGGGATTCCGAGGACCCAAGGAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAAT
ACAGCCAGCGGAAAATACCCGCGAAAGTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGG
GTTGTAGTATCCCCGCCATACTTTTCCTCCCGAGAAAACGGAGCCAGGCCGAACTGTGCGCTGAC
CCTAAGGAACTTTGGGTGCAACAACTTATGCAACACCTGGATAAGACACCTTCTCCTCAAAAGC
CAGCTCAGGGCTGCCGAAAAGATAGAGGCGCCTCAAAAACCGGAAAAAAGGGCAAAGGTTCTA
AAGGATGTAAGCGGACTGAACGCTCTCAAACGCCTAAAGGGCCGtaG

IL-12_2A_CCL21a (Mouse) (SEQ ID NO: 62)

ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCCTCCTGGTGAGCCCACTCATGGC
AATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAGGG
GAGACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACCAAC
GCCATGGCGTAATAGGGAGTGGGAAAACACTCACGATCACAGTTAAAGAGTTCTTGGATGCTGG
TCAATATACTTGCCATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCATAAAAAAG
AGAATGGCATTTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAGACATTTCTCAAGTGTGA
GGCCCCTAATTACAGCGGCAGGTTCACGTGCTCATGGCTGGTCCAGCGCAACATGGACCTCAAG
TTTAACATAAAATCTTCTTCCTCTTCACCTGACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTG
AGCGCAGAAAAAGTAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGTCAAGAGG
ATGTTACGTGCCCGACGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACA
AAACAAGTATGAAAACTACAGTACAAGCTTCTTTATACGGGATATAATTAAACCCGATCCCCCC
AAGAACTTGCAAATGAAACCACTTAAGAACAGCCAGGTGGAAGTTTCCTGGGAGTATCCAGACT
CATGGAGTACTCCTCACAGCTATTTTTCTCTGAAATTCTTTGTAAGGATACAACGGAAGAAAGAG
AAGATGAAAGAGACCGAGGAGGGTTGTAATCAGAAGGGAGCGTTTCTCGTGGAGAAAACGTCT
ACCGAAGTCCAATGTAAAGGTGGCAATGTGTGCGTCCAAGCTCAGGATAGATACTATAATTCAA
GTTGCTCCAAGTGGGCCTGTGTTCCATGCCGCGTTCGGAGCGGGGGAGGTAGCGGAGGAGGTAG
TGGGGGTGGGTCAGGAGGAGGGAGTCGAGTTATCCCGGTGTCAGGCCCCGCACGCTGCTTGAGC
CAGAGTCGCAACCTCCTTAAGACAACAGATGACATGGTGAAAACAGCACGCGAAAAGCTTAAA
CACTACTCTTGTACGGCGGAGGATATTGATCACGAGGATATTACCCGAGACCAAACTAGCACTTT
GAAAACCTGTCTGCCCCTTGAACTTCATAAAAATGAGAGCTGTCTGGCTACACGAGAGACGTCA
AGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAAGACAAGCCTCATGATGACGCTCTGTTTGG
GTTCCATTTACGAGGACTTGAAAATGTATCAAACGGAGTTCCAGGCTATAAATGCGGCGTTGCA
GAACCATAACCATCAACAAATTATACTTGATAAAGGCATGTTGGTGGCGATTGATGAACTCATG
CAGAGTCTCAATCACAACGGGGAAACGTTGAGACAGAAACCCCCAGTCGGTGAAGCGGACCCA
TATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACGCATTCAGCACTAGAGTTGTTACCATCAA
CCGGGTAATGGGATATCTCTCCAGTGCGCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAG
TCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTGGTCCCATGGCGCAAATGATGACCCTTTCCC
TGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCAGGGGTCTGATGGGGGGGGCCAAGA
CTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCTATTGTCAGAGGGTACAGGAAGCAA
GAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTCCCCACGCAAGCATTCCAAACCAGA
ACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTGATGCGAAGGCTTGACCAGCCCCCA
GCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAACAGAGGTACTTCAAAGAGCGGCAAGAAA
GGCAAAGGGAGTAAAGGATGTAAAAGAACGGAGCAGACCCAGCCTTCACGAGGCtaG

CCL21a_2A_IL-12 (Mouse) (SEQ ID NO: 63)

ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCA
GGGGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCT
ATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTC
CCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTG

ATGCGAAGGCTTGACCAGCCCCCAGCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAACAGA
GGTACTTCAAAGAGCGGCAAGAAAGGCAAAGGGAGTAAAGGATGTAAAAGAACGGAGCAGAC
CCAGCCTTCACGAGGCCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGC
GGCGATGTTGAAGAAAATCCTGGTCCCATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCA
TTGTCCTCCTGGTGAGCCCACTCATGGCAATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGA
AGTAGATTGGACTCCAGACGCGCCAGGGGAGACAGTGAATTTGACATGTGACACACCAGAAGA
AGATGACATTACATGGACATCTGACCAACGCCATGGCGTAATAGGGAGTGGGAAAACACTCACG
ATCACAGTTAAAGAGTTCTTGGATGCTGGTCAATATACTTGCCATAAAGGCGGCGAGACACTCA
GCCACTCACATTTGCTTTTGCATAAAAAAGAGAATGGCATTTGGAGCACTGAAATACTTAAGAA
CTTTAAGAACAAGACATTTCTCAAGTGTGAGGCCCCTAATTACAGCGGCAGGTTCACGTGCTCAT
GGCTGGTCCAGCGCAACATGGACCTCAAGTTTAACATAAAATCTTCTTCCTCTTCACCTGACTCC
AGAGCTGTTACTTGCGGCATGGCTTCTCTGAGCGCAGAAAAAGTAACGTTGGATCAAAGAGACT
ACGAAAAGTACTCTGTTTCTTGTCAAGAGGATGTTACGTGCCCGACGGCCGAAGAAACGCTTCC
AATTGAACTCGCGTTGGAAGCTCGCCAACAAAACAAGTATGAAAACTACAGTACAAGCTTCTTT
ATACGGGATATAATTAAACCCGATCCCCCCAAGAACTTGCAAATGAAACCACTTAAGAACAGCC
AGGTGGAAGTTTCCTGGGAGTATCCAGACTCATGGAGTACTCCTCACAGCTATTTTTCTCTGAAA
TTCTTTGTAAGGATACAACGGAAGAAAGAGAAGATGAAAGAGACCGAGGAGGGTTGTAATCAG
AAGGGAGCGTTTCTCGTGGAGAAAACGTCTACCGAAGTCCAATGTAAAGGTGGCAATGTGTGCG
TCCAAGCTCAGGATAGATACTATAATTCAAGTTGCTCCAAGTGGGCCTGTGTTCCATGCCGCGTT
CGGAGCGGGGGAGGTAGCGGAGGAGGTAGTGGGGGTGGGTCAGGAGGAGGGAGTCGAGTTATC
CCGGTGTCAGGCCCCGCACGCTGCTTGAGCCAGAGTCGCAACCTCCTTAAGACAACAGATGACA
TGGTGAAAACAGCACGCGAAAAGCTTAAACACTACTCTTGTACGGCGGAGGATATTGATCACGA
GGATATTACCCGAGACCAAACTAGCACTTTGAAAACCTGTCTGCCCCTTGAACTTCATAAAAATG
AGAGCTGTCTGGCTACACGAGAGACGTCAAGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAA
GACAAGCCTCATGATGACGCTCTGTTTGGGTTCCATTTACGAGGACTTGAAAATGTATCAAACGG
AGTTCCAGGCTATAAATGCGGCGTTGCAGAACCATAACCATCAACAAATTATACTTGATAAAGG
CATGTTGGTGGCGATTGATGAACTCATGCAGAGTCTCAATCACAACGGGGAAACGTTGAGACAG
AAACCCCCAGTCGGTGAAGCGGACCCATATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACG
CATTCAGCACTAGAGTTGTTACCATCAACCGGGTAATGGGATATCTCTCCAGTGCGtaG

IL7 (Mouse) (SEQ ID NO: 64)

ATGTTTCATGTGTCCTTCAGGTACATATTTGGTATCCCACCACTTATATTGGTGCTCTTGCCTGTA
ACCAGCTCTGAATGTCATATAAAAGACAAGGAGGGCAAAGCATACGAGTCCGTATTGATGATCT
CAATCGATGAACTTGACAAGATGACAGGGACCGATTCTAATTGTCCAAATAACGAGCCAAACTT
CTTTCGGAAACACGTGTGTGATGATACAAAAGAAGCTGCTTTTCTTAACAGAGCTGCCAGAAAA
CTCAAGCAGTTCCTCAAGATGAATATATCCGAGGAATTTAACGTGCATCTCCTCACAGTATCTCA
GGGAACTCAAACCCTTGTAAACTGCACTTCTAAGGAGGAGAAGAATGTCAAAGAGCAGAAGAA
AAATGATGCATGTTTTTTGAAACGGCTGTTGAGGGAGATCAAAACATGCTGGAATAAAATCCTC
AAGGGCTCAATTtaG

IL-15 (Human) (SEQ ID NO: 65)

ATGGAAACAGACACATTGCTGCTTTGGGTATTGTTGCTCTGGGTGCCTGGATCAACAGGAAACTG
GGTAAACGTAATTTCAGATCTGAAGAAGATCGAGGACCTTATTCAATCCATGCACATCGATGCC
ACTCTCTACACCGAAAGCGACGTTCACCCATCTTGCAAGGTGACCGCTATGAAATGTGAATTGTT
GGAACTTCAGGTAATTTCTCTGGAGAGCGGCGATGCCTCAATACATGACACCGTTGAAAATCTTA
TCATCCTTGCTAATGATTCACTCTCTAGTAATGGGAACGTAACAGAGAGCGGGTGTAAGGAGTG
TGAAGAACTGGAGGAGAAAAACATTAAGGAATTTTTGCAGTCATTCGTCCATATAGTGCAAATG
TTCATAAACACTTCCAGAAGAAAGCGAGGCTCTGGGGAGGGGCGAGGCTCTCTGCTGACCTGTG
GGGATGTAGAAGAGAATCCAGGTCCCATGGACCGGCTGACCAGCTCATTCCTGCTTCTGATTGTG
CCAGCCTACGTGCTCTCCATCACATGTCCTCCCCCAATGAGCGTCGAGCATGCTGACATCTGGGT
GAAGTCATACTCCTTGTACAGCAGAGAGAGATACATTTGTAATTCCGGATTCAAGCGCAAGGCC
GGCACCTCCTCTCTGACAGAGTGCGTCCTTAACAAAGCAACCAACGTAGCACATTGGACCACAC
CATCCTTGAAGTGCATACGAGAACCTAAATCTTGCGATAAGACTCATACTTGTCCACCTTGTCCA
GCCCCAGAACTGCTTGGCGGACCCTCAGTATTTTTGTTCCCACCAAAGCCAAAAGACACACTCAT
GATATCCAGAACTCCTGAGGTGACCTGTGTCGTTGTAGACGTTTCCCACGAAGATCCTGAAGTAA
AATTCAACTGGTACGTGGATGGGGTCGAAGTCCATAACGCCAAGACTAAACCAAGGGAGGAAC
AGTATAACTCTACTTACCGAGTAGTTTCTGTGTTGACCGTGCTGCACCAGGACTGGTTGAACGGG
AAGGAGTACAAATGCAAGGTGAGCAATAAAGCTCTGCCCGCACCAATCGAAAAGACAATATCT
AAGGCCAAGGGGCAGCCACGAGAGCCCCAGGTATACACACTGCCACCCTCACGCGATGAATTG
ACTAAGAACCAGGTTTCCCTGACCTGTCTTGTAAAAGGTTTCTACCCTTCCGACATAGCTGTTGA
GTGGGAAAGTAACGGGCAGCCAGAGAACAATTACAAGACAACTCCACCCGTTCTTGATAGCGAT

GGATCATTTTTTCTGTATTCCAAACTCACTGTCGATAAAAGTCGCTGGCAGCAAGGCAATGTTTT TAGCTGCTCAGTCATGCACGAAGCACTGCATAATCACTACACACAAAAAAGTTTGTCCCTTAGCC CTGGTAAGtaG
IL-15 (Human) (SEQ ID NO: 66)
ATGTACTCAATGCAGTTGGCCTCCTGTGTAACATTGACCTTGGTCCTCTTGGTCAACAGCAATTG GATCGATGTACGCTACGACTTGGAGAAGATTGAGTCCCTTATACAGAGTATACACATAGATACA ACCTTGTATACTGACAGTGACTTCCATCCCAGCTGTAAAGTGACTGCAATGAACTGTTTTTTGTT GGAGTTGCAAGTAATTCTGCATGAATACAGCAACATGACCCTCAATGAAACCGTTAGGAATGTC CTTTATCTCGCAAATTCTACTCTGAGTAGCAATAAGAATGTTGCCGAAAGCGGCTGCAAGGAGT GCGAAGAACTGGAGGAAAAAACTTTCACCGAGTTTCTCCAGAGTTTCATCAGAATTGTCCAAAT GTTCATTAATACAAGTAGTGGTGGTGGGAGCGGGGGTGGAGGCAGTGGGGGAGGTGGGAGCGG AGGTGGAGGGTCCGGAGGGGGGAGCCTTCAAGGCACTACTTGTCCTCCACCCGTATCCATCGAG CACGCCGATATTCGAGTTAAAAATTATAGTGTTAATAGCAGAGAACGATACGTCTGCAACTCAG GGTTTAAGAGAAAGGCCGGAACTTCAACTCTCATAGAATGCGTGATTAATAAGAATACTAACGT CGCACATTGGACTACTCCCAGTCTCAAGTGCATACGCGATCCATCTCTCGCTCATTACTCACCAG TACCTACAGTGGTTACTCCTAAGGTGACCTCTCAGCCCGAATCACCATCTCCCAGCGCAAAAGAG CCTGAGGCCTTTTCTCCTAAATCAGACACTGCTATGACTACAGAAACAGCCATAATGCCAGGAA GCCGGCTGACACCATCTCAAACTACCAGCGCAGGCACAACTGGGACTGGCTCCCACAAAAGCTC ACGCGCACCAAGTCTCGCCGCAACAATGACATTGGAGCCTACAGCCAGCACATCTCTTAGAATC ACAGAAATTTCTCCCCACAGTAGCAAGATGACCAAGGTGGCAATTAGTACCAGCGTCCTTCTTGT AGGAGCTGGAGTTGTGATGGCATTTTTGGCATGGTATATCAAAAGCAGGtaG
IL-15 (Mouse) (SEQ ID NO: 67)
ATGAAGATCCTCAAGCCATACATGCGAAACACTAGTATTAGCTGTTACTTGTGTTTTCTGCTGAA TAGTCATTTTTTGACTGAAGCAGGAATCCATGTATTTATACTCGGTTGTGTGTCTGTAGGTCTGCC AAAGACTGAGGCTAATTGGATTGACGTGCGCTATGATCTTGAAAAAATAGAGTCCTTGATTCAA TCAATACACATCGATACCACTCTCTACACCGACAGTGATTTCCATCCTTCCTGCAAGGTAACAGC TATGAATTGCTTCCTCCTGGAGCTCCAAGTCATTCTCCATGAGTACTCCAACATGACTTTGAACG AAACTGTAAGAAACGTATTGTATCTGGCTAATAGCACCTTGTCTAGTAACAAAAATGTGGCAGA GAGCGGCTGCAAAGAATGTGAAGAATTGGAAGAGAAAACATTTACAGAGTTCCTGCAATCCTTT ATTCGCATCGTCCAAATGTTTATCAATACCTCTtaG
IL-15 (Mouse) (SEQ ID NO: 68)
ATGTATTCCATGCAACTTGCCAGTTGTGTAACCCTTACTCTCGTCCTGCTCGTTAATTCCGCTGGT GCTAACTGGATAGATGTTCGATACGATCTGGAAAAGATTGAGTCCCTTATCCAATCCATTCATAT AGATACCACCCTTTATACTGACAGCGACTTCCATCCTTCTTGCAAGGTGACCGCTATGAATTGTT TCCTGCTGGAACTCCAAGTTATTCTGCATGAATACTCTAATATGACACTTAACGAGACCGTAAGA AATGTTCTCTATCTCGCTAATAGTACTTTGAGCTCAAATAAGAACGTGGCCGAGTCTGGGTGTAA GGAATGCGAAGAGCTGGAAGAAAAGACATTCACCGAGTTTCTCCAGTCTTTCATACGGATTGTG CAGATGTTTATCAACACATCAGATTACAAAGACGACGATGATAAGtaG
IL-18 (Mouse) (SEQ ID NO: 69)
ATGGCAGCCATGTCTGAGGACTCTTGTGTGAACTTTAAAGAAATGATGTTCATAGACAATACACT CTACTTTATACCTGAGGAGAATGGAGATTTGGAATCTGACAACTTTGGCAGGCTGCATTGCACTA CCGCAGTTATCCGAAACATCAACGATCAGGTACTGTTTGTTGATAAAAGACAACCTGTATTCGAG GACATGACCGACATAGATCAGTCTGCCTCAGAGCCCCAGACTAGGCTTATCATCTATATGTACAA GGACAGCGAAGTACGAGGCCTGGCTGTTACACTCTCAGTCAAAGACTCTAAGATGAGCACCCTG TCATGCAAGAACAAAATTATCAGTTTTGAGGAGATGGACCCACCTGAAAACATAGATGACATTC AGTCAGACCTCATTTTTTTTCAAAAGCGGGTACCAGGACACAACAAAATGGAATTTGAATCATC ACTCTACGAAGGACATTTCCTTGCATGCCAGAAAGAGGATGACGCATTCAAATTGATCCTGAAA AAAAAGGACGAAAATGGTGATAAATCAGTCATGTTTACATTGACCAATCTTCACCAAAGTtaG
IL-18 (Mouse) (SEQ ID NO: 70)
ATGGCTGCAATGTCTGAAGATAGCTGTGTCAACTTTAAGGAGATGATGTTCATTGATAATACTTT GTACTTTATACCTGAAGAAAATGGAGACCTTGAGTCAGACAACTTCGGGAGACTGCACTGCACA ACTGCCGTTATCCGAAACATAAATGATCAAGTATTGTTCGTGGACAAAAGACAACCAGTCTTTG AGGATATGACAGACATCGACCAATCCGCATCTGAACCTCAGACTAGGCTGATCATCTATATGTA CGCCGACTCCGAAGTAAGAGGCCTTGCTGTGACACTTAGTGTTAAGGATAGTAAGATGAGCACA CTGTCCTGTAAGAATAAGATTATATCTTTTGAAGAGATGGACCCTCCCGAGAACATAGATGACAT

CCAGAGCGACTTGATCTTCTTTCAGAAGCGAGTGCCAGGCCATAACAAGATGGAATTTGAATCA TCTCTTTATGAAGGCCATTTCCTCGCATGTCAAAAGGAGGACGATGCCTTCAAGCTCATTCTGAA AAAAAAAGACGAGAACGGTGATAAGAGCGTGATGTTCACTCTGACAAATCTGCACCAGTCAtaG
IL-18 (Human) (SEQ ID NO: 71)
ATGTATCGCATGCAACTCCTGTCCTGCATTGCTCTGAGCTTGGCTTTGGTAACCAACTCATACTTC GGGAAACTGGAGAGTAAACTCTCCGTAATCAGGAATCTTAATGACCAAGTATTGTTTATTGACC AGGGCAACCGCCCGTTGTTCGAGGATATGACTGATTCTGACTGTCGGGATAACGCTCCGAGAAC TATCTTTATCATTTCAATGTACAAGGACAGCCAACCGCGGGGTATGGCTGTGACAATCAGTGTCA AATGTGAGAAGATTTCCACGCTGTCCTGCGAAAACAAGATAATTTCTTTCAAAGAAATGAACCC CCCTGACAATATAAAGGATACAAAGAGTGATATCATCTTCTTTCAGAGGTCCGTGCCCGGCCAC GATAATAAGATGCAATTTGAAAGTTCATCTTATGAGGGGTACTTTTTGGCATGCGAGAAAGAAA GGGATCTCTTCAAGTTGATCCTGAAGAAGGAGGACGAATTGGGCGACCGCTCCATCATGTTCAC AGTCCAGAACGAGGACtaG
IL-18 (Human) (SEQ ID NO: 72)
ATGTACCGCATGCAGCTCCTGAGTTGTATTGCCCTTTCCCTCGCTCTCGTTACCAATTCTTACTTC GGTAAGCTTGCCTCTAAACTCTCTGTTATTAGGAACTTGAACGACCAAGTCCTTTTCATAGACCA AGGGAACAGACCACTGTTTGAAGATATGACGGATAGCGATTGCCGAGATAATGCCCCTAGGACG ATTTTTATCATTAGTATGTATGCGGACTCTCAACCGAGGGGGATGGCCGTTACTATAAGTGTGAA ATGCGAGAAAATATCAACGCTCAGTTGTGAGAACAAAATCATAAGTTTCAAGGAGATGAATCCA CCTGATAACATCAAAGACACTAAGTCTGATATTATATTTTTCCAACGAAGTGTTCCGGGACACGA TAACAAAATGCAATTTGAGAGCTCCTCATACGAGGGCTACTTCCTCGCGTGTGAGAAAGAAAGG GATTTGTTTAAGCTTATCCTCAAGAAAGAGGACGAGTTGGGGGATCGGAGCATAATGTTTACCG TACAGAATGAGGACtaG
IL-21 (Mouse) (SEQ ID NO: 73)
ATGGAGCGGACACTCGTGTGTCTTGTCGTAATTTTTCTCGGGACAGTCGCACACAAGTCCTCACC CCAGGGTCCTGATCGCCTTCTCATACGCCTCCGACATTTGATCGACATTGTAGAGCAGCTCAAAA TTTACGAGAATGACCTCGATCCCGAGCTTTTGAGTGCTCCCCAAGACGTTAAGGGTCATTGCGAG CACGCAGCTTTTGCTTGCTTCCAGAAGGCCAAGTTGAAACCAAGCAACCCTGGTAATAATAAGA CTTTCATCATCGACTTGGTCGCCCAACTCCGAAGGAGGCTGCCTGCCCGGCGCGGAGGAAAAAA ACAAAAGCATATTGCAAAGTGTCCTTCATGTGATTCATACGAAAAGCGGACTCCCAAAGAGTTC TTGGAAAGGTTGAAATGGCTTCTTCAGAAGATGATTCATCAACATTTGTCAtaG
IFN-beta (Human) (SEQ ID NO: 74)
ATGACCAACAAATGCCTTTTGCAAATTGCCCTGCTTTTGTGTTTTAGCACTACCGCATTGAGCAT GTCATATAACCTCCTCGGCTTCCTTCAGAGATCATCAAACTTTCAGTGTCAGAAACTGCTTTGGC AACTTAATGGCAGGCTCGAATATTGTCTGAAAGATCGGATGAATTTCGACATTCCAGAAGAAAT AAAACAGCTTCAACAATTCCAGAAAGAGGACGCCGCCCTGACTATTTACGAGATGCTCCAGAAT ATCTTCGCCATTTTCCGGCAGGACAGCTCATCCACGGGGTGGAATGAGACTATTGTAGAAAATCT TCTGGCTAATGTGTACCATCAAATTAATCACCTCAAAACGGTGCTTGAGGAAAAACTTGAAAAG GAAGATTTCACACGGGGCAAGTTGATGTCCTCCCTGCACCTTAAACGATACTACGGCAGGATTCT TCATTACTTGAAGGCTAAGGAGTATAGCCATTGCGCGTGGACAATTGTACGGGTAGAAATACTG CGAAACTTTTATTTCATCAACCGGCTCACTGGATACCTTAGAAATtaG
IFN-beta (Mouse) (SEQ ID NO: 75)
ATGAACAATCGGTGGATACTCCACGCCGCATTTCTCCTCTGCTTTAGCACGACGGCCCTGTCCAT CAACTACAAACAGCTTCAGTTGCAGGAGCGGACTAACATAAGGAAGTGCCAGGAACTGCTGGA ACAGCTTAATGGTAAAATTAATCTTACATACCGAGCTGACTTCAAAATTCCTATGGAAATGACCG AGAAGATGCAGAAATCCTACACGGCATTCGCCATCCAGGAAATGCTCCAGAACGTATTTCTCGT GTTCCGCAATAATTTCTCTTCTACGGGTTGGAACGAAACCATTGTTGTTAGACTGCTTGACGAAC TGCATCAGCAAACCGTGTTCCTTAAAACCGTGCTTGAGGAGAAGCAGGAGGAGCGCCTGACTTG GGAGATGTCTAGTACCGCACTTCACTTGAAATCCTACTACTGGCGCGTTCAGCGGTATCTGAAGC TGATGAAGTATAACTCATACGCCTGGATGGTAGTGCGCGCAGAGATCTTCAGAAACTTTCTTATC ATCCGGCGACTGACCCGAAACTTTCAGAATtaG
IFN-gamma (Human) (SEQ ID NO: 76)

ATGAAGTACACTAGCTATATATTGGCCTTCCAGCTTTGCATCGTATTGGGTAGCCTCGGATGCTA TTGCCAAGACCCGTATGTCAAAGAAGCCGAAAATCTCAAAAAGTATTTCAATGCCGGACACTCA GACGTCGCGGATAACGGTACACTGTTTCTTGGCATCCTGAAAAATTGGAAGGAAGAGAGTGACA GAAAAATAATGCAGTCACAAATAGTGTCCTTTTACTTTAAGCTGTTCAAAAATTTCAAGGATGAC CAAAGTATCCAGAAGAGTGTTGAAACTATCAAAGAGGACATGAATGTGAAATTCTTTAACAGTA ATAAGAAGAAGCGCGATGACTTCGAGAAACTCACTAATTACAGCGTAACGGATCTTAACGTCCA ACGCAAGGCAATCCACGAGCTTATACAGGTAATGGCTGAGCTTAGTCCCGCAGCCAAGACAGGG AAGAGAAAAAGGTCTCAAATGCTTTTTCGGGGCCGGCGAGCTTCACAAtaG
IFN-gamma (Mouse) (SEQ ID NO: 77)
ATGAACGCTACGCATTGCATCCTCGCACTCCAATTGTTCCTCATGGCTGTGTCAGGGTGTTACTG TCACGGTACTGTCATAGAAAGCCTCGAATCCCTGAATAACTATTTTAACAGTAGCGGTATAGATG TAGAAGAAAAGTCTCTCTTTCTTGACATCTGGAGGAATTGGCAAAAGGATGGAGACATGAAGAT TCTCCAATCTCAGATTATATCATTTTACTTGAGGCTTTTTGAGGTTCTGAAGGATAACCAGGCGA TCAGCAATAATATCAGCGTAATTGAATCTCACCTTATTACAACATTTTTCTCAAATTCCAAGGCA AAGAAAGATGCTTTCATGTCTATCGCGAAATTTGAGGTGAACAATCCTCAGGTACAAAGGCAAG CCTTTAACGAGCTGATTAGAGTTGTACATCAGTTGTTGCCCGAAAGTAGTCTTAGAAAACGCAAA CGGAGCCGATGCtaG
IFN-alpha (Mouse) (SEQ ID NO: 78)
ATGGCAAGGTTGTGCGCTTTTCTCATGGTACTGGCTGTGCTCTCCTATTGGCCTACTTGTTCTCTG GGATGCGACTTGCCACAGACCCACAATCTGCGGAATAAGAGGGCTCTGACTCTGCTGGTGCAAA TGAGACGGCTCTCTCCACTTAGCTGTTTGAAAGATAGAAAGGATTTCGGGTTCCCCCAGGAGAA GGTGGATGCCCAGCAGATCAAGAAGGCACAGGCTATCCCCGTCCTTTCCGAGCTGACCCAGCAA ATTTTGAACATCTTTACAAGTAAGGATAGTTCAGCTGCATGGAATACCACACTTTTGGATTCTTTT TGTAACGATCTGCATCAGCAGCTGAACGATCTCCAGGGATGCCTGATGCAGCAAGTCGGCGTGC AAGAATTTCCACTCACCCAGGAGGACGCTCTGCTCGCAGTGCGAAAGTATTTTCACCGAATTACC GTGTACCTCCGGGAGAAAAAGCATTCACCCTGCGCTTGGGAAGTAGTCAGGGCCGAAGTATGGA GAGCCCTTAGTAGCTCCGCTAATGTACTGGGCCGGTTGCGGGAAGAGAAAtaG
CCL21 (Human) (SEQ ID NO: 79)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCA AGGAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAATACCCGCGAAA GTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCCT CCCGAGAAAACGGAGCCAGGCCGAACTGTGCGCTGACCCTAAGGAACTTTGGGTGCAACAACTT ATGCAACACCTGGATAAGACACCTTCTCCTCAAAAGCCAGCTCAGGGCTGCCGAAAAGATAGAG GCGCCTCAAAAACCGGAAAAAAGGGCAAAGGTTCTAAAGGATGTAAGCGGACTGAACGCTCTC AAACGCCTAAAGGGCCGtaG
CCL21a (Mouse) (SEQ ID NO: 80)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCA GGGGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCT ATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTC CCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTG ATGCGAAGGCTTGACCAGCCCCCAGCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAACAGA GGTACTTCAAAGAGCGGCAAGAAAGGCAAAGGGAGTAAAGGATGTAAAAGAACGGAGCAGAC CCAGCCTTCACGAGGCtaG
Tail-less CCL21 (Human) (SEQ ID NO: 81)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCA AGGAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAATACCCGCGAAA GTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCCT CCCGAGAAAACGGAGCCAGGCCGAACTGTGCGCTGACCCTAAGGAACTTTGGGTGCAACAACTT ATGCAACACCTGGATAAGACACCTTCTCCTCAAAAGCCAGCTCAGGGCtaG
Tail-less CCL21 (Mouse) (SEQ ID NO: 82)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCA GGGGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAAGATACCGTACTCT ATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTC

CCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTG ATGCGAAGGCTTGACCAGCCCCCAGCCCCTGGCAAGCAGTCACCTGGGtaG
CCL19 (Mouse) (SEQ ID NO: 83)
ATGGCACCCCGCGTCACACCCTTGCTTGCTTTTTCTCTGCTTGTCCTCTGGACCTTCCCCGCTCCT ACCCTTGGAGGAGCCAATGATGCCGAGGATTGCTGCCTGAGTGTTACACAAAGGCCAATACCAG GGAATATAGTGAAGGCATTCCGGTATCTGCTCAATGAAGATGGGTGCAGAGTCCCCGCAGTTGT CTTTACAACATTGCGAGGTTACCAGCTTTGTGCTCCCCCAGACCAGCCTTGGGTAGATCGCATTA TTCGCCGGTTGAAGAAGAGCTCAGCAAAGAATAAGGGCAATTCCACACGGAGAAGCCCCGTCTC CtaG
CCL19 (Mouse) (SEQ ID NO: 84)
ATGAAATCAGCAGTCCTTTTCTTGCTCGGGATTATTTTTCTGGAACAATGTGGAGTGAGGGGAAC ACTCGTAATAAGAAACGCTCGGTGCTCATGCATATCAACATCACGGGGCACTATCCACTACAAA TCCCTGAAGGATCTGAAGCAGTTCGCCCCAAGCCCTAACTGTAACAAGACCGAAATTATCGCAA CTCTCAAAAATGGAGATCAGACTTGTCTTGACCCAGATTCAGCAAATGTCAAGAAGCTGATGAA AGAGTGGGAAAAGAAGATTTCACAAAAAAAAAAGCAAAAACGCGGCAAGAAACATCAAAAGA ACATGAAAAACAGGAAACCTAAGACTCCCCAGTCAAGGAGAAGATCCCGCAAGACAACCtaG
CXCL11 (Mouse) (SEQ ID NO: 85)
ATGAACAGAAAAGTTACCGCTATAGCACTTGCTGCCATAATATGGGCCACCGCAGCTCAAGGGT TCCTGATGTTCAAGCAGGGCCGATGCCTCTGCATTGGCCCTGGAATGAAGGCCGTGAAAATGGC CGAAATAGAAAAAGCTAGTGTCATATACCCCTCTAACGGTTGCGATAAAGTCGAGGTTATAGTC ACAATGAAAGCTCATAAACGCCAACGCTGCCTCGACCCCCGGTCTAAGCAGGCTAGGCTCATAA TGCAAGCAATCGAGAAGAAAAACTTTCTTAGACGGCAAAACATGtaG
CXCL10 (Mouse) (SEQ ID NO: 86)
ATGAACCCATCTGCCGCCGTTATTTTCTGTCTGATACTCCTTGGGCTGAGTGGCACACAAGGCAT ACCCCTCGCCCGCACAGTCCGGTGTAATTGTATACATATTGACGACGGCCCTGTTAGAATGCGGG CCATCGGTAAGCTGGAGATTATACCAGCAAGCCTTAGTTGTCCCAGGGTTGAAATCATAGCAAC TATGAAAAAAAACGACGAACAAAGATGTTTGAATCCCGAGAGCAAGACAATCAAAAACCTTAT GAAAGCATTTAGTCAAAAACGCTCTAAACGCGCTCCAtaG
CXCL10 (Human) (SEQ ID NO: 87)
ATGAATCAGACGGCAATCCTTATATGCTGCCTTATATTCCTTACTCTCTCAGGGATACAAGGGGT ACCACTTTCTCGGACTGTTCGCTGCACTTGCATTTCAATATCTAACCAACCTGTAAATCCGCGGA GCCTGGAAAAATTGGAGATTATACCTGCTTCTCAATTCTGCCCTCGGGTGGAAATCATCGCCACT ATGAAGAAGAAGGGCGAGAAAAGGTGTCTGAATCCAGAGTCAAAGGCAATCAAAAACCTGCTG AAAGCGGTGTCAAAGGAACGGTCCAAGAGATCACCCtaG
CXCL11-CXCL10 (Mouse) (SEQ ID NO: 88)
ATGAACAGGAAAGTAACAGCCATTGCATTGGCTGCCATCATCTGGGCCACCGCAGCACAGGGTT TTCTGATGTTTAAGCAAGGGCGCTGTCTCTGTATAGGCCCAGGCATGAAGGCCGTGAAGATGGC AGAGATTGAGAAGGCATCTGTGATTTATCCTTCTAACGGGTGCGATAAAGTCGAAGTTATTGTGA CAATGAAGGCACACAAACGCCAACGGTGTTTGGACCCACGATCTAAACAGGCAAGATTGATTAT GCAAGCCATCGAGAAAAAGAACTTTCTCCGAAGGCAAAATATGATCCCTTTGGCTCGGACAGTG CGGTGTAACTGTATTCACATCGACGATGGGCCAGTACGGATGAGAGCAATAGGAAAGCTCGAAA TCATACCCGCCTCATTGTCTTGTCCCAGGGTGGAAATAATCGCCACTATGAAAAAGAACGATGA ACAGAGGTGTCTCAACCCAGAGAGTAAGACTATCAAGAACCTTATGAAGGCATTCAGTCAGAAG AGGTCAAAGCGAGCACCAtaG
XCL1 (Human) (SEQ ID NO: 89)
ATGAGACTTCTCATATTGGCGCTTCTCGGGATATGTTCTCTTACGGCATACATAGTTGAGGGGGT GGGATCTGAGGTTAGCGATAAACGAACTTGTGTTAGTCTTACAACACAGAGGCTTCCAGTCTCCA GGATAAAAACATATACGATAACTGAGGGATCTCTCAGAGCGGTCATCTTCATAACGAAGAGGGG CCTGAAGGTCTGTGCTGACCCACAAGCGACTTGGGTAAGGGACGTTGTGCGGAGCATGGACAGG AAGAGCAATACTCGCAACAACATGATCCAAACCAAACCTACGGGCACCCAACAGTCAACCAAT ACTGCGGTAACATTGACGGGGtaG

XCL1 (Mouse) (SEQ ID NO: 90)
ATGCGCCTCCTTCTGCTGACTTTTCTGGGTGTATGTTGCCTGACACCCTGGGTCGTAGAAGGAGT AGGAACCGAGGTTCTGGAAGAGTCCTCATGTGTAAACTTGCAGACACAACGACTCCCCGTCCAA AAAATCAAGACCTATATAATCTGGGAGGGGGCAATGCGGGCCGTCATTTTCGTGACTAAACGAG GTCTCAAAATCTGCGCCGACCCCGAGGCTAAGTGGGTGAAGGCAGCCATTAAGACCGTGGATGG GAGAGCCAGCACCAGAAAGAACATGGCCGAAACAGTACCTACTGGCGCACAGCGGTCAACCTC AACTGCTATAACCTTGACAGGAtaG
m_sCD40L #1 (SEQ ID NO: 91)
ATGGAGACTGACACTCTGCTTCTGTGGGTGTTGCTGCTGTGGGTGCCTGGCAGTACAGGCGATAT GCAACGAGGTGACGAGGACCCTCAAATCGCCGCCCATGTAGTCTCTGAAGCTAATAGCAACGCT GCATCCGTCTTGCAGTGGGCAAAGAAAGGCTACTATACTATGAAGTCCAACTTGGTAATGCTTG AAAACGGCAAGCAGTTGACTGTCAAGAGAGAGGGACTTTATTACGTCTATACCCAAGTCACATT CTGTAGCAATCGAGAACCCTCCTCACAGAGGCCTTTTATAGTGGGACTCTGGCTTAAACCAAGTA GCGGCTCTGAGCGCATACTGTTGAAAGCCGCAAACACACACAGCTCTTCCCAACTCTGCGAGCA GCAATCCGTGCATCTCGGTGGAGTATTTGAGCTTCAAGCCGGTGCCTCAGTGTTTGTGAACGTCA CTGAGGCCTCCCAGGTCATACATCGAGTTGGGTTCAGCTCCTTCGGCTTGCTCAAGCTCtaG
m_sCD40L #2 (SEQ ID NO: 92)
ATGGAAACTGATACATTGCTGCTCTGGGTTTTGCTGCTCTGGGTGCCTGGGAGTACAGGCGACAT GAGGAGGCAGTTCGAGGATCTCGTTAAGGATATTACCCTTAATAAGGAGGAGAAGAAAGAAAA CTCTTTTGAGATGCAACGAGGGGACGAAGATCCTCAGATCGCTGCTCACGTGGTCTCTGAAGCTA ACAGCAACGCCGCTTCTGTCCTCCAGTGGGCCAAGAAAGGTTATTACACCATGAAATCAAACCT TGTAATGCTTGAAAACGGGAAACAGCTTACAGTGAAGAGGGAAGGTCTTTACTACGTCTATACC CAGGTAACCTTCTGCTCAAACAGAGAACCATCAAGCCAGAGGCCATTCATAGTGGGGCTCTGGC TCAAACCTTCCAGTGGCAGCGAGAGAATCTTGTTGAAAGCTGCTAATACACATAGTAGTAGCCA GCTTTGCGAGCAACAGTCAGTCCACCTCGGGGGGGTGTTTGAGTTGCAAGCAGGGGCCTCAGTA TTCGTGAATGTCACTGAGGCTTCCCAGGTAATTCACAGGGTAGGCTTTAGTTCATTCGGTTTGCT GAAGCTTtaG
m_sCD40L #3 (SEQ ID NO: 93)
ATGCGAAGAATGCAGCTTCTGCTCCTTATTGCTCTGAGTCTCGCCCTTGTCACCAACTCCGGGGA CAGAATGAAACAAATCGAGGACAAAATTGAAGAAATACTGAGTAAAATATATCACATCGAAAA CGAAATTGCACGCATTAAGAAATTGATTGGCGAACGCACCAGTGGCGGCTCTGGTGGCACCGGA GGTTCAGGCGGGACCGGGGGCTCTGACAAAGTCGAAGAGGAGGTTAACCTTCATGAGGACTTTG TGTTCATCAAGAAGCTGAAACGGTGCAATAAAGGAGAAGGTTCTTTGAGCCTCCTTAATTGCGA AGAGATGCGACGACAGTTCGAGGATCTGGTTAAGGACATTACACTTAATAAGGAAGAGAAAAA GGAGAACTCTTTCGAAATGCAGCGCGGCGATGAAGATCCCCAGATAGCCGCCCATGTCGTCTCT GAGGCCAACTCTAACGCAGCATCCGTCCTCCAGTGGGCTAAGAAAGGATATTATACTATGAAAA GCAATTTGGTCATGCTCGAAAACGGTAAACAGCTCACTGTTAAGAGAGAAGGCCTCTATTACGT ATATACTCAAGTAACTTTCTGTTCTAATAGGGAACCCTCCTCTCAAAGACCTTTTATCGTAGGAC TCTGGTTGAAACCAAGTAGCGGTAGTGAAAGGATTCTGCTCAAAGCAGCTAATACTCACTCCAG CAGTCAACTGTGCGAACAACAAAGCGTTCACCTCGGGGGCGTCTTTGAACTTCAGGCAGGTGCC AGTGTTTTCGTCAACGTAACAGAAGCATCCCAGGTAATTCATCGAGTAGGGTTTTCTAGCTTTGG TTTGCTGAAGCTGtaG
anti-CD40_FGK4.5 (SEQ ID NO: 94)
ATGGAAACTGATCGCCTGTTGCTCTGGGTACTTCTTCTGTGGGTGCCTGGGTCCACTGGTGACAC TGTACTTACACAATCACCCGCTTTGGCCGTTTCTCCTGGTGAACGGGTCACAATTAGTTGCCGAG CTTCCGATTCTGTATCTACTCTTATGCATTGGTATCAACAAAAACCTGGTCAGCAGCCAAAATTG CTCATTTATCTTGCTAGTCACTTGGAGTCCGGCGTACCTGCTCGATTCAGCGGTAGTGGGTCTGG CACAGATTTCACTTTGACCATAGATCCCGTGGAGGCCGATGACACTGCAACCTACTATTGCCAGC AATCCTGGAACGACCCTTGGACTTTCGGCGGCGGCACCAAGCTGGAACTCAAGCGAGCAGATGC TGCCCCAACCGTTAGTATATTCCCACCCTCAACCGAACAACTCGCCACAGGAGGCGCTAGTGTC GTGTGTCTTATGAACAATTTCTATCCACGAGACATTAGCGTCAAGTGGAAAATTGATGGGACAG AAAGGCGAGATGGAGTTTTGGATTCAGTAACAGACCAGGATTCAAAGGATTCTACCTATAGCAT GAGCTCCACCTTGAGCCTGACCAAAGCTGATTATGAATCTCATAACCTGTATACTTGTGAAGTGG TGCATAAGACTTCTAGCTCACCAGTGGTTAAATCTTTTAACCGCAACGAATGTCGGCGCAAGAG GGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTGGTCCC

ATGGACATTCGGCTCTCTTTGGTATTCCTGGTACTTTTTATAAAGGGGGTGCAATGTGAAGTCCA GCTCGTGGAAAGCGGTGGGGGCCTGGTTCAGCCCGGTCGCAGCCTTAAACTTAGTTGCGCAGCA TCCGGATTTACATTTTCTGACTATAACATGGCCTGGGTTCGACAGGCACCCAAAAAAGGGCTGG AGTGGGTCGCAACTATCATATACGATGGTTCCCGGACATACTATAGAGATTCAGTGAAGGGGCG CTTTACAATAAGCAGGGACAATGCTAAGTCTACCTTGTATCTTCAGATGGACTCCCTGAGGAGCG AAGATACAGCAACATATTATTGTGCTACAAACCGCTGGTTGCTGCTTCATTATTTCGACTACTGG GGTCAGGGCGTCATGGTAACTGTATCAAGCGCCGAGACCACAGCCCCTTCTGTATATCCATTGGC ACCAGGTACTGCTCTGAAATCCAACTCAATGGTAACCCTTGGATGTCTGGTTAAGGGTTATTTTC CCGAGCCCGTCACAGTTACTTGGAACTCTGGGGCCCTTTCTAGCGGAGTCCATACCTTTCCCGCC GTTTTGCAGAGTGGTCTGTACACCCTTACCTCAAGCGTCACAGTTCCATCTAGCACATGGAGCTC CCAGGCAGTAACTTGTAATGTGGCCCATCCAGCCTCCTCAACTAAGGTAGATAAAAAGATCGTT CCCAGAGAATGCAATCCATGTGGATGCACCGGGTCTGAGGTCAGCAGTGTGTTCATTTTCCCACC CAAGACTAAAGATGTATTGACTATTACTCTTACACCCAAAGTAACCTGCGTGGTGGTTGATATTA GTCAAAATGATCCCGAGGTACGGTTCTCTTGGTTTATCGACGACGTCGAAGTACATACAGCTCAG ACACACGCTCCCGAGAAACAAAGCAATTCCACTCTTAGGAGCGTGTCCGAGTTGCCAATCGTAC ATAGGGATTGGCTTAATGGCAAGACCTTTAAGTGTAAGGTCAATTCAGGGGCATTCCCCGCACC AATAGAGAAGAGTATAAGCAAACCCGAGGGGACACCCAGAGGTCCACAGGTCTATACAATGGC TCCCCCCAAGGAAGAGATGACCCAAAGTCAAGTCTCAATTACATGTATGGTGAAGGGCTTTTAT CCACCCGACATATACACTGAGTGGAAGATGAATGGACAGCCCCAAGAGAATTATAAAAACACTC CCCCTACCATGGACACCGACGGGTCCTATTTTCTTTATAGTAAATTGAACGTGAAAAAGGAGACC TGGCAACAAGGCAACACTTTCACCTGCTCCGTTCTTCACGAGGGCCTGCATAATCATCATACCGA AAAGTCTCTCAGTCATTCTCCAGGTAAGtaG
CD40L_2 (Human) (SEQ ID NO: 95)
ATGGAAACAGATACGTTGCTGTTGTGGGTACTTCTCCTTTGGGTCCCTGGCAGCACAGGGGACGA GAATAGTTTCGAAATGCAGAAGGGCGACCAGAACCCACAGATCGCGGCTCACGTTATATCAGAA GCAAGTAGTAAGACCACTTCCGTACTTCAGTGGGCTGAAAAAGGATATTACACCATGTCCAACA ATCTCGTGACACTGGAGAACGGTAAACAACTTACGGTGAAACGACAGGGCCTCTATTACATCTA CGCTCAGGTGACATTCTGCTCAAATAGGGAGGCTTCTAGTCAAGCGCCCTTCATCGCCAGCCTGT GCCTCAAATCTCCCGGCCGGTTCGAACGAATCCTGTTGCGAGCGGCCAATACCCATAGCTCAGCT AAACCTTGCGGCCAGCAGAGTATTCATCTTGGTGGTGTGTTTGAACTTCAGCCGGGAGCATCTGT GTTCGTCAACGTAACGGACCCTAGCCAAGTGTCTCATGGGACAGGTTTTACATCCTTCGGACTCC TCAAGTTGtaG
Flt3L (Human) (SEQ ID NO: 96)
ATGACAGTTCTCGCGCCAGCTTGGAGTCCCACCACATACTTGCTTTTGCTTCTGCTTCTGTCCTCT GGCCTGAGTGGGACCCAAGATTGTTCCTTTCAACATTCCCCAATTAGTTCTGATTTTGCAGTGAA GATTAGAGAGCTCTCAGACTATCTGCTGCAAGATTATCCTGTCACAGTCGCTTCAAACCTGCAAG ACGAAGAGCTCTGCGGTGCCTTGTGGCGGTTGGTCTTGGCTCAAAGATGGATGGAGAGACTGAA AACCGTAGCAGGCAGCAAGATGCAGGGTCTCCTGGAAAGGGTGAACACGGAAATCCATTTTGTG ACCAAGTGCGCGTTCCAGCCCCCACCGAGTTGTCTCCGGTTTGTTCAAACGAATATATCCCGGTT GCTCCAGGAAACCTCAGAACAACTGGTGGCTTTGAAACCCTGGATCACAAGACAAAACTTTAGT CGGTGCCTCGAACTCCAGTGCCAACCAGATTCTTCTACACTTCCCCCCCCCGTGGTCCCCGCGCCC GTTGGAAGCAACGGCCCCAtaG
TGFb TRAP (Human) (SEQ ID NO: 97)
ATGGCCTGGAGTCCTCTGTTTCTGACTCTTATAACTCACTGTGCCGGCAGTTGGGCTATACCCCCT CATGTACAGAAGTCTGTAAACAACGACATGATTGTAACCGACAATAATGGCGCAGTGAAATTCC CACAACTGTGTAAGTTCTGTGATGTACGGTTTAGTACATGCGACAATCAAAAAAGCTGTATGTCT AACTGCTCTATTACATCCATATGTGAAAAACCTCAGGAGGTGTGTGTTGCCGTTTGGCGAAAAAA TGATGAGAATATCACACTGGAGACAGTATGTCATGACCCTAAACTGCCATACCATGATTTCATAC TGGAGGACGCCGCCAGTCCTAAGTGCATTATGAAAGAGAAAAAGAAACCCGGTGAAACATTCTT TATGTGCTCTTGTAGCTCTGACGAGTGTAACGACAACATTATATTCAGCGAGGAGTACAATACAA GCAACCCCGATATACCACCTCACGTACAAAAAAGTGTCAACAACGATATGATTGTTACCGACAA TAACGGAGCTGTTAAGTTTCCTCAGTTGTGCAAGTTCTGCGATGTACGATTCTCTACCTGCGACA ACCAAAAGTCATGTATGTCTAACTGTTCCATAACCTCCATCTGCGAGAAGCCCCAGGAAGTCTGC GTCGCCGTGTGGCGGAAAAACGACGAGAATATCACTCTTGAAACCGTTTGTCATGATCCTAAAC TGCCCTATCACGACTTTATTCTGGAAGATGCTGCTTCCCCTAAGTGTATCATGAAAGAAAAGAAG AAACCTGGGGAGACATTCTTTATGTGTTCATGCTCCTCCGATGAGTGTAACGACAATATCATCTT CTCTGAGGAATACAACACTTCTAACCCTGATtaG

Fresolimumab (Human) (SEQ ID NO: 98)
ATGGCCTGGTCCCCTCTTTTTCTGACCCTCATCACACACTGTGCAGGCTCATGGGCTGAGACCGT CTTGACCCAGTCCCCAGGAACTTTGTCTCTGTCTCCTGGTGAAAGAGCTACCCTTAGTTGTCGAG CCTCTCAGTCCCTTGGTTCTAGCTATCTCGCTTGGTACCAGCAAAAGCCAGGCCAGGCCCCACGA CTGCTGATCTACGGAGCATCTTCACGGGCTCCCGGCATTCCCGATCGATTTTCCGGATCTGGTAG TGGTACAGATTTCACACTGACCATATCTCGCCTGGAGCCCGAGGACTTTGCTGTTTATTATTGTC AGCAGTACGCCGATTCTCCTATCACTTTTGGACAGGGAACCCGCCTGGAGATTAAGCGCACAGT AGCAGCTCCATCCGTCTTTATCTTTCCACCATCAGATGAACAGCTCAAGAGTGGGACCGCAAGTG TAGTATGCCTGCTGAACAATTTTTACCCTAGAGAGGCCAAAGTGCAGTGGAAGGTGGATAACGC CCTCCAGAGTGGCAATAGTCAAGAAAGTGTTACTGAGCAAGATAGTAAGGACTCTACATACTCT TTGAGTTCTACTTTGACCCTGTCAAAAGCAGATTATGAAAAACATAAGGTGTATGCATGTGAAGT TACACACCAAGGGTTGTCCTCTCCAGTTACAAAATCTTTTAATAGAGGAGAGTGCCGCCGCAAA CGCGGTAGTGGAGAAGGTCGAGGCTCACTCTTGACCTGTGGCGACGTGGAAGAAAATCCCGGTC CTATGGATTGGACTTGGAGGGTATTTTGTCTTTTGGCAGTAACACCTGGAGCTCACCCCCAAGTA CAGCTCGTCCAATCTGGTGCCGAGGTTAAAAAGCCTGGAAGTTCAGTGAAGGTCTCTTGCAAGG CATCTGGATACACCTTTTCATCTAACGTCATATCCTGGGTACGGCAAGCCCCAGGACAGGGACTT GAGTGGATGGGAGGGGTCATCCCCATCGTGGACATTGCTAATTACGCTCAGCGATTCAAAGGGC GGGTTACTATAACTGCCGACGAGTCTACCTCAACTACCTACATGGAGTTGTCCTCTCTCCGCTCC GAGGACACTGCTGTATATTACTGTGCCAGCACTCTCGGGTTGGTGTTGGATGCCATGGACTATTG GGGACAAGGAACCCTGGTGACAGTTAGCTCCGCAAGCACTAAAGGCCCTTCTGTTTTTCCCTTGG CACCTTGTAGTAGGTCTACCTCTGAGTCTACAGCAGCACTTGGATGCTTGGTTAAGGACTATTTT CCCGAGCCAGTTACAGTCTCTTGGAACAGTGGTGCCCTCACAAGTGGGGTTCATACCTTCCCCGC AGTCCTCCAGAGTAGTGGCCTTTACAGCCTCTCATCAGTTGTGACTGTTCCTAGTTCATCACTCGG TACTAAGACATATACATGTAACGTAGACCACAAGCCAAGCAACACAAAAGTAGACAAACGAGT CGAATCTAAGTATGGACCCCCTTGTCCCTCCTGTCCTGCTCCCGAGTTCCTTGGGGGCCCTTCCGT GTTCTTGTTTCCTCCCAAGCCCAAGGATACCCTCATGATCTCACGAACCCCAGAGGTAACATGTG TGGTTGTTGACGTAAGTCAGGAAGATCCCGAAGTGCAATTTAATTGGTACGTGGATGGCGTCGA AGTCCATAACGCTAAAACAAAACCCCGAGAGGAACAATTCAATTCCACATATCGGGTGGTGAGT GTATTGACCGTTCTTCACCAAGATTGGCTGAACGGCAAGGAGTATAAGTGTAAAGTAAGCAACA AAGGTCTGCCAAGTAGCATAGAAAAAACAATATCTAAAGCTAAGGGCCAACCAAGGGAACCAC AAGTATATACATTGCCCCCCTCTCAGGAAGAGATGACAAAGAATCAAGTTAGCCTGACCTGTTT GGTAAAGGGGTTCTATCCCTCAGATATAGCAGTCGAGTGGGAATCTAACGGCCAGCCCGAGAAT AATTATAAAACAACCCCCCCTGTGTTGGACTCAGACGGCAGCTTCTTTCTCTATTCACGGCTCAC TGTTGATAAGTCCCGATGGCAGGAGGGGAATGTTTTCAGCTGTAGCGTGATGCACGAAGCTCTC CACAACCACTATACACAGAAAAGTTTGTCTTTGTCCCTTGGAAAAtaG
TGFb neutralizing peptide (Human) (SEQ ID NO: 99)
ATGAGTACATCCTTTCCAGAGCTGGATCTGGAGAATTTTGAGTATGACGACAGTGCCGAAGCCT GCTACCTCGGGGACATAGTCGCATTCGGGACAATCTTTTTGTCTGTATTTTACGCCCTGGTGTTTA CATTTGGCCTGGTTGGAAATCTGTTGGTCGTACTCGCTCTCACCAATTCCCGAAAACCCAAAAGT ATAACAGACATATACCTGTTGAATCTGGCACTGAGTGACCTTTTGTTCGTCGCCACCCTTCCTTTT TGGACACACTACCTTATCAGTCACGAGGGGCTTCATAATGCTATGTGCAAGCTCACTACTGCCTT CTTCTTTATCGGATTCTTCGGGGGTATCTTTTTTATCACAGTTATTAGCATTGACCGATACCTTGC CATAGTGCTCGCAGCCAACTCAATGAACAACCGCACCGTGCAGCATGGAGTGACTATTTCCTTG GGTGTGTGGGCCGCTGCTATACTTGTCGCCAGCCCTCAATTCATGTTTACCAAAAGGAAAGACAA TGAGTGCCTCGGAGATTACCCTGAGGTGTTGCAAGAAATGTGGCCTGTACTTCGAAATAGCGAA GTGAATATACTCGGCTTTGCTCTTCCTCTGCTCATCATGTCATTCTGTTATTTTCGAATAATCCAA ACATTGTTCAGCTGTAAGAACCGAAAGAAAGCCCGCGCCGTACGCCTGATTCTGCTCGTTGTGTT CGCCTTTTTTTCTGTTTTGGACTCCTTACAACATAATGATATTCCTGGAGACTCTCAAATTCTATAA CTTTTTTCCCTCCTGTGATATGAAAAGGGACCTTAGATTGGCTCTCAGTGTCACTGAAACAGTAG CCTTTAGCCATTGTTGTCTCAACCCTTTCATATATGCATTTGCAGGGGAAAAGTTCCGGCGGTAT CTCGGACATTTGTATCGGAAGTGCTTGGCCGTGTTGTGTGGTCATCCTGTCCATACCGGATTCTCT CCTGAGAGTCAACGGAGCCGCCAAGATTCAATCCTGTCCAGTTTCACTCACTATACTTCAGAGGG GGATGGCAGCCTTCTGCTC
Kyneurinase #1 (SEQ ID NO: 100)
ATGGAGACCGACACTTTGTTGCTGTGGGTACTTTTGTTGTGGGTCCCAGGATCTACCGGGGATAT GGAACCCTCTCCTCTTGAACTGCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATTGCG ATCCAACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAATTGTCACATTTCAGGAA CTGCTTTTACATTCCAAAGATGAGGGATCTTCCATCCATAGATCTTAGCCTCGTGTCCGAGGATG

ACGATGCCATATATTTTCTTGGGAACAGTCTTGGGTTGCAGCCAAAAATGGTACGGACATATCTC GAAGAGGAGCTGGACAAATGGGCTAAAATGGGTGCTTACGGCCACGACGTGGGAAAACGCCCC TGGATAGTTGGCGACGAATCTATCGTGAGTCTTATGAAAGATATAGTTGGAGCACATGAGAAAG AAATTGCACTGATGAATGCCCTTACTATCAATCTGCATCTCCTCTTGCTTTCATTCTTTAAGCCCA CTCCTAAACGCCACAAAATACTTTTGGAAGCAAAAGCCTTTCCAAGCGACCACTACGCTATTGA GTCACAAATACAACTCCATGGACTTGATGTGGAAAAGTCTATGCGGATGGTAAAACCACGCGAA GGCGAGGAGACCCTTCGAATGGAGGACATACTTGAGGTCATCGAAGAAGAAGGAGATAGTATA GCAGTTATCCTTTTCAGCGGGCTGCACTTCTACACAGGTCAACTCTTTAACATTCCAGCTATTACT AAGGCAGGCCACGCTAAAGGATGCTTCGTGGGCTTTGACCTTGCACACGCAGTAGGAAACGTAG AGCTCCGCTTGCACGATTGGGGCGTTGATTTCGCCTGCTGGTGTTCATATAAGTATCTTAACTCA GGAGCTGGTGGGTTGGCAGGCGCATTCGTACACGAGAAACACGCTCATACCGTAAAGCCTGCAC TGGTAGGGTGGTTCGGACACGATCTCTCTACCCGCTTCAATATGGATAATAAACTCCAGCTTATA CCTGGCGCCAATGGATTCAGGATCTCAAATCCTCCTATTTTGCTCGTTTGCAGTTTGCACGCATCT CTTGAGGTGTTCCAGCAGGCTACCATGACTGCACTCCGCCGGAAGTCAATCCTTTTGACCGGATA CTTGGAGTATATGCTGAAACATTATCACTCAAAAGATAACACTGAGAATAAGGGCCCCATAGTA AACATTATCACTCCATCTCGGGCTGAAGAGCGCGGCTGCCAACTCACATTGACTTTTTCCATTCC CAAGAAGTCAGTGTTCAAAGAGTTGGAGAAACGGGGGGTTGTATGTGATAAGCGGGAGCCAGA TGGAATCCGCGTTGCCCCAGTCCCCCTCTATAATTCTTTTCACGATGTATACAAGTTTATTAGACT GCTGACAAGTATCTTGGACTCATCTGAGCGATCTtaG
Kyneurinase #2 (SEQ ID NO: 101)
ATGGAACCCTCTCCTCTTGAACTGCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATTG CGATCCAACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAATTGTCACATTTCAGG AACTGCTTTTACATTCCAAAGATGAGGGATCTTCCATCCATAGATCTTAGCCTCGTGTCCGAGGA TGACGATGCCATATATTTTCTTGGGAACAGTCTTGGGTTGCAGCCAAAAATGGTACGGACATATC TCGAAGAGGAGCTGGACAAATGGGCTAAAATGGGTGCTTACGGCCACGACGTGGGAAAACGCC CCTGGATAGTTGGCGACGAATCTATCGTGAGTCTTATGAAAGATATAGTTGGAGCACATGAGAA AGAAATTGCACTGATGAATGCCCTTACTATCAATCTGCATCTCCTCTTGCTTTCATTCTTTAAGCC CACTCCTAAACGCCACAAAATACTTTTGGAAGCAAAAGCCTTTCCAAGCGACCACTACGCTATTG AGTCACAAATACAACTCCATGGACTTGATGTGGAAAAGTCTATGCGGATGGTAAAACCACGCGA AGGCGAGGAGACCCTTCGAATGGAGGACATACTTGAGGTCATCGAAGAAGAAGGAGATAGTAT AGCAGTTATCCTTTTCAGCGGGCTGCACTTCTACACAGGTCAACTCTTTAACATTCCAGCTATTAC TAAGGCAGGCCACGCTAAAGGATGCTTCGTGGGCTTTGACCTTGCACACGCAGTAGGAAACGTA GAGCTCCGCTTGCACGATTGGGGCGTTGATTTCGCCTGCTGGTGTTCATATAAGTATCTTAACTC AGGAGCTGGTGGGTTGGCAGGCGCATTCGTACACGAGAAACACGCTCATACCGTAAAGCCTGCA CTGGTAGGGTGGTTCGGACACGATCTCTCTACCCGCTTCAATATGGATAATAAACTCCAGCTTAT ACCTGGCGCCAATGGATTCAGGATCTCAAATCCTCCTATTTTGCTCGTTTGCAGTTTGCACGCATC TCTTGAGGTGTTCCAGCAGGCTACCATGACTGCACTCCGCCGGAAGTCAATCCTTTTGACCGGAT ACTTGGAGTATATGCTGAAACATTATCACTCAAAAGATAACACTGAGAATAAGGGCCCCATAGT AAACATTATCACTCCATCTCGGGCTGAAGAGCGCGGCTGCCAACTCACATTGACTTTTTCCATTC CCAAGAAGTCAGTGTTCAAAGAGTTGGAGAAACGGGGGGTTGTATGTGATAAGCGGGAGCCAG ATGGAATCCGCGTTGCCCCAGTCCCCCTCTATAATTCTTTTCACGATGTATACAAGTTTATTAGAC TGCTGACAAGTATCTTGGACTCATCTGAGCGATCTtaG
VEGF (SEQ ID NO: 102)
ATGAATTTCTTGCTGAGCTGGGTGCATTGGACACTCGCATTGTTGCTGTACTTGCACCATGCCAA GTGGTCCCAGGCTGCACCCACTACTGAGGGCGAGCAAAAGTCTCATGAGGTGATTAAATTTATG GACGTTTACCAACGATCATACTGTCGGCCAATCGAAACCCTCGTAGATATATTCCAGGAGTACCC AGACGAGATCGAATACATTTTCAAGCCCTCATGTGTCCCATTGATGCGATGTGCTGGGTGCTGTA ACGACGAAGCACTTGAATGTGTCCCCACCTCCGAGAGTAACATCACAATGCAAATAATGAGAAT CAAGCCCCACCAATCCCAACATATCGGTGAAATGTCATTCCTTCAGCATTCCCGCTGCGAGTGCC GGCCTAAGAAGGACCGCACCAAACCAGAGAACCATTGTGAACCCTGTTCTGAGAGACGGAAGC ACTTGTTCGTACAGGACCCTCAAACATGCAAGTGCAGCTGTAAGAATACCGACTCACGGTGTAA AGCTAGGCAACTGGAGCTTAATGAAAGGACCTGCCGATGCGATAAACCCAGGAGGtaa
GM-CSF (SEQ ID NO: 103)
ATGTGGTTGCAGAATTTGCTCTTCCTGGGGATTGTGGTCTACAGCCTCTCCGCACCTACCCGCTCT CCTATCACAGTTACAAGACCCTGGAAACATGTGGAGGCCATTAAAGAAGCATTGAATTTGTTGG ACGATATGCCCGTCACCCTGAATGAAGAAGTAGAAGTTGTTTCTAATGAGTTCAGCTTTAAAAA ATTGACCTGTGTGCAGACACGGCTTAAAATTTTTGAACAGGGACTTAGAGGAAACTTTACTAAG

CTGAAGGGGGCACTTAACATGACAGCTTCTTATTATCAGACCTATTGTCCTCCAACACCTGAAAC CGACTGTGAAACACAGGTAACCACTTACGCCGATTTTATTGATTCTTTGAAAACATTCCTCACCG ATATACCATTTGAGTGTAAGAAGCCAGGCCAAAAGtaG
Anti-PD1 (SEQ ID NO: 104)
ATGGAAACTGACACACTTCTTCTGTGGGTCTTGCTCCTGTGGGTCCCAGGCTCTACTGGTGACAG TCCTGATAGGCCATGGAACCCACCTACCTTTAGTCCAGCCTTGCTCGTCGTAACCGAAGGGGACA ACGCTACATTCACCTGCTCTTTTAGCAATACTTCTGAGAGTTTTCATGTAGTCTGGCATCGGGAG AGTCCATCCGGACAAACAGATACTTTGGCCGCTTTTCCAGAGGATAGGTCTCAACCTGGGCAAG ACGCAAGGTTTCGAGTCACACAGCTTCCTAACGGGAGAGATTTTCACATGTCTGTAGTTCGGGCA CGCCGAAATGATTCTGGCACATATGTTTGCGGTGTGATCTCACTTGCTCCAAAGATTCAAATAAA GGAGAGCCTTCGCGCCGAGTTGCGGGTGACTGAGCGGGAGCCCAAGTCCTGCGACAAAACCCAT ACTTGTCCACCCTGTGGCGGCGGGTCATCCGGTGGCGGGTCTGGGGGGCAACCAAGAGAGCCAC AGGTATATACTCTTCCCCCCAGCAGAGAAGAAATGACAAAAAACCAAGTGTCCCTGACATGTCT GGTTAAAGGATTTTATCCCAGTGACATTGCTGTAGAATGGGAATCCAATGGTCAACCCGAGAAT AACTACAAAACCACTCCTCCAGTATTGGACAGTGACGGTTCCTTCTTCCTCTATTCCAAACTTAC AGTGGATAAATCCCGCTGGCAGCAAGGGAATGTATTCAGCTGTAGTGTCATGCACGAAGCTCTT CATAACCATTATACACAGAAATCTCTTTCCCTGAGCCCAGGTAAAtaG
Adenosine Deaminase (ADA) #1 (Mouse) (SEQ ID NO: 105)
ATGGAGACTGATACACTTTTGCTCTGGGTTTTGCTCTTGTGGGTACCAGGGTCTACTGGAGATGC ACAAACTCCTGCATTCAACAAGCCTAAGGTAGAGCTTCATGTCCATTTGGACGGAGCCATAAAA CCTGAAACCATACTCTATTTCGGCAAGAAACGGGGTATAGCACTTCCCGCTGATACCGTGGAAG AGTTGAGAAATATCATTGGCATGGACAAACCTCTTAGCCTGCCTGGCTTTCTTGCAAAGTTCGAC TACTATATGCCAGTTATAGCAGGGTGTAGAGAAGCAATAAAGCGAATCGCCTATGAGTTCGTTG AGATGAAGGCTAAAGAAGGAGTTGTTTACGTGGAAGTCCGGTACTCACCTCATCTGCTTGCTAAT AGCAAGGTGGACCCAATGCCATGGAATCAAACTGAAGGTGATGTAACCCCTGACGATGTGGTCG ATTTGGTCAATCAAGGTCTCCAAGAAGGCGAGCAGGCTTTCGGCATTAAGGTAAGAAGTATATT GTGCTGTATGCGACATCAACCTTCATGGTCCCTGGAGGTCCTCGAATTGTGCAAAAAGTACAATC AAAAAACAGTGGTCGCAATGGATCTCGCTGGAGATGAGACCATAGAAGGTTCCTCTCTTTTCCCC GGTCATGTCGAAGCATATGAAGGGGCTGTCAAAAATGGTATCCACCGCACCGTCCACGCAGGGG AAGTAGGGTCCCCAGAAGTAGTCAGGGAAGCCGTTGACATTTTGAAAACAGAAAGAGTCGGGC ATGGCTACCATACAATAGAGGACGAAGCCTTGTACAATCGACTTTTGAAAGAAAATATGCACTT CGAGGTCTGTCCCTGGAGTTCATATCTCACCGGAGCATGGGACCCCAAAACAACCCACGCCGTC GTACGCTTCAAGAATGATAAGGCAAACTACAGTTTGAATACAGATGATCCACTGATATTCAAGT CAACACTTGACACTGACTACCAGATGACAAAAAAAGATATGGGTTTCACCGAAGAAGAGTTCAA GAGATTGAACATTAACGCAGCAAAAAGCTCCTTCCTGCCAGAGGAAGAGAAAAAAGAATTGCTT GAAAGGTTGTATCGAGAATACCAA
Adenosine Deaminase (ADA) #2 (Mouse) (SEQ ID NO: 106)
ATGGCACAAACTCCAGCTTTTAATAAGCCCAAAGTGGAACTTCATGTTCATCTGGATGGGGCAAT TAAGCCCGAAACTATATTGTACTTTGGCAAAAAGAGGGGTATTGCCCTGCCAGCAGATACCGTT GAGGAGCTTCGCAACATCATTGGGATGGACAAGCCCCTCTCTCTGCCAGGTTTTCTCGCTAAATT CGATTATTATATGCCTGTTATTGCTGGTTGCCGGGAGGCCATCAAGAGGATAGCCTACGAGTTTG TTGAGATGAAGGCCAAAGAGGGCGTGGTGTACGTAGAGGTCAGATACAGCCCTCACCTGCTTGC CAACAGCAAGGTGGACCCAATGCCCTGGAACCAAACCGAGGGGGATGTCACTCCCGACGACGTT GTAGACCTCGTAAATCAGGGCCTTCAAGAGGGCGAGCAGGCATTTGGCATAAAAGTCCGGTCTA TACTCTGCTGTATGAGGCACCAACCCTCCTGGTCTTTGGAGGTACTTGAGTTGTGTAAGAAATAC AATCAAAAGACTGTAGTCGCCATGGATCTTGCAGGCGATGAAACCATCGAGGGTAGCTCCTTGT TCCCTGGACATGTTGAAGCCTACGAGGGGGCCGTAAAAAATGGGATACACAGGACTGTCCACGC TGGTGAAGTCGGAAGCCCAGAGGTGGTAAGGGAGGCAGTTGACATACTCAAGACAGAGCGGGT TGGACACGGATACCACACAATTGAGGACGAGGCCCTGTATAACCGCCTCCTCAAAGAGAACATG CATTTTGAGGTGTGTCCTTGGTCCAGCTACCTGACTGGTGCTTGGGACCCTAAAACAACTCACGC CGTGGTCCGGTTCAAGAACGATAAAGCCAATTACTCTTTGAATACCGACGACCCCCTCATATTCA AATCAACATTGGATACCGACTACCAAATGACCAAAAAGGATATGGGGTTTACTGAAGAGGAGTT CAAGAGGCTCAACATAAATGCCGCTAAATCCTCCTTTCTCCCCGAGGAAGAAAAAAAAGAACTC CTTGAGCGGCTGTATAGGGAGTATCAA
4-1BBL #1 (Mouse) (SEQ ID NO: 107)

ATGGAAACAGATACACTCTTGCTCTGGGTACTGCTTCTGTGGGTCCCCGGCTCTACTGGGGATGA AGATGATGTAACTACTACAGAAGAACTCGCTCCCGCTCTTGTCCCCCCACCCAAGGGTACCTGCG CCGGTTGGATGGCTGGCATCCCAGGACATCCAGGTCACAACGGTACCCCCGGAAGAGATGGTCG GGATGGAACTCCCGGCGAGAAGGGCGAAAAAGGGGATGCAGGGCTTCTGGGACCTAAAGGTGA AACAGGGGACGTTGGAATGACTGGTGCAGAAGGGCCTCGCGGCTTTCCTGGCACCCCTGGGAGG AAAGGAGAGCCCGGAGAGCTCCAGAGAACTGAACCTCGGCCTGCACTCACTATAACTACTTCCC CTAATCTTGGGACCCGCGAGAACAACGCCGATCAGGTTACACCTGTAAGCCATATCGGGTGCCC CAATACTACCCAGCAAGGGAGTCCCGTGTTCGCAAAGCTTTTGGCTAAAAACCAAGCATCCCTG TGTAACACTACTCTTAATTGGCATTCACAAGACGGTGCTGGTAGCTCTTATCTTTCTCAGGGGCT GCGGTACGAAGAAGATAAGAAGGAATTGGTTGTGGATTCTCCAGGACTCTATTATGTCTTTCTCG AATTGAAGCTCAGTCCCACCTTCACAAACACTGGACACAAAGTCCAGGGCTGGGTAAGTCTGGT ACTCCAAGCAAAGCCCCAGGTTGACGATTTCGACAATTTGGCACTCACCGTAGAGCTTTTCCCAT GCTCCATGGAAAATAAACTTGTTGATCGGTCATGGTCACAGCTCTTGCTGCTTAAGGCAGGGCAT CGCCTCTCAGTGGGTCTGAGAGCTTATTTGCATGGTGCACAAGATGCTTACAGGGATTGGGAATT GTCCTACCCAAACACTACAAGTTTCGGGTTGTTCCTTGTCAAACCTGATAACCCATGGGAGtaG
4-1BBL #2 (Mouse) (SEQ ID NO: 108)
ATGGAAACTGATACACTCCTCCTGTGGGTCCTTCTTTTGTGGGTGCCCGGATCAACCGGCGATGG CTGGATGGCAGGCATCCCAGGACACCCAGGACACAACGGTACTCCAGGTCGAGACGGTCGGGAT GGGACTCCTGGGGAGAAAGGCGAGAAAGGGGACGCTGGTTTGCTCGGTCCTAAGGGGGAAACC GGGGATGTAGGAATGACAGGGGCTGAAGGGCCTCGGGGATTTCCTGGGACACCAGGCAGGAAG GGTGAACCAGGGGAGGCCCTCCAGCGCACCGAGCCACGGCCAGCTCTGACCATAACAACAAGT CCAAACCTGGGCACACGCGAAAACAATGCTGACCAGGTGACTCCTGTAAGTCACATCGGATGCC CTAACACTACACAACAGGGCTCTCCTGTATTTGCAAAGCTTCTCGCAAAAAATCAAGCATCACTT TGTAATACAACCCTGAACTGGCATTCTCAGGACGGAGCAGGGTCCTCTTATTTGTCTCAAGGGCT CCGCTACGAAGAAGATAAAAAGGAATTGGTTGTTGACAGTCCAGGTTTGTATTATGTGTTTTTGG AACTTAAGCTGTCACCAACCTTCACTAACACCGGCCACAAGGTCCAAGGCTGGGTTAGTCTTGTT TTGCAAGCCAAACCTCAAGTGGATGATTTTGACAATCTGGCTTTGACTGTTGAGCTTTTTCCATG CAGTATGGAGAATAAACTGGTTGATCGGTCATGGTCACAGCTCCTTCTGCTCAAGGCCGGACAT AGGCTGAGTGTGGGACTTCGGGCCTACTTGCACGGCGCCCAGGACGCATACCGAGACTGGGAAC TCAGCTACCCTAACACAACTTCTTTTGGGTTGTTCCTTGTCAAACCCGATAATCCTTGGGAAtaG
HPGE2 #1 (Mouse) (SEQ ID NO: 109)
ATGGAGACTGATACTTTGCTCCTGTGGGTTCTTCTCCTGTGGGTTCCTGGTTCCACAGGGGATAT GCATGTCAATGGCAAGGTAGCACTCGTGACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTGCC GAGGCCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACTTGGAGGCTGGAGTTA AATGCAAAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCCTCTTTGTGCAGTGTGACGTT GCTGACCAAAAGCAACTCAGGGACACATTCAGGAAGGTCGTAGACCATTTCGGACGCCTCGATA TACTCGTTAATAATGCCGGGGTAAACAACGAAAAGAACTGGGAACAAACATTGCAAATCAACCT GGTAAGTGTCATTAGCGGAACTTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGGGCGAG GGCGGGATCATTATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCCTGTTTA CTGTGCCAGCAAGCACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAATCTTATGA AGAGTGGGGTAAGACTGAATGTTATCTGCCCAGGTTTCGTAGATACCCCAATCCTGGAGAGCAT CGAGAAGGAGGAAAATATGGGACAATACATTGAATATAAAGATCAAATCAAGGCTATGATGAA GTTCTACGGGGTTCTGCATCCATCCACAATTGCCAACGGGCTCATTAATCTGATTGAGGACGACG CCTTGAACGGAGCTATAATGAAAATCACAGCTTCCAAAGGCATTCACTTCCAAGATTATGATATA TCACCCTTGCTTGTCAAGGCTCCTCTGACAAGT
HPGE2 #2 (Mouse) (SEQ ID NO: 110)
ATGCATGTCAATGGCAAGGTAGCACTCGTGACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTG CCGAGGCCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACTTGGAGGCTGGAGTT AAATGCAAAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCCTCTTTGTGCAGTGTGACG TTGCTGACCAAAAGCAACTCAGGGACACATTCAGGAAGGTCGTAGACCATTTCGGACGCCTCGA TATACTCGTTAATAATGCCGGGGTAAACAACGAAAAGAACTGGGAACAAACATTGCAAATCAAC CTGGTAAGTGTCATTAGCGGAACTTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGGGCG AGGGCGGGATCATTATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCCTGTT TACTGTGCCAGCAAGCACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAATCTTAT GAAGAGTGGGGTAAGACTGAATGTTATCTGCCCAGGTTTCGTAGATACCCCAATCCTGGAGAGC ATCGAGAAGGAGGAAAATATGGGACAATACATTGAATATAAAGATCAAATCAAGGCTATGATG AAGTTCTACGGGGTTCTGCATCCATCCACAATTGCCAACGGGCTCATTAATCTGATTGAGGACGA

CGCCTTGAACGGAGCTATAATGAAAATCACAGCTTCCAAAGGCATTCACTTCCAAGATTATGAT ATATCACCCTTGCTTGTCAAGGCTCCTCTGACAAGT
Human IL-15 Polypeptide Sequence (SEQ ID NO: 199)
MVLGTIDLCSCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS
Human IL-15 Polypeptide Sequence (SEQ ID NO: 224)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS
Human IL-15Rα Polypeptide Sequence (SEQ ID NO: 200)
MAPRRARGCRTLGLPALLLLLLLRPPATRGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSN NTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT VAISTSTVLLCGLSAVSLLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL
Human IL-15Rα sushi domain Polypeptide Sequence (SEQ ID NO: 201)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
Human IL-15/IL-15Rα sushi domain Polypeptide Sequence (SEQ ID NO: 202)
MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQ VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSSGGSG GGGSGGGSGGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATN VAHWTTPSLKCIR
IL-12 (IL-12p70) Polypeptide Sequence (SEQ ID NO: 203)
MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSS EVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEA KNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACP AAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSY FSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGSGGG SGGGSGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTS TVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLM DPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYL NAS

Secretion Signals and Signal-Anchors

The one or more effector molecules of the chimeric proteins provided for herein can be secretable effector molecules having a secretion signal peptide (also referred to as a signal peptide or signal sequence) at the chimeric protein's N-terminus (*e.g.*, an effector molecule's N-terminus for **S – C – MT**) that direct newly synthesized proteins destined for secretion or membrane localization (also referred to as membrane insertion) to the proper protein processing pathways. For chimeric proteins having the formula **MT – C – S**, a membrane tethering domain generally has a signal-anchor sequence (*e.g.*, signal-anchor sequences of a Type II transmembrane protein) that direct newly synthesized proteins destined for membrane localization to the proper protein processing pathways. For chimeric proteins having the formula **S – C – MT**, a membrane tethering domain having a reverse signal-anchor sequence (*e.g.*, signal-anchor sequences of certain Type III transmembrane proteins) can be used, generally

without a separate secretion signal peptide, that direct newly synthesized proteins destined for membrane localization to the proper protein processing pathways.

In general, for all membrane-cleavable chimeric proteins described herein, the one or more effector molecules are secretable effector molecules (referred to as "S" in the formula **S – C – MT** or **MT – C – S**). In embodiments with two or more chimeric proteins, each chimeric protein can comprise a secretion signal. In embodiments with two or more chimeric proteins, each chimeric protein can comprise a secretion signal such that each effector molecule is capable of secretion from an engineered cell following cleavage of the protease cleavage site.

The secretion signal peptide operably associated with an effector molecule can be a native secretion signal peptide (*e.g.*, the secretion signal peptide generally endogenously associated with the given effector molecule). The secretion signal peptide operably associated with an effector molecule can be a non-native secretion signal peptide native secretion signal peptide. Non-native secretion signal peptides can promote improved expression and function, such as maintained secretion, in particular environments, such as tumor microenvironments. Non-limiting examples of non-native secretion signal peptide are shown in **Table 3**.

A secretion signal peptide can be an IgE signal peptide. An IgE signal peptide can include the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228). An IgE signal peptide can be encoded by a polynucleotide sequence that includes the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229). An IgE signal peptide can be encoded by a polynucleotide sequence that includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229).

A secretion signal peptide of the membrane-cleavable chimeric protein (*e.g.*, IL-15) can be an IgE signal peptide. An IgE signal peptide of the membrane-cleavable chimeric protein (*e.g.*, IL-15) can include the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228). An IgE signal peptide of the membrane-cleavable chimeric protein (*e.g.*, IL-15) can be encoded by a polynucleotide sequence that includes the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229). An IgE signal peptide of the membrane-cleavable chimeric protein (*e.g.*, IL-15) can be encoded by a polynucleotide sequence that includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229).

Table 3. Exemplary Signal Secretion Peptides

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
IL-12	MCHQQLVISWFSLV FLASPLVA (SEQ ID NO: 112)	P29460	ATGTGTCACCAGCAGCTCGTTATATC CTGGTTTAGTTTGGTGTTTCTCGCTT CACCCCTGGTGGCA (SEQ ID NO: 31)
IL-12 (Codon Optimized)	MCHQQLVISWFSLV FLASPLVA (SEQ ID NO: 112)	-	ATGTGCCATCAGCAACTCGTCATCTC CTGGTTCTCCCTTGTGTTCCTCGCTTC CCCTCTGGTCGCC (SEQ ID NO: 32)
IL-2 (Optimized)	MQLLSCIALILALV (SEQ ID NO: 113)	-	ATGCAACTGCTGTCATGTATCGCACT CATCCTGGCGCTGGTA (SEQ ID NO: 33)
IL-2 (Native)	MYRMQLLSCIALSL ALVTNS (SEQ ID NO: 114)	P60568	ATGTATCGGATGCAACTTTTGAGCTG CATCGCATTGTCTCTGGCGCTGGTGA CAAATTCC (SEQ ID NO: 34)
Trypsinogen-2	MNLLLILTFVAAAV A (SEQ ID NO: 115)	P07478	ATGAATCTCTTGCTCATACTTACGTT TGTCGCTGCTGCCGTTGCG (SEQ ID NO: 35)
Gaussia Luciferase	MGVKVLFALICIAV AEA (SEQ ID NO: 116)	-	ATGGGCGTGAAGGTCTTGTTTGCCCT TATCTGCATAGCTGTTGCGGAGGCG (SEQ ID NO: 36)
CD5	MPMGSLQPLATLYL LGMLVASCLG (SEQ ID NO: 117)	P06127	ATGCCGATGGGGAGCCTTCAACCTTT GGCAACGCTTTATCTTCTGGGGATGT TGGTTGCTAGTTGCCTTGGG (SEQ ID NO: 37)
IgKVII (mouse)	METDTLLLWVLLL WVPGSTGD (SEQ ID NO: 118)		ATGGAAACTGACACGTTGTTGCTGT GGGTATTGCTCTTGTGGGTCCCAGGA TCTACGGGCGAC (SEQ ID NO: 38)
IgKVII (human)	MDMRVPAQLLGLL LLWLRGARC (SEQ ID NO: 119)	P01597	ATGGATATGAGGGTTCCCGCCCAGC TTTTGGGGCTGCTTTTGTTGTGGCTT CGAGGGGCTCGGTGT (SEQ ID NO: 39)
VSV-G	MKCLLYLAFLFIGV NC (SEQ ID NO: 120)	-	ATGAAGTGTCTGTTGTACCTGGCGTT TCTGTTCATTGGTGTAAACTGT (SEQ ID NO: 40)
Prolactin	MNIKGSPWKGSLLL LLVSNLLLCQSVAP (SEQ ID NO: 121)	P01236	ATGAATATCAAAGGAAGTCCGTGGA AGGGTAGTCTCCTGCTGCTCCTCGTA TCTAACCTTCTCCTTTGTCAATCCGT GGCACCC (SEQ ID NO: 41)
Serum albumin preproprotein	MKWVTFISLLFLFSS AYS (SEQ ID NO: 122)	P02768	ATGAAATGGGTAACATTCATATCAC TTCTCTTTCTGTTCAGCTCTGCGTATT CT (SEQ ID NO: 42)
Azurocidin preproprotein	MTRLTVLALLAGLL ASSRA (SEQ ID NO: 123)	20160	ATGACAAGGCTTACTGTTTTGGCTCT CCTCGCTGGACTCTTGGCTTCCTCCC GAGCA (SEQ ID NO: 43)

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
Osteonectin (BM40)	MRAWIFFLLCLAGR ALA (SEQ ID NO: 124)	P09486	ATGAGGGCTTGGATTTTTTTTCTGCT CTGCCTTGCCGGTCGAGCCCTGGCG (SEQ ID NO: 44)
CD33	MPLLLLLPLLWAGA LA (SEQ ID NO: 125)	P20138	ATGCCTCTTCTGCTTTTGCTTCCTCTT TTGTGGGCAGGTGCCCTCGCA (SEQ ID NO: 45)
IL-6	MNSFSTSAFGPVAFS LGLLLVLPAAFPAP (SEQ ID NO: 126)	P05231	ATGAACTCTTTCTCAACCTCTGCGTT TGGTCCGGTCGCTTTCTCCCTTGGGC TCCTGCTTGTCTTGCCAGCAGCGTTT CCTGCGCCA (SEQ ID NO: 46)
IL-8	MTSKLAVALLAAFL ISAALC (SEQ ID NO: 127)	P10145	ATGACAAGTAAACTGGCGGTAGCCT TGCTCGCGGCCTTTTTGATTTCCGCA GCCCTTTGT (SEQ ID NO: 47)
CCL2	MKVSAALLCLLLIA ATFIPQGLA (SEQ ID NO: 128)	P13500	ATGAAGGTAAGTGCAGCGTTGCTTT GCCTTCTCCTCATTGCAGCGACCTTT ATTCCTCAAGGGCTGGCC (SEQ ID NO: 48)
TIMP2	MGAAARTLRLALGL LLLATLLRPADA (SEQ ID NO: 129)	P16035	ATGGGAGCGGCAGCTAGAACACTTC GACTTGCCCTTGGGCTCTTGCTCCTT GCAACCCTCCTTAGACCTGCCGACG CA (SEQ ID NO: 49)
VEGFB	MSPLLRRLLLAALL QLAPAQA (SEQ ID NO: 130)	P49765	ATGTCACCGTTGTTGCGGAGATTGCT GTTGGCCGCACTTTTGCAACTGGCTC CTGCTCAAGCC (SEQ ID NO: 50)
Osteoprotegerin	MNNLLCCALVFLDI SIKWTTQ (SEQ ID NO: 131)	O00300	ATGAATAACCTGCTCTGTTGTGCGCT CGTGTTCCTGGACATTTCTATAAAAT GGACAACGCAA (SEQ ID NO: 51)
Serpin E1	MQMSPALTCLVLGL ALVFGEGSA (SEQ ID NO: 132)	P05121	ATGCAAATGTCTCCTGCCCTTACCTG TCTCGTACTTGGTCTTGCGCTCGTAT TTGGAGAGGGATCAGCC (SEQ ID NO: 52)
GROalpha	MARAALSAAPSNPR LLRVALLLLLLVAA GRRAAG (SEQ ID NO: 133)	P09341	ATGGCAAGGGCTGCACTCAGTGCTG CCCCGTCTAATCCCAGATTGCTTCGA GTTGCATTGCTTCTTCTGTTGCTGGT TGCAGCTGGTAGGAGAGCAGCGGGT (SEQ ID NO: 53)
CXCL12	MNAKVVVVLVLVL TALCLSDG (SEQ ID NO: 134)	P48061	ATGAATGCAAAAGTCGTGGTCGTGC TGGTTTTGGTTCTGACGGCGTTGTGT CTTAGTGATGGG (SEQ ID NO: 54)
IL-21 (Codon Optimized)	MERIVICLMVIFLGT LVHKSSS (SEQ ID NO: 135)	Q9HBE4	ATGGAACGCATTGTGATCTGCCTGAT GGTCATCTTCCTGGGCACCTTAGTGC ACAAGTCGAGCAGC (SEQ ID NO: 55)
CD8	MALPVTALLLPLAL LLHAARP (SEQ ID NO: 136)	-	ATGGCCTTACCAGTGACCGCCTTGCT CCTGCCGCTGGCCTTGCTGCTCCACG CCGCCAGGCCG (SEQ ID NO: 139)

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
CD8 (Codon Optimized)	MALPVTALLLPLAL LLHAARP (SEQ ID NO: 137)	-	ATGGCGCTCCCGGTGACAGCACTTCT CTTGCCTCTTGCCCTGCTGTTGCATG CCGCGCGCCCA (SEQ ID NO: 140) OR ATGGCTCTGCCCGTGACAGCTTTGCT GCTGCCTTTGGCACTGCTGCTGCATG CTGCTAGACCA (SEQ ID NO: 416)
GMCSF	MLLVTSLLLCELPHP AFLLIP (SEQ ID NO: 138)	-	ATGTTGCTCGTGACATCCCTCTTGCT TTGTGAGTTGCCTCATCCCGCATTCC TGCTCATCCCA (SEQ ID NO: 141)
NKG2D	PFFFCCFIAVAMGIR FIIMVA (SEQ ID NO: 192)		CCCTTCTTCTTCTGTTGCTTTATCGCC GTGGCCATGGGCATCCGCTTCATCAT TATGGTGGCC (SEQ ID NO: 193)
IgE	MDWTWILFLVAAA TRVHS (SEQ ID NO: 228)		ATGGACTGGACTTGGATACTCTTTCT GGTCGCTGCCGCCACACGGGTGCAC TCT (SEQ ID NO: 229)
GM-CSFRa	MLLLVTSLLLCELPH PAFLLIP (SEQ ID 423).		ATGCTGCTGCTGGTTACATCTCTGCT GCTGTGCGAGCTGCCCCATCCTGCCT TTCTGCTTATTCCT (SEQ ID NO: 424)

Protease Cleavage Site

In certain embodiments, the chimeric proteins provided for herein (*e.g.*, in general, for all membrane-cleavable chimeric proteins described herein) contain a protease cleavage site (*e.g.*, referred to as "C" in the formula **S – C – MT** or **MT – C – S** for membrane-cleavable chimeric proteins described herein). In general, the protease cleavage site can be any amino acid sequence motif capable of being cleaved by a protease. Examples of protease cleavage sites include, but are not limited to, a Type 1 transmembrane protease cleavage site, a Type II transmembrane protease cleavage site, a GPI anchored protease cleavage site, an ADAM8 protease cleavage site, an ADAM9 protease cleavage site, an ADAM10 protease cleavage site, an ADAM12 protease cleavage site, an ADAM15 protease cleavage site, an ADAM17 protease cleavage site, an ADAM19 protease cleavage site, an ADAM20 protease cleavage site, an ADAM21 protease cleavage site, an ADAM28 protease cleavage site, an ADAM30 protease cleavage site, an ADAM33 protease cleavage site, a BACE1 protease cleavage site, a BACE2 protease cleavage site, a SIP protease cleavage site, an MT1-MMP protease cleavage site, an MT3-MMP protease cleavage site, an MT5-MMP protease cleavage site, a furin protease cleavage site, a PCSK7 protease cleavage site, a matriptase protease cleavage site, a matriptase-2 protease cleavage site, an MMP9 protease cleavage site, or an NS3 protease cleavage site.

One example of a protease cleavage site is a hepatitis C virus (HCV) nonstructural protein 3 (NS3) protease cleavage site, including, but not limited to, a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B cleavage site. For a description of NS3 protease

and representative sequences of its cleavage sites for various strains of HCV, see, e.g., Hepatitis C Viruses: Genomes and Molecular Biology (S.L. Tan ed., Taylor & Francis, 2006), Chapter 6, pp. 163-206; herein incorporated by reference in its entirety. For example, the sequences of HCV NS4A/4B protease cleavage site; HCV NS5A/5B protease cleavage site; C-terminal degron with NS4A/4B protease cleavage site; N-terminal degron with HCV NS5A/5B protease cleavage site are provided. Representative NS3 sequences are listed in the National Center for Biotechnology Information (NCBI) database. See, for example, NCBI entries: Accession Nos. YP_001491553, YP_001469631, YP_001469632, NP_803144, NP_671491, YP_001469634, YP_001469630, YP_001469633, ADA68311, ADA68307, AFP99000, AFP98987, ADA68322, AFP99033, ADA68330, AFP99056, AFP99041, CBF60982, CBF60817, AHH29575, AIZ00747, AIZ00744, ABI36969, ABN05226, KF516075, KF516074, KF516056, AB826684, AB826683, JX171009, JX171008, JX171000, EU847455, EF154714, GU085487, JX171065, JX171063; all of which sequences (as entered by the date of filing of this application) are herein incorporated by reference.

Another example of a protease cleavage site is an ADAM17-specific protease (also referred to as Tumor Necrosis Factor-α Converting Enzyme [TACE]) cleavage site. An ADAM17-specific protease cleavage site can be an endogenous sequence of a substrate naturally cleaved by ADAM17. An ADAM17-specific protease cleavage site can be an engineered sequence capable of being cleaved by ADAM17. An engineered ADAM17-specific protease cleavage site can be an engineered for specific desired properties including, but not limited to, optimal expression of the chimeric proteins, specificity for ADAM17, rate-of-cleavage by ADAM17, ratio of secreted and membrane-bound chimeric protein levels, and cleavage in different cell states. A protease cleavage site can be selected for specific cleavage by ADAM17. For example, certain protease cleavage sites capable of being cleaved by ADAM17 are also capable of cleavage by additional ADAM family proteases, such as ADAM10. Accordingly, an ADAM17-specific protease cleavage site can be selected and/or engineered such that cleavage by other proteases, such as ADAM10, is reduced or eliminated. A protease cleavage site can be selected for rate-of-cleavage by ADAM17. For example, it can be desirable to select a protease cleavage site demonstrating a specific rate-of-cleavage by ADAM17, such as reduced cleavage kinetics relative to an endogenous sequence of a substrate naturally cleaved by ADAM17. In such cases, in general, a specific rate-of-cleavage can be selected to regulate the rate of processing of the chimeric protein, which in turn regulates the rate of release/secretion of the payload effector molecule. Accordingly, an ADAM17-specific protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage by ADAM17. A protease cleavage site can be selected for both specific cleavage by ADAM17 and

rate-of-cleavage by ADAM17. Exemplary ADAM17-specific protease cleavage sites, including those demonstrating particular specificity and rate-of-cleavage kinetics, are shown in **Table 4A** below with reference to the site of cleavage (P5-P1: N-terminal; P1'-P5': C-terminal). Further details of ADAM17 and ADAM10, including expression and protease cleavage sites, are described in Sharma, *et al.* (J Immunol October 15, 2017, 199 (8) 2865-2872), Pham *et al.* (Anticancer Res. 2017 Oct;37(10):5507-5513), Caescu *et al.* (Biochem J. 2009 Oct 23; 424(1): 79–88), and Tucher *et al.* (J. Proteome Res. 2014, 13, 4, 2205–2214), each herein incorporated by reference for purposes.

Table 4A – Various ADAM17 Protease Cleavage Site Sequences

P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'	P5'		FULL SEQ	SEQ ID NO
P	R	A	E	A	V	K	G	G			PRAEAVKGG	179
P	R	A	E	A	L	K	G	G			PRAEALKGG	180
P	R	A	E	Y	S	K	G	G			PRAEYSKGG	181
P	R	A	E	P	I	K	G	G			PRAEPIKGG	182
P	R	A	E	A	Y	K	G	G			PRAEAYKGG	183
P	R	A	E	S	S	K	G	G			PRAESSKGG	184
P	R	A	E	F	T	K	G	G			PRAEFTKGG	185
D	E	P	H	Y	S	Q	R	R			DEPHYSQRR	187
P	P	L	G	P	I	F	N	P	G		PPLGPIFNPG	188
P	L	A	Q	A	Y	R	S	S			PLAQAYRSS	189
T	P	I	D	S	S	F	N	P	D		TPIDSSFNPD	190
V	T	P	E	P	I	F	S	L	I		VTPEPIFSLI	191

In some embodiments, the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176). In some embodiments, the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177). In some embodiments, the first region is located N-terminal to the second region. In some embodiments, the protease cleavage site comprises the amino acid sequence of $PRAEX_1X_2KGG$ (SEQ ID NO: 178), wherein X_1 is A, Y, P, S, or F, and wherein X_2 is V, L, S, I, Y, T, or A. In some embodiments, the protease cleavage site comprises the amino acid sequence of $PRAEX_1X_2KGG$ (SEQ ID NO: 178), wherein X_1 is A, Y, P, S, or F, and wherein X_2 is V, L, S, I, Y, or T. In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179). In some embodiments, the protease cleavage site

comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186).

In some embodiments, the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187). In some embodiments, the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188). In some embodiments, the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189). In some embodiments, the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190). In some embodiments, the protease cleavage site comprises the amino acid sequence of VTPEPIFSLI (SEQ ID NO: 191). The protease cleavage sites of SEQ ID NOs: 187, 189, and 191 are cleavable by ADAM17.

In some embodiments, the protease cleavage site can include an N-terminal peptide linker, such as SGGGGSGGGGSG (SEQ ID NO: 230). In some embodiments, the protease cleavage site can include a C-terminal peptide linker, such as GGGSGGGGSGGGSLQ (SEQ ID NO: 231). In some embodiments, the protease cleavage site can include an N-terminal peptide linker and a C-terminal peptide linker, such as both table (SEQ ID NO: 230) and GGGSGGGGSGGGSLQ (SEQ ID NO: 231).

In some embodiments, the protease cleavage site can include a Tumor Necrosis Factor-α Converting Enzyme (TACE)-specific cleavage site (also referred to as ADAM17), an N-terminal peptide linker, and a C-terminal peptide linker. In some embodiments, the protease cleavage site can include the TACE -specific cleavage site VTPEPIFSLI (SEQ ID NO: 191), an N-terminal peptide linker, and a C-terminal peptide linker. In some embodiments, the protease cleavage site can include the TACE -specific cleavage site, an N-terminal peptide linker, and a C-terminal peptide linker and have the amino acid sequence SGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQ (SEQ ID NO: 250). In some embodiments, the protease cleavage site can include the TACE -specific cleavage site, an N-terminal peptide linker, and a C-terminal peptide linker encoded by a polynucleotide sequence comprising the sequence TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTT

CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAA (SEQ ID NO: 251). In some embodiments, the protease cleavage site can include the TACE-specific cleavage site, an N-terminal peptide linker, and a C-terminal peptide linker encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTT CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAA (SEQ ID NO: 251).

In some embodiments, the protease cleavage site comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198), which is a cleavage site that is native to CD16 and is cleavable by ADAM17.

The protease cleavage site can be C-terminal of the secretable effector molecule. The protease cleavage site can be N-terminal of the secretable effector molecule. In general, for all membrane-cleavable chimeric proteins described herein, the protease cleavage site is either: (1) C-terminal of the secretable effector molecule and N-terminal of the cell membrane tethering domain (in other words, the protease cleavage site is in between the secretable effector molecule and the cell membrane tethering domain); or (2) N-terminal of the secretable effector molecule and C-terminal of the cell membrane tethering domain (also between the secretable effector molecule and the cell membrane tethering domain with domain orientation inverted). The protease cleavage site can be connected to the secretable effector molecule by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the effector molecule or protease cleavage site. The protease cleavage site can be connected to the cell membrane tethering domain by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or protease cleavage site. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, $[GS]_4GG$ [SEQ ID NO: 182]), $A(EAAAK)_3A$ (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a "KEGS" linker such as the amino acid sequence KESGSVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional exemplary polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art.

In the Membrane-Cleavable system, following expression and localization of the chimeric protein into the cell membrane, the protease cleavage site directs cleavage of the chimeric protein such that the effector molecule is released ("secreted") into the extracellular space of a cell.

In general, a protease that cleaves the protease cleavage site is a protease specific for that specific protease cleavage site. For example, in the case of a disintegrin and metalloproteinase ("ADAM") family protease, the protease that cleaves a specific ADAM protease cleavage site is generally limited to the ADAM protease(s) that specifically recognize the specific ADAM protease cleavage site motif. A protease cleavage site can be selected and/or engineered such that cleavage by undesired proteases is reduced or eliminated. Proteases can be membrane-bound or membrane-associated. Proteases can be secreted, *e.g.*, secreted in a specific cellular environment, such as a tumor microenvironment ("TME").

A protease that cleaves the protease cleavage site of the chimeric protein can be expressed in the same cell that expresses the chimeric protein. A protease that cleaves the protease cleavage site of the chimeric protein can be endogenous to a cell expressing the chimeric protein. In other words, a cell engineered to express the chimeric protein can endogenously express the protease specific for the protease cleavage site present in the chimeric protein. Endogenous expression of the protease refers to both expression under generally homeostatic conditions (*e.g.*, a cell generally considered to be healthy), and also to differential expression under non-homeostatic conditions (*e.g.*, upregulated expression in a tumor cell). The protease cleavage site can be selected based on the known proteases endogenously expressed by a desired cell population. In such cases, in general, the cleavage of the protease cleavage site (and thus release/secretion of a payload) can be restricted to only those cells of interest due to the cell-restricted protease needing to come in contact with the protease cleavage site of chimeric protein expressed in the same cell. For example, and without wishing to be bound by theory, ADAM17 is believed to be restricted in its endogenous expression to NK cell and T cells. Thus, selection of an ADAM17-specific protease cleavage site may restrict the cleavage of the protease cleavage site to NK cell and T cells co-expressing the chimeric protein. In other examples, a protease cleavage site can be selected for a specific tumor-associated protease known to be expressed in a particular tumor population of interest (*e.g.*, in a specific tumor cell engineered to express the chimeric protein). Protease and/or expression databases can be used to select an appropriate protease cleavage site, such as selecting a protease cleavage site cleaved by a tumor-associated proteases through consulting Oncomine (www.oncomine.org), the European Bioinformatic Institute (www.ebi.ac.uk) in particular (www.ebi.ac.uk/gxa), PMAP (www.proteolysis.org), ExPASy Peptide Cutter (ca.expasy.org/tools/peptide cutter) and

PMAP.Cut DB (cutdb.burnham.org), each of which is incorporated by reference for all purposes.

A protease that cleaves the protease cleavage site of the chimeric protein can be heterologous to a cell expressing the chimeric protein. For example, a cell engineered to express the chimeric protein can also be engineered to express a protease not generally expressed by the cell that is specific for the protease cleavage site present in the chimeric protein. A cell engineered to express both the chimeric protein and the protease can be engineered to express each from separate engineered nucleic acids or from a multicistronic systems (multicistronic and multi-promoter systems are described in greater detail in the Section herein titled "Multicistronic and Multiple Promoter Systems"). Heterologous proteases and their corresponding protease cleavage site can be selected as described above with reference to endogenous proteases.

A protease that cleaves the protease cleavage site of the chimeric protein can be expressed on a separate distinct cell than the cell that expresses the chimeric protein. For example, the protease can be generally expressed in a specific cellular environment, such as a tumor microenvironment. In such cases, in general, the cleavage of the protease cleavage site can be restricted to only those cellular environments of interest (*e.g.*, a tumor microenvironment) due to the environment-restricted protease needing to come in contact with the protease cleavage site. In embodiments having membrane-cleavable chimeric proteins, in general, the secretion of the effector molecule can be restricted to only those cellular environments of interest (*e.g.*, a tumor microenvironment) due to the environment-restricted protease needing to come in contact with the protease cleavage site. A protease that cleaves the protease cleavage site of the chimeric protein can be endogenous to the separate distinct cell. A protease that cleaves the protease cleavage site of the chimeric protein can be heterologous to the separate distinct cell. For example, the separate distinct cell can be engineered to express a protease not generally expressed by the separate distinct cell.

Proteases include, but are not limited to, a Type 1 transmembrane protease, a Type II transmembrane protease, a GPI anchored protease, an ADAM8 protease, an ADAM9 protease, an ADAM10 protease, an ADAM12 protease, an ADAM15 protease, an ADAM17 protease, an ADAM19 protease, an ADAM20 protease, an ADAM21 protease, an ADAM28 protease, an ADAM30 protease, an ADAM33 protease, a BACE1 protease, a BACE2 protease, a SIP protease, an MT1-MMP protease, an MT3-MMP protease, an MT5-MMP protease, a furin protease, a PCSK7 protease, a matriptase protease, a matriptase-2 protease, and an MMP9 protease. A protease can be an NS3 protease. A protease can be an ADAM17 protease.

Proteases can be tumor associated proteases, such as, a cathepsin, a cysteine protease, an aspartyl protease, a serine protease, or a metalloprotease. Specific examples of tumor associated

proteases include Cathepsin B, Cathepsin L, Cathepsin S, Cathepsin D, Cathepsin E, Cathepsin A, Cathepsin G, Thrombin, Plasmin, Urokinase, Tissue Plasminogen Activator, Metalloproteinase 1 (MMP1), MMP2, MMP3, MMP4, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP20, MMP21, MMP23, MMP24, MMP25, MMP26, MMP28, ADAM, ADAMTS, CD10 (CALLA), or prostate specific antigen. Proteases can also include, but are not limited to, proteases listed in **Table 4B** below. Exemplary cognate protease cleavage sites for certain proteases are also listed in **Table 4B**.

Table 4B: Exemplary Proteases with Cognate Cleavage Sites and Inhibitors

Protease (UniProt Accession No.)	**Cognate cleavage site**	**Protease inhibitors**
HCV NS4A/4B	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS5A/5B	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS3	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS2-3	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HIV-1 protease (SEQ ID NO: 144)		Amprenavir, Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir
Signal peptidase (P67812, P15367, P00804, P0803)	preference of eukaryotic signal peptidase for cleavage after residue 20 ($Xaa^{20}\downarrow$) of pre(Δpro)apoA-II: Ala, Cys > Gly > Ser, Thr > Pro > Asn, Val, Ile, Leu, Tyr, His, Arg, Asp.	
proprotein convertases cleaving at hydrophobic residues (e.g., Leu, Phe, Val, or Met) (Q16549,	(R/K)-X-(hydrophobic)-X↓, where X is any amino acid	

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
Q8NBP7, Q92824, P29120, Q6UW60, P29122, Q9QXV0)		
proprotein convertases cleaving at small amino acid residues such as Ala or Thr (Q16549, Q8NBP7, Q92824, P29120, Q6UW60, P29122)	(K/R)-(X)n-(K/R)↓, where n is 0, 2, 4 or 6 and X is any amino acid	
proopiomelanocortin converting enzyme (PCE) (Q9UO77615, O776133)	Cleavage at paired basic residues in certain prohormones, either between them, or on the carboxyl side	
chromaffin granule aspartic protease (CGAP)	tends to cleave dipeptide bonds that have hydrophobic residues as well as a beta-methylene group	
prohormone thiol protease (cathepsin L1) (P07154, P07711, P06797, P25975, Q28944)		
carboxypeptidases (e.g., carboxypeptidase E/H, carboxypeptidase D and carboxypeptidase Z) (Q9M099, P15169, Q04609, P08819, P08818, O77564, P70627, O35409, P07519, Q8VZU3, P22792, P15087, P16870, Q9JHH6, Q96IY4, Q7L8A9)	cleaves a peptide bond at the carboxy-terminal (C-terminal) end of a protein or peptide	
aminopeptidases (e.g., arginine aminopeptidase, lysine aminopeptidase, aminopeptidase B)	cleaves a peptide bond at the amino-terminal (N-terminal) end of a protein or peptide	
prolyl endopeptidase (Q12884, P48147, P97321, Q4J6C6)	Hydrolysis of Pro-|-Xaa >> Ala-|-Xaa in oligopeptides. Release of an N-terminal dipeptide, Xaa-Yaa-|-Zaa-, from a polypeptide, preferentially when Yaa is Pro, provided Zaa is neither Pro nor hydroxyproline	
aminopeptidase N (P97449, P15144, P15145, P15684)	Release of an N-terminal amino acid, Xaa-|-Yaa- from a peptide, amide or arylamide. Xaa is preferably Ala, but may be most amino acids including Pro (slow	

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	action). When a terminal hydrophobic residue is followed by a prolyl residue, the two may be released as an intact Xaa-Pro dipeptide	
insulin degrading enzyme (P14735, P35559, Q9JHR7, P22817, Q24K02)	Degradation of insulin, glucagon and other polypeptides. No action on proteins. Cleaves multiple short polypeptides that vary considerably in sequence	
Calpain (O08529, P17655, Q07009, Q27971, P20807, P07384, O35350, O14815, P04632, Q9Y6Q1, O15484, Q9HC96, A6NHC0, Q9UMQ6)	No specific amino acid sequence is uniquely recognized by calpains. Amongst protein substrates, tertiary structure elements rather than primary amino acid sequences appear to be responsible for directing cleavage to a specific substrate. Amongst peptide and small-molecule substrates, the most consistently reported specificity is for small, hydrophobic amino acids (e.g., leucine, valine and isoleucine) at the P2 position, and large hydrophobic amino acids (e.g., phenylalanine and tyrosine) at the P1 position. One fluorogenic calpain substrate is (EDANS)-Glu-Pro-Leu-Phe=Ala-Glu-Arg-Lys-(DABCYL), (EDANSEPLFAERKDABCYL (SEQ ID NO: 145)) with cleavage occurring at the Phe=Ala bond.	
caspase 1 (P29466, P29452)	Strict requirement for an Asp residue at position P1 and has a preferred cleavage sequence of Tyr-Val-Ala-Asp-|- (YVAD; SEQ ID NO: 146).	
caspase 2 (P42575, P29594)	Strict requirement for an Asp residue at P1, with 316-asp being essential for proteolytic activity and has a preferred cleavage sequence of Val-Asp-Val-Ala-Asp-|- (VDVAD; SEQ ID NO: 147).	
caspase 3 (P42574, P70677)	Strict requirement for an Asp residue at positions P1 and P4. It has a preferred cleavage sequence of Asp-Xaa-Xaa-Asp-|- with a hydrophobic amino-acid residue at P2 and a hydrophilic amino-acid	

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	residue at P3, although Val or Ala are also accepted at this position.	
caspase 4 (P70343, P49662)	Strict requirement for Asp at the P1 position. It has a preferred cleavage sequence of Tyr-Val-Ala-Asp-|-(YVAD; SEQ ID NO: 146) but also cleaves at Asp-Glu-Val-Asp-|-(DEVD; SEQ ID NO: 148).	
caspase 5 (P51878)	Strict requirement for Asp at the P1 position. It has a preferred cleavage sequence of Tyr-Val-Ala-Asp-|-(YVAD; SEQ ID NO: 146) but also cleaves at Asp-Glu-Val-Asp-|- -|-(DEVD; SEQ ID NO: 148).	
caspase 6 (P55212)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of Val-Glu-His-Asp-|-(VEHD; SEQ ID NO: 149).	
caspase 7 (P97864, P55210)	Strict requirement for an Asp residue at position P1 and has a preferred cleavage sequence of Asp-Glu-Val-Asp-|- (DEVD; SEQ ID NO: 148).	
caspase 8 (Q8IRY7, O89110, Q14790)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of (Leu/Asp/Val)-Glu-Thr-Asp-|-(Gly/Ser/Ala).	
caspase 9 (P55211, Q8C3Q9, Q5IS54)	Strict requirement for an Asp residue at position P1 and with a marked preference for His at position P2. It has a preferred cleavage sequence of Leu-Gly-His-Asp-|-Xaa (LGHD; SEQ ID NO: 150).	
caspase 10 (Q92851)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of Leu-Gln-Thr-Asp-|-Gly (LQTDG; SEQ ID NO: 151).	
puromycin sensitive aminopeptidase (P55786, Q11011)	Release of an N-terminal amino acid, preferentially alanine, from a wide range of peptides, amides and arylamides.	
angiotensin converting enzyme (ACE) (P12821, P09470, Q9BYF1) SEQ ID NO: 156	Release of a C-terminal dipeptide, oligopeptide-|-Xaa-Yaa, when Xaa is not Pro, and Yaa is neither Asp nor Glu.	Benazepril (Lotensin), Captopril, Enalapril (Vasotec), Fosinopril, Lisinopril (Prinivil, Zestril), Moexipril,

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
		Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), Trandolapril (Mavik), Zofenopril
pyroglutamyl peptidase II (Q9NXJ5)	Release of the N-terminal pyroglutamyl group from pGlu--His-Xaa tripeptides and pGlu--His-Xaa-Gly tetrapeptides	
dipeptidyl peptidase IV (P27487, P14740, P28843)	Release of an N-terminal dipeptide, Xaa-Yaa-|-Zaa-, from a polypeptide, preferentially when Yaa is Pro, provided Zaa is neither Pro nor hydroxyproline	
N-arginine dibasic convertase (O43847, Q8BHG1)	Hydrolysis of polypeptides, preferably at -Xaa-|-Arg-Lys-, and less commonly at -Arg-|-Arg-Xaa-, in which Xaa is not Arg or Lys	
endopeptidase 24.15 (thimet oligopeptidase) (P52888, P24155)	Preferential cleavage of bonds with hydrophobic residues at P1, P2 and P3' and a small residue at P1' in substrates of 5 to 15 residues	
endopeptidase 24.16 (neurolysin) (Q9BYT8, Q91YP2)	Preferential cleavage in neurotensin: 10-Pro-|-Tyr-11	
amyloid precursor protein secretase alpha (P05067, P12023, Q9Y5Z0, P56817)	Endopeptidase of broad specificity.	
amyloid precursor protein secretase beta (P05067, P12023, Q9Y5Z0, P56817)	Broad endopeptidase specificity. Cleaves Glu-Val-Asn-Leu-|-Asp-Ala-Glu-Phe (EVNLDAEF; SEQ ID NO: 152) in the Swedish variant of Alzheimer's amyloid precursor protein	
amyloid precursor protein secretase gamma (P05067, P12023, Q9Y5Z0, P56817)	intramembrane cleavage of integral membrane proteins	
MMP 1 (P03956, Q9EPL5uy)	Cleavage of the triple helix of collagen at about three-quarters of the length of the molecule from the N-terminus, at 775-Gly-|-Ile-776 in the alpha-1(I) chain. Cleaves synthetic substrates and alpha-macroglobulins at bonds where P1' is a hydrophobic residue.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 2 (P08253, P33434)	Cleavage of gelatin type I and collagen types IV, V, VII, X.	SB-3CT *p*-OH SB-3CT

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	Cleaves the collagen-like sequence Pro-Gln-Gly-|-Ile-Ala-Gly-Gln (PQGIAGQ; SEQ ID NO: 153).	*O*-phosphate SB-3CT RXP470.1
MMP 3 (P08254, P28862)	Preferential cleavage where P1', P2' and P3' are hydrophobic residues.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 7 (P09237, Q10738)	Cleavage of 14-Ala-|-Leu-15 and 16-Tyr-|-Leu-17 in B chain of insulin. No action on collagen types I, II, IV, V. Cleaves gelatin chain alpha-2(I) > alpha-1(I).	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 8 (P22894, O70138)	Can degrade fibrillar type I, II, and III collagens. Cleavage of interstitial collagens in the triple helical domain. Unlike EC 3.4.24.7, this enzyme cleaves type III collagen more slowly than type I.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 9 (P14780, P41245)	Cleavage of gelatin types I and V and collagen types IV and V. Cleaves KiSS1 at a Gly-|-Leu bond. Cleaves type IV and type V collagen into large C-terminal three quarter fragments and shorter N-terminal one quarter fragments. Degrades fibronectin but not laminin or Pz-peptide.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 10 (P09238, O55123)	Can degrade fibronectin, gelatins of type I, III, IV, and V; weakly collagens III, IV, and V.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 11 (P24347, Q02853)	A(A/Q)(N/A)↓(L/Y)(T/V/M/R)(R/K) G(G/A)E↓LR ↓ denotes the cleavage site	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 12 (P39900, P34960)	Hydrolysis of soluble and insoluble elastin. Specific cleavages are also produced at 14-Ala-|-Leu-15 and 16-Tyr-|-Leu-17 in the B chain of insulin Has significant elastolytic activity. Can accept large and small amino acids at the P1' site, but has a	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	preference for leucine. Aromatic or hydrophobic residues are preferred at the P1 site, with small hydrophobic residues (preferably alanine) occupying P3	
MMP 13 (P45452, P33435)	Cleaves triple helical collagens, including type I, type II and type III collagen, but has the highest activity with soluble type II collagen. Can also degrade collagen type IV, type XIV and type X	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 14 (P50281, P53690)	Activates progelatinase A by cleavage of the propeptide at 37-Asn-|-Leu-38. Other bonds hydrolyzed include 35-Gly-|-Ile-36 in the propeptide of collagenase 3, and 341-Asn-|-Phe-342, 441-Asp-|-Leu-442 and 354-Gln-|-Thr-355 in the aggrecan interglobular domain.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
urokinase plasminogen activator (uPA) (P00749, P06869)	Specific cleavage of Arg-|-Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
tissue plasminogen activator (tPA) (P00750, P11214)	Specific cleavage of Arg-|-Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
tissue plasminogen activator (tPA) (P00750, P11214)	Specific cleavage of Arg-|-Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
Plasmin (P00747, P20918)	Preferential cleavage: Lys-|-Xaa > Arg-|-Xaa, higher selectivity than trypsin. Converts fibrin into soluble products.	α-2-antiplasmin (AP)
Thrombin (P00734, P19221)	Cleaves bonds after Arg and Lys Converts fibrinogen to fibrin and activates factors V, VII, VIII, XIII, and, in complex with thrombomodulin, protein C.	
BMP-1 (procollagen C-peptidase) (P13497, P98063)	Cleavage of the C-terminal propeptide at Ala-|-Asp in type I and II procollagens and at Arg-|-Asp in type III.	
ADAM (Q9P0K1, Q9UKQ2, Q9JLN6, O14672, Q13444, P78536, Q13443, O43184, P78325, Q9UKF5, Q9BZ11, Q9H2U9, Q99965,		SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
O75077, Q9H013, O43506)		
granzyme A (P12544, P11032)	Preferential cleavage: -Arg-|-Xaa-, -Lys-|-Xaa- >> -Phe-|-Xaa- in small molecule substrates.	
granzyme B (P10144, P04187)	Preference for bulky and aromatic residues at the P1 position and acidic residues at the P3' and P4' sites.	
granzyme M (P51124, Q03238)	Cleaves peptide substrates after methionine, leucine, and norleucine.	
tobacco Etch virus (TEV) protease (P04517, P0CK09)	E-Xaa-Xaa-Y -Xaa-Q-(G/S), with cleavage occurring between Q and G/S. The most common sequence is ENLYFQS (SEQ ID NO: 154)	
chymotrypsin-like serine protease (P08217, Q9UNI1, Q91X79, P08861, P09093, P08218)		-*Thermobifida fusca* Thermopin -*Pyrobaculum aerophilum* Aeropin -*Thermococcus kodakaraensis* Tk-serpin -*Alteromonas sp.* Marinostatin -*Streptomyces misionensis* SMTI -*Streptomyces sp.* chymostatin
alphavirus proteases (P08411, P03317, P13886, Q8JUX6, Q86924, Q4QXJ8, Q8QL53, P27282, Q5XXP4)		
chymotrypsin-like cysteine proteases (Q86TL0, Q14790, Q99538, O15553)		-*Thermobifida fusca* Thermopin -*Pyrobaculum aerophilum* Aeropin -*Thermococcus kodakaraensis* Tk-serpin -*Alteromonas sp.* Marinostatin -*Streptomyces misionensis* SMTI -*Streptomyces sp.* chymostatin
papain-like cysteine proteases (P25774, P53634, Q96K76)		

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
picornavirus leader proteases (P03305, P03311, P13899)		
HIV proteases (P04585, P03367, P04584, P03369, P12497, P03366, P04587)		
Herpesvirus proteases (P10220, Q2HRB6, O40922, Q69527)		
adenovirus proteases (P03252, P24937, Q83906, P68985, P09569, P11825, P10381)		
Streptomyces griseus protease A (SGPA) (P00776)		
Streptomyces griseus protease B (SGPB) (P00777)		
alpha-lytic protease (P85142, P00778)		
serine proteases (P48740, P98064, Q9UL52, P05981, O60235)		
cysteine proteases (Q86TL0, Q14790, Q8WYN0, Q96DT6, P55211)		
aspartic proteases (Q9Y5Z0, P56817, Q00663, Q53RT3, P0CY27)		
threonine proteases (Q9UI38, Q16512, Q9H6P5, Q8IWU2)		
Mast cell (MC) chymase (CMA1) (NM_001836)	Abz-HPFHL(SEQ ID NO: 155)-Lys(Dnp)-NH2	BAY 1142524 SUN13834
Rat mast cell protease-1, -2, -3, -4, -5 (NM_017145, NM_172044, NM_001170466, NM_019321, NM_013092)	Abz-HPFHL(SEQ ID NO: 155)-Lys(Dnp)-NH2	TY-51469
Rat vascular chymase (RVCH) (O70500)	Abz-HPFHL(SEQ ID NO: 155)-Lys(Dnp)-NH2	
DENV NS3pro (NS2B/NS3) SEQ ID NOs: 157, 158, 159, 160	A strong preference for basic amino acid residues (Arg/Lys) at the P1 positions was observed, whereas the preferences for the P2– 4 sites were	Anthraquinone BP13944 ZINC04321905 MB21

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	in the order of Arg > Thr > Gln/Asn/Lys for P2, Lys > Arg > Asn for P3, and Nle > Leu > Lys > Xaa for P4. The prime site substrate specificity was for small and polar amino acids in P1 and P3.	Policresulen SK-12 NSC135618 Biliverdin

A protease can be any of the following human proteases (MEROPS peptidase database number provided in parentheses; Rawlings N. D., Morton F. R., Kok, C. Y., Kong, J. & Barrett A. J. (2008) MEROPS: the peptidase database. Nucleic Acids Res. 36 Database issue, D320-325; herein incorporated by reference for all purposes): pepsin A (MER000885), gastricsin (MER000894), memapsin-2 (MER005870), renin (MER000917), cathepsin D (MER000911), cathepsin E (MER000944), memapsin-1 (MER005534), napsin A (MER004981), Mername-AA034 peptidase (MER014038), pepsin A4 (MER037290), pepsin A5 (Homo sapiens) (MER037291), hCG1733572 (Homo sapiens)-type putative peptidase (MER107386), napsin B pseudogene (MER004982), CYMP g.p. (Homo sapiens) (MER002929), subfamily A1A unassigned peptidases (MER181559), mouse mammary tumor virus retropepsin (MER048030), rabbit endogenous retrovirus endopeptidase (MER043650), S71-related human endogenous retropepsin (MER001812), RTVL-H-type putative peptidase (MER047117), RTVL-H-type putative peptidase (MER047133), RTVL-H-type putative peptidase (MER047160), RTVL-H-type putative peptidase (MER047206), RTVL-H-type putative peptidase (MER047253), RTVL-H-type putative peptidase (MER047260), RTVL-H-type putative peptidase (MER047291), RTVL-H-type putative peptidase (MER047418), RTVL-H-type putative peptidase (MER047440), RTVL-H-type putative peptidase (MER047479), RTVL-H-type putative peptidase (MER047559), RTVL-H-type putative peptidase (MER047583), RTVL-H-type putative peptidase (MER015446), human endogenous retrovirus retropepsin homologue 1 (MER015479), human endogenous retrovirus retropepsin homologue 2 (MER015481), endogenous retrovirus retropepsin pseudogene 1 (Homo sapiens chromosome 14) (MER029977), endogenous retrovirus retropepsin pseudogene 2 (Homo sapiens chromosome 8) (MER029665), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER002660), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER030286), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER047144), endogenous retrovirus retropepsin pseudogene 5 (Homo sapiens chromosome 12) (MER029664), endogenous retrovirus retropepsin pseudogene 6 (Homo sapiens chromosome 7) (MER002094), endogenous retrovirus retropepsin pseudogene

7 (Homo sapiens chromosome 6) (MER029776), endogenous retrovirus retropepsin pseudogene 8 (Homo sapiens chromosome Y) (MER030291), endogenous retrovirus retropepsin pseudogene 9 (Homo sapiens chromosome 19) (MER029680), endogenous retrovirus retropepsin pseudogene 10 (Homo sapiens chromosome 12) (MER002848), endogenous retrovirus retropepsin pseudogene 11 (Homo sapiens chromosome 17) (MER004378), endogenous retrovirus retropepsin pseudogene 12 (Homo sapiens chromosome 11) (MER003344), endogenous retrovirus retropepsin pseudogene 13 (Homo sapiens chromosome 2 and similar) (MER029779), endogenous retrovirus retropepsin pseudogene 14 (Homo sapiens chromosome 2) (MER029778), endogenous retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER047158), endogenous retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER047332), endogenous retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER003182), endogenous retrovirus retropepsin pseudogene 16 (MER047165), endogenous retrovirus retropepsin pseudogene 16 (MER047178), endogenous retrovirus retropepsin pseudogene 16 (MER047200), endogenous retrovirus retropepsin pseudogene 16 (MER047315), endogenous retrovirus retropepsin pseudogene 16 (MER047405), endogenous retrovirus retropepsin pseudogene 16 (MER030292), endogenous retrovirus retropepsin pseudogene 17 (Homo sapiens chromosome 8) (MER005305), endogenous retrovirus retropepsin pseudogene 18 (Homo sapiens chromosome 4) (MER030288), endogenous retrovirus retropepsin pseudogene 19 (Homo sapiens chromosome 16) (MER001740), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047222), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047454), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047477), endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER004403), endogenous retrovirus retropepsin pseudogene 22 (Homo sapiens chromosome X) (MER030287), subfamily A2A non-peptidase homologues (MER047046), subfamily A2A non-peptidase homologues (MER047052), subfamily A2A non-peptidase homologues (MER047076), subfamily A2A non-peptidase homologues (MER047080), subfamily A2A non-peptidase homologues (MER047088), subfamily A2A non-peptidase homologues (MER047089), subfamily A2A non-peptidase homologues (MER047091), subfamily A2A non-peptidase homologues (MER047092), subfamily A2A non-peptidase homologues (MER047093), subfamily A2A non-peptidase homologues (MER047094), subfamily A2A non-peptidase homologues (MER047097), subfamily A2A non-peptidase homologues (MER047099), subfamily A2A non-peptidase homologues MER047101), subfamily A2A non-peptidase homologues (MER047102), subfamily A2A non-peptidase homologues (MER047107), subfamily A2A non-peptidase homologues (MER047108), subfamily A2A non-

peptidase homologues (MER047109), subfamily A2A non-peptidase homologues (MER047110), subfamily A2A non-peptidase homologues MER047111), subfamily A2A non-peptidase homologues (MER047114), subfamily A2A non-peptidase homologues (MER047118), subfamily A2A non-peptidase homologues (MER047121), subfamily A2A non-peptidase homologues (MER047122), subfamily A2A non-peptidase homologues (MER047126), subfamily A2A non-peptidase homologues (MER047129), subfamily A2A non-peptidase homologues (MER047130), subfamily A2A non-peptidase homologues (MER047134), subfamily A2A non-peptidase homologues (MER047135), subfamily A2A non-peptidase homologues (MER047137), subfamily A2A non-peptidase homologues (MER047140), subfamily A2A non-peptidase homologues (MER047141), subfamily A2A non-peptidase homologues (MER047142), subfamily A2A non-peptidase homologues (MER047148), subfamily A2A non-peptidase homologues (MER047149), subfamily A2A non-peptidase homologues (MER047151), subfamily A2A non-peptidase homologues (MER047154), subfamily A2A non-peptidase homologues (MER047155), subfamily A2A non-peptidase homologues (MER047156), subfamily A2A non-peptidase homologues (MER047157), subfamily A2A non-peptidase homologues (MER047159), subfamily A2A non-peptidase homologues (MER047161), subfamily A2A non-peptidase homologues (MER047163), subfamily A2A non-peptidase homologues (MER047166), subfamily A2A non-peptidase homologues (MER047171), subfamily A2A non-peptidase homologues (MER047173), subfamily A2A non-peptidase homologues (MER047174), subfamily A2A non-peptidase homologues (MER047179), subfamily A2A non-peptidase homologues (MER047183), subfamily A2A non-peptidase homologues (MER047186), subfamily A2A non-peptidase homologues (MER047190), subfamily A2A non-peptidase homologues (MER047191), subfamily A2A non-peptidase homologues (MER047196), subfamily A2A non-peptidase homologues (MER047198), subfamily A2A non-peptidase homologues (MER047199), subfamily A2A non-peptidase homologues (MER047201), subfamily A2A non-peptidase homologues (MER047202), subfamily A2A non-peptidase homologues (MER047203), subfamily A2A non-peptidase homologues (MER047204), subfamily A2A non-peptidase homologues (MER047205), subfamily A2A non-peptidase homologues (MER047207), subfamily A2A non-peptidase homologues (MER047208), subfamily A2A non-peptidase homologues (MER047210), subfamily A2A non-peptidase homologues (MER047211), subfamily A2A non-peptidase homologues (MER047212), subfamily A2A non-peptidase homologues (MER047213), subfamily A2A non-peptidase homologues (MER047215), subfamily A2A non-peptidase homologues (MER047216), subfamily A2A non-peptidase homologues (MER047218), subfamily A2A non-peptidase homologues

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(MER004980), cathepsin L (MER000622), cathepsin S (MER000633), cathepsin O (MER001690), cathepsin K (MER000644), cathepsin W (MER003756), cathepsin H (MER000629), cathepsin B (MER000686), dipeptidyl-peptidase I (MER001937), bleomycin hydrolase (animal) (MER002481), tubulointerstitial nephritis antigen (MER016137), tubulointerstitial nephritis antigen-related protein (MER021799), cathepsin L-like pseudogene 1 (Homo sapiens) (MER002789), cathepsin B-like pseudogene (chromosome 4, Homo sapiens) (MER029469), cathepsin B-like pseudogene (chromosome 1, Homo sapiens) (MER029457), CTSLL2 g.p. (Homo sapiens) (MER005210), CTSLL3 g.p. (Homo sapiens) (MER005209), calpain-1 (MER000770), calpain-2 (MER000964), calpain-3 (MER001446), calpain-9 (MER004042), calpain-8 (MER021474), calpain-15 (MER004745), calpain-5 (MER002939), calpain-11 (MER005844), calpain-12 (MER029889), calpain-10 (MER013510), calpain-13 (MER020139), calpain-14 (MER029744), Mername-AA253 peptidase (MER005537), calpamodulin (MER000718), hypothetical protein 940251 (MER003201), ubiquitinyl hydrolase-L1 (MER000832), ubiquitinyl hydrolase-L3 (MER000836), ubiquitinyl hydrolase-BAP1 (MER003989), ubiquitinyl hydrolase-UCH37 (MER005539), ubiquitin-specific peptidase 5 (MER002066), ubiquitin-specific peptidase 6 (MER000863), ubiquitin-specific peptidase 4 (MER001795), ubiquitin-specific peptidase 8 (MER001884), ubiquitin-specific peptidase 13 (MER002627), ubiquitin-specific peptidase 2 (MER004834), ubiquitin-specific peptidase 11 (MER002693), ubiquitin-specific peptidase 14 (MER002667), ubiquitin-specific peptidase 7 (MER002896), ubiquitin-specific peptidase 9X (MER005877), ubiquitin-specific peptidase 10 (MER004439), ubiquitin-specific peptidase 1 (MER004978), ubiquitin-specific peptidase 12 (MER005454), ubiquitin-specific peptidase 16 (MER005493), ubiquitin-specific peptidase 15 (MER005427), ubiquitin-specific peptidase 17 (MER002900), ubiquitin-specific peptidase 19 (MER005428), ubiquitin-specific peptidase 20 (MER005494), ubiquitin-specific peptidase 3 (MER005513), ubiquitin-specific peptidase 9Y (MER004314), ubiquitin-specific peptidase 18 (MER005641), ubiquitin-specific peptidase 21 (MER006258), ubiquitin-specific peptidase 22 (MER012130), ubiquitin-specific peptidase 33 (MER014335), ubiquitin-specific peptidase 29 (MER012093), ubiquitin-specific peptidase 25 (MER011115), ubiquitin-specific peptidase 36 (MER014033), ubiquitin-specific peptidase 32 (MER014290), ubiquitin-specific peptidase 26 (Homo sapiens-type) (MER014292), ubiquitin-specific peptidase 24 (MER005706), ubiquitin-specific peptidase 42 (MER011852), ubiquitin-specific peptidase 46 (MER014629), ubiquitin-specific peptidase 37 (MER014633), ubiquitin-specific peptidase 28 (MER014634), ubiquitin-specific peptidase 47 (MER014636), ubiquitin-specific peptidase 38 (MER014637), ubiquitin-specific peptidase 44 (MER014638), ubiquitin-specific peptidase 50 (MER030315), ubiquitin-specific peptidase 35 (MER014646), ubiquitin-specific peptidase 30 (MER014649), Mername-

AA091 peptidase (MER014743), ubiquitin-specific peptidase 45 (MER030314), ubiquitin-specific peptidase 51 (MER014769), ubiquitin-specific peptidase 34 (MER014780), ubiquitin-specific peptidase 48 (MER064620), ubiquitin-specific peptidase 40 (MER015483), ubiquitin-specific peptidase 41 (MER045268), ubiquitin-specific peptidase 31 (MER015493), Mername-AA129 peptidase (MER016485), ubiquitin-specific peptidase 49 (MER016486), Mername-AA187 peptidase (MER052579), USP17-like peptidase (MER030192), ubiquitin-specific peptidase 54 (MER028714), ubiquitin-specific peptidase 53 (MER027329), ubiquitin-specific endopeptidase 39 [misleading] (MER064621), Mername-AA090 non-peptidase homologue (MER014739), ubiquitin-specific peptidase 43 [misleading] (MER030140), ubiquitin-specific peptidase 52 [misleading] (MER030317), NEK2 pseudogene (MER014736), C19 pseudogene (Homo sapiens: chromosome 5) (MER029972), Mername-AA088 peptidase (MER014750), autophagin-2 (MER013564), autophagin-1 (MER013561), autophagin-3 (MER014316), autophagin-4 (MER064622), Cezanne deubiquitinylating peptidase (MER029042), Cezanne-2 peptidase (MER029044), tumor necrosis factor alpha-induced protein 3 (MER029050), trabid peptidase (MER029052), VCIP135 deubiquitinating peptidase (MER152304), otubain-1 (MER029056), otubain-2 (MER029061), CylD protein (MER030104), UfSP1 peptidase (MER042724), UfSP2 peptidase (MER060306), DUBA deubiquitinylating enzyme (MER086098), KIAA0459 (Homo sapiens)-like protein (MER122467), Otud1 protein (MER125457), glycosyltransferase 28 domain containing 1, isoform CRA_c (Homo sapiens)-like (MER123606), hin1L g.p. (Homo sapiens) (MER139816), ataxin-3 (MER099998), ATXN3L putative peptidase (MER115261), Josephin domain containing 1 (Homo sapiens) (MER125334), Josephin domain containing 2 (Homo sapiens) (MER124068), YOD1 peptidase (MER116559), legumain (plant alpha form) (MER044591), legumain (MER001800), glycosylphosphatidylinositol:protein transamidase (MER002479), legumain pseudogene (Homo sapiens) (MER029741), family C13 unassigned peptidases (MER175813), caspase-1 (MER000850), caspase-3 (MER000853), caspase-7 (MER002705), caspase-6 (MER002708), caspase-2 (MER001644), caspase-4 (MER001938), caspase-5 (MER002240), caspase-8 (MER002849), caspase-9 (MER002707), caspase-10 (MER002579), caspase-14 (MER012083), paracaspase (MER019325), Mername-AA143 peptidase (MER021304), Mername-AA186 peptidase (MER020516), putative caspase (Homo sapiens) (MER021463), FLIP protein (MER003026), Mername-AA142 protein (MER021316), caspase-12 pseudogene (Homo sapiens) (MER019698), Mername-AA093 caspase pseudogene (MER014766), subfamily C14A non-peptidase homologues (MER185329), subfamily C14A non-peptidase homologues (MER179956), separase (Homo sapiens-type) (MER011775), separase-like pseudogene (MER014797), SENP1 peptidase (MER011012), SENP3 peptidase (MER011019), SENP6

peptidase (MER011109), SENP2 peptidase (MER012183), SENP5 peptidase (MER014032), SENP7 peptidase (MER014095), SENP8 peptidase (MER016161), SENP4 peptidase (MER005557), pyroglutamyl-peptidase I (chordate) (MER011032), Mername-AA073 peptidase (MER029978), Sonic hedgehog protein (MER002539), Indian hedgehog protein (MER002538), Desert hedgehog protein (MER012170), dipeptidyl-peptidase III (MER004252), Mername-AA164 protein (MER020410), LOC138971 g.p. (Homo sapiens) (MER020074), Atp23 peptidase (MER060642), prenyl peptidase 1 (MER004246), aminopeptidase N (MER000997), aminopeptidase A (MER001012), leukotriene A4 hydrolase (MER001013), pyroglutamyl-peptidase II (MER012221), cytosol alanyl aminopeptidase (MER002746), cystinyl aminopeptidase (MER002060), aminopeptidase B (MER001494), aminopeptidase PILS (MER005331), arginyl aminopeptidase-like 1 (MER012271), leukocyte-derived arginine aminopeptidase (MER002968), aminopeptidase Q (MER052595), aminopeptidase O (MER019730), Tata binding protein associated factor (MER026493), angiotensin-converting enzyme peptidase unit 1 (MER004967), angiotensin-converting enzyme peptidase unit 2 (MER001019), angiotensin-converting enzyme-2 (MER011061), Mername-AA153 protein (MER020514), thimet oligopeptidase (MER001737), neurolysin (MER010991), mitochondrial intermediate peptidase (MER003665), Mername-AA154 protein (MER021317), leishmanolysin-2 (MER014492), leishmanolysin-3 (MER180031), matrix metallopeptidase-1 (MER001063), matrix metallopeptidase-8 (MER001084), matrix metallopeptidase-2 (MER001080), matrix metallopeptidase-9 (MER001085), matrix metallopeptidase-3 (MER001068), matrix metallopeptidase-10 (Homo sapiens-type) (MER001072), matrix metallopeptidase-11 (MER001075), matrix metallopeptidase-7 (MER001092), matrix metallopeptidase-12 (MER001089), matrix metallopeptidase-13 (MER001411), membrane-type matrix metallopeptidase-1 (MER001077), membrane-type matrix metallopeptidase-2 (MER002383), membrane-type matrix metallopeptidase-3 (MER002384), membrane-type matrix metallopeptidase-4 (MER002595), matrix metallopeptidase-20 (MER003021), matrix metallopeptidase-19 (MER002076), matrix metallopeptidase-23B (MER004766), membrane-type matrix metallopeptidase-5 (MER005638), membrane-type matrix metallopeptidase-6 (MER012071), matrix metallopeptidase-21 (MER006101), matrix metallopeptidase-22 (MER014098), matrix metallopeptidase-26 (MER012072), matrix metallopeptidase-28 (MER013587), matrix metallopeptidase-23A (MER037217), macrophage elastase homologue (chromosome 8, Homo sapiens) (MER030035), Mername-AA156 protein (MER021309), matrix metallopeptidase-like 1 (MER045280), subfamily M10A non-peptidase homologues (MER175912), subfamily M10A non-peptidase homologues (MER187997), subfamily M10A non-peptidase homologues (MER187998), subfamily M10A non-peptidase homologues

(MER180000), meprin alpha subunit (MER001111), meprin beta subunit (MER005213), procollagen C-peptidase (MER001113), mammalian tolloid-like 1 protein (MER005124), mammalian-type tolloid-like 2 protein (MER005866), ADAMTS9 peptidase (MER012092), ADAMTS14 peptidase (MER016700), ADAMTS15 peptidase (MER017029), ADAMTS16 peptidase (MER015689), ADAMTS17 peptidase (MER016302), ADAMTS18 peptidase (MER016090), ADAMTS19 peptidase (MER015663), ADAM8 peptidase (MER003902), ADAM9 peptidase (MER001140), ADAM10 peptidase (MER002382), ADAM12 peptidase (MER005107), ADAM19 peptidase (MER012241), ADAM15 peptidase (MER002386), ADAM17 peptidase (MER003094), ADAM20 peptidase (MER004725), ADAMDEC1 peptidase (MER000743), ADAMTS3 peptidase (MER005100), ADAMTS4 peptidase (MER005101), ADAMTS1 peptidase (MER005546), ADAM28 peptidase (Homo sapiens-type) (MER005495), ADAMTS5 peptidase (MER005548), ADAMTS8 peptidase (MER005545), ADAMTS6 peptidase (MER005893), ADAMTS7 peptidase (MER005894), ADAM30 peptidase (MER006268), ADAM21 peptidase (Homo sapiens-type) (MER004726), ADAMTS10 peptidase (MER014331), ADAMTS12 peptidase (MER014337), ADAMTS13 peptidase (MER015450), ADAM33 peptidase (MER015143), ovastacin (MER029996), ADAMTS20 peptidase (Homo sapiens-type) (MER026906), procollagen I N-peptidase (MER004985), ADAM2 protein (MER003090), ADAM6 protein (MER047044), ADAM7 protein (MER005109), ADAM18 protein (MER012230), ADAM32 protein (MER026938), non-peptidase homologue (Homo sapiens chromosome 4) (MER029973), family M12 non-peptidase homologue (Homo sapiens chromosome 16) (MER047654), family M12 non-peptidase homologue (Homo sapiens chromosome 15) (MER047250), ADAM3B protein (Homo sapiens-type) (MER005199), ADAM11 protein (MER001146), ADAM22 protein (MER005102), ADAM23 protein (MER005103), ADAM29 protein (MER006267), protein similar to ADAM21 peptidase preproprotein (Homo sapiens) (MER026944), Mername-AA225 peptidase homologue (Homo sapiens) (MER047474), putative ADAM pseudogene (chromosome 4, Homo sapiens) (MER029975), ADAM3A g.p. (Homo sapiens) (MER005200), ADAM1 g.p. (Homo sapiens) (MER003912), subfamily M12B non-peptidase homologues (MER188210), subfamily M12B non-peptidase homologues (MER188211), subfamily M12B non-peptidase homologues (MER188212), subfamily M12B non-peptidase homologues (MER188220), neprilysin (MER001050), endothelin-converting enzyme 1 (MER001057), endothelin-converting enzyme 2 (MER004776), DINE peptidase (MER005197), neprilysin-2 (MER013406), Kell blood-group protein (MER001054), PHEX peptidase (MER002062), i-AAA peptidase (MER001246), i-AAA peptidase (MER005755), paraplegin (MER004454), Afg3-like protein 2 (MER005496), Afg3-like protein 1A (MER014306), pappalysin-1 (MER002217), pappalysin-2 (MER014521),

farnesylated-protein converting enzyme 1 (MER002646), metalloprotease-related protein-1 (MER030873), aminopeptidase AMZ2 (MER011907), aminopeptidase AMZ1 (MER058242), carboxypeptidase A1 (MER001190), carboxypeptidase A2 (MER001608), carboxypeptidase B (MER001194), carboxypeptidase N (MER001198), carboxypeptidase E (MER001199), carboxypeptidase M (MER001205), carboxypeptidase U (MER001193), carboxypeptidase A3 (MER001187), metallocarboxypeptidase D peptidase unit 1 (MER003781), metallocarboxypeptidase Z (MER003428), metallocarboxypeptidase D peptidase unit 2 (MER004963), carboxypeptidase A4 (MER013421), carboxypeptidase A6 (MER013456), carboxypeptidase A5 (MER017121), metallocarboxypeptidase O (MER016044), cytosolic carboxypeptidase-like protein 5 (MER033174), cytosolic carboxypeptidase 3 (MER033176), cytosolic carboxypeptidase 6 (MER033178), cytosolic carboxypeptidase 1 (MER033179), cytosolic carboxypeptidase 2 (MER037713), metallocarboxypeptidase D non-peptidase unit (MER004964), adipocyte-enhancer binding protein 1 (MER003889), carboxypeptidase-like protein X1 (MER013404), carboxypeptidase-like protein X2 (MER078764), cytosolic carboxypeptidase (MER026952), family M14 non-peptidase homologues (MER199530), insulysin (MER001214), mitochondrial processing peptidase beta-subunit (MER004497), nardilysin (MER003883), eupitrilysin (MER004877), mitochondrial processing peptidase non-peptidase alpha subunit (MER001413), ubiquinol-cytochrome c reductase core protein I (MER003543), ubiquinol-cytochrome c reductase core protein II (MER003544), ubiquinol-cytochrome c reductase core protein domain 2 (MER043998), insulysin unit 2 (MER046821), nardilysin unit 2 (MER046874), insulysin unit 3 (MER078753), mitochondrial processing peptidase subunit alpha unit 2 (MER124489), nardilysin unit 3 (MER142856), LOC133083 g.p. (Homo sapiens) (MER021876), subfamily M16B non-peptidase homologues (MER188757), leucyl aminopeptidase (animal) (MER003100), Mername-AA040 peptidase (MER003919), leucyl aminopeptidase-1 (Caenorhabditis-type) (MER013416), methionyl aminopeptidase 1 (MER001342), methionyl aminopeptidase 2 (MER001728), aminopeptidase P2 (MER004498), Xaa-Pro dipeptidase (eukaryote) (MER001248), aminopeptidase P1 (MER004321), mitochondrial intermediate cleaving peptidase 55 kDa (MER013463), mitochondrial methionyl aminopeptidase (MER014055), Mername-AA020 peptidase homologue (MER010972), proliferation-association protein 1 (MER005497), chromatin-specific transcription elongation factor 140 kDa subunit (MER026495), proliferation-associated protein 1-like (Homo sapiens chromosome X) (MER029983), Mername-AA226 peptidase homologue (Homo sapiens) (MER056262), Mername-AA227 peptidase homologue (Homo sapiens) (MER047299), subfamily M24A non-peptidase homologues (MER179893), aspartyl aminopeptidase (MER003373), Gly-Xaa carboxypeptidase (MER033182), carnosine dipeptidase II

(MER014551), carnosine dipeptidase I (MER015142), Mername-AA161 protein (MER021873), aminoacylase (MER001271), glutamate carboxypeptidase II (MER002104), NAALADASE L peptidase (MER005239), glutamate carboxypeptidase III (MER005238), plasma glutamate carboxypeptidase (MER005244), Mername-AA103 peptidase (MER015091), Fxna peptidase (MER029965), transferrin receptor protein (MER002105), transferrin receptor 2 protein (MER005152), glutaminyl cyclise (MER015095), glutamate carboxypeptidase II (Homo sapiens)-type non-peptidase homologue (MER026971), nicalin (MER044627), membrane dipeptidase (MER001260), membrane-bound dipeptidase-2 (MER013499), membrane-bound dipeptidase-3 (MER013496), dihydro-orotase (MER005767), dihydropyrimidinase (MER033266), dihydropyrimidinase related protein-1 (MER030143), dihydropyrimidinase related protein-2 (MER030155), dihydropyrimidinase related protein-3 (MER030151), dihydropyrimidinase related protein-4 (MER030149), dihydropyrimidinase related protein-5 (MER030136), hypothetical protein like 5730457F11RIK (MER033184), 1300019j08rik protein (MER033186)), guanine aminohydrolase (MER037714), Kae1 putative peptidase (MER001577), OSGEPL1-like protein (MER013498), S2P peptidase (MER004458), subfamily M23B non-peptidase homologues (MER199845), subfamily M23B non-peptidase homologues (MER199846), subfamily M23B non-peptidase homologues (MER199847), subfamily M23B non-peptidase homologues (MER137320), subfamily M23B non-peptidase homologues (MER201557), subfamily M23B non-peptidase homologues (MER199417), subfamily M23B non-peptidase homologues (MER199418), subfamily M23B non-peptidase homologues (MER199419), subfamily M23B non-peptidase homologues (MER199420), subfamily M23B non-peptidase homologues (MER175932), subfamily M23B non-peptidase homologues (MER199665), Poh1 peptidase (MER020382), Jab1/MPN domain metalloenzyme (MER022057), Mername-AA165 peptidase (MER021865), Brcc36 isopeptidase (MER021890), histone H2A deubiquitinase MYSM1 (MER021887), AMSH deubiquitinating peptidase (MER030146), putative peptidase (Homo sapiens chromosome 2) (MER029970), Mername-AA168 protein (MER021886), COP9 signalosome subunit 6 (MER030137), 26S proteasome non-ATPase regulatory subunit 7 (MER030134), eukaryotic translation initiation factor 3 subunit 5 (MER030133), IFP38 peptidase homologue (MER030132), subfamily M67A non-peptidase homologues (MER191181), subfamily M67A unassigned peptidases (MER191144), granzyme B (Homo sapiens-type) (MER000168), testisin (MER005212), tryptase beta (MER000136), kallikrein-related peptidase 5 (MER005544), corin (MER005881), kallikrein-related peptidase 12 (MER006038), DESC1 peptidase (MER006298), tryptase gamma 1 (MER011036), kallikrein-related peptidase 14 (MER011038), hyaluronan-binding peptidase (MER003612), transmembrane peptidase, serine 4 (MER011104), intestinal serine peptidase

(rodent) (MER016130), adrenal secretory serine peptidase (MER003734), tryptase delta 1 (Homo sapiens) (MER005948), matriptase-3 (MER029902), marapsin (MER006119), tryptase-6 (MER006118), ovochymase-1 domain 1 (MER099182), transmembrane peptidase, serine 3 (MER005926), kallikrein-related peptidase 15 (MER000064), Mername-AA031 peptidase (MER014054), TMPRSS13 peptidase (MER014226), Mername-AA038 peptidase (MER062848), Mername-AA204 peptidase (MER029980), cationic trypsin (Homo sapiens-type) (MER000020), elastase-2 (MER000118), mannan-binding lectin-associated serine peptidase-3 (MER031968), cathepsin G (MER000082), myeloblastin (MER000170), granzyme A (MER001379), granzyme M (MER001541), chymase (Homo sapiens-type) (MER000123), tryptase alpha (MER000135), granzyme K (MER001936), granzyme H (MER000166), chymotrypsin B (MER000001), elastase-1 (MER003733), pancreatic endopeptidase E (MER000149), pancreatic elastase II (MER000146), enteropeptidase (MER002068), chymotrypsin C (MER000761), prostasin (MER002460), kallikrein 1 (MER000093), kallikrein-related peptidase 2 (MER000094), kallikrein-related peptidase 3 (MER000115), mesotrypsin (MER000022), complement component C1r-like peptidase (MER016352), complement factor D (MER000130), complement component activated C1r (MER000238), complement component activated C1s (MER000239), complement component C2a (MER000231), complement factor B (MER000229), mannan-binding lectin-associated serine peptidase 1 (MER000244), complement factor I (MER000228), pancreatic endopeptidase E form B (MER000150), pancreatic elastase IIB (MER000147), coagulation factor XIIa (MER000187), plasma kallikrein (MER000203) coagulation factor Xia (MER000210), coagulation factor IXa (MER000216), coagulation factor Vila (MER000215), coagulation factor Xa (MER000212), thrombin (MER000188), protein C (activated) (MER000222), acrosin (MER000078), hepsin (MER000156), hepatocyte growth factor activator (MER000186), mannan-binding lectin-associated serine peptidase 2 (MER002758), u-plasminogen activator (MER000195), t-plasminogen activator (MER000192), plasmin (MER000175), kallikrein-related peptidase 6 (MER002580), neurotrypsin (MER004171), kallikrein-related peptidase 8 (MER005400), kallikrein-related peptidase 10 (MER003645), epitheliasin (MER003736), kallikrein-related peptidase 4 (MER005266), prosemin (MER004214), chymopasin (MER001503), kallikrein-related peptidase 11 (MER004861), kallikrein-related peptidase 11 (MER216142), trypsin-2 type A (MER000021), HtrA1 peptidase (Homo sapiens-type) (MER002577), HtrA2 peptidase (MER208413), HtrA2 peptidase (MER004093), HtrA3 peptidase (MER014795), HtrA4 peptidase (MER016351), Tysnd1 peptidase (MER050461), TMPRSS12 peptidase (MER017085), HAT-like putative peptidase 2 (MER021884), trypsin C (MER021898), kallikrein-related peptidase 7 (MER002001), matriptase (MER003735), kallikrein-related peptidase 13 (MER005269),

kallikrein-related peptidase 9 (MER005270), matriptase-2 (MER005278), umbilical vein peptidase (MER005421), LCLP peptidase (MER001900), spinesin (MER014385), marapsin-2 (MER021929), complement factor D-like putative peptidase (MER056164), ovochymase-2 (MER022410), HAT-like 4 peptidase (MER044589), ovochymase 1 domain 1 (MER022412), epidermis-specific SP-like putative peptidase (MER029900), testis serine peptidase 5 (MER029901), Mername-AA258 peptidase (MER000285), polyserase-IA unit 1 (MER030879), polyserase-IA unit 2 (MER030880), testis serine peptidase 2 (human-type) (MER033187), hypothetical acrosin-like peptidase (Homo sapiens) (MER033253), HAT-like 5 peptidase (MER028215), polyserase-3 unit 1 (MER061763), polyserase-3 unit 2 (MER061748), peptidase similar to tryptophan/serine protease (MER056263), polyserase-2 unit 1 (MER061777), Mername-AA123 peptidase (MER021930), HAT-like 2 peptidase (MER099184), hCG2041452-like protein (MER099172), hCG22067 (Homo sapiens) (MER099169), brain-rescue-factor-1 (Homo sapiens) (MER098873), hCG2041108 (Homo sapiens) (MER099173), polyserase-2 unit 2 (MER061760), polyserase-2 unit 3 (MER065694), Mername-AA201 (peptidase homologue) MER099175, secreted trypsin-like serine peptidase homologue (MER030000), polyserase-1A unit 3 (MER029880), azurocidin (MER000119), haptoglobin-1 (MER000233), haptoglobin-related protein (MER000235), macrophage-stimulating protein (MER001546), hepatocyte growth factor (MER000185), protein Z (MER000227), TESP1 protein (MER047214), LOC136242 protein (MER016132), plasma kallikrein-like protein 4 (MER016346), PRSS35 protein (MER016350), DKFZp586H2123-like protein (MER066474), apolipoprotein (MER000183), psi-KLK1 pseudogene (Homo sapiens) (MER033287), tryptase pseudogene I (MER015077), tryptase pseudogene II (MER015078), tryptase pseudogene III (MER015079), subfamily S1A unassigned peptidases (MER216982), subfamily S1A unassigned peptidases (MER216148), amidophosphoribosyltransferase precursor (MER003314), glutamine-fructose-6-phosphate transaminase 1 (MER003322), glutamine:fructose-6-phosphate amidotransferase (MER012158), Mername-AA144 protein (MER021319), asparagine synthetase (MER033254), family C44 non-peptidase homologues (MER159286), family C44 unassigned peptidases (MER185625) family C44 unassigned peptidases (MER185626), secernin 1 (MER045376), secernin 2 (MER064573), secernin 3 (MER064582), acid ceramidase precursor (MER100794), N-acylethanolamine acid amidase precursor (MER141667), proteasome catalytic subunit 1 (MER000556), proteasome catalytic subunit 2 (MER002625), proteasome catalytic subunit 3 (MER002149), proteasome catalytic subunit 1i (MER000552), proteasome catalytic subunit 2i (MER001515), proteasome catalytic subunit 3i (MER000555), proteasome catalytic subunit 5t (MER026203), protein serine kinase c17 (MER026497), proteasome subunit alpha 6 (MER000557), proteasome subunit alpha 2 (MER000550), proteasome subunit alpha 4

(MER000554), proteasome subunit alpha 7 (MER033250), proteasome subunit alpha 5 (MER000558), proteasome subunit alpha 1 (MER000549), proteasome subunit alpha 3 (MER000553), proteasome subunit XAPC7 (MER004372), proteasome subunit beta 3 (MER001710), proteasome subunit beta 2 (MER002676), proteasome subunit beta 1 (MER000551), proteasome subunit beta 4 (MER001711), Mername-AA230 peptidase homologue (Homo sapiens) (MER047329), Mername-AA231 pseudogene (Homo sapiens) (MER047172), Mername-AA232 pseudogene (Homo sapiens) (MER047316), glycosylasparaginase precursor (MER003299), isoaspartyl dipeptidase (threonine type) (MER031622), taspase-1 (MER016969), gamma-glutamyltransferase 5 (mammalian-type) (MER001977), gamma-glutamyltransferase 1 (mammalian-type) (MER001629), gamma-glutamyltransferase 2 (Homo sapiens) (MER001976), gamma-glutamyltransferase-like protein 4 (MER002721), gamma-glutamyltransferase-like protein 3 (MER016970), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026204), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026205), Mername-AA211 putative peptidase (MER026207), gamma-glutamyltransferase 6 (MER159283), gamma-glutamyl transpeptidase homologue (chromosome 2, Homo sapiens) (MER037241), polycystin-1 (MER126824), KIAA1879 protein (MER159329), polycystic kidney disease 1-like 3 (MER172554), gamma-glutamyl hydrolase (MER002963), guanine 5″-monophosphate synthetase (MER043387), carbamoyl-phosphate synthase (Homo sapiens-type) (MER078640), dihydro-orotase (N-terminal unit) (Homo sapiens-type) (MER060647), DJ-1 putative peptidase (MER003390), Mername-AA100 putative peptidase (MER014802), Mername-AA101 non-peptidase homologue (MER014803), KIAA0361 protein (Homo sapiens-type) (MER042827), F1134283 protein (Homo sapiens) (MER044553), non-peptidase homologue chromosome 21 open reading frame 33 (Homo sapiens) (MER160094), family C56 non-peptidase homologues (MER177016), family C56 non-peptidase homologues (MER176613), family C56 non-peptidase homologues (MER176918), EGF-like module containing mucin-like hormone receptor-like 2 (MER037230), CD97 antigen (human type) (MER037286), EGF-like module containing mucin-like hormone receptor-like 3 (MER037288), EGF-like module containing mucin-like hormone receptor-like 1 (MER037278), EGF-like module containing mucin-like hormone receptor-like 4 (MER037294), cadherin EGF LAG seven-pass G-type receptor 2 precursor (Homo sapiens) (MER045397), Gpr64 (Mus musculus)-type protein (MER123205), GPR56 (Homo sapiens)-type protein (MER122057), latrophilin 2 (MER122199), latrophilin-1 (MER126380), latrophilin 3 (MER124612), protocadherin Flamingo 2 (MER124239), ETL protein (MER126267), G protein-coupled receptor 112 (MER126114), seven transmembrane helix receptor (MER125448), Gpr114 protein (MER159320), GPR126 vascular inducible G

protein-coupled receptor (MER140015), GPR125 (Homo sapiens)-type protein (MER159279), GPR116 (Homo sapiens)-type G-protein coupled receptor (MER159280), GPR128 (Homo sapiens)-type G-protein coupled receptor (MER162015), GPR133 (Homo sapiens)-type protein (MER159334), GPR110 G-protein coupled receptor (MER159277), GPR97 protein (MER159322), KPG_006 protein (MER161773), KPG_008 protein (MER161835), KPG_009 protein (MER159335), unassigned homologue (MER166269), GPR113 protein (MER159352), brain-specific angiogenesis inhibitor 2 (MER159746), PIDD auto-processing protein unit 1 (MER020001), PIDD auto-processing protein unit 2 (MER063690), MUC1 self-cleaving mucin (MER074260), dystroglycan (MER054741), proprotein convertase 9 (MER022416), site-1 peptidase (MER001948), furin (MER000375), proprotein convertase 1 (MER000376), proprotein convertase 2 (MER000377), proprotein convertase 4 (MER028255), PACE4 proprotein convertase (MER000383), proprotein convertase 5 (MER002578), proprotein convertase 7 (MER002984), tripeptidyl-peptidase II (MER000355), subfamily S8A non-peptidase homologues (MER201339), subfamily S8A non-peptidase homologues (MER191613), subfamily S8A unassigned peptidases (MER191611), subfamily S8A unassigned peptidases (MER191612), subfamily S8A unassigned peptidases (MER191614), tripeptidyl-peptidase I (MER003575), prolyl oligopeptidase (MER000393), dipeptidyl-peptidase IV (eukaryote) (MER000401), acylaminoacyl-peptidase (MER000408), fibroblast activation protein alpha subunit (MER000399), PREPL A protein (MER004227), dipeptidyl-peptidase 8 (MER013484), dipeptidyl-peptidase 9 (MER004923), FLJ1 putative peptidase (MER017240), Mername-AA194 putative peptidase (MER017353), Mername-AA195 putative peptidase (MER017367), Mername-AA196 putative peptidase (MER017368), Mername-AA197 putative peptidase (MER017371), C14orf29 protein (MER033244), hypothetical protein (MER033245), hypothetical esterase/lipase/thioesterase (MER047309), protein bat5 (MER037840), hypothetical protein flj40219 (MER033212), hypothetical protein flj37464 (MER033240), hypothetical protein flj33678 (MER033241), dipeptidylpeptidase homologue DPP6 (MER000403), dipeptidylpeptidase homologue DPP10 (MER005988), protein similar to Mus musculus chromosome 20 open reading frame 135 (MER037845), kynurenine formamidase (MER046020), thyroglobulin precursor (MER011604), acetylcholinesterase (MER033188), cholinesterase (MER033198), carboxylesterase D1 (MER033213), liver carboxylesterase (MER033220), carboxylesterase 3 (MER033224), carboxylesterase 2 (MER033226), bile salt-dependent lipase (MER033227), carboxylesterase-related protein (MER033231), neuroligin 3 (MER033232), neuroligin 4, X-linked (MER033235), neuroligin 4, Y-linked (MER033236), esterase D (MER043126), arylacetamide deacetylase (MER033237), KIAA1363-like protein (MER033242), hormone-sensitive lipase (MER033274), neuroligin 1 (MER033280), neuroligin

2 (MER033283), family S9 non-peptidase homologues (MER212939), family S9 non-peptidase homologues (MER211490), subfamily S9C unassigned peptidases (MER192341), family S9 unassigned peptidases (MER209181), family S9 unassigned peptidases (MER200434), family S9 unassigned peptidases (MER209507), family S9 unassigned peptidases (MER209142), serine carboxypeptidase A (MER000430), vitellogenic carboxypeptidase-like protein (MER005492), RISC peptidase (MER010960), family S15 unassigned peptidases (MER199442), family S15 unassigned peptidases (MER200437), family S15 unassigned peptidases (MER212825), lysosomal Pro-Xaa carboxypeptidase (MER000446), dipeptidyl-peptidase II (MER004952), thymus-specific serine peptidase (MER005538), epoxide hydrolase-like putative peptidase (MER031614), Loc328574-like protein (MER033246), abhydrolase domain-containing protein 4 (MER031616), epoxide hydrolase (MER000432), mesoderm specific transcript protein (MER199890), mesoderm specific transcript protein (MER017123), cytosolic epoxide hydrolase (MER029997), cytosolic epoxide hydrolase (MER213866), similar to hypothetical protein FLJ22408 (MER031608), CGI-58 putative peptidase (MER030163), Williams-Beuren syndrome critical region protein 21 epoxide hydrolase (MER031610), epoxide hydrolase (MER031612), hypothetical protein 922408 (epoxide hydrolase) (MER031617), monoglyceride lipase (MER033247), hypothetical protein (MER033249), valacyclovir hydrolase (MER033259), Ccg1-interacting factor b (MER210738), glycosylasparaginase precursor (MER003299), isoaspartyl dipeptidase (threonine type) (MER031622). taspase-1 (MER016969), gamma-glutamyltransferase 5 (mammalian-type) (MER001977), gamma-glutamyltransferase 1 (mammalian-type) (MER001629), gamma-glutamyltransferase 2 (Homo sapiens) (MER001976), gamma-glutamyltransferase-like protein 4 (MER002721). gamma-glutamyltransferase-like protein 3 (MER016970). similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026204). similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026205). Mername-AA211 putative peptidase (MER026207). gamma-glutamyltransferase 6 (MER159283). gamma-glutamyl transpeptidase homologue (chromosome 2, Homo sapiens) (MER037241). polycystin-1 (MER126824), KIAA1879 protein (MER159329). polycystic kidney disease 1-like 3 (MER172554). gamma-glutamyl hydrolase (MER002963). guanine 5″-monophosphate synthetase (MER043387). carbamoyl-phosphate synthase (Homo sapiens-type) (MER078640). dihydro-orotase (N-terminal unit) (Homo sapiens-type) (MER060647). DJ-1 putative peptidase (MER003390). Mername-AA100 putative peptidase (MER014802). Mername-AA101 non-peptidase homologue (MER014803). KIAA0361 protein (Homo sapiens-type) (MER042827). F1134283 protein (Homo sapiens) (MER044553). non-peptidase homologue chromosome 21 open reading frame 33 (Homo sapiens) (MER160094). family C56 non-peptidase homologues (MER177016), family C56 non-

peptidase homologues (MER176613). family C56 non-peptidase homologues (MER176918). EGF-like module containing mucin-like hormone receptor-like 2 (MER037230). CD97 antigen (human type) (MER037286). EGF-like module containing mucin-like hormone receptor-like 3 (MER037288). EGF-like module containing mucin-like hormone receptor-like 1 (MER037278). EGF-like module containing mucin-like hormone receptor-like 4 (MER037294). cadherin EGF LAG seven-pass G-type receptor 2 precursor (Homo sapiens) (MER045397), Gpr64 (Mus musculus)-type protein (MER123205). GPR56 (Homo sapiens)-type protein (MER122057). latrophilin 2 (MER122199). latrophilin-1 (MER126380). latrophilin 3 (MER124612). protocadherin Flamingo 2 (MER124239). ETL protein (MER126267). G protein-coupled receptor 112 (MER126114). seven transmembrane helix receptor (MER125448). Gpr114 protein (MER159320). GPR126 vascular inducible G protein-coupled receptor (MER140015). GPR125 (Homo sapiens)-type protein (MER159279). GPR116 (Homo sapiens)-type G-protein coupled receptor (MER159280). GPR128 (Homo sapiens)-type G-protein coupled receptor (MER162015). GPR133 (Homo sapiens)-type protein (MER159334) GPR110 G-protein coupled receptor (MER159277), GPR97 protein (MER159322), KPG_006 protein (MER161773) KPG_008 protein (MER161835), KPG_009 protein (MER159335), unassigned homologue (MER166269), GPR113 protein (MER159352), brain-specific angiogenesis inhibitor 2 (MER159746), PIDD auto-processing protein unit 1 (MER020001), PIDD auto-processing protein unit 2 (MER063690), MUC1 self-cleaving mucin (MER074260), dystroglycan (MER054741), proprotein convertase 9 (MER022416), site-1 peptidase (MER001948), furin (MER000375), proprotein convertase 1 (MER000376), proprotein convertase 2 (MER000377), proprotein convertase 4 (MER028255), PACE4 proprotein convertase (MER000383), proprotein convertase 5 (MER002578), proprotein convertase 7 (MER002984), tripeptidyl-peptidase II (MER000355), subfamily S8A non-peptidase homologues (MER201339), subfamily S8A non-peptidase homologues (MER191613), subfamily S8A unassigned peptidases (MER191611), subfamily S8A unassigned peptidases (MER191612), subfamily S8A unassigned peptidases (MER191614), tripeptidyl-peptidase I (MER003575), prolyl oligopeptidase (MER000393), dipeptidyl-peptidase IV (eukaryote) (MER000401), acylaminoacyl-peptidase (MER000408), fibroblast activation protein alpha subunit (MER000399), PREPL A protein (MER004227), dipeptidyl-peptidase 8 (MER013484), dipeptidyl-peptidase 9 (MER004923), FLJ1 putative peptidase (MER017240), Mername-AA194 putative peptidase (MER017353), Mername-AA195 putative peptidase (MER017367), Mername-AA196 putative peptidase (MER017368), Mername-AA197 putative peptidase (MER017371), C14orf29 protein (MER033244), hypothetical protein (MER033245), hypothetical esterase/lipase/thioesterase (MER047309), protein bat5 (MER037840), hypothetical protein flj40219 (MER033212), hypothetical protein

flj37464 (MER033240), hypothetical protein flj33678 (MER033241), dipeptidylpeptidase homologue DPP6 (MER000403), dipeptidylpeptidase homologue DPP10 (MER005988), protein similar to Mus musculus chromosome 20 open reading frame 135 (MER037845), kynurenine formamidase (MER046020), thyroglobulin precursor (MER011604), acetylcholinesterase (MER033188), cholinesterase (MER033198), carboxylesterase D1 (MER033213), liver carboxylesterase (MER033220), carboxylesterase 3 (MER033224), carboxylesterase 2 (MER033226), bile salt-dependent lipase (MER033227), carboxylesterase-related protein (MER033231), neuroligin 3 (MER033232), neuroligin 4, X-linked (MER033235), neuroligin 4, Y-linked (MER033236), esterase D (MER043126), arylacetamide deacetylase (MER033237), KIAA1363-like protein (MER033242), hormone-sensitive lipase (MER033274), neuroligin 1 (MER033280), neuroligin 2 (MER033283), family S9 non-peptidase homologues (MER212939), family S9 non-peptidase homologues (MER211490), subfamily S9C unassigned peptidases (MER192341), family S9 unassigned peptidases (MER209181), family S9 unassigned peptidases (MER200434), family S9 unassigned peptidases (MER209507), family S9 unassigned peptidases (MER209142), serine carboxypeptidase A (MER000430), vitellogenic carboxypeptidase-like protein (MER005492), RISC peptidase (MER010960), family S15 unassigned peptidases (MER199442), family S15 unassigned peptidases (MER200437), family S15 unassigned peptidases (MER212825), lysosomal Pro-Xaa carboxypeptidase (MER000446), dipeptidyl-peptidase II (MER004952), thymus-specific serine peptidase (MER005538), epoxide hydrolase-like putative peptidase (MER031614), Loc328574-like protein (MER033246), abhydrolase domain-containing protein 4 (MER031616), epoxide hydrolase (MER000432), mesoderm specific transcript protein (MER199890), mesoderm specific transcript protein (MER017123), cytosolic epoxide hydrolase (MER029997), cytosolic epoxide hydrolase (MER213866), similar to hypothetical protein FLJ22408 (MER031608), CGI-58 putative peptidase (MER030163), Williams-Beuren syndrome critical region protein 21 epoxide hydrolase (MER031610), epoxide hydrolase (MER031612), hypothetical protein flj22408 (epoxide hydrolase) (MER031617), monoglyceride lipase (MER033247), hypothetical protein (MER033249), valacyclovir hydrolase (MER033259), Ccg1-interacting factor b (MER210738).

Protease enzymatic activity can be regulated. For example, certain proteases can be inactivated by the presence or absence of a specific agent (e.g., that binds to the protease, such as specific small molecule inhibitors). Such proteases can be referred to as a "repressible protease." Exemplary inhibitors for certain proteases are listed in **Table 4B**. For example, an NS3 protease can be repressed by a protease inhibitor including, but not limited to, simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir. In another example, protease activity can be regulated through

regulating expression of the protease itself, such as engineering a cell to express a protease using an inducible promoter system (*e.g.*, Tet On/Off systems) or cell-specific promoters (promoters that can be used to express a heterologous protease are described in more detail in the Section herein titled "Promoters"). A protease can also contain a degron, such as any of the degrons described herein, and can be regulated using any of the degron systems described herein.

Protease enzymatic activity can also be regulated through selection of a specific protease cleavage site. For example, a protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage by a desired protease, such as reduced cleavage kinetics relative to an endogenous sequence of a substrate naturally cleaved by the desired protease. As another example, a protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage in a cell-state specific manner. For example, various cell states (*e.g.*, following cellular signaling, such as immune cell activation) can influence the expression and/or localization of certain proteases. As an illustrative example, ADAM17 protein levels and localization is known to be influenced by signaling, such as through Protein kinase C (PKC) signaling pathways (*e.g.*, activation by the PKC activator Phorbol-12-myristat-13-acetat [PMA]). Accordingly, a protease cleavage site can be selected and/or engineered such that cleavage of the protease cleavage site and subsequent release of an effector molecule is increased or decreased, as desired, depending on the protease properties (*e.g.*, expression and/or localization) in a specific cell state. As another example, a protease cleavage site (particularly in combination with a specific membrane tethering domain) can be selected and/or engineered for optimal protein expression of the chimeric protein.

Cell Membrane Tethering Domain

The membrane-cleavable chimeric proteins provided for herein contain a cell-membrane tethering domain (referred to as "MT" in the formula **S – C – MT** or **MT – C – S**). In general, the cell-membrane tethering domain can be any amino acid sequence motif capable of directing the chimeric protein to be localized to (*e.g.*, inserted into), or otherwise associated with, the cell membrane of the cell expressing the chimeric protein. The cell-membrane tethering domain can be a transmembrane-intracellular domain. The cell-membrane tethering domain can be a transmembrane domain. The cell-membrane tethering domain can be an integral membrane protein domain (*e.g.*, a transmembrane domain). The cell-membrane tethering domain can be derived from a Type I, Type II, or Type III transmembrane protein. The cell-membrane tethering domain can include post-translational modification tag, or motif capable of post-translational modification to modify the chimeric protein to include a post-translational modification tag,

where the post-translational modification tag allows association with a cell membrane. Examples of post-translational modification tags include, but are not limited to, lipid-anchor domains (*e.g.*, a GPI lipid-anchor, a myristoylation tag, or palmitoylation tag). Examples of cell-membrane tethering domains include, but are not limited to, a transmembrane-intracellular domain and/or transmembrane domain derived from PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, or BTLA. The cell membrane tethering domain can be a cell surface receptor or a cell membrane-bound portion thereof.

In some embodiments, the cell-membrane tethering domain comprises a transmembrane domain derived from a B7-1 polypeptide. In some embodiments, the B7-1 transmembrane domain comprises the sequence LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204). In some embodiments, the B7-1 transmembrane domain is encoded by a polynucleotide sequence having the sequence TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252). In some embodiments, the B7-1 transmembrane domain is encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252).

In some embodiments, the cell-membrane tethering domain of the membrane-cleavable chimeric protein (*e.g.*, IL-15) includes a transmembrane domain derived from a B7-1 polypeptide. In some embodiments, the B7-1 transmembrane domain of the membrane-cleavable chimeric protein (*e.g.*, IL-15) includes the sequence LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204). In some embodiments, the B7-1 transmembrane domain of the membrane-cleavable chimeric protein (*e.g.*, IL-15) is encoded by a polynucleotide sequence having the sequence TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252). In some embodiments, the B7-1 transmembrane domain of the membrane-cleavable chimeric protein (*e.g.*, IL-15) is encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252).

In some embodiments, the cell-membrane tethering domain comprises a transmembrane domain derived from a CD8 polypeptide. Any suitable CD8 polypeptide may be used. Exemplary CD8 polypeptides include, without limitation, NCBI Reference Nos. NP_001139345 and AAA92533.1. Examples of CD8 transmembrane domains include IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 205), IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206), and IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 207). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 205). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 207). In some embodiments, the cell-membrane tethering domain comprises a hinge and transmembrane domain derived from CD8.In some embodiments, the CD8 hinge comprises the sequence TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271). In some embodiments, the CD8 hinge comprises the sequence AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 209).

In general, for all membrane-cleavable chimeric proteins described herein, the cell membrane tethering domain is either: (1) C-terminal of the protease cleavage site and N-terminal of any intracellular domain, if present (in other words, the cell membrane tethering domain is in between the protease cleavage site and, if present, an intracellular domain); or (2) N-terminal of the protease cleavage site and C-terminal of any intracellular domain, if present (also between the protease cleavage site and, if present, an intracellular domain with domain orientation inverted). In embodiments featuring a degron associated with the chimeric protein, the degron domain is the terminal cytoplasmic-oriented domain, specifically relative to the cell membrane tethering (in other words, the cell membrane tethering domain is in between the protease cleavage site and the degron). The cell membrane tethering domain can be connected to the protease cleavage site by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of cell membrane tethering domain or protease cleavage site. The cell membrane tethering domain can be connected to an intracellular domain, if present, by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell

membrane tethering domain or the intracellular domain. The cell membrane tethering domain can be connected to the degron, if present, by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or degron. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, $[GS]_4GG$ [SEQ ID NO: 182]), $A(EAAAK)_3A$ (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a "KEGS" linker such as the amino acid sequence KESGSVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art.

In general, the cell-membrane tethering domain is oriented such that the secreted effector molecule and the protease cleavage site are extracellularly exposed following insertion into, or association with, the cell membrane, such that the protease cleavage site is capable of being cleaved by its respective protease and releasing ("secreting") the effector molecule into the extracellular space.

Degron Systems and Domains

In some embodiments, any of the proteins described herein can include a degron domain including, but not limited to, a protease, a transcription factor, a promoter or constituent of a promoter system (*e.g.*, an ACP), and/or any of the membrane-cleavable chimeric protein described herein. In general, the degron domain can be any amino acid sequence motif capable of directing regulated degradation, such as regulated degradation through a ubiquitin-mediated pathway. In the presence of an immunomodulatory drug (IMiD), the degron domain directs ubiquitin-mediated degradation of a degron-fusion protein.

The degron domain can be a cereblon (CRBN) polypeptide substrate domain capable of binding CRBN in response to an immunomodulatory drug (IMiD) including, but not limited to, IKZF1, IKZF3, CKla, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN. The CRBN polypeptide substrate domain can be a chimeric fusion product of native CRBN polypeptide sequences, such as a IKZF3/ZFP91/IKZF3 chimeric fusion product

having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRD AL (SEQ ID NO: 175). Degron domains, and in particular CRBN degron systems, are described in more detail in International Application Pub. No. WO2019/089592A1, herein incorporated by reference for all purposes. Other examples of degron domains include, but are not limited to HCV NS4 degron, PEST (two copies of residues 277-307 of human IκBα; SEQ ID NO: 161), GRR (residues 352-408 of human p105; SEQ ID NO: 162), DRR (residues 210-295 of yeast Cdc34; SEQ ID NO: 163), SNS (tandem repeat of SP2 and NB (SP2-NB-SP2 of influenza A or influenza B; *e.g.*, SEQ ID NO: 164), RPB (four copies of residues 1688-1702 of yeast RPB; SEQ ID NO: 165), SPmix (tandem repeat of SP1 and SP2 (SP2-SP1-SP2-SP1-SP2 of influenza A virus M2 protein; SEQ ID NO: 166), NS2 (three copies of residues 79-93 of influenza A virus NS protein; SEQ ID NO: 167), ODC (residues 106-142 of ornithine decarboxylase; SEQ ID NO: 168), Nek2A, mouse ODC (residues 422–461; SEQ ID NO: 169), mouse ODC_DA (residues 422-461 of mODC including D433A and D434A point mutations), an APC/C degron, a COP1 E3 ligase binding degron motif, a CRL4-Cdt2 binding PIP degron, an actinfilin-binding degron, a KEAP1 binding degron, a KLHL2 and KLHL3 binding degron, an MDM2 binding motif, an N-degron, a hydroxyproline modification in hypoxia signaling, a phytohormone-dependent SCF-LRR-binding degron, an SCF ubiquitin ligase binding phosphodegron, a phytohormone-dependent SCF-LRR-binding degron, a DSGxxS phospho-dependent degron, an Siah binding motif, an SPOP SBC docking motif, or a PCNA binding PIP box.

Regulated degradation can be drug-inducible. Drugs capable of mediating/regulating degradation can be small-molecule compounds. Drugs capable of mediating/regulating degradation can include an "immunomodulatory drug" (IMiD). In general, as used herein, IMiDs refer to a class of small-molecule immunomodulatory drugs containing an imide group. Cereblon (CRBN) is known target of IMiDs and binding of an IMiD to CRBN or a CRBN polypeptide substrate domain alters the substrate specificity of the CRBN E3 ubiquitin ligase complex leading to degradation of proteins having a CRBN polypeptide substrate domain (*e.g.*, any of secretable effector molecules or other proteins of interest described herein). For degron domains having a CRBN polypeptide substrate domain, examples of imide-containing IMiDs include, but are not limited to, a thalidomide, a lenalidomide, or a pomalidomide. The IMiD can be an FDA-approved drug.

Chimeric proteins described herein can contain a degron domain (*e.g.*, referred to as "D" in the formula **S – C – MT – D** or **D – MT – C – S** for membrane-cleavable chimeric proteins described herein). In the absence of an IMiD, degron/ubiquitin-mediated degradation of the chimeric protein does not occur. Following expression and localization of the chimeric protein

into the cell membrane, the protease cleavage site directs cleavage of the chimeric protein such that the effector molecule is released ("secreted") into the extracellular space. In the presence of an immunomodulatory drug (IMiD), the degron domain directs ubiquitin-mediated degradation of the chimeric protein such that secretion of the effector molecule is reduced or eliminated. In general, for membrane-cleavable chimeric proteins fused to a degron domain, the degron domain is the terminal cytoplasmic-oriented domain, specifically relative to the cell membrane tethering domain, *e.g.*, the most C-terminal domain in the formula **S – C – MT – D** or the most N-terminal domain in the formula **D – MT – C – S**. The degron domain can be connected to the cell membrane tethering domain by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or the degron domain. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, $[GS]_4GG$ [SEQ ID NO: 182]), $A(EAAAK)_3A$ (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a "KEGS" linker such as the amino acid sequence KESGSVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art. In general, the degron is oriented in relation to the cell membrane tethering domain such that the degron is exposed to the cytosol following localization to the cell membrane such that the degron domain is capable of mediating degradation (*e.g.*, exposure to the cytosol and cytosol) and is capable of mediating ubiquitin-mediated degradation.

For degron-fusion proteins, the degron domain can be N-terminal or C-terminal of the protein of interest, *e.g.*, the effector molecule. The degron domain can be connected to the protein of interest by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the protein of interest or the degron domain. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, $[GS]_4GG$ [SEQ ID NO: 182]), $A(EAAAK)_3A$ (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a "KEGS" linker such as the amino acid sequence KESGSVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional

polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art. A polypeptide linker can be cleavable, *e.g.*, any of the protease cleavage sites described herein.

Homing Molecules

A "tumor microenvironment" is the cellular environment in which a tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix (ECM) (see, *e.g.*, Pattabiraman, D.R. & Weinberg, R.A. *Nature Reviews Drug Discovery* 13, 497–512 (2014); Balkwill, F.R. *et al. J Cell Sci* 125, 5591-5596, 2012; and Li, H. *et al. J Cell Biochem* 101(4), 805-15, 2007).

In some embodiments, engineered nucleic acids are configured to produce at least one homing molecule. For example, in membrane-cleavable chimeric proteins described herein containing a secreted effector molecule, the secreted effector molecule can be a homing molecule. "Homing," refers to active navigation (migration) of a cell to a target site (*e.g.*, a cell, tissue (*e.g.*, tumor), or organ). A "homing molecule" refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate interaction of an engineered cell to a target site. Non-limiting examples of homing molecules include CXCR1, CCR9, CXCR2, CXCR3, CXCR4, CCR2, CCR4, FPR2, VEGFR, IL6R, CXCR1, CSCR7, and PDGFR.

In some embodiments, a homing molecule is a chemokine receptor (cell surface molecule that binds to a chemokine). Chemokines are small cytokines or signaling proteins secreted by cells that can induce directed chemotaxis in cells. Chemokines can be classified into four main subfamilies: CXC, CC, CX3C and XC, all of which exert biological effects by binding selectively to chemokine receptors located on the surface of target cells. In some embodiments, engineered nucleic acids are configured to produce CXCR4, a chemokine receptor which allows engineered cells to home along a chemokine gradient towards a stromal cell-derived factor 1 (also known as SDF1, C-X-C motif chemokine 12, and CXCL12)-expressing cell, tissue, or tumor. Non-limiting examples of chemokine receptors that may be encoded by the engineered nucleic acids of the present disclosure include: CXC chemokine receptors (*e.g.*, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7), CC chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, and CCR11), CX3C chemokine receptors (*e.g.*, CX3CR1, which binds to CX3CL1),

and XC chemokine receptors (*e.g.*, XCR1). In some embodiments, a chemokine receptor is a G protein-linked transmembrane receptor, or a member of the tumor necrosis factor (TNF) receptor superfamily (including but not limited to TNFRSF1A, TNFRSF1B). In some embodiments, engineered nucleic acids are configured to produce CXCL8, CXCL9, and/or CXCL10 (promote T-cell recruitment), CCL3 and/or CXCL5, CCL21 (Th1 recruitment and polarization).

In some embodiments, engineered nucleic acids are configured to produce G-protein coupled receptors (GPCRs) that detect N-formylated-containing oligopeptides (including but not limited to FPR2 and FPRL1).

In some embodiments, engineered nucleic acids are configured to produce receptors that detect interleukins (including but not limited to IL6R).

In some embodiments, engineered nucleic acids are configured to produce receptors that detect growth factors secreted from other cells, tissues, or tumors (including but not limited to FGFR, PDGFR, EGFR, and receptors of the VEGF family, including but not limited to VEGF-C and VEGF-D).

In some embodiments, a homing molecule is an integrin. Integrins are transmembrane receptors that facilitate cell-extracellular matrix (ECM) adhesion. Integrins are obligate heterodimers having two subunits: α (alpha) and β (beta). The α subunit of an integrin may be, without limitation: ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, IGTA7, ITGA8, ITGA9, IGTA10, IGTA11, ITGAD, ITGAE, ITGAL, ITGAM, ITGAV, ITGA2B, ITGAX. The β subunit of an integrin may be, without limitation: ITGB1, ITGB2, ITGB3, ITGB4, ITGB5, ITGB6, ITGB7, and ITGB8. Engineered nucleic acids can be configured to produce any combination of the integrin α and β subunits.

In some embodiments, a homing molecule is a matrix metalloproteinase (MMP). MMPs are enzymes that cleave components of the basement membrane underlying the endothelial cell wall. Non-limiting examples of MMPs include MMP-2, MMP-9, and MMP. In some embodiments, engineered nucleic acids are configured to produce an inhibitor of a molecule (*e.g.*, protein) that inhibits MMPs. For example, engineered nucleic acids can be configured to express an inhibitor (*e.g.*, an RNAi molecule) of membrane type 1 MMP (MT1-MMP) or TIMP metallopeptidase inhibitor 1 (TIMP-1).

In some embodiments, a homing molecule is a ligand that binds to selectin (*e.g.*, hematopoietic cell E-/L-selectin ligand (HCELL), Dykstran e*t al.*, Stem Cells. 2016 Oct;34(10):2501-2511) on the endothelium of a target tissue, for example.

The term "homing molecule" also encompasses transcription factors that regulate the production of molecules that improve/enhance homing of cells.

In some embodiments, a homing molecule includes an antibody, such as anti-integrin alpha4,beta7 or anti-MAdCAM.

Chimeric Antigen Receptors (CARs)

Certain aspects of the present disclosure relate to chimeric receptors that have any one of the antigen-specific binding domains described herein (*e.g.*, EMCN-specific, FLT3-specific, and/or CD33-specific) and are capable of specifically binding to a protein, an antigen-derived antigen, or an antigen-derived epitope.

In some embodiments, the chimeric receptor is a chimeric antigen receptor (CAR). In general, CARs are chimeric proteins that include an antigen-binding domain and polypeptide molecules that are heterologous to the antigen-binding domain, such as peptides heterologous to an antibody that an antigen-binding domain may be derived from. Polypeptide molecules that are heterologous to the antigen-binding domain can include, but are not limited to, a transmembrane domain, one or more intracellular signaling domains, a hinge domain, a spacer region, one or more peptide linkers, or combinations thereof.

In some embodiments, CARs are engineered receptors that graft or confer a specificity of interest (*e.g.*, EMCN, FLT3, or CD33) onto an immune effector cell. In certain embodiments, CARs can be used to graft the specificity of an antibody onto an immunoresponsive cell, such as a T cell. In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain (e.g., an scFv) fused to a transmembrane domain, fused to one or more intracellular signaling domains.

In some embodiments, the chimeric antigen receptor is an activating chimeric antigen receptor (aCAR and also generally referred to as CAR unless otherwise specified). In some embodiments, binding of the chimeric antigen receptor to its cognate ligand is sufficient to induce activation of the immunoresponsive cell. In some embodiments, binding of the chimeric antigen receptor to its cognate ligand is sufficient to induce stimulation of the immunoresponsive cell. In some embodiments, activation of an immunoresponsive cell results in killing of target cells. In some embodiments, activation of an immunoresponsive cell results in cytokine or chemokine expression and/or secretion by the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell results in cytokine or chemokine expression and/or secretion by the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell induces differentiation of the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell induces proliferation of the immunoresponsive cell. In some embodiments, activation and/or stimulation of the immunoresponsive cell can be combinations of the above responses.

The number of ABDs in a binding molecule, such as the chimeric proteins described herein, defines the "valency" of the binding molecule. A binding molecule having a single ABD is "monovalent". A binding molecule having a plurality of ABDs is said to be "multivalent". A multivalent binding molecule having two ABDs is "bivalent." A multivalent binding molecule having three ABDs is "trivalent." A multivalent binding molecule having four ABDs is "tetravalent." In various multivalent embodiments, all of the plurality of ABDs have the same recognition specificity and can be referred to as a "monospecific multivalent" binding molecule. In other multivalent embodiments, at least two of the plurality of ABDs have different recognition specificities. Such binding molecules are multivalent and "multispecific." In multivalent embodiments in which the ABDs collectively have two recognition specificities, the binding molecule is "bispecific." In multivalent embodiments in which the ABDs collectively have three recognition specificities, the binding molecule is "trispecific." In multivalent embodiments in which the ABDs collectively have a plurality of recognition specificities for different epitopes present on the same antigen, the binding molecule is "multiparatopic." Multivalent embodiments in which the ABDs collectively recognize two epitopes on the same antigen are "biparatopic."

In various multivalent embodiments, multivalency of the binding molecule improves the avidity of the binding molecule for a specific target. As described herein, "avidity" refers to the overall strength of interaction between two or more molecules, e.g. a multivalent binding molecule for a specific target, wherein the avidity is the cumulative strength of interaction provided by the affinities of multiple ABDs. Avidity can be measured by the same methods as those used to determine affinity, as described above. In certain embodiments, the avidity of a binding molecule for a specific target is such that the interaction is a specific binding interaction, wherein the avidity between two molecules has a KD value below 10^{-6}M, 10^{-7}M, 10^{-8}M, 10^{-9}M, or 10^{-10}M. In certain embodiments, the avidity of a binding molecule for a specific target has a KD value such that the interaction is a specific binding interaction, wherein the one or more affinities of individual ABDs do not have has a KD value that qualifies as specifically binding their respective antigens or epitopes on their own. In certain embodiments, the avidity is the cumulative strength of interaction provided by the affinities of multiple ABDs for separate antigens on a shared specific target or complex, such as separate antigens found on an individual cell. In certain embodiments, the avidity is the cumulative strength of interaction provided by the affinities of multiple ABDs for separate epitopes on a shared individual antigen.

In some embodiments, an aCAR can be a bivalent-bispecific CAR. In some embodiments, an aCAR can be a bivalent-bispecific CAR expressed on a cell of the present disclosure (e.g., an immunoresponsive cell) as OR-logic gates to increase the potential targets of

an activating chimeric receptor (*e.g.*, an OR-logic gate targeting multiple tumor targets). A bivalent-bispecific aCAR can include an antigen-binding domain specific for FLT3 and an antigen-binding domain specific for CD33. A bivalent-bispecific aCAR can include i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response, and (iv) one or more polypeptides including, but not limited to, a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

A CAR of the present disclosure may be a first, second, or third generation CAR. "First generation" CARs comprise a single intracellular signaling domain, generally derived from a T cell receptor chain. "First generation" CARs generally have the intracellular signaling domain from the CD3-zeta (CD3ζ) chain, which is the primary transmitter of signals from endogenous TCRs. "First generation" CARs can provide de novo antigen recognition and cause activation of both $CD4^+$ and $CD8^+$ T cells through their CD3ζ chain signaling domain in a single fusion molecule, independent of HLA-mediated antigen presentation. "Second generation" CARs add a second intracellular signaling domain from one of various co-stimulatory molecules (e.g., CD28, 4-1BB, ICOS, OX40) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. "Second generation" CARs provide both co-stimulation (e.g., CD28 or 4- 1BB) and activation (CD3ζ). Preclinical studies have indicated that "Second Generation" CARs can improve the anti-tumor activity of immunoresponsive cell, such as a T cell. "Third generation" CARs have multiple intracellular co-stimulation signaling domains (e.g., CD28 and 4-1BB) and an intracellular activation signaling domain (CD3ζ).

In some embodiments, the chimeric antigen receptor is a chimeric inhibitory receptor (iCAR). In some embodiments, the one or more chimeric inhibitory receptors bind antigens that are expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

In some embodiments, a chimeric inhibitory receptor (*e.g.*, an EMCN-specific chimeric inhibitory receptor) may be used, for example, with one or more activating chimeric receptors (e.g., activating chimeric TCRs or CARs, such as FLT3 and/or CD33 aCARs) expressed on a cell of the present disclosure (e.g., an immunoresponsive cell) as NOT-logic gates to control, modulate, or otherwise inhibit one or more activities of the one or more activating chimeric receptors. For instance, if a healthy cell expresses both an antigen that is recognized by a tumor-targeting chimeric receptor and an antigen that is recognized by an chimeric inhibitory receptor,

an immunoresponsive cell expressing the tumor antigen may bind to the healthy cell. In such a case, the inhibitory chimeric antigen will also bind its cognate ligand on the healthy cell and the inhibitory function of the chimeric inhibitory receptor will reduce, decrease, prevent, or inhibit the activation of the immunoresponsive cell via the tumor-targeting chimeric receptor ("NOT-logic gating"). In some embodiments, a chimeric inhibitory receptor of the present disclosure may inhibit one or more activities of a cell of the present disclosure (e.g., an immunoresponsive cell). In some embodiments, an immunoresponsive cell may comprise one or more tumor-targeting chimeric receptors and one or more chimeric inhibitory receptors that targets an antigen that is not expressed, or generally considered to be expressed, on the tumor (*e.g.*, EMCN). Combinations of tumor-targeting chimeric receptors and chimeric inhibitory receptors in the same immunoresponsive cell may be used to reduce on-target off-tumor toxicity.

In some embodiments, the extracellular antigen-binding domain of a CAR of the present disclosure binds to one or more antigens (*e.g.*, EMCN, FLT3, or CD33) with a dissociation constant (K_d) of about 2 x 10^{-7} M or less, about 1 x 10^{-7} M or less, about 9 x 10^{-8} M or less, about 1 x 10^{-8} M or less, about 9 x 10^{-9} M or less, about 5 x 10^{-9} M or less, about 4 x 10^{-9} M or less, about 3 x 10^{-9} M or less, about 2 x 10^{-9} M or less, or about 1 x 10^{-9} M or less. In some embodiments, the K_d ranges from about is about 2 x 10^{-7} M to about 1 x 10^{-9} M.

Binding of the extracellular antigen-binding domain of a CAR of the present disclosure can be determined by, for example, an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), FACS analysis, a bioassay (e.g., growth inhibition), bio-layer interferometry (e.g., Octet/FORTEBIO®), surface plasmon resonance (SPR) technology (e.g., Biacore®), or a Western Blot assay. Each of these assays generally detect the presence of protein-antibody complexes of particular interest by employing a labeled reagent (e.g., an antibody or scFv) specific for the complex of interest. For example, the scFv can be radioactively labeled and used in an RIA assay. The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography. In certain embodiments, the extracellular antigen-binding domain of the CAR is labeled with a fluorescent marker. Non-limiting examples of fluorescent markers include green fluorescent protein (GFP), blue fluorescent protein (e.g., EBFP, EBFP2, Azurite, and mKalamal), cyan fluorescent protein (e.g., ECFP, Cerulean, and CyPet), and yellow fluorescent protein (e.g., YFP, Citrine, Venus, and YPet). In certain embodiments, the extracellular antigen-binding domain of the CAR is labeled with a secondary antibody specific for the extracellular antigen-binding domain and wherein the secondary antibody is labeled (*e.g.*, radioactively or with a fluorescent marker).

In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain that binds to EMCN, FLT3, or CD33. In some embodiments, CARs of the

present disclosure comprise an extracellular antigen-binding domain that binds to an EMCN protein, an EMCN-derived antigen, or an EMCN-derived epitope. In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain that binds to a FLT3 protein, a FLT3-derived antigen, or a FLT3-derived epitope. In some embodiments. In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain that binds to an CD33 protein, a CD33-derived antigen, or a CD33-derived epitope. CARs of the present disclosure comprise an extracellular antigen-binding domain, a transmembrane domain, and one or more intracellular signaling domains. In some embodiments, the extracellular antigen-binding domain comprises an scFv. In some embodiments, the extracellular antigen-binding domain comprises a Fab fragment, which may be crosslinked. In certain embodiments, the extracellular binding domain is a $F(ab)_2$ fragment.

Extracellular Antigen-binding Domain

Antigen-binding domains of the present disclosure can include any domain that binds to the antigen including, without limitation, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a bispecific antibody, a conjugated antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody (sdAb) such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen-binding domain, such as a recombinant fibronectin domain, a T cell receptor (TCR), a recombinant TCR with enhanced affinity, or a fragment thereof, e.g., single chain TCR, and the like. In some instances, it is beneficial for the antigen-binding domain to be derived from the same species in which the CAR will ultimately be used in.

In some embodiments, the extracellular antigen-binding domain comprises an antibody. In certain embodiments, the antibody is a human antibody. In certain embodiments, the antibody is a chimeric antibody. In some embodiments, the extracellular antigen-binding domain comprises an antigen-binding fragment of an antibody.

In some embodiments, the extracellular antigen-binding domain comprises a F(ab) fragment. In certain embodiments, the extracellular antigen-binding domain comprises a F(ab') fragment.

In some embodiments, the extracellular antigen-binding domain comprises an scFv. In some embodiments, the extracellular antigen-binding domain comprises two single chain variable fragments (scFvs). In some embodiments, each of the two scFvs binds to a distinct epitope on the same antigen. In some embodiments, the extracellular antigen-binding domain

comprises a first scFv and a second scFv. In some embodiments, the first scFv and the second scFv bind distinct epitopes on the same antigen. In certain embodiments, the scFv is a mammalian scFv. In certain embodiments, the scFv is a chimeric scFv. In certain embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

In certain embodiments, the VH and VL are separated by a peptide linker. In certain embodiments, the peptide linker comprises any of the amino acid sequences shown in **Table 6**.

In certain embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain. In some embodiments, each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain. When there are two or more scFv linked together, each scFv can be linked to the next scFv with a peptide linked. In some embodiments, each of the one or more scFvs is separated by a peptide linker.

In some embodiments, a peptide linker includes the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 244). In some embodiments, a peptide linker between antigen binding domains of an iCAR comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 244). In some embodiments, a peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253).

In some embodiments, a peptide linker includes the amino acid sequence GGGGS (SEQ ID NO: 242). In some embodiments, a peptide linker between antigen binding domains of an aCAR comprises the amino acid sequence GGGGS (SEQ ID NO: 242). In some embodiments, a peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCGGCGGTGGCTCT (SEQ ID NO: 254) or GGTGGCGGCGGATCC (SEQ ID NO: 255). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GGCGGCGGTGGCTCT (SEQ ID NO: 254) or GGTGGCGGCGGATCC (SEQ ID NO: 255).

In some embodiments, a peptide linker includes the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243). In some embodiments, a peptide linker between antigen

binding domains of an aCAR comprises the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243). In some embodiments, a peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), or GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), or GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258).

In some embodiments, a peptide linker includes the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247). In some embodiments, a peptide linker between antigen binding domains of an aCAR comprises the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247). In some embodiments, a peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGGGC (SEQ ID NO: 249). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGGGC (SEQ ID NO: 249).

Table 6. Peptide Linkers

Linker	Amino Acid Sequence	SEQ ID NO:
GS-linker	SGGGGSGGGGSG	230
GS-linker	GGGSGGGGSGGGSLQ	231
$(G_2S)_1$ scFv linker	GGS	232
$(G_2S)_2$ scFv linker	GGSGGS	233
$(G_2S)_3$ scFv linker	GGSGGSGGS	234
$(G_2S)_4$ scFv linker	GGSGGSGGSGGS	235
$(G_2S)_5$ scFv linker	GGSGGSGGSGGSGGS	236
$(G_3S)_1$ scFv linker	GGGS	237
$(G_3S)_2$ scFv linker	GGGSGGGS	238
$(G_3S)_3$ scFv linker	GGGSGGGSGGGS	239
$(G_3S)_4$ scFv linker	GGGSGGGSGGGSGGGS	240

$(G_3S)_5$ scFv linker	GGGSGGGSGGGSGGGSGGGS	241
$(G_4S)_1$ linker	GGGGS	242
$(G_4S)_2$ linker	GGGGSGGGGS	243
$(G_4S)_3$ linker	GGGGSGGGGSGGGGS	244
$(G_4S)_4$ linker	GGGGSGGGGSGGGGSGGGGS	245
$(G_4S)_5$ linker	GGGGSGGGGSGGGGSGGGGSGGGGS	246
Whitlow linker ["Loop 6" linker]	GSTSGSGKPGSGEGSTKG	247
scFv linker 2	EAAAKEAAAKEAAAKEAAAK	248

In some embodiments, the immune effector cell comprises a first chimeric receptor and a second chimeric receptor. The antigen-binding domain of the first chimeric receptor and the antigen-binding domain of the second chimeric receptor can be an appropriate antigen biding domain described herein or known in the art. For example, the first or second antigen-binding domain can be one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs). In some embodiments, the antigen-binding domain of the first chimeric receptor and/or the second chimeric receptor comprises two single chain variable fragments (scFvs). In some embodiments, each of the two scFvs binds to a distinct epitope on the same antigen. In some embodiments, the antigen-binding domain of the first chimeric receptor can be specific for EMCN and the chimeric receptor can be specific for a second distinct antigen, such as a cancer antigen (*e.g.*, an antigen expressed on a myeloid cell, such as an AML cell).

In some embodiments, the extracellular antigen-binding domain comprises a single-domain antibody (sdAb). In certain embodiments, the sdAb is a humanized sdAb. In certain embodiments, the sdAb is a chimeric sdAb.

In some embodiments, a CAR of the present disclosure may comprise two or more antigen-binding domains, three or more antigen-binding domains, four or more antigen-binding domains, five or more antigen-binding domains, six or more antigen-binding domains, seven or more antigen-binding domains, eight or more antigen-binding domains, nine or more antigen-binding domains, or ten or more antigen-binding domains. In some embodiments, each of the two or more antigen-binding domains binds the same antigen. In some embodiments, each of the two or more antigen-binding domains binds a different epitope of the same antigen. In some embodiments, each of the two or more antigen-binding domains binds a different antigen.

In some embodiments, the CAR comprises two antigen-binding domains. In some embodiments, the two antigen-binding domains are attached to one another via a flexible linker. In some embodiments, each of the two-antigen-binding domains may be independently selected

from an antibody, an antigen-binding fragment of an antibody, an scFv, a sdAb, a recombinant fibronectin domain, a T cell receptor (TCR), a recombinant TCR with enhanced affinity, and a single chain TCR. In some embodiments, the CAR comprising two antigen-binding domains is a bispecific CAR or a tandem CAR (tanCAR).

In certain embodiments, the bispecific CAR or tanCAR comprises an antigen-binding domain comprising a bispecific antibody or antibody fragment (e.g., scFv). In some embodiments, within each antibody or antibody fragment (e.g., scFv) of a bispecific antibody molecule, the VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_1) upstream of its VL (VL_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_2) upstream of its VH (VH_2), such that the overall bispecific antibody molecule has the arrangement VH_1-VL_1-VL_2-VH_2. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_1) upstream of its VH (VH_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_2) upstream of its VL (VL_2), such that the overall bispecific antibody molecule has the arrangement VL_1 VH_1-VH_2-VL_2. In some embodiments, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), for example, between VL_1 and VL_2 if the construct is arranged as VH_1-VL_1-VL_2-VH_2, or between VH_1 and VH_2 if the construct is arranged as VL_1-VH_1-VH_2-VL_2. The linker may be a linker as described herein, e.g., a (Gly_4-Ser)n linker, wherein n is 1 , 2, 3, 4, 5, or 6. In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. In some embodiments, a linker is disposed between the VL and VH of the first scFv. In some embodiments, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers may be the same or different. Accordingly, in some embodiments, a bispecific CAR or tanCAR comprises VLs, VHs, and may further comprise one or more linkers in an arrangement as described herein.

In an illustrative non-limiting example, a CD33/FLT3 bispecific bivalent aCAR can have antigen binding domains encoded in the following order: (FLT3-VH) – L1 – (CD33-VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a third peptide linker, respectively. L1, L2, and L3 can be the same peptide linker. L1, L2, and L3 can be the same peptide linker encoded by the same polynucleotide sequence. L1, L2, and L3 can be the same peptide linker encoded by different polynucleotide sequences. L1 and L3 can be the same peptide linker and L2 a different peptide linker. L1 and L3 can be the same peptide linker encoded by the same polynucleotide sequence. L1 and L3 can be the same peptide linker

encoded by different polynucleotide sequences. L1, L2, and L3 can each be different peptide linkers.

CAR Transmembrane Domains

In some embodiments, the transmembrane domain of a CAR of the present disclosure (*e.g.,* the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) comprises a hydrophobic alpha helix that spans at least a portion of a cell membrane. It has been shown that different transmembrane domains can result in different receptor stability. After antigen recognition, receptors cluster and a signal is transmitted to the cell. In some embodiments, the transmembrane domain of a CAR of the present disclosure can comprise the transmembrane domain of a CD8 polypeptide, a CD28 polypeptide, a CD3-zeta polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a CTLA-4 polypeptide, a PD-1 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, a BTLA polypeptide, a LIR-1 (LILRB1) polypeptide, or can be a synthetic peptide, or any combination thereof.

In some embodiments, the transmembrane domain is derived from a CD8 polypeptide. Any suitable CD8 polypeptide may be used. Exemplary CD8 polypeptides include, without limitation, NCBI Reference Nos. NP_001139345 and AAA92533.1. In some embodiments, the transmembrane domain is derived from a CD28 polypeptide. Any suitable CD28 polypeptide may be used. Exemplary CD28 polypeptides include, without limitation, NCBI Reference Nos. NP_006130.1 and NP_031668.3. In some embodiments, the transmembrane domain is derived from a CD3-zeta polypeptide. Any suitable CD3-zeta polypeptide may be used. Exemplary CD3-zeta polypeptides include, without limitation, NCBI Reference Nos. NP_932170.1 and NP_001106862.1. In some embodiments, the transmembrane domain is derived from a CD4 polypeptide. Any suitable CD4 polypeptide may be used. Exemplary CD4 polypeptides include, without limitation, NCBI Reference Nos. NP_000607.1 and NP_038516.1. In some embodiments, the transmembrane domain is derived from a 4-1BB polypeptide. Any suitable 4-1BB polypeptide may be used. Exemplary 4-1BB polypeptides include, without limitation, NCBI Reference Nos. NP_001552.2 and NP_001070977.1. In some embodiments, the transmembrane domain is derived from an OX40 polypeptide. Any suitable OX40 polypeptide may be used. Exemplary OX40 polypeptides include, without limitation, NCBI Reference Nos. NP_003318.1 and NP_035789.1. In some embodiments, the transmembrane domain is derived from an ICOS polypeptide. Any suitable ICOS polypeptide may be used. Exemplary ICOS polypeptides include, without limitation, NCBI Reference Nos. NP_036224 and NP_059508. In some embodiments, the transmembrane domain is derived from a CTLA-4 polypeptide. Any

suitable CTLA-4 polypeptide may be used. Exemplary CTLA-4 polypeptides include, without limitation, NCBI Reference Nos. NP_005205.2 and NP_033973.2. In some embodiments, the transmembrane domain is derived from a PD-1 polypeptide. Any suitable PD-1 polypeptide may be used. Exemplary PD-1 polypeptides include, without limitation, NCBI Reference Nos. NP_005009 and NP_032824. In some embodiments, the transmembrane domain is derived from a LAG-3 polypeptide. Any suitable LAG-3 polypeptide may be used. Exemplary LAG-3 polypeptides include, without limitation, NCBI Reference Nos. NP_002277.4 and NP_032505.1. In some embodiments, the transmembrane domain is derived from a 2B4 polypeptide. Any suitable 2B4 polypeptide may be used. Exemplary 2B4 polypeptides include, without limitation, NCBI Reference Nos. NP_057466.1 and NP_061199.2. In some embodiments, the transmembrane domain is derived from a BTLA polypeptide. Any suitable BTLA polypeptide may be used. Exemplary BTLA polypeptides include, without limitation, NCBI Reference Nos. NP_861445.4 and NP_001032808.2. Any suitable LIR-1 (LILRB1) polypeptide may be used. Exemplary LIR-1 (LILRB1) polypeptides include, without limitation, NCBI Reference Nos. NP_001075106.2 and NP_001075107.2.

In some embodiments, the transmembrane domain comprises a polypeptide comprising an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the sequence of NCBI Reference No. NP_001139345, AAA92533.1, NP_006130.1, NP_031668.3, NP_932170.1, NP_001106862.1, NP_000607.1, NP_038516.1, NP_001552.2, NP_001070977.1, NP_003318.1, NP_035789.1, NP_036224, NP_059508, NP_005205.2, NP_033973.2, NP_005009, NP_032824, NP_002277.4, NP_032505.1, NP_057466.1, NP_061199.2, NP_861445.4, or NP_001032808.2, or fragments thereof. In some embodiments, the homology may be determined using standard software such as BLAST or FASTA. In some embodiments, the polypeptide may comprise one conservative amino acid substitution, up to two conservative amino acid substitutions, or up to three conservative amino acid substitutions. In some embodiments, the polypeptide can have an amino acid sequence that is a consecutive portion of NCBI Reference No. NP_001139345, AAA92533.1, NP_006130.1, NP_031668.3, NP_932170.1, NP_001106862.1, NP_000607.1, NP_038516.1, NP_001552.2, NP_001070977.1, NP_003318.1, NP_035789.1, NP_036224, NP_059508, NP_005205.2, NP_033973.2, NP_005009, NP_032824, NP_002277.4, NP_032505.1, NP_057466.1, NP_061199.2, NP_861445.4, or NP_001032808.2 that is at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 210, at least 220, at least 230, or at least 240 amino acids in length.

Further examples of suitable polypeptides from which a transmembrane domain may be derived include, without limitation, the transmembrane region(s) of the alpha, beta or zeta chain of the T-cell receptor, CD27, CD3 epsilon, CD45, CD5, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, CD2, CD27, LFA-1 (CD11a, CD18), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7Rα, ITGA1, VLA1 , CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, and NG2C.

In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 205). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 207).

In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain. In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain having the sequence VIGILVAVILLLLLLLLLLFLI (SEQ ID NO: 259). In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain encoded by a polynucleotide sequence having the sequence GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCC TGATC (SEQ ID NO: 260). In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCC TGATC (SEQ ID NO: 260).

In some embodiments, an aCAR transmembrane domain includes a CD8 transmembrane domain. In some embodiments, an aCAR transmembrane domain includes a CD8 transmembrane domain having the sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206). In some embodiments, an aCAR transmembrane domain includes a CD8 transmembrane domain encoded by a polynucleotide sequence having the sequence ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCTGGTC

ATCACCCTGTACTGCAACCACAGA (SEQ ID NO: 261). In some embodiments, an aCAR transmembrane domain includes a CD8 transmembrane domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCTGGTC ATCACCCTGTACTGCAACCACAGA (SEQ ID NO: 261).

Spacer Region

In some embodiments, a CAR of the present disclosure (*e.g.,* the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) can also comprise a spacer region that links the extracellular antigen-binding domain to the transmembrane domain. The spacer region may be flexible enough to allow the antigen-binding domain to orient in different directions to facilitate antigen recognition. In some embodiments, the spacer region may be a hinge from a human protein. For example, the hinge may be a human Ig (immunoglobulin) hinge, including without limitation an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge. In some embodiments, the spacer region may comprise an IgG4 hinge, an IgG2 hinge, an IgD hinge, a CD28 hinge, a KIR2DS2 hinge, an LNGFR hinge, or a PDGFR-beta extracellular linker. In some embodiments, the spacer region is localized between the antigen-binding domain and the transmembrane domain. In some embodiments, a spacer region may comprise any of the amino acid sequences listed in **Table 7**, or an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of the amino acid sequences listed in **Table 7**. In some embodiments, nucleic acids encoding any of the spacer regions of the present disclosure may comprise any of the nucleic acid sequences listed in **Table 8**, or a nucleic acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of the nucleic acid sequences listed in **Table 8**.

In some embodiments, an aCAR hinge domain includes a CD8 hinge. In some embodiments, an aCAR hinge domain includes a CD8 hinge having the amino acid sequence ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACD (SEQ ID NO: 272). In some embodiments, an aCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence having the sequence GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCCCGCC AAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAG CCAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAGCTGCTGGCGGAGCCGTGC ATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284). In some embodiments, an

aCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCCCGCC AAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAG CCAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAGCTGCTGGCGGAGCCGTGC ATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284).

In some embodiments, an iCAR hinge domain includes a CD8 hinge. In some embodiments, an iCAR hinge domain includes a CD8 hinge having the amino acid sequence TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271). In some embodiments, an iCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence having the sequence ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGCAACC CCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCACACTA GAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283). In some embodiments, an iCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGCAACC CCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCACACTA GAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283).

In some embodiments, an iCAR hinge domain includes a LIR1 hinge. In some embodiments, an iCAR hinge domain includes a LIR1 hinge having the amino acid sequence HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 417). In some embodiments, an iCAR hinge domain includes a LIR1 hinge encoded by a polynucleotide sequence having the sequence CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAGCAGC CCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAAC AGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418). In some embodiments, an iCAR hinge domain includes a LIR1 hinge encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAGCAGC CCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAAC AGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418).

Table 7. Spacer Amino Acid Sequences

Amino Acid Sequence	SEQ ID NO:	Description
AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP	262	CD28 hinge
ESKYGPPCPSCP	263	IgG4 minimal hinge
ESKYGPPAPSAP	264	IgG4 minimal hinge, no disulfides
ESKYGPPCPPCP	265	IgG4 S228P minimal hinge, enhanced disulfide formation
EPKSCDKTHTCP	266	IgG1 minimal hinge
AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRN	267	Extended CD8a hinge
ACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQNTVCEECPDGTYSDEADAEC	268	LNGFR hinge
ACPTGLYTHSGECCKACNLGEGVAQPCGANQTVC	269	Truncated LNGFR hinge (TNFR-Cys1)
AVGQDTQEVIVVPHSLPFKV	270	PDGFR-beta extracellular linker
TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD	271	Example spacer derived from CD8 hinge
ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	272	Example spacer derived from CD8 hinge
FVPVFLPAKPTTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD	273	Example spacer
HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV	417	LIR1 Hinge

Table 8. Spacer Nucleic Acid Sequences

Nucleic Acid Sequence	SEQ ID NO:	Description
GCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAATGAAAAGAGTAATGGGACTATCATTCATGTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGTCCGTCTAAACCT	274	CD28 hinge
GAAAGCAAGTACGGTCCACCTTGCCCTAGCTGTCCG	275	IgG4 minimal hinge
GAATCCAAGTACGGCCCCCCAGCGCCTAGTGCCCCA	276	IgG4 minimal hinge, no disulfides
GAATCTAAATATGGCCCGCCATGCCCGCCTTGCCCA	277	IgG4 S228P minimal hinge, enhanced disulfide formation
GAACCGAAGTCTTGTGATAAAACTCATACGTGCCCG	278	IgG1 minimal hinge

Nucleic Acid Sequence	SEQ ID NO:	Description
GCTGCTGCTTTCGTACCCGTGTTCCTCCCTGCTAAGCC TACGACTACCCCCGCACCGAGACCACCCACGCCAGCA CCCACGATTGCTAGCCAGCCCCTTAGTTTGCGACCAGA AGCTTGTCGGCCTGCTGCTGGTGGCGCGGTACATACCC GCGGCCTTGATTTTGCTTGCGATATATATATCTGGGCG CCTCTGGCCGGAACATGCGGGGTCCTCCTCCTTTCTCT GGTTATTACTCTCTACTGTAATCACAGGAAT	279	Extended CD8a hinge
GCCTGCCCGACCGGGCTCTACACTCATAGCGGGGAAT GTTGTAAGGCATGTAACTTGGGTGAGGGCGTCGCACA GCCCTGCGGAGCTAACCAAACAGTGTGCGAACCCTGC CTCGATAGTGTGACGTTCTCTGATGTTGTATCAGCTAC AGAGCCTTGCAAACCATGTACTGAGTGCGTTGGACTT CAGTCAATGAGCGCTCCATGTGTGGAGGCAGATGATG CGGTCTGTCGATGTGCTTACGGATACTACCAAGACGA GACAACAGGGCGGTGCGAGGCCTGTAGAGTTTGTGAG GCGGGCTCCGGGCTGGTGTTTTCATGTCAAGACAAGC AAAATACGGTCTGTGAAGAGTGCCCTGATGGCACCTA CTCAGACGAAGCAGATGCAGAATGC	280	LNGFR hinge
GCCTGCCCTACAGGACTCTACACGCATAGCGGTGAGT GTTGTAAAGCATGCAACCTCGGGGAAGGTGTAGCCCA GCCATGCGGGGCTAACCAAACCGTTTGC	281	Truncated LNGFR hinge (TNFR-Cys1)
GCTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTGC CACACTCCTTGCCCTTTAAGGTG	282	PDGFR-beta extracellular linker
ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTC CAACAATTGCACTGCAACCCCTGAGTCTGAGGCCCGA GGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCACACT AGAGGCCTGGACTTTGCCTGTGAC	283	Example spacer derived from CD8 hinge
GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGT GCCCGTGTTTCTGCCCGCCAAGCCTACAACAACCCCTG CTCCTAGACCACCTACACCAGCTCCTACAATCGCCAGC CAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAG CTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTT TGCCTGCGAC	284	Example spacer derived from CD8 hinge
CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACC TTCTGGCGGCCCTAGCAGCCCTACAACAGGACCTACA AGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAA CAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCT GGGCGTT	418	LIR1 Hinge

In some embodiments, a CAR of the present disclosure may further include a short oligopeptide or polypeptide linker that is between 2 amino acid residues and 10 amino acid residues in length, and that may form the linkage between the transmembrane domain and the cytoplasmic region of the CAR. A non-limiting example of a suitable linker is a glycine-serine doublet. In some embodiments, the linker comprises the ammo acid sequence of GGCKJSGGCKJS (SEQ ID NO: 208).

In some aspects, the transmembrane domain further comprises at least a portion of an extracellular domain of the same protein.

Intracellular Signaling Domains

In some embodiments, a CAR of the present disclosure (*e.g.*, the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) comprises one or more cytoplasmic domains or regions. The cytoplasmic domain or region of the CAR may include an intracellular signaling domain.

Examples of suitable intracellular signaling domains that may be used in CARs of the present disclosure include, without limitation, cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to modulate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

Without wishing to be bound by theory, it is believed that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or co-stimulatory signal is thus also typically required for full activation. Accordingly, T cell activation may be mediated by two distinct classes of cytoplasmic signaling sequences, those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic domain, e.g., a co-stimulatory domain). In addition, T cell signaling and function (*e.g.*, an activating signaling cascade) can be negatively regulated by inhibitory receptors present in a T cell through intracellular inhibitory co-signaling domains.

In some embodiments, the intracellular signaling domain of a CAR of the present disclosure can include an inhibitory intracellular signaling domains. Examples of inhibitory intracellular domains that can be used include PD-1, CTLA4, TIGIT, BTLA, and LIR-1 (LILRB1), TIM3, KIR3DL1, NKG2A, LAG3, SLAP1, SLAP2, Dok-1, Dok-2, LAIR1, GRB-2, CD200R, SIRPα, HAVR, GITR, PD-L1, KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL2, CD94, KLRG-1, CEACAM1, LIR2, LIR3, LIR5, SIGLEC-2, and SIGLEC-10. **Table 10** and **Table 11** provide the amino acid and nucleotide sequneces, respectively, of the exemplary inhibitory intracellular signaling domains. In some embodiments, the inhibitory intracellular signaling domain includes one or more intracellular inhibitory co-signaling domains (e.g., see **Table 10** and **Table 11**. In some embodiments, the one or more intracellular inhibitory co-signaling domains are linked to other domains (*e.g.*, a transmembrane domain) through a peptide linker (*e.g.*, *see* **Table 6**) or a spacer or hinge sequence (*e.g.*, *see* **Tables 7 and 8**). In some embodiments, when two or more intracellular inhibitory co-signaling domains are present, the two or more intracellular inhibitory co-signaling domains can be linked through a peptide linker (*e.g.*, *see* **Table 6**) or a spacer or hinge sequence (*e.g.*, *see* **Tables 7 and 8**). In some

embodiments, the intracellular inhibitory co-signaling domain is an inhibitory domain. In some embodiments, the one or more intracellular inhibitory co-signaling domains of a chimeric protein comprises one or more ITIM-containing protein, or fragment(s) thereof. ITIMs are conserved amino acid sequences found in cytoplasmic tails of many inhibitory immune receptors. Examples of ITIM-containing proteins include, but are not limited to, PD-1, TIGIT, BTLA, and LIR-1 (LILRB1), TIM3, KIR3DL1, NKG2A, LAG3, LAIR1, SIRPα, KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL2, CD94, KLRG-1, CEACAM1, LIR2, LIR3, LIR5, SIGLEC-2, and SIGLEC-10.

Table 10 - Exemplary inhibitory intracellular signaling domain amino acid sequences

Amino Acid Sequence	SEQ ID NO:	Description
CSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVP CVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL	435	PD-1 intracellular signaling domain
AVSLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPYFIPIN	436	CTLA4 intracellular signaling domain
RRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYD NDPDLCFRMQEGSEVYSNPCLEENKPGIVYASLNHSVIGPNSRLARNVK EAPTEYASICVRS	437	BTLA intracellular signaling domain
LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQE ENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASP PSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREA TEPPPSQEGPSPAVPSIYATLAIH	285	LIR1 intracellular signaling domain
HLWCSNKKNAAVMDQEPAGNRTANSEDSDEQDPEEVTYAQLDHCVFT QRKITRPSQRPKTPPTDTILYTELPNAKPRSKVVSCP	438	KIR3DL1 intracellular signaling domain
AVSLSKMLKKRSPLTTGVGVKMPPTEPECEKQFQPYFIPIN	439	CTLA4 intracellular signaling domain
KEPASPLDKCHYTKDNGQFDQSAKQLNLEAYTIEQETALISNKNGKPKR QQRKPNPPLNLDSYIVGQNDM	440	NKG2A (reversed) intracellular signaling domain
LTRKKKALRIHSVEGDLRRKSAGQEEWSPSAPSPPGSCVQAEAAPAGLC GEQRGEDCAELHDYFNVLSYRSLGNCSFFTETG	441	TIGIT intracellular signaling domain
MDNQGVIYSDLNLPPNPKRQQRKPKGNKNSILATEQEITYAELNLQKAS QDFQGNDKTYHCKDLPSAPEK	442	NKG2A intracellular signaling domain
MEAIAKYDFKATADDELSFKRGDILKVLNEECDQNWYKAELNGKDGFI PKNYIEMKPHPWFFGKIPRAKAEEMLSKQRHDGAFLIRESESAPGDFSLS VKFGNDVQHFKVLRDGAGKYFLWVVKFNSLNELVDYHRSTSVSRNQQI FLRDIEQVPQQPTYVQALFDFDPQEDGELGFRRGDFIHVMDNSDPNWW KGACHGQTGMFPRNYVTPVNRNV	443	GRB2 intracellular signaling domain

MDGAVMEGPLFLQSQRFGTKRWRKTWAVLYPASPHGVARLEFFDHKG SSSGGGRGSSRRLDCKVIRLAECVSVAPVTVETPPEPGATAFRLDTAQRS HLLAADAPSSAAWVQTLCRNAFPKGSWTLAPTDNPPKLSALEMLENSL YSPTWEGSQFWVTVQRTEAAERCGLHGSYVLRVEAERLTLLTVGAQSQ ILEPLLSWPYTLLRRYGRDKVMFSFEAGRRCPSGPGTFTFQTAQGNDIFQ AVETAIHRQKAQGKAGQGHDVLRADSHEGEVAEGKLPSPPGPQELLDS PPALYAEPLDSLRIAPCPSQDSLYSDPLDSTSAQAGEGVQRKKPLYWDL YEHAQQQLLKAKLTDPKEDPIYDEPEGLAPVPPQGLYDLPREPKDAWW CQARVKEEGYELPYNPATDDYAVPPPRSTKPLLAPKPQGPAFPEPGTAT GSGIKSHNSALYSQVQKSGASGSWDCGLSRVGTDKTGVKSEGST	444	Dok-1 intracellular signaling domain
MGDGAVKQGFLYLQQQQTFGKKWRRFGASLYGGSDCALARLELQEGP EKPRRCEAARKVIRLSDCLRVAEAGGEASSPRDTSAFFLETKERLYLLAA PAAERGDWVQAICLLAFPGQRKELSGPEGKQSRPCMEENELYSSAVTVG PHKEFAVTMRPTEASERCHLRGSYTLRAGESALELWGGPEPGTQLYDW PYRFLRRFGRDKVTFSFEAGRRCVSGEGNFEFETRQGNEIFLALEEAISA QKNAAPATPQPQPATIPASLPRPDSPYSRPHDSLPPPSPTTPVPAPRPRGQ EGEYAVPFDAVARSLGKNFRGILAVPPQLLADPLYDSIEETLPPRPDHIY DEPEGVAALSLYDSPQEPRGEAWRRQATADRDPAGLQHVQPAGQDFSA SGWQPGTEYDNVVLKKGPK	448	Dok-2 intracellular signaling domain
PAPAERPLPNPEGLDSDFLAVLSDYPSPDISPPIFRRGEKLRVISDEGGWW KAISLSTGRESYIPGICVARVYHGWLFEGLGRDKAEELLQLPDTKVGSF MIRESETKKGFYSLSVRHRQVKHYRIFRLPNNWYYISPRLTFQCLEDLVN HYSEVADGLCCVLTTPCLTQSTAAPAVRASSSPVTLRQKTVDWRRVSRL QEDPEGTENPLGVDESLFSYGLRESIASYLSLTSEDNTSFDRKKKSISLMY GGSKRKSSFFSSPPYFED	446	$SLAP1_{aa8\text{-}aa276}$ intracellular signaling domain
PAPAERPLPNPEGLDSDFLAVLSDYPSPDISPPIFRRGEKLRVISDEGGWW KAISLSTGRESYIPGICVARVYHGWLFEGLGRDKAEELLQLPDTKVGSF MIRESETKKGFYSLSVRHRQVKHYRIFRLPNNWYYISPRLTFQCLEDLVN HYSEVADGLCCVLTTPCLTQSTAAPAVRASSSPVTLRQKTVDWRRVSRL QEDPEGTENPLGVDESLFSYGLRESIASYLSLTSEDNTSF	447	$SLAP1_{aa8\text{-}aa247}$ intracellular signaling domain
RKSLPSPSLSSSVQGQGPVTMEAERSKATAVALGSFPAGGPAELSLRLGE PLTIVSEDGDWWTVLSEVSGREYNIPSVHVAKVSHGWLYEGLSREKAEE LLLLPGNPGGAFLIRESQTRRGSYSLSVRLSRPASWDRIRHYRIHCLDNG WLYISPRLTFPSLQALVDHYSELADDICCLLKEPCVLQRAGPLPGKDIPLP VTVQRTPLNWKELDSSLLFSEAATGEESLLSEGLRESLSFYISLNDEAVSL DDA	448	$SLAP2_{aa8\text{-}aa261}$ intracellular signaling domain
KVNGCRKYKLNKTESTPVVEEDEMQPYASYTEKNNPLYDTTNKVKASE ALQSEVDTDLHTL	449	CD200R intracellular signaling domain
RIRQKKAQGSTSSTRLHEPEKNAREITQDTNDITYADLNLPKGKKPAPQA AEPNNHTEYASIQTSPQPASEDTLTYADLDMVHLNRTPKQPAPKPEPSFS EYASVQVPRK	450	SIRPα intracellular signaling domain
HRWCSNKKNAAVMDQESAGNRTANSEDSDEQDPQEVTYTQLNHCVFT QRKITRPSQRPKTPPTDIIVYTELPNAESRSKVVSCP	451	KIR2DL1 intracellular signaling domain
MTDSVIYSMLELPTATQAQNDYGPQQKSSSSRPSCSCLGSG	452	KLRG1 intracellular signaling domain
HRQNQIKQGPPRSKDEEQKPQQRPDLAVDVLERTADKATVNGLPEKDR ETDTSALAAGSSQEVTYAQLDHWALTQRTARAVSPQSTKPMAESITYA AVARH	453	LAIR1 intracellular signaling domain
LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQE ENLYAAVKDTQPEDGVEMDTRAAASEAPQDVTYAQLHSLTLRRKATEP PPSQEREPPAEPSIYATLAIH	454	LIR2 intracellular signaling domain

RRQRHSKHRTSDQRKTDFQRPAGAAETEPKDRGLLRRSSPAADVQEEN LYAAVKDTQSEDRVELDSQSPHDEDPQAVTYAPVKHSSPRREMASPPSS LSGEFLDTKDRQVEEDRQMDTEAAASEASQDVTYAQLHSLTLRRKATE PPPSQEGEPPAEPSIYATLAIH	455	LIR3 intracellular signaling domain
QHWRQGKHRTLAQRQADFQRPPGAAEPEPKDGGLQRRSSPAADVQGE NFCAAVKNTQPEDGVEMDTRQSPHDEDPQAVTYAKVKHSRPRREMAS PPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSFTLRQK ATEPPPSQEGASPAEPSVYATLAIH	456	LIR5 intracellular signaling domain
KLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNP MMEDGISYTTLRFPEMNIPRTGDAESSEMQRPPPDCDDTVTYSALHKRQ VGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENVDYVILKH	457	SIGLEC-2 intracellular signaling domain
KILPKRRTQTETPRPRFSRHSTILDYINVVPTAGPLAQKRNQKATPNSPRT PLPPGAPSPESKKNQKKQYQLPSFPEPKSSTQAPESQESQEELHYATLNFP GVRPRPEARMPKGTQADYAEVKFQ	458	SIGLEC-10 intracellular signaling domain

Table 11 - Exemplary inhibitory intracellular signaling domain nucleic acid sequences

Nucleic Acid Sequence	SEQ ID NO:	Description
TGTAGCAGAGCCGCCAGAGGAACAATCGGCGCCAGAAGAACAGGCC AGCCTCTGAAAGAGGACCCCTCTGCCGTTCCTGTGTTCAGCGTGGAC TATGGCGAGCTGGATTTCCAGTGGCGGGAAAAGACACCCGAGCCTCC AGTGCCTTGTGTGCCTGAGCAGACAGAGTACGCCACCATCGTGTTCC CTAGCGGCATGGGCACATCTAGCCCTGCCAGAAGAGGATCTGCCGAC GGACCTAGATCTGCCCAGCCTCTTAGACCTGAGGACGGCCACTGTTC TTGGCCTCTT	459	PD-1 intracellular signaling domain
TGTAGCCGAGCGGCCAGAGGCACAATCGGGGCAAGACGAACAGGAC AGCCGCTCAAAGAGGACCCCAGTGCGGTCCCCGTTTTCTCCGTGGAT TACGGAGAACTGGATTTCCAGTGGCGGGAGAAGACACCAGAGCCCC CGGTGCCCTGCGTGCCGGAGCAGACTGAGTACGCCACGATTGTGTTT CCCTCTGGAATGGGGACTTCATCCCCCGCTAGGCGCGGCTCAGCTGA TGGCCCAAGATCCGCTCAACCGTTGCGGCCAGAGGACGGGCATTGCA GTTGGCCTCTG	460	PD-1 intracellular signaling domain
GCCGTGTCTCTGAGCAAGATGCTGAAGAAGCGGAGCCCTCTGACCAC CGGCGTGTACGTGAAAATGCCTCCTACCGAGCCTGAGTGCGAGAAGC AGTTCCAGCCTTACTTCATCCCCATCAAC	461	CTLA4 intracellular signaling domain
AGGAGACATCAGGGGAAGCAGAATGAACTCAGCGATACAGCAGGGC GAGAAATTAATTTGGTAGACGCGCATCTGAAGTCCGAACAGACAGA GGCTTCTACTAGACAGAACTCCCAAGTTTTGTTGAGTGAGACGGGGA TCTATGATAATGATCCCGATCTGTGTTTTAGAATGCAGGAGGGTAGT GAAGTCTACTCAAACCCGTGCCTGGAAGAAAATAAGCCCGGCATTGT TTACGCTAGTTTGAATCATTCTGTAATAGGCCCGAACTCCAGACTGG CTCGCAATGTGAAGGAGGCCCCAACTGAGTATGCGTCCATTTGCGTG CGGTCT	462	BTLA intracellular signaling domain
AGAAGACATCAGGGGAAGCAGAATGAACTCAGCGATACAGCAGGGC GAGAAATTAATTTGGTAGACGCGCATCTGAAGTCCGAACAGACAGA GGCTTCTACTAGACAGAACTCCCAAGTTTTGTTGAGTGAGACGGGGA TCTATGATAATGATCCCGATCTGTGTTTTAGAATGCAGGAGGGTAGT GAAGTCTACTCAAACCCGTGCCTGGAAGAAAATAAGCCCGGCATTGT TTACGCTAGTTTGAATCATTCTGTAATAGGCCCGAACTCCAGACTGG CTCGCAATGTGAAGGAGGCCCCAACTGAGTATGCGTCCATTTGCGTG CGGTCT	463	BTLA intracellular signaling domain
AGAAGGCACCAGGGAAAGCAGAACGAGCTGAGCGATACCGCCGGCA GAGAAATCAACCTGGTGGACGCCCACCTGAAAAGCGAGCAGACAGA GGCCAGCACCAGACAGAATAGCCAGGTGCTGCTGAGCGAGACAGGC	464	BTLA intracellular signaling domain

ATCTACGACAACGACCCCGACCTGTGCTTCCGGATGCAAGAGGGAAG CGAGGTGTACAGCAACCCCTGCCTGGAAGAGAACAAGCCCGGCATC GTGTACGCTAGCCTGAACCACTCTGTGATCGGCCCCAATTCCAGACT GGCCCGGAACGTGAAAGAGGCCCCTACAGAGTACGCCAGCATCTGC GTCAGAAGC		
TTGCGCCACAGACGGCAGGGAAAGCACTGGACTAGTACGCAGAGGA AAGCGGACTTCCAGCATCCCGCAGGAGCCGTGGGGCCTGAACCCACT GATCGCGGCCTTCAATGGAGGTCTAGCCCGGCGGCAGACGCACAAG AGGAAAACTTGTACGCAGCCGTTAAGCACACCCAACCGGAGGACGG CGTTGAGATGGATACCCGCTCCCCTCACGATGAAGACCCTCAAGCAG TCACTTACGCGGAAGTAAAGCATAGCCGCCCCAGACGGGAAATGGC TAGCCCGCCGTCCCCCCTTAGCGGGGAATTTCTGGACACTAAAGATA GGCAGGCGGAAGAGGACCGCCAAATGGATACAGAGGCGGCGGCAA GTGAAGCACCTCAAGACGTTACTTACGCTCAACTTCACAGCCTTACC CTCAGGCGAGAAGCGACTGAACCACCCCCTTCCCAAGAAGGGCCAA GCCCAGCGGTTCCTTCTATCTATGCTACTCTTGCTATTCAC	465	LIR1 intracellular signaling domain
CTGCGGCACAGACGGCAGGGCAAGCACTGGACAAGCACACAGAGAA AGGCCGACTTTCAGCACCCTGCTGGTGCCGTTGGACCTGAGCCTACA GATAGAGGACTGCAGTGGCGGTCTAGCCCTGCCGCTGATGCTCAAGA GGAAAACCTGTACGCCGCCGTGAAGCACACCCAACCTGAAGATGGC GTGGAAATGGACACCAGATCTCCCCACGATGAGGACCCTCAGGCCGT GACATATGCCGAAGTGAAGCACTCCCGGCCTCGGAGAGAAATGGCT AGCCCTCCAAGTCCTCTGAGCGGCGAGTTCCTGGACACCAAGGATAG ACAGGCCGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCATCT GAGGCCCCACAGGATGTGACTTATGCCCAGCTGCACAGCCTGACACT GCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAGGGCCCATCTC CAGCCGTGCCTAGCATCTATGCCACACTGGCCATCCAC	466	LIR1 intracellular signaling domain
GCCGTGTCACTTAGTAAGATGCTGAAGAAGAGGTCACCACTGACGAC AGGGGTTGGAGTGAAGATGCCACCCACAGAACCCGAATGTGAGAAG CAATTCCAGCCTTATTTCATTCCAATAAAT	467	CTLA4 intracellular signaling domain
CATCTGTGGTGTTCTAATAAGAAGAATGCTGCTGTGATGGATCAAGA GCCCGCTGGTAACAGAACGGCCAACAGTGAAGATAGCGATGAGCAG GACCCAGAAGAAGTGACCTACGCCCAACTCGACCACTGTGTTTTTAC GCAGCGGAAAATCACTCGACCCTCTCAACGACCCAAAACGCCGCCTA CGGACACCATACTCTACACCGAACTGCCGAACGCCAAACCACGGTCC AAGGTGGTATCATGTCCG	468	KIR3DL1 intracellular signaling domain
CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAA AGGCCGATTTTCAGCACCCTGCTGGCGCCGTTGGACCTGAGCCTACA GATAGAGGACTGCAGTGGCGGTCTAGCCCTGCTGCCGATGCTCAAGA GGAAAACCTGTACGCCGCCGTGAAGCACACCCAACCTGAAGATGGC GTGGAAATGGACACCAGATCTCCCCACGATGAGGACCCTCAGGCCGT GACATACGCTGAAGTGAAGCACTCCCGGCCTCGGAGAGAAATGGCT AGCCCTCCAAGTCCTCTGAGCGGCGAGTTCCTGGACACCAAGGATAG ACAGGCCGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCCTCT GAAGCCCCACAGGATGTGACATATGCCCAGCTGCATAGCCTGACACT GCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAGGGCCCATCTC CAGCCGTGCCTAGCATCTATGCCACACTGGCCATTCAC	469	LIR1 intracellular signaling domain
AAGGAGCCTGCGTCCCCGTTGGATAAATGCCACTATACTAAGGATAA CGGTCAGTTCGATCAGAGTGCAAAGCAACTTAACTTGGAGGCTTACA CTATAGAGCAAGAAACAGCGCTGATAAGTAATAAGAACGGTAAGCC AAAGCGACAGCAGAGGAAACCCAATCCTCCGCTTAACTTGGATAGCT ACATCGTCGGGCAAAATGACATG	470	NKG2A (reversed) intracellular signaling domain
CTGACCAGAAAGAAGAAGGCCCTGAGAATCCACAGCGTGGAAGGCG ACCTGCGGAGAAAGTCTGCCGGACAAGAAGAGTGGTCCCCTAGCGC TCCATCTCCACCTGGATCTTGTGTGCAGGCCGAAGCAGCTCCTGCTG GACTGTGTGGCGAACAGAGAGGCGAAGATTGCGCCGAGCTGCACGA	471	TIGIT intracellular signaling domain

CTACTTCAACGTGCTGAGCTACAGAAGCCTGGGCAACTGCAGCTTCT TCACCGAGACAGGA		
ATGGACAACCAGGGCGTGATCTACAGCGACCTGAACCTGCCTCCTAA TCCTAAGCGGCAGCAGAGAAAGCCCAAGGGCAACAAGAACAGCATC CTGGCCACCGAGCAAGAGATCACCTACGCCGAGCTGAATCTGCAGA AGGCCAGCCAGGACTTCCAGGGCAACGACAAGACCTACCACTGCAA GGACCTGCCTAGCGCTCCCGAGAAG	472	NKG2A intracellular signaling domain
ATGGACAACCAGGGCGTCATCTACAGCGACCTGAACCTGCCTCCTAA TCCAAAGCGGCAGCAGCGGAAGCCCAAGGGCAACAAGAATAGCATC CTGGCCACCGAGCAAGAGATCACCTACGCCGAGCTGAATCTGCAGA AGGCCAGCCAGGATTTCCAGGGCAACGACAAGACCTACCACTGCAA GGACCTGCCTAGCGCTCCTGAGAAA	473	NKG2A intracellular signaling domain (codon optimized)
ATGGAAGCCATTGCCAAATACGACTTCAAGGCCACCGCCGACGACG AGCTGAGCTTCAAGAGAGGCGACATCCTGAAGGTGCTGAACGAGGA ATGCGACCAGAACTGGTACAAGGCCGAGCTGAACGGCAAGGACGGC TTCATCCCCAAGAACTACATCGAGATGAAGCCCCATCCATGGTTCTT CGGCAAGATCCCCAGAGCCAAGGCCGAAGAGATGCTGAGCAAGCAG AGACACGACGGCGCCTTTCTGATCCGGGAATCTGAATCTGCCCCTGG CGACTTCAGCCTGAGCGTGAAGTTCGGCAACGACGTGCAGCACTTCA AGGTCCTGAGAGATGGCGCCGGAAAGTACTTCCTGTGGGTCGTGAAG TTTAACAGCCTGAACGAGCTGGTGGACTACCACAGATCCACCAGCGT GTCCCGGAACCAGCAGATCTTCCTGCGGGACATCGAACAGGTGCCAC AGCAGCCAACATACGTGCAGGCCCTGTTCGACTTCGACCCTCAAGAG GATGGCGAGCTGGGCTTTAGACGGGGCGATTTCATCCACGTGATGGA CAACAGCGACCCCAACTGGTGGAAGGGCGCTTGTCATGGACAGACC GGCATGTTCCCCAGAAACTACGTGACCCCTGTGAACCGGAACGTG	474	GRB2 intracellular signaling domain
ATGGATGGCGCCGTGATGGAAGGCCCTCTGTTTCTCCAGAGCCAGAG ATTCGGCACCAAGCGGTGGCGGAAAACATGGGCCGTTCTGTACCCTG CCTCTCCTCATGGCGTGGCCCGGCTGGAATTTTTCGATCACAAGGGCT CTAGCAGCGGCGGAGGCAGAGGATCTAGTAGACGGCTGGACTGCAA AGTGATCCGGCTGGCCGAGTGTGTGTCTGTGGCTCCTGTGACCGTGG AAACCCCTCCTGAACCTGGCGCCACAGCCTTCAGACTGGATACAGCC CAGAGAAGCCATCTGCTGGCCGCCGATGCTCCTTCTTCTGCTGCTTGG GTGCAGACCCTGTGCCGGAACGCTTTTCCTAAAGGCAGCTGGACACT GGCCCCTACCGACAATCCTCCTAAGCTGAGCGCCCTGGAAATGCTGG AAAACAGCCTGTACAGCCCCACCTGGGAGGGCTCTCAGTTTTGGGTC ACCGTGCAGAGAACAGAGGCCGCCGAAAGATGTGGCCTGCACGGCT CTTATGTGCTGAGAGTGGAAGCCGAGAGACTGACCCTGCTGACAGTG GGAGCCCAGTCTCAGATCCTGGAACCTCTGCTGAGCTGGCCCTACAC ACTGCTGAGAAGATACGGCCGGGACAAAGTGATGTTCAGCTTCGAG GCCGGCAGAAGATGTCCTTCTGGCCCTGGCACATTCACATTTCAGAC AGCCCAGGGCAACGACATCTTCCAGGCTGTGGAAACCGCCATCCACA GACAGAAGGCCCAGGGAAAAGCCGGCCAGGGACACGATGTTCTGAG AGCCGATTCTCACGAGGGCGAAGTGGCCGAGGGAAAGCTTCCTTCTC CACCTGGACCTCAAGAGCTGCTGGATAGCCCTCCTGCTCTGTATGCC GAGCCTCTGGACAGCCTGAGAATCGCCCCTTGTCCAAGCCAGGACTC TCTGTACAGCGATCCCCTGGATAGCACATCTGCCCAAGCTGGCGAAG GCGTGCAGAGGAAGAAGCCCCTGTACTGGGATCTGTACGAGCACGCT CAGCAGCAACTGCTGAAGGCCAAGCTGACAGACCCCAAAGAGGACC CCATCTACGACGAGCCTGAAGGACTTGCTCCAGTGCCACCTCAGGGC CTGTACGATCTGCCTAGAGAGCCTAAAGACGCCTGGTGGTGTCAGGC CAGAGTGAAAGAGGAAGGCTACGAGCTGCCTTACAACCCCGCCACC GATGATTATGCCGTGCCTCCTCCAAGAAGCACCAAACCACTGCTGGC CCCAAAGCCTCAGGGACCTGCTTTTCCTGAGCCTGGAACAGCCACAG GCAGCGGCATCAAGAGCCACAATAGCGCCCTGTATAGCCAGGTGCA GAAAAGCGGCGCCAGCGGCTCTTGGGATTGTGGACTTAGCAGAGTG GGCACCGACAAGACCGGCGTGAAGTCTGAGGGAAGCACA	475	Dok-1 intracellular signaling domain

ATGGGAGATGGCGCCGTGAAGCAGGGCTTTCTGTATCTCCAGCAGCA GCAGACCTTCGGCAAGAAGTGGCGGAGATTTGGCGCCTCTCTGTACG GCGGCTCTGATTGTGCTCTGGCCCGACTGGAACTGCAAGAGGGACCT GAGAAGCCCAGAAGATGCGAGGCCGCCAGAAAAGTGATCCGGCTGA GCGATTGTCTGAGAGTGGCTGAAGCAGGCGGCGAAGCCAGCTCTCCT AGAGATACCAGCGCATTCTTCCTGGAAACAAAAGAGCGGCTGTACCT GCTGGCCGCTCCTGCTGCTGAAAGAGGCGATTGGGTCCAAGCCATCT GCCTGCTGGCTTTTCCCGGCCAGAGAAAAGAGCTGTCTGGCCCTGAG GGCAAGCAGAGCAGGCCTTGCATGGAAGAGAACGAGCTGTACTCCA GCGCCGTGACAGTGGGCCCTCACAAAGAATTTGCCGTGACCATGAGG CCCACCGAGGCCAGCGAAAGATGTCACCTGAGAGGCAGCTACACCC TGAGAGCCGGCGAATCTGCTCTGGAACTTTGGGGAGGACCTGAGCCT GGCACACAGCTGTACGACTGGCCCTACAGATTCCTGCGGAGATTCGG CCGGGACAAAGTGACCTTCAGCTTTGAGGCTGGCCGCAGATGTGTGT CCGGCGAGGGCAATTTCGAGTTCGAGACAAGACAGGGCAACGAGAT CTTCCTGGCTCTGGAAGAGGCCATCAGCGCCCAGAAAAATGCTGCCC CTGCTACACCTCAGCCTCAGCCTGCTACAATCCCTGCCTCTCTGCCCA GACCTGACAGCCCTTATAGCAGACCCCACGACTCTCTGCCTCCACCTT CTCCAACAACACCCGTGCCTGCTCCTAGACCTAGAGGACAAGAGGGC GAGTACGCCGTGCCTTTTGATGCCGTGGCTAGAAGCCTGGGCAAGAA CTTCAGAGGCATCCTGGCTGTGCCTCCACAGCTGCTGGCTGACCCTCT GTACGACAGCATCGAGGAAACCCTGCCTCCAAGACCTGACCACATCT ACGACGAGCCTGAAGGCGTGGCAGCCCTGTCTCTGTATGACTCCCCT CAAGAGCCTAGAGGCGAAGCCTGGCGTAGACAGGCTACCGCCGATA GAGATCCTGCCGGACTGCAACATGTGCAGCCAGCCGGCCAGGATTTT TCTGCCTCTGGATGGCAGCCAGGCACCGAGTACGATAACGTGGTGCT GAAGAAGGGCCCCAAG	476	Dok-2 intracellular signaling domain
CCAGCCCCAGCGGAGCGACCGCTGCCAAACCCTGAAGGGCTCGACA GTGACTTTTTGGCTGTCCTCTCCGACTATCCTAGTCCCGATATCAGCC CCCCGATATTCAGGCGCGGTGAAAAACTCCGAGTCATCAGCGATGAG GGGGGTTGGTGGAAAGCCATCAGCCTGAGTACCGGACGAGAGTCAT ACATTCCTGGAATATGTGTAGCGAGGGTGTACCACGGTTGGCTGTTC GAGGGTCTGGGAAGAGATAAGGCCGAGGAACTTCTCCAACTCCCAG ATACAAAAGTCGGTAGTTTTATGATTCGGGAAAGTGAAACTAAAAAG GGGTTCTATAGCCTCTCAGTTCGGCATAGGCAGGTCAAGCATTATCG GATATTTCGCTTGCCTAACAACTGGTACTACATAAGTCCCCGACTCAC ATTCCAATGCCTGGAGGACCTCGTGAATCACTATTCAGAAGTTGCAG ATGGCCTCTGCTGCGTACTCACGACACCCTGCCTTACCCAGAGTACA GCGGCCCCGGCTGTTCGGGCATCTTCCAGCCCAGTAACACTCAGGCA AAAAACTGTGGATTGGCGCAGAGTCTCACGCCTTCAGGAAGATCCTG AGGGTACGGAAAATCCGCTCGGTGTGGACGAGTCACTGTTCTCCTAT GGCTTGAGGGAATCTATAGCGTCTTACCTTTCCTTGACGTCTGAAGAT AATACGTCTTTCGATCGCAAAAAAAAATCAATATCTCTTATGTATGG CGGAAGCAAAAGGAAATCATCATTCTTTTCCTCTCCACCATATTTCGA AGAT	477	$SLAP1_{aa8\text{-}aa276}$ intracellular signaling domain
CCAGCCCCAGCGGAGCGACCGCTGCCAAACCCTGAAGGGCTCGACA GTGACTTTTTGGCTGTCCTCTCCGACTATCCTAGTCCCGATATCAGCC CCCCGATATTCAGGCGCGGTGAAAAACTCCGAGTCATCAGCGATGAG GGGGGTTGGTGGAAAGCCATCAGCCTGAGTACCGGACGAGAGTCAT ACATTCCTGGAATATGTGTAGCGAGGGTGTACCACGGTTGGCTGTTC GAGGGTCTGGGAAGAGATAAGGCCGAGGAACTTCTCCAACTCCCAG ATACAAAAGTCGGTAGTTTTATGATTCGGGAAAGTGAAACTAAAAAG GGGTTCTATAGCCTCTCAGTTCGGCATAGGCAGGTCAAGCATTATCG GATATTTCGCTTGCCTAACAACTGGTACTACATAAGTCCCCGACTCAC ATTCCAATGCCTGGAGGACCTCGTGAATCACTATTCAGAAGTTGCAG ATGGCCTCTGCTGCGTACTCACGACACCCTGCCTTACCCAGAGTACA GCGGCCCCGGCTGTTCGGGCATCTTCCAGCCCAGTAACACTCAGGCA AAAAACTGTGGATTGGCGCAGAGTCTCACGCCTTCAGGAAGATCCTG AGGGTACGGAAAATCCGCTCGGTGTGGACGAGTCACTGTTCTCCTAT	478	$SLAP1_{aa8\text{-}aa247}$ intracellular signaling domain

GGCTTGAGGGAATCTATAGCGTCTTACCTTTCCTTGACGTCTGAAGAT AATACGTCTTTC		
AGAAAATCCCTCCCCAGTCCAAGCCTGTCTAGTAGCGTTCAGGGTCA GGGCCCAGTTACTATGGAAGCGGAACGATCCAAGGCTACCGCAGTTG CTCTGGGTTCATTCCCGGCTGGAGGGCCAGCGGAACTCTCCTTGCGC CTGGGTGAGCCACTTACCATCGTTTCTGAGGACGGAGATTGGTGGAC GGTTCTTTCTGAAGTATCTGGGAGAGAATACAACATACCGTCAGTTC ATGTCGCAAAAGTTTCACACGGTTGGCTTTACGAGGGACTCAGCAGG GAAAAAGCGGAGGAATTGTTGCTTTTGCCCGGCAATCCTGGCGGAGC ATTTTTGATAAGAGAGAGTCAGACCCGGAGAGGCAGTTATTCCCTGT CAGTTAGACTCAGCAGACCTGCGAGTTGGGATAGGATTCGGCACTAC AGGATTCACTGCCTTGATAACGGCTGGCTGTATATATCTCCTCGCCTG ACATTCCCTAGCCTCCAAGCGTTGGTTGATCATTACTCTGAACTGGCA GACGATATCTGCTGCCTGCTCAAGGAACCGTGCGTCCTCCAGAGGGC AGGCCCACTTCCTGGCAAGGATATTCCACTTCCAGTCACGGTTCAAA GAACCCCCCTCAATTGGAAGGAGCTGGATAGCTCTCTCCTCTTTTCCG AGGCCGCTACAGGTGAAGAATCTCTGTTGTCTGAAGGATTGAGAGAG AGTCTCTCCTTCTACATCTCTCTGAATGATGAAGCAGTGTCATTGGAC GACGCA	479	$SLAP2_{aa8\text{-}aa261}$ intracellular signaling domain
AAAGTGAACGGCTGCCGGAAGTACAAGCTGAACAAGACCGAGAGCA CCCCTGTGGTGGAAGAGGACGAGATGCAGCCTTACGCCAGCTACACC GAGAAGAACAACCCTCTGTACGACACCACCAACAAAGTGAAGGCCA GCGAGGCCCTCCAGAGCGAGGTTGACACAGATCTGCACACCCTG	480	CD200R intracellular signaling domain
CACCGGTGGTGCAGCAACAAGAAAAACGCCGCCGTGATGGACCAAG AGAGCGCCGGAAATAGAACCGCCAACAGCGAGGATAGCGACGAGCA GGACCCTCAAGAAGTGACCTACACACAGCTGAACCACTGCGTGTTCA CCCAGCGGAAGATCACCAGACCTAGCCAGCGGCCTAAGACACCACC TACCGACATCATCGTGTACACCGAGCTGCCCAACGCCGAGAGCAGAT CCAAGGTCGTGTCCTGTCCT	481	KIR2DL1 intracellular signaling domain
ATGACCGACAGCGTGATCTACAGCATGCTCGAGCTGCCTACAGCCAC ACAGGCCCAGAATGATTACGGCCCTCAGCAGAAGTCCAGCTCCAGCA GACCTAGCTGTAGCTGTCTTGGCTCCGGC	482	KLRG1 intracellular signaling domain
CACCGGCAGAACCAGATCAAGCAGGGCCCTCCTAGAAGCAAGGACG AGGAACAGAAGCCTCAGCAGAGGCCTGATCTGGCCGTGGACGTGCT GGAAAGAACAGCCGATAAGGCCACCGTGAACGGCCTGCCTGAGAAG GACAGAGAGACAGACACATCTGCCCTGGCCGCTGGCAGCTCCCAAG AAGTGACATACGCCCAGCTGGACCACTGGGCCCTGACACAGAGAAC TGCCAGAGCTGTGTCCCCTCAGAGCACCAAACCTATGGCCGAGAGCA TCACCTATGCCGCCGTGGCCAGACAT	483	LAIR1 intracellular signaling domain
CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAA AGGCCGATTTTCAGCACCCTGCTGGCGCCGTTGGACCTGAGCCTACA GATAGAGGACTGCAGTGGCGGTCTAGCCCTGCTGCCGATGCTCAAGA GGAAAACCTGTACGCCGCCGTGAAGGACACCCAACCTGAAGATGGC GTGGAAATGGACACCAGAGCCGCCGCATCTGAAGCCCCTCAGGATGT GACATACGCCCAGCTGCATAGCCTGACACTGAGGCGGAAAGCCACA GAGCCTCCACCTAGCCAAGAGAGAGAGCCTCCTGCCGAGCCTAGCAT CTATGCCACACTGGCCATTCAC	484	LIR2 intracellular signaling domain
CGGAGACAGAGACACAGCAAGCACAGAACCAGCGACCAGAGAAAG ACCGACTTCCAGAGGCCTGCTGGCGCCGCTGAGACAGAGCCTAAAG ATAGAGGACTGCTGCGGAGAAGCAGCCCTGCTGCCGATGTGCAAGA GGAAAATCTGTACGCCGCCGTGAAGGACACCCAGAGCGAGGATAGA GTGGAACTGGACAGCCAGTCTCCTCACGACGAAGATCCTCAGGCCGT GACATACGCCCCTGTGAAGCACAGCAGCCCTCGGAGAGAAATGGCC TCTCCACCTTCTAGCCTGAGCGGCGAGTTCCTGGACACCAAGGACAG ACAGGTGGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCCTCT GAAGCCAGCCAGGATGTGACATATGCCCAGCTGCATAGCCTGACACT	485	LIR3 intracellular signaling domain

GCGGCGGAAAGCTACAGAGCCTCCTCCATCTCAAGAGGGCGAGCCTC CAGCCGAGCCTAGCATCTATGCCACACTGGCCATTCAC		
CAGCATTGGAGACAGGGAAAGCACAGAACACTGGCCCAGCGGCAGG CCGATTTCCAAAGACCTCCAGGTGCCGCCGAACCTGAGCCTAAAGAT GGTGGCCTGCAGCGGAGATCTTCTCCTGCTGCCGATGTGCAGGGCGA GAATTTTTGTGCCGCCGTGAAGAACACCCAGCCTGAGGATGGCGTGG AAATGGACACCAGACAGAGCCCTCACGACGAGGATCCACAGGCCGT GACATACGCCAAAGTGAAGCACAGCCGGCCTCGGAGAGAAATGGCT AGCCCTCCAAGTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAG ACAGGCCGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCCTCT GAAGCCCCTCAGGATGTGACATATGCCCAGCTGCATAGCTTCACCCT GCGGCAGAAAGCCACAGAGCCTCCACCTTCTCAAGAGGGCGCTTCTC CAGCCGAGCCATCTGTGTATGCCACACTGGCCATTCAC	486	LIR5 intracellular signaling domain
AAGCTGCAGCGGAGATGGAAGAGAACACAGAGCCAGCAGGGCCTGC AAGAGAATAGCAGCGGCCAGAGCTTCTTCGTGCGGAACAAGAAAGT GCGGAGAGCCCCTCTGTCTGAGGGACCTCATAGCCTGGGCTGTTACA ACCCCATGATGGAAGATGGCATCAGCTACACCACACTGCGGTTCCCC GAGATGAACATCCCTAGAACAGGCGACGCCGAGAGCAGCGAAATGC AAAGACCTCCTCCTGACTGCGACGACACCGTGACATATAGCGCCCTG CACAAGAGACAAGTGGGCGACTACGAGAACGTGATCCCTGACTTCCC TGAGGACGAGGGCATCCACTACAGCGAGCTGATCCAGTTTGGCGTGG GCGAAAGACCCCAGGCTCAAGAGAATGTGGACTACGTGATCCTGAA GCAC	487	SIGLEC-2 intracellular signaling domain
AAGATCCTGCCTAAGCGGCGCACCCAGACCGAGACTCCTAGACCTAG ATTCAGCCGGCACAGCACCATCCTGGACTACATCAACGTGGTGCCTA CCGCCGGACCACTGGCTCAGAAGAGAAACCAGAAGGCCACACCTAA CAGCCCCAGAACACCTCTTCCACCTGGCGCACCTTCTCCAGAGAGCA AGAAGAACCAGAAGAAGCAGTACCAGCTGCCTAGCTTCCCCGAGCC TAAGAGCAGTACACAGGCCCCTGAGAGCCAAGAGTCCCAAGAGGAA CTGCACTACGCCACACTGAACTTCCCCGGCGTCAGACCTAGACCTGA GGCCAGAATGCCTAAGGGCACCCAGGCCGATTACGCCGAAGTGAAG TTTCAA	488	SIGLEC-10 intracellular signaling domain

In some embodiments, an iCAR includes a LIR1 intracellular inhibitory domain. In some embodiments, an iCAR includes a LIR1 intracellular inhibitory domain having the amino acid sequence

LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKH TQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEED RQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 285). In some embodiments, an iCAR includes a LIR1 intracellular inhibitory domain encoded by a polynucleotide sequence comprising the sequence

CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACT TTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGG CGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCA CACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACC CTCAGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGC AAGCCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCG

AAGAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGATGT
GACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCAC
CTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTC
AC (SEQ ID NO: 286). In some embodiments, an iCAR includes a LIR1 intracellular inhibitory domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACT
TTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGG
CGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCA
CACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACC
CTCAGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGC
AAGCCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCG
AAGAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGATGT
GACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCAC
CTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTC
AC (SEQ ID NO: 286).

In some embodiments, the one or more intracellular inhibitory co-signaling domains comprise one or more non-ITIM scaffold proteins, or a fragment(s) thereof. In some embodiments, the one or more non-ITIM scaffold proteins, or fragments thereof, are selected from GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, GITR, and PD-L1.

The inhibitory intracellular signaling domain can further include an enzymatic inhibitory domain. In some embodiments, the enzymatic inhibitory domain comprises an enzyme catalytic domain. In some embodiments, the enzyme catalytic domain is derived from an enzyme selected from the group consisting of: CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP. Examples of enzymatic regulation of signaling is described in more detail in Pavel Otáhal et al. (Biochim Biophys Acta. 2011 Feb;1813(2):367-76), Kosugi A., et al. (Involvement of SHP-1 tyrosine phosphatase in TCR-mediated signaling pathways in lipid rafts, Immunity, 2001 Jun; 14(6): 669-80), and Stanford, et al. (Regulation of TCR signaling by tyrosine phosphatases: from immune homeostasis to autoimmunity, Immunology, 2012 Sep; 137(1): 1-19), each of which is incorporated herein by reference for all purposes.

In some embodiments, the intracellular signaling domain of a CAR of the present disclosure can comprise a primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way or in an inhibitory way. Primary intracellular signaling

domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs (ITAMs). Examples of suitable ITAM-containing primary intracellular signaling domains that that may be used in the CARs of the present disclosure include, without limitation, those of CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, DAP10, DAP12, and CD66d.

In some embodiments, a CAR of the present disclosure comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta polypeptide. A CD3-zeta polypeptide of the present disclosure may have an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the sequence of NCBI Reference No. NP_932170 or NP_001106864.2, or fragments thereof. In some embodiments, the CD3-zeta polypeptide may comprise one conservative amino acid substitution, up to two conservative amino acid substitutions, or up to three conservative amino acid substitutions. In some embodiments, the polypeptide can have an amino acid sequence that is a consecutive portion of NCBI Reference No. NP_932170 or NP_001106864.2 that is at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, or at least 160, at least 170, or at least 180 amino acids in length.

In other embodiments, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In one embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

In some embodiments, the intracellular signaling domain of a CAR of the present disclosure can comprise the CD3-zeta signaling domain by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the present disclosure. For example, the intracellular signaling domain of the CAR can comprise a CD3-zeta chain portion and a costimulatory signaling domain. The costimulatory signaling domain may refer to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule of the present disclosure is a cell surface molecule other than an antigen receptor or its ligands that may be required for an efficient response of lymphocytes to an antigen. Examples of suitable costimulatory molecules include, without limitation, CD97, CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10,

DAP12, MyD88, 2B4, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLAl, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and the like.

In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain. In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain having the amino acid sequence RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 287). In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain encoded by a polynucleotide sequence comprising the sequence AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCTAGAC GGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCC GCCTACCGGTCC (SEQ ID NO: 288). In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCTAGAC GGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCC GCCTACCGGTCC (SEQ ID NO: 288).

In some embodiments, aCAR intracellular signaling domains include a CD3ζ signaling domain. In some embodiments, aCAR intracellular signaling domains include a CD3ζ signaling domain having the amino acid sequence RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 289). In some embodiments, aCAR intracellular signaling domains include a CD3ζ signaling domain encoded by a polynucleotide sequence having the sequence

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACC
AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG
AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT
TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC
TCGC (SEQ ID NO: 290). In some embodiments, aCAR intracellular signaling domains include a CD3ζ signaling domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACC
AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG
AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT
TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC
TCGC (SEQ ID NO: 290).

In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain and a CD3ζ signaling domain. In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain having the amino acid sequence RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 287) and a CD3ζ signaling domain having the amino acid sequence RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 289).

In some embodiments, the intracellular signaling sequences within the cytoplasmic portion of a CAR of the present disclosure may be linked to each other in a random or specified order. In some embodiments, a short oligopeptide or polypeptide linker, for example, between 2 amino acids and 10 amino acids (e.g., 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, or 10 amino acids) in length may form the linkage between intracellular signaling sequences. In one embodiment, a glycine-serine doublet can be used as a suitable linker. In one embodiment, a single ammo acid, e.g., an alanine or a glycine, can be used as a suitable linker.

In some embodiments, the intracellular signaling domain comprises two or more costimulatory signaling domains, e.g., two costimulatory signaling domains, three costimulatory

signaling domains, four costimulatory signaling domains, five costimulatory signaling domains, six costimulatory signaling domains, seven costimulatory signaling domains, eight costimulatory signaling domains, nine costimulatory signaling domains, 10 costimulatory signaling domains, or more costimulatory signaling domains. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the two or more costimulatory signaling domains are separated by a linker of the present disclosure (*e.g.*, any of the linkers described in **Table 6**). In one embodiment, the linker is a glycine residue. In another embodiment, the linker is an alanine residue.

In some embodiments, a cell of the present disclosure expresses a CAR that includes an antigen-binding domain, a transmembrane domain, a primary signaling domain, and one or more costimulatory signaling domains.

In some embodiments, the transmembrane domain is derived from the same protein as one of the one or more intracellular signaling domains. In some embodiments, the CAR is an inhibitory CAR and includes a transmembrane domain and at least one intracellular inhibitory co-signaling domain each derived from a protein selected from PD-1, CTLA4, TIGIT, BTLA, and LIR1 (LILRB1), TIM3, KIR3DL1, NKG2A, LAG3, SLAP1, SLAP2, Dok-1, Dok-2, LAIR1, GRB-2, CD200R, SIRPα, HAVR, GITR, PD-L1, KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL2, CD94, KLRG-1, CEACAM1, LIR2, LIR3, LIR5, SIGLEC-2, and SIGLEC-10.

In some embodiments, the transmembrane domain is derived from a first protein and the one or more intracellular signaling domains are derived from a second protein that are distinct from the first protein.

Natural Killer Cell Receptor (NKR) CARs

In some embodiments, a CAR of the present disclosure comprises one or more components of a natural killer cell receptor (NKR), thereby forming an NKR-CAR. The NKR component may be a transmembrane domain, a hinge domain, or a cytoplasmic domain from any suitable natural killer cell receptor, including without limitation, a killer cell immunoglobulin-like receptor (KIR),such as KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, DIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3, KIR2DP1, and KIRS DPI; a natural cytotoxicity receptor (NCR), such as NKp30, NKp44, NKp46; a signaling lymphocyte activation molecule (SLAM) family of immune cell receptor, such as CD48, CD229, 2B4, CD84, NTB-A, CRACC, BLAME, and CD2F-10; an Fc receptor (FcR), such as CD16, and CD64; and an Ly49 receptor, such as LY49A and LY49C. In some embodiments, the NKR-CAR may interact with an adaptor molecule or intracellular signaling domain, such as DAP12. Exemplary configurations and sequences of CARs

comprising NKR components are described in International Patent Publication WO2014/145252, published September 18, 2014.

Immunoresponsive cells

Certain aspects of the present disclosure relate to a cell, such as an immunoresponsive cell, that has been genetically engineered to comprise one or more chimeric receptors of the present disclosure or one or more nucleic acids encoding such chimeric receptors, and to methods of using such cells for treating myeloid malignancies (e.g., AML).

In some embodiments, the cell is a mammalian cell. In some embodiments, the mammalian cell is a primary cell. In some embodiments, the mammalian cell is a cell line. In some embodiments, the mammalian cell, a bone marrow cell, a blood cell, a skin cell, bone cell, a muscle cell, a neuronal cell, a fat cell, a liver cell, or a heart cell. In some embodiments, the cell is a stem cell. Exemplary stem cells include, without limitation embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), adult stem cells, and tissue-specific stem cells, such as hematopoietic stem cells (blood stem cells), mesenchymal stem cells (MSC), neural stem cells, epithelial stem cells, or skin stem cells. In some embodiments, the cell is a cell that is derived or differentiated from a stem cell of the present disclosure. In some embodiments, the cell is an immune cell. Immune cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary immune cells include, without limitation, T cells (*e.g.*, helper T cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cells, alpha beta T cells, and gamma delta T cells), B cells, natural killer (NK) cells, dendritic cells, myeloid cells, macrophages, and monocytes. In some embodiments, the cell is a neuronal cell. Neuronal cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary neuronal cells include, without limitation, neural progenitor cells, neurons (*e.g.*, sensory neurons, motor neurons, cholinergic neurons, GABAergic neurons, glutamatergic neurons, dopaminergic neurons, or serotonergic neurons), astrocytes, oligodendrocytes, and microglia.

In some embodiments, the cell is an immunoresponsive cell. Immunoresponsive cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary immunoresponsive cells of the present disclosure include, without limitation, cells of the lymphoid lineage. The lymphoid lineage, comprising B cells, T cells, and natural killer (NK) cells, provides for the production of antibodies, regulation of the cellular immune system, detection of foreign agents in the blood, detection of cells foreign to the host, and the like. Examples of immunoresponsive cells of the lymphoid lineage include,

without limitation, T cells, Natural Killer (NK) cells, embryonic stem cells, pluripotent stem cells, and induced pluripotent stem cells (e.g., those from which lymphoid cells may be derived or differentiated). T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. In some embodiments, T cells of the present disclosure can be any type of T cells, including, without limitation, T helper cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: e.g., T_{EM} cells and T_{EMRA} cells, regulatory T cells (also known as suppressor T cells), natural killer T cells, mucosal associated invariant T cells, and γδ T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. A patient's own T cells may be genetically modified to target specific antigens through the introduction of one or more chimeric receptors, such as a chimeric TCRs or CARs.

Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune response. NK cells do not require prior activation in order to perform their cytotoxic effect on target cells.

In some embodiments, an immunoresponsive cell of the present disclosure is a T cell. T cells of the present disclosure may be autologous, allogeneic, or derived in vitro from engineered progenitor or stem cells.

In some embodiments, an immunoresponsive cell of the present disclosure is a universal T cell with deficient TCR-αβ. Methods of developing universal T cells are described in the art, for example, in Valton et al., Molecular Therapy (2015); 23 9, 1507-1518, and Torikai et al., Blood 2012 119:5697-5705.

In some embodiments, an immunoresponsive cell of the present disclosure is an isolated immunoresponsive cell comprising one or more chimeric receptors of the present disclosure. In some embodiments, the immunoresponsive cell comprises one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more chimeric receptors of the present disclosure.

In some embodiments, an immunoresponsive cell is a T cell. In some embodiments, an immunoresponsive cell is a Natural Killer (NK) cell.

In some embodiments, an immunoresponsive cell expresses or is capable of expressing an immune receptor. Immune receptors generally are capable of inducing signal transduction or changes in protein expression in the immune receptor-expressing cell that results in the modulation of an immune response upon binding to a cognate ligand (*e.g.*, regulate, activate, initiate, stimulate, increase, prevent, attenuate, inhibit, reduce, decrease, inhibit, or suppress an

immune response). For example, when CD3 chains present in a TCR/CAR cluster in response to ligand binding, an immunoreceptor tyrosine-based activation motifs (ITAMs)-meditated signal transduction cascade is produced. Specifically, in certain embodiments, when an endogenous TCR, exogenous TCR, chimeric TCR, or a CAR (specifically an activating CAR) binds their respective antigen, a formation of an immunological synapse occurs that includes clustering of many molecules near the bound receptor (e.g., CD4 or CD8, CD3γ/δ/ε/ζ, etc.). This clustering of membrane bound signaling molecules allows for ITAM motifs contained within the CD3 chains to become phosphorylated that in turn can initiate a T cell activation pathway and ultimately activates transcription factors, such as NF-κB and AP-1. These transcription factors are capable of inducing global gene expression of the T cell to increase IL-2 production for proliferation and expression of master regulator T cell proteins in order to initiate a T cell mediated immune response, such as cytokine production and/or T cell mediated killing.

Cells Expressing Multiple Chimeric Receptors

In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors of the present disclosure. In some embodiments, the cell comprises two or more chimeric receptors, wherein one of the two or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises three or more chimeric receptors, wherein at least one of the three or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises four or more chimeric receptors, wherein at least one of the four or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises five or more chimeric receptors, wherein at least one of the five or more chimeric receptors is a chimeric inhibitory receptor.

In some embodiments, each of the two or more chimeric receptors comprise a different antigen-binding domain, e.g., that binds to the same antigen or to a different antigen. In some embodiments each antigen bound by the two or more chimeric receptors are expressed on the same cell, such as a myeloid cell type (e.g., same AML cell type). In some embodiments each antigen bound by the two or more chimeric receptors is an AML-associated antigen (e.g., FLT3, CD33, CD123, CLEC12A, CXCR4, EphA3, etc.).

In embodiments where a cell of the present disclosure (e.g., an immunoresponsive cell) expresses two or more distinct chimeric receptors, the antigen-binding domain of each of the different chimeric receptors may be designed such that the antigen-binding domains do not interact with one another. For example, a cell of the present disclosure (e.g., an immunoresponsive cell) expressing a first chimeric receptor (*e.g.*, an EMCN-specific chimeric receptor) and a second chimeric receptor may comprise a first chimeric receptor that comprises

an antigen-binding domain that does not form an association with the antigen-binding domain of the second chimeric receptor. For example, the antigen-binding domain of the first chimeric receptor may comprise an antibody fragment, such as an scFv, while the antigen-binding domain of the second chimeric receptor may comprise a VHH.

Without wishing to be bound by theory, it is believed that in cells having a plurality of chimeric membrane embedded receptors that each comprise an antigen-binding domain, interactions between the antigen-binding domains of each of the receptors can be undesirable, because such interactions may inhibit the ability of one or more of the antigen-binding domains to bind their cognate antigens. Accordingly, in embodiments where cells of the present disclosure (e.g., immunoresponsive cells) express two or more chimeric receptors, the chimeric receptors comprise antigen-binding domains that minimize such inhibitory interactions. In one embodiment, the antigen-binding domain of one chimeric receptor comprises an scFv and the antigen-binding domain of the second chimeric receptor comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence.

In some embodiments, when present on the surface of a cell, binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen is not substantially reduced by the presence of the second chimeric receptor. In some embodiments, binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen in the presence of the second chimeric receptor is 85%, 90%, 95%, 96%, 97%, 98%, or 99% of binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen in the absence of the second chimeric receptor. In some embodiments, when present on the surface of a cell, the antigen-binding domains of the first chimeric receptor and the second chimeric receptor associate with one another less than if both were scFv antigen-binding domains. In some embodiments, the antigen-binding domains of the first chimeric receptor and the second chimeric receptor associate with one another 85%, 90%, 95%, 96%, 97%, 98%, or 99% less than if both were scFv antigen-binding domains.

Chimeric Inhibitory Receptors

In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises one or more chimeric inhibitory receptors of the present disclosure. In some embodiments, each of the one or more chimeric inhibitory receptors comprises an antigen-binding domain that binds an antigen generally expressed on normal cells (*e.g.*, cells generally considered to be healthy) but not on tumor cells, such as AML cells. In some embodiments, a chimeric inhibitory receptor includes an antigen-binding domain that binds EMCN (*e.g.*, an

EMCN-specific antigen-binding domain having one or more of the amino acid sequences listed in Table 1).

In some embodiments, the one or more chimeric inhibitory receptors bind antigens that are expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

In some embodiments, a chimeric inhibitory receptor (*e.g.*, an EMCN-specific chimeric inhibitory receptor) may be used, for example, with one or more activating chimeric receptors (e.g., activating chimeric TCRs or CARs) expressed on a cell of the present disclosure (e.g., an immunoresponsive cell) as NOT-logic gates to control, modulate, or otherwise inhibit one or more activities of the one or more activating chimeric receptors. In some embodiments, a chimeric inhibitory receptor of the present disclosure may inhibit one or more activities of a cell of the present disclosure (e.g., an immunoresponsive cell).

In some embodiments, a cell of the present disclosure comprises one or more chimeric inhibitory receptors of the present disclosure and further comprises a tumor-targeting chimeric receptor that binds to one or more tumor-associated antigens. In some embodiments, the one or more tumor-associated antigens include an AML-associated antigen. In some embodiments, the one or more tumor-associated antigens include CD33. In some embodiments, the one or more tumor-associated antigens include FLT3. In some embodiments, the one or more tumor-associated antigens include CD33 and FLT3.

Co-stimulatory ligands

In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) can further include one or more recombinant or exogenous co-stimulatory ligands. For example, the cell can be further transduced with one or more co-stimulatory ligands, such that the cell co-expresses or is induced to co-express one or more chimeric receptors of the present disclosure (*e.g.*, the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) and one or more co-stimulatory ligands. Without wishing to be bound by theory, it is believed that the interaction between the one or more chimeric receptors and the one or more co-stimulatory ligands may provide a non-antigen-specific signal important for full activation of the cell. Examples of suitable co-stimulatory ligands include, without limitation, members of the tumor necrosis factor (TNF) superfamily, and immunoglobulin (Ig) superfamily ligands. TNF is a cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary role is in the regulation of immune cells. Members of TNF superfamily share a number of

common features. The majority of TNF superfamily members are synthesized as type II transmembrane proteins (extracellular C-terminus) containing a short cytoplasmic segment and a relatively long extracellular region. Examples of suitable TNF superfamily members include, without limitation, nerve growth factor (NGF), CD40L (CD40L)/CD 154, CD137L/4-1BBL, TNF-a, CD134L/OX40L/CD252, CD27L/CD70, Fas ligand (FasL), CD30L/CD153, tumor necrosis factor beta (TNFP)/lymphotoxin- alpha (LTa), lymphotoxin-beta (LTP), CD257/B cell-activating factor (B AFF)/Bly s/THANK/Tall- 1, glucocorticoid-induced TNF Receptor ligand (GITRL), and TNF-related apoptosis-inducing ligand (TRAIL), LIGHT (TNFSF 14). The immunoglobulin (Ig) superfamily is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. These proteins share structural features with immunoglobulins and possess an immunoglobulin domain (fold). Examples of suitable immunoglobulin superfamily ligands include, without limitation, CD80 and CD86, both ligands for CD28, PD-L1/(B7-H1) that ligands for PD-1. In certain embodiments, the one or more co-stimulatory ligands are selected from 4-1BBL, CD80, CD86, CD70, OX40L, CD48, TNFRSF14, PD-L1, and combinations thereof.

Chemokine receptor

In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises one or more chimeric receptors (*e.g.,* the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) and may further include one or more chemokine receptors. For example, transgenic expression of chemokine receptor CCR2b or CXCR2 in cells, such as T cells, enhances trafficking to CCL2-secreting or CXCL1-secreting solid tumors (Craddock et al, J Immunother. 2010 Oct; 33(8):780-8 and Kershaw et al. Hum Gene Ther. 2002 Nov 1; 13(16): 1971 -80). Without wishing to be bound by theory, it is believed that chemokine receptors expressed on chimeric receptor-expressing cells of the present disclosure may recognize chemokines secreted by tumors and improve targeting of the cell to the tumor, which may facilitate the infiltration of the cell to the tumor and enhance the antitumor efficacy of the cell. Chemokine receptors of the present disclosure may include a naturally occurring chemokine receptor, a recombinant chemokine receptor, or a chemokine-binding fragment thereof. Examples of suitable chemokine receptors that may expressed on a cell of the present disclosure include, without limitation, a CXC chemokine receptor, such as CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, or CXCR7; a CC chemokine receptor, such as CCR1 , CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, or CCR11; a CX3C chemokine receptor, such as CX3CR1; an XC chemokine receptor, such as XCR1; and

chemokine-binding fragments thereof. In some embodiments, the chemokine receptor to be expressed on the cell is chosen based on the chemokines secreted by the tumor.

Chimeric Receptor Regulation

Some embodiments of the present disclosure relate to regulating one or more chimeric receptor activities of chimeric receptor-expressing cells of the present disclosure (*e.g.,* the EMCN-specific CARs described herein). There are several ways chimeric receptor activities can be regulated. In some embodiments, a regulatable chimeric receptor, wherein one or more chimeric receptor activities can be controlled, may be desirable to optimize the safety and/or efficacy of the chimeric receptor therapy. For example, inducing apoptosis using a caspase fused to a dimerization domain (see, e.g., Di et al., N Engl. J. Med. 2011 Nov. 3; 365(18): 1673-1683) can be used as a safety switch in the chimeric receptor therapy. In some embodiments, a chimeric receptor-expressing cell of the present disclosure can also express an inducible Caspase-9 (iCaspase-9) that, upon administration of a dimerizer drug, such as rimiducid (IUPAC name: [(1R)-3-(3,4-dimethoxyphenyl)-1-[3-[2-[2-[[2-[3-[(1R)-3-(3,4-dimethoxyphenyl)-1-[(2S)-1-[(2S)-2-(3,4,5-trimethoxyphenyl)butanoyl]piperidine-2-carbonyl]oxypropyl]phenoxy]acetyl]amino]ethylamino]-2-oxoethoxy]phenyl]propyl] (2S)-1-[(2S)-2-(3,4,5-trimethoxyphenyl)butanoyl]piperidine-2-carboxylate), induces activation of the Caspase-9 and results in apoptosis of the cells. In some embodiments, the iCaspase-9 contains a binding domain that comprises a chemical inducer of dimerization (CID) that mediates dimerization in the presence of the CID, which results in inducible and selective depletion of the chimeric receptor-expressing cells.

Alternatively, in some embodiments a chimeric receptor of the present disclosure may be regulated by utilizing a small molecule or an antibody that deactivates or otherwise inhibits chimeric receptor activity. For example, an antibody may delete the chimeric receptor-expressing cells by inducing antibody dependent cell-mediated cytotoxicity (ADCC). In some embodiments, a chimeric receptor-expressing cell of the present disclosure may further express an antigen that is recognized by a molecule that is capable of inducing cell death by ADCC or complement-induced cell death. For example, a chimeric receptor-expressing cell of the present disclosure may further express a receptor capable of being targeted by an antibody or antibody fragment. Examples of suitable receptors that may be targeted by an antibody or antibody fragment include, without limitation, EpCAM, VEGFR, integrins (e.g., αvβ3, α4, αI¾β3, α4β7, α5β1, αvβ3, αv), members of the TNF receptor superfamily (e.g., TRAIL-R1 and TRAIL-R2), PDGF receptor, interferon receptor, folate receptor, GPNMB, ICAM-1 , HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD11,

CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41 , CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof.

In some embodiments, a chimeric receptor-expressing cell of the present disclosure may also express a truncated epidermal growth factor receptor (EGFR) that lacks signaling capacity but retains an epitope that is recognized by molecules capable of inducing ADCC (e.g., WO2011/056894).

In some embodiments, a chimeric receptor-expressing cell of the present disclosure further includes a highly expressing compact marker/suicide gene that combines target epitopes from both CD32 and CD20 antigens in the chimeric receptor-expressing cell, which binds an anti-CD20 antibody (e.g., rituximab) resulting in selective depletion of the chimeric receptor-expressing cell by ADCC. Other methods for depleting chimeric receptor-expressing cells of the present disclosure my include, without limitation, administration of a monoclonal anti-CD52 antibody that selectively binds and targets the chimeric receptor-expressing cell for destruction by inducing ADCC. In some embodiments, the chimeric receptor-expressing cell can be selectively targeted using a chimeric receptor ligand, such as an anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody can cause effector cell activity, such as ADCC or ADC activity. In some embodiments, the chimeric receptor ligand can be further coupled to an agent that induces cell killing, such as a toxin. In some embodiments, a chimeric receptor-expressing cell of the present disclosure may further express a target protein recognized by a cell depleting agent of the present disclosure. In some embodiments, the target protein is CD20 and the cell depleting agent is an anti-CD20 antibody. In such embodiments, the cell depleting agent is administered once it is desirable to reduce or eliminate the chimeric receptor-expressing cell. In some embodiments, the cell depleting agent is an anti-CD52 antibody.

In some embodiments, a regulated chimeric receptor comprises a set of polypeptides, in which the components of a chimeric receptor of the present disclosure are partitioned on separate polypeptides or members. For example, the set of polypeptides may include a dimerization switch that, when in the presence of a dimerization molecule, can couple the polypeptides to one another to form a functional chimeric receptor.

Endomucin-Specific Antigen-binding Domains

The present disclosure provides antigen-binding domains (e.g., single-chain variable fragments) that bind to endomucin (EMCN), chimeric proteins including antigen-binding domains that bind to EMCN (*e.g.*, any of the iCARs described herein), and nucleic acids

encoding such antigen-binding domains and chimeric proteins. Without wishing to be bound by theory, EMCN is a sialoglycoprotein that interferes with assembly of focal adhesion complexes and inhibits interaction between cells and extracellular matrix. EMCN-specific antigen-binding domains bind to human EMCN (*e.g.*, Uniprot Q9ULC0, herein incorporated by reference for all purposes) or an epitope fragment thereof. EMCN can be expressed on cells generally considered to be healthy, such as healthy hematopoietic stem cells (HSCs), HSPCs, healthy multipotent progenitors (MPPs), healthy lympho-myeloid primed progenitor (LMPPs), and healthy hematopoietic progenitor cells (HPCs). EMCN can be expressed on hematopoietic stem and progenitor cells (HSPCs). EMCN can be expressed on HSCs. EMCN can be expressed on MPPs. EMCN can be expressed on LMPPs. EMCN can be expressed on HPCs. EMCN-specific antibodies have been previously described, including CBFYE-0213, V.7.C7.1, L4B1, L5F12, L10B5, L3F12, L6H3, , L9H8, and L10F12, as described in Samulowitz U. et al., Am. J. Path., 2002 May, 160(5):1669-1681, herein incorporated by reference for all purposes.

The present disclosure provides an EMCN-specific antigen-binding domain including one or more of the amino acid sequences listed in **Table 1**.

Table 1.

Amino Acid Sequence	SEQ ID NO:	Description
RYDMH	291	CDR-H1 of Kabat-annotated CDR
GFTFSRY	292	CDR-H1 Version 1 of Chothia-annotated CDR
GFTLSRY	293	CDR-H1 Version 2 of Chothia-annotated CDR
GFSFSRY	294	CDR-H1 Version 3 of Chothia-annotated CDR
GFSLSRY	295	CDR-H1 Version 4 of Chothia-annotated CDR
VIWGNGNTHYHSALKS	296	CDR-H2 of Kabat-annotated CDR
WGNGN	297	CDR-H2 of Chothia-annotated CDR
RIKD	298	CDR-H3 (Kabat- and Chothia-annotated CDR)
KSSQSLVASDENTYLN	299	CDR-L1 (Kabat- and Chothia-annotated CDR)
QVSKLDS	300	CDR-L2 (Kabat- and Chothia-annotated CDR)
LQGIHLPWT	301	CDR-L3(Kabat- and Chothia-annotated CDR)

Amino Acid Sequence	SEQ ID NO:	Description
EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPG KGLEWVSVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNS LRAEDTAVYYCTLRIKDWGQGTMVTVSS	302	VH 3-23 Version 1
EVQLVESGGGLVQPGGSLRLSCAAS GFTLSRY DMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS	303	VH 3-23 Version 2
EVQLVESGGGLVQPGGSLRLSCAAS GFSFSRY DMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS	304	VH 3-23 Version 3
EVQLVESGGGLVQPGGSLRLSCAAS GFSLSRY DMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS	305	VH 3-23 Version 4
QVQLVESGGGVVQPGRSLRLSCAASGFTFSRYDMHWVRQAPG KGLEWVAVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNS LRAEDTAVYYCTLRIKDWGQGTMVTVSS	306	VH 3-33 Version 1
QVQLVESGGGVVQPGRSLRLSCAAS GFTLSRY DMHWVRQAPGKGLEWVAVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS	307	VH 3-33 Version 2
QVQLVESGGGVVQPGRSLRLSCAAS GFSFSRY DMHWVRQAPGKGLEWVAVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS	308	VH 3-33 Version 3
QVQLVESGGGVVQPGRSLRLSCAAS GFSLSRY DMHWVRQAPGKGLEWVAVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS	309	VH 3-33 Version 4
DVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQ RPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCLQGIHLPWTFGQGTKLEIK	310	VL 2-30

In some embodiments, the antigen-binding domain specific for EMCN includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the EMCN-VH includes a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 296), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and the EMCN-VL includes a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 299), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 300), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 301), and where the amino acid sequences of the CDR-H1, the

CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

In some embodiments, the antigen-binding domain specific for EMCN includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the EMCN-VH includes the amino acid sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNT HYHSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS (SEQ ID NO: 302); and the EMCN-VL includes the amino acid sequence DVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKL DSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQGTKLEIK (SEQ ID NO: 310).

In some embodiments, the antigen-binding domain specific for EMCN includes the amino acid sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNT HYHSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSSGGG GSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPG QSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQ GTKLEIK (SEQ ID NO: 311). In some embodiments, the antigen-binding domain specific for EMCN is encoded by a polynucleotide sequence comprising the sequence GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAG ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTGGGTCCG ACAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACGGCAACA CACACTACCACAGCGCCCTGAAGTCCCGGTTCACCATCTCCAGAGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTA CTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTG GAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCAT GACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCT GCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTC CAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGGA TAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAA GATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGGCATCC ATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAG (SEQ ID NO: 312). In some embodiments, the antigen-binding domain specific for EMCN is encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTGGGTCCG
ACAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACGGCAACA
CACACTACCACAGCGCCCTGAAGTCCCGGTTCACCATCTCCAGAGACAACAGCAAG
AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTA
CTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTG
GAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCAT
GACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCT
GCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTC
CAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGGA
TAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAA
GATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGGCATCC
ATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAG (SEQ ID NO: 312).

FLT3 and CD33-Specific Antigen-binding Domains

The present disclosure provides antigen-binding domains (e.g., single-chain variable fragments) that bind to FLT3 and CD33, chimeric proteins including antigen-binding domains that bind to FLT3 and CD33 (*e.g.*, any of the aCARs described herein), and nucleic acids encoding such antigen-binding domains and chimeric proteins.

In some embodiments, chimeric receptors comprise one or more of the amino acid sequences listed in Table A1 or Table A2. In some embodiments, activating chimeric receptors (aCARS) comprise one or more of the amino acid sequences listed in Table A1 or Table A2. In some embodiments, bispecific-bivalent aCARS comprise one or more of the amino acid sequences listed in Table A1 or Table A2. Table A1 provides the variable domains of an antibody heavy chain or light chain. The CDRs were determined using the Kabat method and are in ***bold italic*** in Table A1 for each variable heavy chain or variable light chain and shown in Table A2. In some embodiments, nucleic acids encoding any of the chimeric receptors of the present disclosure comprise one or more of the nucleic acid sequences listed in Table B.

Table A1

Amino Acid Sequence	SEQ ID NO:	Description
EVQLVQSGAEVKKPGASVKVSCKAS***GYTFTSYYMH***WARQAP GQGLEWMG***IINPSGGSTSYAQKFQG***RVTMTRDTSTSTVYMEL SSLRSEDTAVYYCAR***VVAAAVADY***WGQGTLVTVSS	313	Heavy chain variable domain of anti-FLT3 antibody D4-3
DVVMTQSPLSLPVTPGEPASISC***RSSQSLLHSNGYNYLD***WYLQ KPGQSPQLLIY***LGSNRAS***GVPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQSLQTPFTFGPGTKVDIK	314	Light chain variable domain of anti-FLT3 antibody D4-3

Table A1		
Amino Acid Sequence	**SEQ ID NO:**	**Description**
EVQLVQSGAEVKKPGSSVKVSCKAS***GGTFSSYAIS***WVRQAPG QGLEWMG***GIIPIFGTANYAQKFQG***RVTITADKSTSTAYMELSS LRSEDTAVYYCAT***FALFGFREQAFDI***WGQGTTVTVSS	315	Heavy chain variable domain of anti-FLT3 antibody NC7
DIQMTQSPSSLSASVGDRVTITC***RASQSISSYLN***WYQQKPGKA PKLLIY***AASSLQS***GVPSRFSGSGSGTDFTLTISSLQPEDLATYYC ***QQSYSTPFT***FGPGTKVDIK	316	Light chain variable domain of anti-FLT3 antibody NC7
EVQLVQSGAEVKKPGASVKVSCKAS***GYTFTSYYMH***WVRQAP GQGLEWMG***IINPSGGSTSYAQKFQG***RVTMTRDTSTSTVYMEL SSLRSEDTAVYYCAR***GVGAHDAFDI***WGQGTTVTVSS	317	Heavy chain variable domain of anti-FLT3 antibody EB10
DVVMTQSPLSLPVTPGEPASISC***RSSQSLLHSNGNNYLD***WYLQ KPGQSPQLLIY***LGSNRAS***GVPDRFSGSGSDTDFTLQISRVEAED VGVYYC***MQGTHPAIS***FGQGTRLEIK	318	Light chain variable domain of anti-FLT3 antibody EB10
QVQLQQPGAELVKPGASLKLSCKSSGYTFT***SYWMH***WVRQRP GHGLEWIG***EIDPSDSYKDYNQKFKD***KATLTVDRSSNTAYMHL SSLTSDDSAVYYCAR***AITTTPFDF***WGQGTTLTVSS	319	Heavy chain variable domain of anti-FLT3 antibody 4G8
DIVLTQSPATLSVTPGDSVSLSC***RASQSISNNLH***WYQQKSHES PRLLIK***YASQSIS***GIPSRFSGSGSGTDFTLSINSVETEDFGVYFC***Q QSNTWPYT***FGGGTKLEIKR	320	Light chain variable domain of anti-FLT3 antibody 4G8
QVTLKESGPTLVKPTETLTLTCTLSGFSLN***NARMGVS***WIRQPP GKCLEWLA***HIFSNDEKSYSTSLKN***RLTISKDSSKTQVVLTMTN VDPVDTATYYCAR***IVGYGSGWYGFFDY***WGQGTLVTVSS	321	Heavy chain variable domain of anti-FLT3 antibody FL_39
DIQMTQSPSSLSASVGDRVTITC***RASQGIRNDLG***WYQQKPGK APKRLIY***AASTLQS***GVPSRFSGSGSGTEFTLTISSLQPEDFATYY C***LQHNSYPLT***FGCGTKVEIK	322	Light chain variable domain of anti-FLT3 antibody FL_39
QVTLKESGPVLVKPTETLTLTCTVSGFSLR***NARMAVS***WIRQPP GKTLEWLA***HIFSNDEKSYSTSLKS***RLTISKDTSKSQVVLTMTN MDPVDTATYYCAR***IVGYGSGWYGYFDY***WGQGTLVTVSS	323	Heavy chain variable domain of anti-FLT3 antibody FL_16
DIQMTQSPSSVSASVGDRVTITC***RASQDIRYDLA***WYQQKPGK APKRLIY***AASSLQS***GVPSRFSGSGSGTEFTLTISSLQPEDFATYY CLQHNFYPLTFGGGTKVEIK	324	Light chain variable domain of anti-FLT3 antibody FL_16
EVQLVESGGGLVQPGGSLRLSCAAS***GFTFSSYG***MHWVRQAP GKGLEWVAV***ISYDGSNK***YYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYC***ANLAPWAAYW***GQGTLVTVSS	325	Heavy chain variable domain of anti-FLT3 antibody ml0006
EIVLTQSPLSLPVTPGEPASISCRSS***QSLLHSNGYNY***LDWYLQK PGQSPQLLIY***LGS***NRASGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYC***MQALQTPHT***FGQGTKLEIK	326	Light chain variable domain of anti-FLT3 antibody ml0006
QVQLKQSGPGLVQPSQSLSITCTVSGFSLT***NYGLH***WVRQSPGK GLEWLG***VIWSGGSTDYNAAFIS***RLSISKDNSKSQVFFKMNSLQ ADDTAIYYCAR***KGGIYYANHYYAMDY***WGQGTSVTVSS	327	Heavy chain variable domain of anti-FLT3 antibody BV10
DIVMTQSPSSLSVSAGEKVTMSC***KSSQSLLNSGNQKNYMA***WY QQKPGQPPKLLIY***GASTRES***GVPDRFTGSGSGTDFTLTISSVQA EDLAVYYC***QNDHSYPLT***FGAGTKLELKR	328	Light chain variable domain of anti-FLT3 antibody BV10
QVQLVQSGAEVKKPGSSVKVSCKASGYTFT***DYNMH***WVRQAP GQGLEWIG***YIYPYNGGTGYNQKFKSK***ATITADESTNTAYMELS SLRSEDTAVYYCAR***GRPAMDYWGQ***GTLVTVSS	329	Heavy chain variable domain of anti-CD33 antibody lintuzumab

Table A1		
Amino Acid Sequence	**SEQ ID NO:**	**Description**
DIQMTQSPSSLSASVGDRVTITC***RASESVDNYGISFMN***WFQQK PGKAPKLLIY***AASNQGS***GVPSRFSGSGSGTDFTLTISSLQPDDF ATYYC***QQSKEVPWT***FGQGTKVEIK	330	Light chain variable domain of anti-CD33 antibody lintuzumab
EVQLVQSGAEVKKPGSSVKVSCKAS***GYTITDSN***IHWVRQAPG QSLEWIGY***IYPYNGGT***DYNQKFKNRATLTVDNPTNTAYMELS SLRSEDTAFYYC***VNGNPWLAY***WGQGTLVTVSS	331	Heavy chain variable domain of anti-CD33 antibody gemtuzumab
DIQLTQSPSTLSASVGDRVTITCRAS***ESLDNYGIRF***LTWFQQKP GKAPKLLMY***AAS***NQGSGVPSRFSGSGSGTEFTLTISSLQPDDF ATYYC***QQTKEVPWS***FGQGTKVEVKR	332	Light chain variable domain of anti-CD33 antibody gemtuzumab

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAGCCAGGCGCTTC CGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATAT GCACTGGGCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTA TAAATCCTTCTGGCGGGTCAACCAGCTACGCACAAAAATTTCAAGGTCGG GTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAATTGTC TAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGC TGCTGCTGTGGCAGACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCA GC	333	Heavy chain variable domain of anti-FLT3 antibody D4-3
GACGTAGTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAG CCCGCCTCCATCTCATGTAGATCCTCCCAATCTCTCCTTCATTCAAACGGGT ATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGCCCTCAACTGC TCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCG GGAGTGGATCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCA GAAGATGTTGGTGTGTATTACTGTATGCAATCACTCCAGACCCCGTTTACC TTTGGGCCTGGAACAAAGGTAGATATTAAA	334	Light chain variable domain of anti-FLT3 antibody D4-3
GAGGTTCAACTGGTACAAAGCGGAGCCGAGGTAAAGAAACCAGGGAGTA GCGTCAAAGTGTCCTGCAAAGCCTCAGGCGGCACATTCAGTAGCTATGCT ATTTCATGGGTACGCCAAGCACCAGGACAGGGGCTGGAGTGGATGGGCGG GATTATCCCCATCTTCGGTACGGCAAACTATGCACAAAAGTTCCAGGGAC GAGTCACCATCACGGCTGATAAGTCCACCTCCACCGCCTATATGGAGCTGA GTTCCCTTCGGAGCGAGGATACTGCTGTGTATTATTGTGCCACGTTCGCAC TGTTCGGTTTTCGGGAGCAGGCGTTTGATATTTGGGGACAAGGCACAACG GTCACGGTCAGTTCA	335	Heavy chain variable domain of anti-FLT3 antibody NC7
GACATTCAGATGACCCAGAGTCCCTCTTCATTGAGTGCGAGCGTCGGTGAT CGGGTTACGATAACCTGTAGGGCCTCCCAAAGTATATCATCATATTTGAACC TGGTACCAACAGAAACCTGGGAAAGCGCCGAAGCTCCTTATCTATGCTGC CAGCTCTTTGCAAAGCGGTGTGCCCTCACGGTTCTCCGGTAGTGGGTCCGG GACCGACTTCACTTTGACCATCAGCAGCCTTCAGCCAGAGGATCTTGCCAC TTATTACTGCCAGCAATCTTATAGCACACCGTTTACATTCGGTCCAGGCAC AAAGGTAGACATTAAG	336	Light chain variable domain of anti-FLT3 antibody NC7
GAGGTACAGCTTGTGCAGAGTGGAGCAGAAGTTAAAAAACCCGGAGCTTC CGTGAAGGTAAGCTGCAAGGCTTCAGGATATACATTTACTAGCTACTACAT GCACTGGGTCCGCCAAGCTCCGGGCCAAGGCCTTGAATGGATGGGCATCA TAAATCCCAGTGGAGGCTCAACGAGCTATGCACAAAAGTTCCAAGGGCGC GTTACCATGACGCGCGACACCAGCACGTCCACCGTCTATATGGAACTCTCA AGTTTGCGATCTGAAGATACGGCTGTCTACTATTGCGCACGAGGGGTCGG	337	Heavy chain variable domain of anti-FLT3 antibody EB10

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
AGCGCATGACGCCTTCGACATCTGGGGACAAGGGACTACAGTAACTGTGT CAAGC		
GATGTTGTTATGACACAGTCTCCCCTCTCTTTGCCTGTTACGCCTGGCGAGC CCGCCTCTATTTCTTGTCGATCTAGTCAGAGCCTGCTGCATTCTAATGGAA ACAACTATTTGGACTGGTACTTGCAAAAGCCGGGTCAAAGTCCC	338	Light chain variable domain of anti-FLT3 antibody EB10
CAAGTCCAACTTCAGCAGCCAGGCGCTGAGTTGGTTAAACCGGGCGCAAG CCTCAAACTTAGTTGCAAGTCATCCGGATATACTTTCACGTCTTATTGGAT GCATTGGGTACGACAAAGACCTGGTCACGGCCTCGAATGGATTGGCGAAA TCGACCCGTCAGACAGCTACAAGGATTACAACCAGAAATTCAAAGATAAG GCAACACTTACTGTGGATCGCTCAAGTAACACGGCTTACATGCACCTCTCT TCACTCACGTCTGACGACAGTGCGGTGTATTATTGCGCCCGCGCTATTACA ACAACCCCTTTCGATTTCTGGGGCCAGGGTACTACGCTCACAGTCTCATCC	339	Heavy chain variable domain of anti-FLT3 antibody 4G8
GATATCGTCCTCACCCAATCCCCGGCTACTTTGAGTGTAACACCAGGCGAC AGCGTGTCACTGTCATGCCGAGCCTCCCAGTCAATCAGCAATAATCTGCAT TGGTATCAACAGAAATCACACGAATCCCCCCGACTTTTGATAAAGTATGCG TCACAGTCCATATCAGGCATTCCCAGTAGGTTTTCAGGCAGTGGTTCAGGT ACTGACTTCACCCTCTCCATTAACTCTGTAGAAACAGAGGACTTTGGCGTC TACTTCTGTCAGCAATCCAACACCTGGCCTTATACATTCGGCGGCGGCACT AAGCTGGAAATTAAGAGA	340	Light chain variable domain of anti-FLT3 antibody 4G8
CAAGTAACCCTTAAAGAGTCCGGCCCCACTTTGGTTAAACCTACTGAAACA CTTACACTCACATGCACATTGTCCGGCTTTTCACTCAACAACGCAAGGATG GGTGTGTCCTGGATTCGCCAGCCCCCTGGAAAATGTTTGGAATGGCTCGCT CATATATTTAGCAACGACGAGAAAAGTTACTCAACTTCACTCAAGAACCG CCTCACTATTAGCAAAGATTCCTCCAAAACCCAAGTAGTTCTGACAATGAC GAATGTAGACCCAGTCGATACTGCAACTTACTATTGCGCACGAATAGTCG GTTACGGGAGTGGCTGGTATGGGTTTTTCGACTATTGGGGACAGGGCACTC TTGTAACAGTAAGTAGC	341	Heavy chain variable domain of anti-FLT3 antibody FL39
GACATCCAGATGACTCAATCTCCATCTAGCCTCTCAGCGTCTGTGGGCGAT CGAGTCACCATCACCTGTAGGGCTTCCCAGGGTATAAGGAATGATTTGGG CTGGTACCAGCAAAAACCGGGTAAGGCTCCGAAACGACTGATATACGCAG CTTCTACGTTGCAATCCGGGGTGCCATCCAGATTTAGTGGCAGCGGGAGCG GTACTGAGTTTACGCTGACTATCTCCTCACTTCAGCCAGAGGATTTCGCCA CGTACTATTGTCTGCAACACAACTCCTATCCGCTGACCTTCGGGTGCGGGA CAAAGGTGGAAATTAAA	342	Light chain variable domain of anti-FLT3 antibody FL39
CAAGTGACCTTGAAGGAGTCAGGGCCAGTGTTGGTAAAACCTACTGAGAC TCTCACGTTGACATGCACGGTATCAGGTTTCAGCCTGAGGAACGCTCGGAT GGCCGTCAGTTGGATACGCCAGCCGCCAGGCAAAACTCTTGAATGGTTGG CGCACATATTCAGTAACGACGAGAAATCTTACTCTACATCCCTTAAGTCTC GCCTCACCATTTCTAAAGACACATCCAAATCACAAGTGGTACTCACGATGA CAAACATGGACCCTGTTGACACTGCTACATATTATTGTGCTAGGATAGTGG GCTACGGTAGCGGATGGTACGGTTATTTTGATTACTGGGGACAAGGGACG CTTGTTACGGTGTCCTCA	343	Heavy chain variable domain of anti-FLT3 antibody FL16
GACATTCAGATGACCCAGTCTCCGTCCAGCGTTAGCGCAAGCGTGGGGGA TAGAGTCACTATTACGTGTAGAGCCAGTCAAGATATACGGTACGATCTTGC TTGGTATCAGCAAAAACCGGGAAAAGCCCCGAAGAGACTTATATATGCAG CTTCCTCCTTGCAAAGCGGGGTCCCATCCCGGTTTAGTGGTAGTGGTTCCG GAACAGAGTTCACGCTGACTATTTCATCACTGCAACCCGAAGATTTTGCCA CCTACTACTGCCTTCAACACAATTTCTATCCTCTTACCTTCGGCGGAGGTAC TAAGGTAGAGATTAAG	345	Light chain variable domain of anti-FLT3 antibody FL16

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GAAGTACAGTTGGTTGAGAGTGGTGGAGGACTCGTTCAACCTGGCGGTAG TTTGCGACTCAGCTGCGCGGCTTCCGGTTTCACCTTCTCATCCTATGGGATG CACTGGGTCAGACAAGCCCCTGGAAAGGGCCTCGAATGGGTTGCTGTGAT TAGCTATGACGGCTCTAATAAATACTATGCAGATAGTGTAAAGGGGAGAT TTACGATTTCTCGCGATAATAGCAAAAATACGCTGTACCTGCAAATGGAA ACCAACAGCCTGCGAGCGGAAGATACGGCGGTTTATTACTGCGCGAATCT TGCCCCGTGGGCAGCATACTGGGGACAGGGGACGTTGGTGACGGTAAGCA GT	346	Heavy chain variable domain of anti-FLT3 antibody ml0006
GAGATTGTGCTCACCCAGTCTCCACTCAGCCTTCCTGTAACGCCCGGTGAG CCTGCCTCTATATCATGCCGAAGTTCCCAAAGCCTTCTGCACTCAAACGGC TATAACTACTTGGACTGGTACCTCCAGAAGCCCGGCCAAAGTCCTCAACTG TTGATATACCTGGGGTCCAACCGGGCATCAGGAGTACCTGATAGATTCTCA GGAAGTGGGTCAGGAACCGACTTCACGCTGAAAATTAGTCGCGTAGAGGC GGAAGATGTAGGTGTGTATTACTGTATGCAGGCGTTGCAAACACCGCACA CTTTTGGACAGGGAACCAAACTGGAAATAAAGACCAGTAGTGGT	347	Light chain variable domain of anti-FLT3 antibody ml0006
CAGGTCCAACTGAAACAAAGCGGTCCCGGTCTTGTCCAGCCCTCCCAATCT CTCAGTATTACTTGCACTGTGTCAGGTTTCAGCCTCACGAACTACGGTCTG CATTGGGTCCGCCAGTCTCCAGGAAAAGGCCTGGAGTGGCTCGGTGTTATC TGGAGTGGTGGAAGTACGGATTACAATGCTGCCTTTATCTCTCGGCTCAGT ATCTCCAAAGATAACTCTAAGTCCCAAGTCTTTTTCAAAATGAACTCTTTG CAGGCAGATGATACGGCCATATACTATTGCGCACGCAAGGGTGGGATCTA CTATGCAAACCACTATTACGCGATGGACTACTGGGGCCAAGGCACGAGTG TTACCGTGTCAAGC	348	Heavy chain variable domain of anti-FLT3 antibody BV10
GACATAGTGATGACTCAGTCTCCGTCCTCTCTTTCCGTGAGTGCGGGCGAA AAGGTTACCATGTCCTGCAAAAGTTCACAGTCACTTCTCAATTCTGGCAAC CAAAAAAATTACATGGCATGGTATCAACAGAAACCAGGTCAGCCGCCAAA GCTCCTCATATATGGTGCATCAACGCGAGAGTCAGGCGTACCTGACAGGTT TACCGGATCTGGCAGCGGTACAGACTTTACTCTTACCATATCAAGTGTGCA GGCAGAGGACTTGGCGGTATACTATTGTCAAAACGATCATAGTTACCCTCT TACATTTGGCGCGGGCACTAAACTGGAGCTGAAACGC	349	Light chain variable domain of anti-FLT3 antibody BV10
CAGGTTCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAG CGTGAAGGTGTCCTGCAAGGCCAGCGGCTACACCTTTACCGACTACAACA TGCACTGGGTCCGACAGGCCCCTGGACAAGGACTTGAGTGGATCGGCTAC ATCTACCCCTACAATGGCGGCACCGGCTACAACCAGAAGTTCAAGAGCAA GGCCACCATCACCGCCGACGAGAGCACAAACACCGCCTACATGGAACTGA GCAGCCTGAGAAGCGAGGACACCGCCGTGTACTACTGTGCCAGAGGCAGA CCCGCCATGGATTATTGGGGACAGGGCACCCTGGTCACCGTTTCTAGC	350	Heavy chain variable domain of anti-CD33 antibody lintuzumab
GATATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGA TAGAGTGACCATCACCTGTAGAGCCAGCGAGAGCGTGGACAACTACGGCA TCAGCTTCATGAACTGGTTCCAGCAGAAGCCCGGCAAGGCCCCTAAGCTG CTGATCTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTC CGGCTCTGGCAGCGGCACCGATTTCACCCTGACAATCTCTAGCCTCCAGCC TGACGACTTCGCCACCTACTACTGCCAGCAGAGCAAAGAGGTGCCCTGGA CATTCGGCCAGGGCACAAAGGTGGAAATCAAG	351	Light chain variable domain of anti-CD33 antibody lintuzumab
GAAGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAG CGTGAAGGTGTCCTGCAAGGCCAGCGGCTACACCATCACCGACAGCAACA TCCACTGGGTCCGACAGGCTCCAGGCCAGTCTCTTGAGTGGATCGGCTACA TCTACCCCTACAACGGCGGCACCGACTACAACCAGAAGTTCAAGAACCGG GCCACACTGACCGTGGACAACCCTACCAATACCGCCTACATGGAACTGAG CAGCCTGCGGAGCGAGGACACCGCCTTTTACTACTGCGTGAACGGCAACC CCTGGCTGGCCTATTGGGGACAGGGAACACTGGTCACAGTGTCTAGC	352	Heavy chain variable domain of anti-CD33 antibody gemtuzumab

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GATATTCAGCTGACACAGAGCCCCAGCACACTGTCTGCCTCTGTGGGCGAC AGAGTGACCATCACCTGTAGAGCCAGCGAGAGCCTGGACAACTACGGCAT CAGATTTCTGACCTGGTTCCAGCAGAAGCCCGGCAAGGCTCCTAAGCTGCT GATGTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTCCG GCTCTGGCAGCGGCACAGAGTTCACCCTGACAATCTCTAGCCTCCAGCCTG ACGACTTCGCCACCTACTACTGCCAGCAGACCAAAGAGGTGCCCTGGTCCT TTGGACAGGGCACCAAGGTGGAAGTGAAGCGG	353	Light chain variable domain of anti-CD33 antibody gemtuzumab

Table A2												
Ab	**SEQ ID NO**	**CDR-H1**	**SEQ ID NO**	**CDR-H2**	**SEQ ID NO**	**CDR-H3**	**SEQ ID NO**	**CDR-L1**	**SEQ ID NO**	**CDR-L2**	**SEQ ID NO**	**CDR-L3**
FLT3 D4-3	354	GYTFTSYYMH	355	IINPSGGSTSYAQKFQG	356	VVAAAVADY	357	RSSQSLLHSNGYNYLD	358	LGSNRA	359	MQSLQTPFT
FLT3 NC7	360	GGTFSSYAIS	361	GIIPIFGTANYAQKFQG	362	FALFGFREQAFDI	363	RASQSISSYLN	364	AASSLQS	365	QQSYSTPFT
FLT3 EB10	366	GYTFTSYYMH	367	IINPSGGSTSYAQKFQG	368	GVGAHDAFDI	369	RSSQSLLHSNGNNYLD	370	LGSNRA	371	MQGTHPAIS
FLT3 4G8	372	SYWMH	373	EIDPSDSYKDYNQKFKD	374	AITTTPFDF	375	RASQSISNNLH	376	YASQSIS	377	QQSNTWPYT
FLT3 FL_39	378	NARMGVS	379	HIFSNDEKSYSTSLKN	380	IVGYGSGWYGFFDY	381	RASQGIRNDLG	382	AASTLQS	383	LQHNSYPLT
FLT3 FL_16	384	NARMAVS	385	HIFSNDEKSYSTSLKS	386	IVGYGSGWYGYFDY	387	RASQDIRYDLA	388	AASSLQS	389	LQHNFYPLT
FLT3 ml0006	390	GFTFSSYG	391	ISYDGSNK	392	ANLAPWAAYW	393	QSLLHSNGYNY	394	LGS	395	MQALQTPHT
FLT3 BV10	396	NYGLH	397	VIWSGGSTDYNAAFIS	398	KGGIYYANHYYAMDY	399	KSSQSLLNSGNQKNYMA	400	GASTRES	401	QNDHSYPLT
CD33 antibody lintuzumab	402	DYNMH	403	YIYPYNGGTGYNQKFKSKA	404	GRPAMDYWGQ	405	RASESVDNYGISFMN	406	AASNQGS	407	QQSKEVPWT
CD33 antibody	408	GYTITDSN	409	IYPYNGGT	410	VNGNPWLAY	411	ESLDNYGIRF	412	AAS	413	QQTKEVPWS

gemtuzumab												

In some embodiments, an antigen-binding domain specific for CD33 includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the CD33-VH includes: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and the CD33-VL includes: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

In some embodiments, an antigen-binding domain specific for CD33 includes a CD33-VH having the amino acid sequence QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSS (SEQ ID NO: 329), and a CD33-VL having the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIK (SEQ ID NO: 330).

In some embodiments, an antigen-binding domain specific for FLT3 includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the FLT3-VH includes a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GGTFSSYAIS (SEQ ID NO: 360), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIIPIFGTANYAQKFQG (SEQ ID NO: 361), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQAFDI (SEQ ID NO: 362), and the FLT3-VL includes a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASQSISSYLN (SEQ ID NO: 363), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and a light chain

complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

In some embodiments, an antigen-binding domain specific for FLT3 includes a FLT3-VH having the amino acid sequence EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTAN YAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTV TVSS (SEQ ID NO: 315), and a FLT3-VL having the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS RFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIK (SEQ ID NO: 316).

In some embodiments, a bivalent-bispecific aCAR includes an antigen-binding domain specific for FLT3 and an antigen-binding domain specific for CD33.

In some embodiments, a bivalent-bispecific aCAR specific for FLT3 and CD33 includes the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCA TFALFGFREQAFDIWGQGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGYT FTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSL RSEDTAVYYCARGRPAMDYWGQGTLVTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSS LSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGS GSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKGGGGSDIQMTQSPSSLSA SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTL TISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL YCNHRRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPA YKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 414).

In some embodiments, a bivalent-bispecific aCAR specific for FLT3 and CD33 includes the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVR QAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCA TFALFGFREQAFDIWGQGTTVTVSSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCK ASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAY MELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSDIQMTQSPSSLS

ASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGS
GTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPS
SLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGT
DFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTT
TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYCNHRRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA
DAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
415).

Engineered Nucleic Acids

Provided herein are multicistronic expression systems that include engineered nucleic acids encoding (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR.

In certain embodiments described herein, the multicistronic expression systems included engineered nucleic acids that encode an expression cassette containing a promoter and an exogenous polynucleotide sequence encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR.

The promoter is operably linked to expression cassette of the multicistronic system such that the exogenous polynucleotide sequence encoding each of the membrane-cleavable chimeric protein, the bivalent aCAR, and the iCAR is configured to be expressed as a single polypeptide.

An "engineered nucleic acid" is a nucleic acid that does not occur in nature. It should be understood, however, that while an engineered nucleic acid as a whole is not naturally-occurring, it may include nucleotide sequences that occur in nature. In some embodiments, an engineered nucleic acid comprises nucleotide sequences from different organisms (*e.g.,* from different species). For example, in some embodiments, an engineered nucleic acid includes a murine nucleotide sequence, a bacterial nucleotide sequence, a human nucleotide sequence, and/or a viral nucleotide sequence. The term "engineered nucleic acids" includes recombinant nucleic acids and synthetic nucleic acids. A "recombinant nucleic acid" refers to a molecule that is constructed by joining nucleic acid molecules and, in some embodiments, can replicate in a live cell. A "synthetic nucleic acid" refers to a molecule that is amplified or chemically, or by other means, synthesized. Synthetic nucleic acids include those that are chemically modified, or otherwise modified, but can base pair with naturally- occurring nucleic acid molecules. Modifications include, but are not limited to, one or more modified internucleotide linkages and non-natural nucleic acids. Modifications are described in further detail in U.S. Pat. No.

6,673,611 and U.S. Application Publication 2004/0019001 and, each of which is incorporated by reference in their entirety. Modified internucleotide linkages can be a phosphorodithioate or phosphorothioate linkage. Non-natural nucleic acids can be a locked nucleic acid (LNA), a peptide nucleic acid (PNA), glycol nucleic acid (GNA), a phosphorodiamidate morpholino oligomer (PMO or "morpholino"), and threose nucleic acid (TNA). Non-natural nucleic acids are described in further detail in International Application WO 1998/039352, U.S. Application Pub. No. 2013/0156849, and U.S. Pat. Nos. 6,670,461; 5,539,082; 5,185,444, each herein incorporated by reference in their entirety. Recombinant nucleic acids and synthetic nucleic acids also include those molecules that result from the replication of either of the foregoing. Engineered nucleic acid of the present disclosure may be encoded by a single molecule (*e.g.*, included in the same plasmid or other vector) or by multiple different molecules (*e.g.*, multiple different independently-replicating molecules). Engineered nucleic acids can be an isolated nucleic acid. Isolated nucleic acids include, but are not limited to a cDNA polynucleotide, an RNA polynucleotide, an RNAi oligonucleotide (*e.g.*, siRNAs, miRNAs, antisense oligonucleotides, shRNAs, etc.), an mRNA polynucleotide, a circular plasmid, a linear DNA fragment, a vector, a minicircle, a ssDNA, a bacterial artificial chromosome (BAC), and yeast artificial chromosome (YAC), and an oligonucleotide.

Engineered nucleic acid of the present disclosure may be produced using standard molecular biology methods (*see*, *e.g.*, Green and Sambrook, Molecular Cloning, A Laboratory Manual, 2012, Cold Spring Harbor Press). In some embodiments, engineered nucleic acid constructs are produced using GIBSON ASSEMBLY® Cloning (see, *e.g.*, Gibson, D.G. *et al.* Nature Methods, 343-345, 2009; and Gibson, D.G. *et al.* Nature Methods, 901-903, 2010, each of which is incorporated by reference herein). GIBSON ASSEMBLY® typically uses three enzymatic activities in a single-tube reaction: 5' exonuclease, the ΄Y extension activity of a DNA polymerase and DNA ligase activity. The 5 ' exonuclease activity chews back the 5 ' end sequences and exposes the complementary sequence for annealing. The polymerase activity then fills in the gaps on the annealed regions. A DNA ligase then seals the nick and covalently links the DNA fragments together. The overlapping sequence of adjoining fragments is much longer than those used in Golden Gate Assembly, and therefore results in a higher percentage of correct assemblies. In some embodiments, engineered nucleic acid constructs are produced using IN-FUSION® cloning (Clontech).

Promoters

In general, in all embodiments described herein, the engineered nucleic acids encoding the membrane-cleavable chimeric protein, the bivalent aCAR, and the iCAR encode an expression cassette containing a promoter. In some embodiments, an engineered nucleic acid (*e.g.*, an engineered nucleic acid comprising an expression cassette) comprises a promoter operably linked to a nucleotide sequence (*e.g.*, an exogenous polynucleotide sequence) encoding at least 2 distinct proteins. For example, the engineered nucleic acid may comprise a promoter operably linked to a nucleotide sequence encoding at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10 distinct proteins. In some embodiments, an engineered nucleic acid comprises a promoter operably linked to a nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more distinct proteins. In some embodiments, an engineered nucleic acid (*e.g.*, an engineered nucleic acid comprising an expression cassette) comprises a promoter operably linked to a nucleotide sequence (*e.g.*, an exogenous polynucleotide sequence) encoding at least 2 membrane-cleavable chimeric proteins. For example, the engineered nucleic acid may comprise a promoter operably linked to a nucleotide sequence encoding at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10 membrane-cleavable chimeric proteins. In some embodiments, an engineered nucleic acid comprises a promoter operably linked to a nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more membrane-cleavable chimeric proteins.

A "promoter" refers to a control region of a nucleic acid sequence at which initiation and rate of transcription of the remainder of a nucleic acid sequence are controlled. A promoter may also contain sub-regions at which regulatory proteins and molecules may bind, such as RNA polymerase and other transcription factors. Promoters may be constitutive, inducible, repressible, tissue-specific or any combination thereof. A promoter drives expression or drives transcription of the nucleic acid sequence that it regulates. Herein, a promoter is considered to be "operably linked" when it is in a correct functional location and orientation in relation to a nucleic acid sequence it regulates to control ("drive") transcriptional initiation and/or expression of that sequence.

A promoter may be one naturally associated with a gene or sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment of a given gene or sequence. Such a promoter can be referred to as "endogenous." In some embodiments, a coding nucleic acid sequence may be positioned under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with the encoded sequence in its natural environment. Such promoters may include promoters of other

genes; promoters isolated from any other cell; and synthetic promoters or enhancers that are not "naturally occurring" such as, for example, those that contain different elements of different transcriptional regulatory regions and/or mutations that alter expression through methods of genetic engineering that are known in the art. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. No. 4,683,202 and U.S. Pat. No. 5,928,906).

Promoters of an engineered nucleic acid may be "inducible promoters," which refer to promoters that are characterized by regulating (*e.g.*, initiating or activating) transcriptional activity when in the presence of, influenced by or contacted by a signal. The signal may be endogenous or a normally exogenous condition (*e.g.*, light), compound (*e.g.*, chemical or non-chemical compound) or protein (*e.g.*, cytokine) that contacts an inducible promoter in such a way as to be active in regulating transcriptional activity from the inducible promoter. Activation of transcription may involve directly acting on a promoter to drive transcription or indirectly acting on a promoter by inactivation a repressor that is preventing the promoter from driving transcription. Conversely, deactivation of transcription may involve directly acting on a promoter to prevent transcription or indirectly acting on a promoter by activating a repressor that then acts on the promoter.

A promoter is "responsive to" or "modulated by" a local tumor state (*e.g.*, inflammation or hypoxia) or signal if in the presence of that state or signal, transcription from the promoter is activated, deactivated, increased, or decreased. In some embodiments, the promoter comprises a response element. A "response element" is a short sequence of DNA within a promoter region that binds specific molecules (*e.g.*, transcription factors) that modulate (regulate) gene expression from the promoter. Response elements that may be used in accordance with the present disclosure include, without limitation, a phloretin-adjustable control element (PEACE), a zinc-finger DNA-binding domain (DBD), an interferon-gamma-activated sequence (GAS) (Decker, T. *et al. J Interferon Cytokine Res.* 1997 Mar;17(3):121-34, incorporated herein by reference), an interferon-stimulated response element (ISRE) (Han, K. J. *et al. J Biol Chem.* 2004 Apr 9;279(15):15652-61, incorporated herein by reference), a NF-kappaB response element (Wang, V. *et al.* Cell Reports. 2012; 2(4): 824-839, incorporated herein by reference), and a STAT3 response element (Zhang, D. *et al. J of Biol Chem.* 1996; 271: 9503-9509, incorporated herein by reference). Other response elements are encompassed herein. Response elements can also contain tandem repeats (*e.g.*, consecutive repeats of the same nucleotide sequence encoding the response element) to generally increase sensitivity of the response

element to its cognate binding molecule. Tandem repeats can be labeled 2X, 3X, 4X, 5X, *etc.* to denote the number of repeats present.

Non-limiting examples of responsive promoters (also referred to as "inducible promoters") (*e.g.*, TGF-beta responsive promoters) are listed in **Table 5A**, which shows the design of the promoter and transcription factor, as well as the effect of the inducer molecule towards the transcription factor (TF) and transgene transcription (T) is shown (B, binding; D, dissociation; n.d., not determined) (A, activation; DA, deactivation; DR, derepression) (see Horner, M. & Weber, W. *FEBS Letters* 586 (2012) 20784-2096m, and references cited therein). Non-limiting examples of components of inducible promoters include those presented in **Table 5B**.

Table 5A. Examples of Responsive Promoters

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
				TF	T
Transcriptional activator-responsive promoters					
AIR	PAIR (OalcA-PhCMVmin)	AlcR	Acetaldehyde	n.d.	A
ART	PART (OARG-PhCMVmin)	ArgR-VP16	l-Arginine	B	A
BIT	PBIT3 (OBirA3-PhCMVmin)	BIT (BirA-VP16)	Biotin	B	A
Cumate – activator	PCR5 (OCuO6-PhCMVmin)	cTA (CymR-VP16)	Cumate	D	DA
Cumate – reverse activator	PCR5 (OCuO6-PhCMVmin)	rcTA (rCymR-VP16)	Cumate	B	A
E-OFF	PETR (OETR-PhCMVmin)	ET (E-VP16)	Erythromycin	D	DA
NICE-OFF	PNIC (ONIC-PhCMVmin)	NT (HdnoR-VP16)	6-Hydroxy-nicotine	D	DA
PEACE	PTtgR1 (OTtgR-PhCMVmin)	TtgA1 (TtgR-VP16)	Phloretin	D	DA
PIP-OFF	PPIR (OPIR-Phsp70min)	PIT (PIP-VP16)	Pristinamycin I	D	DA
QuoRex	PSCA (OscbR-PhCMVmin)PSPA (OpapRI-PhCMVmin)	SCA (ScbR-VP16)	SCB1	D	DA
Redox	PROP (OROP-PhCMVmin)	REDOX (REX-VP16)	NADH	D	DA

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
TET-OFF	PhCMV*-1 (OtetO7-PhCMVmin)	tTA (TetR-VP16)	Tetracycline	D	DA
TET-ON	PhCMV*-1 (OtetO7-PhCMVmin)	rtTA (rTetR-VP16)	Doxycycline	B	A
TIGR	PCTA (OrheO-PhCMVmin)	CTA (RheA-VP16)	Heat	D	DA
TraR	O7x(tra box)-PhCMVmin	p65-TraR	3-Oxo-C8-HSL	B	A
VAC-OFF	P1VanO2 (OVanO2-PhCMVmin)	VanA1 (VanR-VP16)	Vanillic acid	D	DA
Transcriptional repressor-responsive promoters					
Cumate - repressor	PCuO (PCMV5-OCuO)	CymR	Cumate	D	DR
E-ON	PETRON8 (PSV40-OETR8)	E-KRAB	Erythromycin	D	DR
NICE-ON	PNIC (PSV40-ONIC8)	NS (HdnoR-KRAB)	6-Hydroxy-nicotine	D	DR
PIP-ON	PPIRON (PSV40-OPIR3)	PIT3 (PIP-KRAB)	Pristinamycin I	D	DR
Q-ON	PSCAON8 (PSV40-OscbR8)	SCS (ScbR-KRAB)	SCB1	D	DR
TET-ON repressor-based	OtetO-PHPRT	tTS-H4 (TetR-HDAC4)	Doxycycline	D	DR
T-REX	PTetO (PhCMV-OtetO2)	TetR	Tetracycline	D	DR
UREX	PUREX8 (PSV40-OhucO8)	mUTS (KRAB-HucR)	Uric acid	D	DR
VAC-ON	PVanON8 (PhCMV-OVanO8)	VanA4 (VanR-KRAB)	Vanillic acid	D	DR
Hybrid promoters					
QuoRexPIP-ON(NOT IF gate)	OscbR8-OPIR3-PhCMVmin	SCAPIT3	SCB1Pristinamycin I	DD	DADR
QuoRexE-ON(NOT IF gate)	OscbR-OETR8-PhCMVmin	SCAE-KRAB	SCB1Erythromycin	DD	DADR
TET-OFFE-ON(NOT IF gate)	OtetO7-OETR8-PhCMVmin	tTAE-KRAB	TetracyclineErythromycin	DD	DADR
TET-OFFPIP-ONE-ON	OtetO7-OPIR3-OETR8-PhCMVmin	tTAPIT3E-KRAB	TetracyclinePristinamycin IErythromycin	DDD	DADRDR

Table 5B. Exemplary Components of Inducible Promoters

Name	DNA SEQUENCE
minimal promoter; minP	AGAGGGTATATAATGGAAGCTCGACTTCCAG (SEQ ID NO: 1)
NFkB response element protein promoter; 5x NFkB-RE	GGGAATTTCCGGGGACTTTCCGGGAATTTCCGGGGACTTTCCGGGAATTTCC (SEQ ID NO: 2)
CREB response element protein promoter; 4x CRE	CACCAGACAGTGACGTCAGCTGCCAGATCCCATGGCCGTCATACTGTGACGTCTTTCAGACACCCCATTGACGTCAATGGGAGAA (SEQ ID NO: 3)
NFAT response element protein promoter; 3x NFAT binding sites	GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGT (SEQ ID NO: 4)
SRF response element protein promoter; 5x SRE	AGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCT (SEQ ID NO: 5)
SRF response element protein promoter 2; 5x SRF-RE	AGTATGTCCATATTAGGACATCTACCATGTCCATATTAGGACATCTACTATGTCCATATTAGGACATCTTGTATGTCCATATTAGGACATCTAAAATGTCCATATTAGGACATCT (SEQ ID NO: 6)
AP1 response element protein promoter; 6x AP1-RE	TGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAG (SEQ ID NO: 7)
TCF-LEF response element promoter; 8x TCF-LEF-RE	AGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGTATAAGATCAAAGGGCCTAAGATCAAAGGGACTAAGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGCCTA (SEQ ID NO: 8)
SBEx4	GTCTAGACGTCTAGACGTCTAGACGTCTAGAC (SEQ ID NO: 9)
SMAD2/3 - CAGACA x4	CAGACACAGACACAGACACAGACA (SEQ ID NO: 10)
STAT3 binding site	Ggatccggtactcgagatctgcgatctaagtaagcttggcattccggtactgttggtaaagccac (SEQ ID NO: 11)
minCMV	taggcgtgtacggtgggaggcctatataagcagagctcgtttagtgaaccgtcagatcgcctgga (SEQ ID NO: 170)
YB_TATA	TCTAGAGGGTATATAATGGGGGCCA (SEQ ID NO: 171)
minTK	Ttcgcatattaaggtgacgcgtgtggcctcgaacaccgagcgaccctgcagcgacccgcttaa (SEQ ID NO: 172)

Non-limiting examples of promoters include the cytomegalovirus (CMV) promoter, the elongation factor 1-alpha (EF1a) promoter, the elongation factor (EFS) promoter, the MND promoter (a synthetic promoter that contains the U3 region of a modified MoMuLV LTR with myeloproliferative sarcoma virus enhancer), the phosphoglycerate kinase (PGK) promoter, the spleen focus-forming virus (SFFV) promoter, the simian virus 40 (SV40) promoter, and the ubiquitin C (UbC) promoter (see **Table 5C**).

Table 5C. Exemplary Constitutive Promoters

Name	DNA SEQUENCE
CMV	GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG CCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT AGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAG TACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG CCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAG TTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATT GACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTC (SEQ ID NO: 12)
EF1a	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGCCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAG TGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAA GTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTT GAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAG GCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTT TTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTT GGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGG GCCTGCGAGCGCGACCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGC TCTGGTGCCTGTCCTCGCGCCGCCGTGTATCGCCCCGCCCCGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGTCCTGCTGCAGGGAGC TCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGA AAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCG TCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGC TTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA (SEQ ID NO: 13)
EFS	GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCC GAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGG TAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAA CCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGA ACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTG AGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTG AACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGG CGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTG CTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC GGCGCCTAC (SEQ ID NO: 14)
MND	TTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAGC TAGGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTG GTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGC CAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGT CCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTG CCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCG CTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCA (SEQ ID NO: 15)
PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACGCGGCTGCT CTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACG TCCGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTGGGCCCCCCGGCGACGCTT CCTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGCGGTTCGCGGCGTGCCGGACGTGACA AACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGACAGCGCCAGGGAGCAATG GCAGCGCGCCGACCGCGATGGGCTGTGGCCAATAGCGGCTGCTCAGCGGGGCGCGCCG

Name	DNA SEQUENCE
	AGAGCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGG CCCTGTTCCTGCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTCGGCA GTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAG (SEQ ID NO: 16)
SFFV	GTAACGCCATTTTGCAAGGCATGGAAAAATACCAAACCAAGAATAGAGAAGTTCAGAT CAAGGGCGGGTACATGAAAATAGCTAACGTTGGGCCAAACAGGATATCTGCGGTGAGC AGTTTCGGCCCCGGCCCGGGGCCAAGAACAGATGGTCACCGCAGTTTCGGCCCCGGCCC GAGGCCAAGAACAGATGGTCCCCAGATATGGCCCAACCCTCAGCAGTTTCTTAAGACCC ATCAGATGTTTCCAGGCTCCCCCAAGGACCTGAAATGACCCTGCGCCTTATTTGAATTAA CCAATCAGCCTGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTTCCCGAGCTCTATAAAAGA GCTCACAACCCCTCACTCGGCGCGCCAGTCCTCCGACAGACTGAGTCGCCCGGG (SEQ ID NO: 17)
SV40	CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG TATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCC CAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCC CTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGC TGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAG AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCT (SEQ ID NO: 18)
UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCACGGCGAGCGCTGCCACGT CAGACGAAGGGCGCAGGAGCGTTCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGC CCGCTGCTCATAAGACTCGGCCTTAGAACCCCAGTATCAGCAGAAGGACATTTTAGGAC GGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAA AAGTAGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGTGAACGCCGATG ATTATATAAGGACGCGCCGGGTGTGGCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGT CGCGGTTCTTGTTTGTGGATCGCTGTGATCGTCACTTGGTGAGTTGCGGGCTGCTGGGCT GGCCGGGGCTTTCGTGGCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTGGAGAGACC GCCAAGGGCTGTAGTCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTTGGGGGG AGCGCACAAAATGGCGGCTGTTCCCGAGTCTTGAATGGAAGACGCTTGTAAGGCGGGCT GTGAGGTCGTTGAAACAAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAGGTCTTGA GGCCTTCGCTAATGCGGGAAAGCTCTTATTCGGGTGAGATGGGCTGGGGCACCATCTGG GGACCCTGACGTGAAGTTTGTCACTGACTGGAGAACTCGGGTTTGTCGTCTGGTTGCGG GGGCGGCAGTTATGCGGTGCCGTTGGGCAGTGCACCCGTACCTTTGGGAGCGCGCGCCT CGTCGTGTCGTGACGTCACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCCACCTGCCG GTAGGTGTGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCGGGCCTAGGGTAGGC TCTCCTGAATCGACAGGCGCCGGACCTCTGGTGAGGGGAGGGATAAGTGAGGCGTCAG TTTCTTTGGTCGGTTTTATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGTTTTGAACTA TGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCACCTTTTGAAATGTAAT CATTTGGGTCAATATGTAATTTTCAGTGTTAGACTAGTAAAGCTTCTGCAGGTCGACTCT AGAAAATTGTCCGCTAAATTCTGGCCGTTTTTGGCTTTTTTGTTAGAC (SEQ ID NO: 19)
hEF1aV1	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAG TGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAA GTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTT GAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAG GCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTT TTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTT GGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGG GCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGC TCTGGTGCCTGGTCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGC TCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGA AAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCG TCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGC TTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT

Name	DNA SEQUENCE
	CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA (SEQ ID NO: 20)
hCAGG	ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCC ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC GTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCAT ATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC CCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGC TATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTC CCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGG GGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGC GAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTT ATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGG AGTCGCTGCGACGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCG CCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGA AAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCG TGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGCCCGGCGGCTGTGA GCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCG GCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGC GGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAACC CCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCC GTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGT GCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGG CCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGT AATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCT GGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCA GGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCCT CTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGG CGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATG CCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTT TGGCAAAGAATTC (SEQ ID NO: 21)
hEF1aV2	Gggcagagcgcacatcgcccacagtccccgagaagttgggggggagggtcggcaattgaaccggtgcctagagaaggtggcgcggggtaa actgggaaagtgatgtcgtgtactggctccgcctttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgc aacgggtttgccgccagaacacag (SEQ ID NO: 22)
hACTb	CCACTAGTTCCATGTCCTTATATGGACTCATCTTTGCCTATTGCGACACACACTCAATGA ACACCTACTACGCGCTGCAAAGAGCCCCGCAGGCCTGAGGTGCCCCCACCTCACCACTC TTCCTATTTTTGTGTAAAAATCCAGCTTCTTGTCACCACCTCCAAGGAGGGGGAGGAGG AGGAAGGCAGGTTCCTCTAGGCTGAGCCGAATGCCCCTCTGTGGTCCCACGCCACTGAT CGCTGCATGCCCACCACCTGGGTACACACAGTCTGTGATTCCCGGAGCAGAACGGACCC TGCCCACCCGGTCTTGTGTGCTACTCAGTGGACAGACCCAAGGCAAGAAAGGGTGACAA GGACAGGGTCTTCCCAGGCTGGCTTTGAGTTCCTAGCACCGCCCCGCCCCCAATCCTCTG TGGCACATGGAGTCTTGGTCCCCAGAGTCCCCCAGCGGCCTCCAGATGGTCTGGGAGGG CAGTTCAGCTGTGGCTGCGCATAGCAGACATACAACGGACGGTGGGCCCAGACCCAGG CTGTGTAGACCCAGCCCCCCCGCCCCGCAGTGCCTAGGTCACCCACTAACGCCCCAGGC CTGGTCTTGGCTGGGCGTGACTGTTACCCTCAAAAGCAGGCAGCTCCAGGGTAAAAGGT GCCCTGCCCTGTAGAGCCCACCTTCCTTCCCAGGGCTGCGGCTGGGTAGGTTTGTAGCCT TCATCACGGGCCACCTCCAGCCACTGGACCGCTGGCCCCTGCCCTGTCCTGGGGAGTGT GGTCCTGCGACTTCTAAGTGGCCGCAAGCCACCTGACTCCCCCAACACCACACTCTACC TCTCAAGCCCAGGTCTCTCCCTAGTGACCCACCCAGCACATTTAGCTAGCTGAGCCCCAC AGCCAGAGGTCCTCAGGCCCTGCTTTCAGGGCAGTTGCTCTGAAGTCGGCAAGGGGGAG TGACTGCCTGGCCACTCCATGCCCTCCAAGAGCTCCTTCTGCAGGAGCGTACAGAACCC AGGGCCCTGGCACCCGTGCAGACCCTGGCCCACCCCACCTGGGCGCTCAGTGCCCAAGA GATGTCCACACCTAGGATGTCCCGCGGTGGGTGGGGGGCCCGAGAGACGGGCAGGCCG GGGGCAGGCCTGGCCATGCGGGGCCGAACCGGGCACTGCCCAGCGTGGGGCGCGGGGG CCACGGCGCGCGCCCCCAGCCCCCGGGCCCAGCACCCCAAGGCGGCCAACGCCAAAAC TCTCCCTCCTCCTCTTCCTCAATCTCGCTCTCGCTCTTTTTTTTTTTCGCAAAAGGAGGGG AGAGGGGGTAAAAAAATGCTGCACTGTGCGGCGAAGCCGGTGAGTGAGCGGCGCGGGG

Name	DNA SEQUENCE
	CCAATCAGCGTGCGCCGTTCCGAAAGTTGCCTTTTATGGCTCGAGCGGCCGCGGCGGCG CCCTATAAAACCCAGCGGCGCGACGCGCCACCACCGCCGAGACCGCGTCCGCCCCGCG AGCACAGAGCCTCGCCTTTGCCGATCCGCCGCCCGTCCACACCCGCCGCCAGgtaagcccggc cagccgaccggggcaggcggctcacggcccggccgcaggcggccgcggcccctcgcccgtgcagagccgccgtctgggccgcagcggg gggcgcatgggggggaaccggaccgccgtgggggcgcgggagaagccccctgggcctccggagatgggggacacccacgccagttc ggaggcgcgaggccgcgctcgggaggcgcgctccgggggtgccgctctcgggcgggggcaaccggcggggtctttgtctgagccgggct cttgccaatggggatcgcagggtgggcgcggcggagccccccgccaggcccggtgggggctgggcgccattgcgcgtgcgcgctggtcctt tgggcgctaactgcgtgcgcgctgggaattggcgctaattgcgcgtgcgcgctgggactcaaggcgctaactgcgcgtgcgttctgggcccg gggtgccgcggcctgggctgggcgaaggcgggctcggccggaaggggtggggtcgccgcggctcccgggcgcttgcgcgcacttcctgc ccgagccgctggccgcccgagggtgtggccgctgcgtgcgcgcgcgccgacccggcgctgtttgaacgggcggaggcggggctggcgcc cggttgggaggggttggggcctggcttcctgccgcgccgccggggacgcctccgaccagtgtttgccttttatggtaataacgcggccggcc cggcttcctttgtcccaatctgggcgcgcgccggcgcccctggcggctaaggactcggcgcgccggaagtggccagggcgggggcgac ctcggctcacagcgcgcccggctat (SEQ ID NO: 23)
heIF4A1	GTTGATTTCCTTCATCCCTGGCACACGTCCAGGCAGTGTCGAATCCATCTCTGCTACAGG GGAAAACAAATAACATTTGAGTCCAGTGGAGACCGGGAGCAGAAGTAAAGGGAAGTGA TAACCCCCAGAGCCCGGAAGCCTCTGGAGGCTGAGACCTCGCCCCCCTTGCGTGATAGG GCCTACGGAGCCACATGACCAAGGCACTGTCGCCTCCGCACGTGTGAGAGTGCAGGGCC CCAAGATGGCTGCCAGGCCTCGAGGCCTGACTCTTCTATGTCACTTCCGTACCGGCGAG AAAGGCGGGCCCTCCAGCCAATGAGGCTGCGGGGCGGGCCTTCACCTTGATAGGCACTC GAGTTATCCAATGGTGCCTGCGGGCCGGAGCGACTAGGAACTAACGTCATGCCGAGTTG CTGAGCGCCGGCAGGCGGGGCCGGGGCGGCCAAACCAATGCGATGGCCGGGGCGGAGT CGGGCGCTCTATAAGTTGTCGATAGGCGGGCACTCCGCCCTAGTTTCTAAGGACCATG (SEQ ID NO: 24)
hGAPDH	AGTTCCCCAACTTTCCCGCCTCTCAGCCTTTGAAAGAAAGAAAGGGGAGGGGGCAGGCC GCGTGCAGTCGCGAGCGGTGCTGGGCTCCGGCTCCAATTCCCCATCTCAGTCGCTCCCA AAGTCCTTCTGTTTCATCCAAGCGTGTAAGGGTCCCCGTCCTTGACTCCCTAGTGTCCTG CTGCCCACAGTCCAGTCCTGGGAACCAGCACCGATCACCTCCCATCGGGCCAATCTCAG TCCCTTCCCCCCTACGTCGGGGCCCACACGCTCGGTGCGTGCCCAGTTGAACCAGGCGG CTGCGGAAAAAAAAAAGCGGGGAGAAAGTAGGGCCCGGCTACTAGCGGTTTTACGGGC GCACGTAGCTCAGGCCTCAAGACCTTGGGCTGGGACTGGCTGAGCCTGGCGGGAGGCG GGGTCCGAGTCACCGCCTGCCGCCGCGCCCCCGGTTTCTATAAATTGAGCCCGCAGCCT CCCGCTTCGCTCTCTGCTCCTCCTGTTCGACAGTCAGCCGCATCTTCTTTTGCGTCGCCAG gtgaagacgggcggagagaaacccgggaggctagggacggcctgaaggcggcaggggcgggcgcaggccggatgtgttcgcgccgctgc ggggtgggcccgggcggcctccgcattgcaggggcgggcggaggacgtgatgcggcgcgggctgggcatggaggcctggtgggggagg ggaggggaggcgtgggtgtcggccggggccactaggcgctcactgttctctcctccgcgcagCCGAGCCACATCGCTGAG ACAC (SEQ ID NO: 25)
hGRP78	AGTGCGGTTACCAGCGGAAATGCCTCGGGGTCAGAAGTCGCAGGAGAGATAGACAGCT GCTGAACCAATGGGACCAGCGGATGGGGCGGATGTTATCTACCATTGGTGAACGTTAGA AACGAATAGCAGCCAATGAATCAGCTGGGGGGGCGGAGCAGTGACGTTTATTGCGGAG GGGGCCGCTTCGAATCGGCGGCGGCCAGCTTGGTGGCCTGGGCCAATGAACGGCCTCCA ACGAGCAGGGCCTTCACCAATCGGCGGCCTCCACGACGGGGCTGGGGGAGGGTATATA AGCCGAGTAGGCGACGGTGAGGTCGACGCCGGCCAAGACAGCACAGACAGATTGACCT ATTGGGGTGTTTCGCGAGTGTGAGAGGGAAGCGCCGCGGCCTGTATTTCTAGACCTGCC CTTCGCCTGGTTCGTGGCGCCTTGTGACCCCGGGCCCCTGCCGCCTGCAAGTCGGAAATT GCGCTGTGCTCCTGTGCTACGGCCTGTGGCTGGACTGCCTGCTGCTGCCCAACTGGCTGG CAC (SEQ ID NO: 26)
hGRP94	TAGTTTCATCACCACCGCCACCCCCCCGCCCCCCCGCCATCTGAAAGGGTTCTAGGGGA TTTGCAACCTCTCTCGTGTGTTTCTTCTTTCCGAGAAGCGCCGCCACACGAGAAAGCTGG CCGCGAAAGTCGTGCTGGAATCACTTCCAACGAAACCCCAGGCATAGATGGGAAAGGG TGAAGAACACGTTGCCATGGCTACCGTTTCCCCGGTCACGGAATAAACGCTCTCTAGGA TCCGGAAGTAGTTCCGCCGCGACCTCTCTAAAAGGATGGATGTGTTCTCTGCTTACATTC ATTGGACGTTTTCCCTTAGAGGCCAAGGCCGCCCAGGCAAAGGGGCGGTCCCACGCGTG AGGGGCCCGCGGAGCCATTTGATTGGAGAAAAGCTGCAAACCCTGACCAATCGGAAGG AGCCACGCTTCGGGCATCGGTCACCGCACCTGGACAGCTCCGATTGGTGGACTTCCGCC CCCCCTCACGAATCCTCATTGGGTGCCGTGGGTGCGTGGTGCGGCGCGATTGGTGGGTT CATGTTTCCCGTCCCCCGCCCGCGAGAAGTGGGGGTGAAAAGCGGCCCGACCTGCTTGG GGTGTAGTGGGCGGACCGCGCGGCTGGAGGTGTGAGGATCCGAACCCAGGGGTGGGGG

Name	DNA SEQUENCE
	GTGGAGGCGGCTCCTGCGATCGAAGGGGACTTGAGACTCACCGGCCGCACGTC (SEQ ID NO: 27)
hHSP70	GGGCCGCCCACTCCCCCTTCCTCTCAGGGTCCCTGTCCCCTCCAGTGAATCCCAGAAGAC TCTGGAGAGTTCTGAGCAGGGGGCGGCACTCTGGCCTCTGATTGGTCCAAGGAAGGCTG GGGGGCAGGACGGGAGGCGAAAACCCTGGAATATTCCCGACCTGGCAGCCTCATCGAG CTCGGTGATTGGCTCAGAAGGGAAAAGGCGGGTCTCCGTGACGACTTATAAAAGCCCA GGGGCAAGCGGTCCGGATAACGGCTAGCCTGAGGAGCTGCTGCGACAGTCCACTACCTT TTTCGAGAGTGACTCCCGTTGTCCCAAGGCTTCCCAGAGCGAACCTGTGCGGCTGCAGG CACCGGCGCGTCGAGTTTCCGGCGTCCGGAAGGACCGAGCTCTTCTCGCGGATCCAGTG TTCCGTTTCCAGCCCCCAATCTCAGAGCGGAGCCGACAGAGAGCAGGGAACCC (SEQ ID NO: 28)
hKINb	GCCCCACCCCCGTCCGCGTTACAACCGGGAGGCCCGCTGGGTCCTGCACCGTCACCCTC CTCCCTGTGACCGCCCACCTGATACCCAAACAACTTTCTCGCCCCTCCAGTCCCCAGCTC GCCGAGCGCTTGCGGGGAGCCACCCAGCCTCAGTTTCCCCAGCCCCGGGCGGGGCGAG GGGCGATGACGTCATGCCGGCGCGCGGCATTGTGGGGCGGGGCGAGGCGGGGCGCCGG GGGGAGCAACACTGAGACGCCATTTTCGGCGGCGGGAGCGGCGCAGGCGGCCGAGCGG GACTGGCTGGGTCGGCTGGGCTGCTGGTGCGAGGAGCCGCGGGGCTGTGCTCGGCGGCC AAGGGGACAGCGCGTGGGTGGCCGAGGATGCTGCGGGGCGGTAGCTCCGGCGCCCCTC GCTGGTGACTGCTGCGCCGTGCCTCACACAGCCGAGGCGGGCTCGGCGCACAGTCGCTG CTCCGCGCTCGCGCCCGGCGGCGCTCCAGGTGCTGACAGCGCGAGAGAGCGCGGCCTC AGGAGCAACAC (SEQ ID NO: 29)
hUBIb	TTCCAGAGCTTTCGAGGAAGGTTTCTTCAACTCAAATTCATCCGCCTGATAATTTTCTTA TATTTTCCTAAAGAAGGAAGAGAAGCGCATAGAGGAGAAGGGAAATAATTTTTTAGGA GCCTTTCTTACGGCTATGAGGAATTTGGGGCTCAGTTGAAAAGCCTAAACTGCCTCTCG GGAGGTTGGGCGCGGCGAACTACTTTCAGCGGCGCACGGAGACGGCGTCTACGTGAGG GGTGATAAGTGACGCAACACTCGTTGCATAAATTTGCGCTCCGCCAGCCCGGAGCATTT AGGGGCGGTTGGCTTTGTTGGGTGAGCTTGTTTGTGTCCCTGTGGGTGGACGTGGTTGGT GATTGGCAGGATCCTGGTATCCGCTAACAGgtactggcccacagccgtaaagacctgcgggggcgtgagagggg ggaatgggtgaggtcaagctggaggcttcttggggttgggtgggccgctgaggggaggggagggcgaggtgacgcgacacccggcctttctg ggagagtgggccttgttgacctaaggggggcgagggcagttggcacgcgcacgcgccgacagaaactaacagacattaaccaacagcgattc cgtcgcgtttacttgggaggaaggcggaaaagaggtagtttgtgtggcttctggaaaccctaaatttggaatcccagtatgagaatggtgtcccttct tgtgtttcaatgggatttttacttcgcgagtcttgtgggtttggttttgtttcagtttgcctaacaccgtgcttaggtttgaggcagattggagttcggtcg ggggagtttgaatatccggaacagttagtggggaaagctgtggacgcttggtaagagagcgctctggattttccgctgttgacgttgaaaccttgaa tgacgaatttcgtattaagtgacttagccttgtaaaattgaggggaggcttgcggaatattaacgtatttaaggcattttgaaggaatagttgctaatttt gaagaatattaggtgtaaaagcaagaaatacaatgatcctgaggtgacacgcttatgttttacttttaaactagGTCACC (SEQ ID NO: 30)
CAG	gacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacata acttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatag taacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgt atcatatgccaagtacgcccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttat gggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtcgaggtgagccccacgttctgcttcact ctccccatctccccccccctccccacccccaattttgtatttatttatttttttaattattttgtgcagcgatgggggcgggggggg gggggggggcgcgcgccaggcggggcggggcggggcgaggggcggggcggggcgaggcggagaggtgcgg cggcagccaatcagagcggcgcgctccgaaagtttccttttatggcgaggcggcggcggcggcggccctataaaaag cgaagcgcgcggcgggcg (SEQ ID NO: 173)
HLP	Tgtttgctgcttgcaatgtttgcccattttagggtggacacaggacgctgtggtttctgagccaggggggcgactcagatcc cagccagtggacttagcccctgtttgctcctccgataactggggtgaccttggttaatattcaccagcagcctcccccgttg cccctctggatccactgcttaaatacggacgaggacagggccctgtctcctcagcttcaggcaccaccactgacctgggg acagtgaat (SEQ ID NO: 174)

The promoter can be a tissue-specific promoter. In general, a tissue-specific promoter directs transcription of a nucleic acid, (*e.g.*, the engineered nucleic acids encoding the chimeric

proteins, such as membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S**) such that expression is limited to a specific cell type, organelle, or tissue. Tissue-specific promoters include, but are not limited to, albumin (liver specific, Pinkert et al., (1987)), lymphoid specific promoters (Calame and Eaton, 1988), particular promoters of T-cell receptors (Winoto and Baltimore, (1989)) and immunoglobulins; Banerji et al., (1983); Queen and Baltimore, 1983), neuron specific promoters (e.g. the neurofilament promoter; Byrne and Ruddle, 1989), pancreas specific promoters (Edlund et al., (1985)) or mammary gland specific promoters (milk whey promoter, U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166) as well as developmentally regulated promoters such as the murine hox promoters (Kessel and Gruss, Science 249:374-379 (1990)) or the α-fetoprotein promoter (Campes and Tilghman, Genes Dev. 3:537-546 (1989)), the contents of each of which are fully incorporated by reference herein. The promoter can be constitutive in the respective specific cell type, organelle, or tissue. Tissue-specific promoters and/or regulatory elements can also include promoters from the liver fatty acid binding (FAB) protein gene, specific for colon epithelial cells; the insulin gene, specific for pancreatic cells; the transphyretin, .alpha.1- antitrypsin, plasminogen activator inhibitor type 1 (PAI-I), apolipoprotein AI and LDL receptor genes, specific for liver cells; the myelin basic protein (MBP) gene, specific for oligodendrocytes; the glial fibrillary acidic protein (GFAP) gene, specific for glial cells; OPSIN, specific for targeting to the eye; and the neural-specific enolase (NSE) promoter that is specific for nerve cells. Examples of tissue-specific promoters include, but are not limited to, the promoter for creatine kinase, which has been used to direct expression in muscle and cardiac tissue and immunoglobulin heavy or light chain promoters for expression in B cells. Other tissue specific promoters include the human smooth muscle alpha-actin promoter. Exemplary tissue-specific expression elements for the liver include but are not limited to HMG-COA reductase promoter, sterol regulatory element 1, phosphoenol pyruvate carboxy kinase (PEPCK) promoter, human C-reactive protein (CRP) promoter, human glucokinase promoter, cholesterol L 7-alpha hydroylase (CYP-7) promoter, beta- galactosidase alpha-2,6 sialylkansferase promoter, insulin-like growth factor binding protein (IGFBP-I) promoter, aldolase B promoter, human transferrin promoter, and collagen type I promoter. Exemplary tissue-specific expression elements for the prostate include but are not limited to the prostatic acid phosphatase (PAP) promoter, prostatic secretory protein of 94 (PSP 94) promoter, prostate specific antigen complex promoter, and human glandular kallikrein gene promoter (hgt-1). Exemplary tissue- specific expression elements for gastric tissue include but are not limited to the human H+/K+-ATPase alpha subunit promoter. Exemplary tissue-specific expression elements for the pancreas include but are not limited to pancreatitis associated protein promoter (PAP), elastase 1 transcriptional enhancer, pancreas

specific amylase and elastase enhancer promoter, and pancreatic cholesterol esterase gene promoter. Exemplary tissue-specific expression elements for the endometrium include, but are not limited to, the uteroglobin promoter. Exemplary tissue-specific expression elements for adrenal cells include, but are not limited to, cholesterol side-chain cleavage (SCC) promoter. Exemplary tissue-specific expression elements for the general nervous system include, but are not limited to, gamma-gamman enolase (neuron- specific enolase, NSE) promoter. Exemplary tissue-specific expression elements for the brain include, but are not limited to, the neurofilament heavy chain (NF-H) promoter. Exemplary tissue-specific expression elements for lymphocytes include, but are not limited to, the human CGL-1/granzyme B promoter, the terminal deoxy transferase (TdT), lambda 5, VpreB, and lck (lymphocyte specific tyrosine protein kinase p561ck) promoter, the humans CD2 promoter and its 3 ' transcriptional enhancer, and the human NK and T cell specific activation (NKG5) promoter. Exemplary tissue-specific expression elements for the colon include, but are not limited to, pp60c-src tyrosine kinase promoter, organ-specific neoantigens (OSNs) promoter, and colon specific antigen-P promoter. Tissue-specific expression elements for breast cells are for example, but are not limited to, the human alpha-lactalbumin promoter. Exemplary tissue-specific expression elements for the lung include, but are not limited to, the cystic fibrosis transmembrane conductance regulator (CFTR) gene promoter.

In some embodiments, a promoter of the present disclosure is modulated by signals within a tumor microenvironment. A tumor microenvironment is considered to modulate a promoter if, in the presence of the tumor microenvironment, the activity of the promoter is increased or decreased by at least 10%, relative to activity of the promoter in the absence of the tumor microenvironment. In some embodiments, the activity of the promoter is increased or decreased by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, relative to activity of the promoter in the absence of the tumor microenvironment. For example, the activity of the promoter is increased or decreased by 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%, relative to activity of the promoter in the absence of the tumor microenvironment.

In some embodiments, the activity of the promoter is increased or decreased by at least 2 fold (*e.g.*, 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold), relative to activity of the promoter in the absence of the tumor microenvironment. For example, the activity of the promoter is increased or decreased by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold, relative to activity of the promoter in the absence of the tumor

microenvironment. In some embodiments, the activity of the promoter is increased or decreased by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold, relative to activity of the promoter in the absence of the tumor microenvironment.

In some embodiments, a promoter of the present disclosure is activated under a hypoxic condition. A "hypoxic condition" is a condition where the body or a region of the body is deprived of adequate oxygen supply at the tissue level. Hypoxic conditions can cause inflammation (*e.g.*, the level of inflammatory cytokines increase under hypoxic conditions). In some embodiments, the promoter that is activated under hypoxic condition is operably linked to a nucleotide encoding a chimeric proteins that decreases the expression of activity of inflammatory cytokines, thus reducing the inflammation caused by the hypoxic condition. In some embodiments, the promoter that is activated under hypoxic conditions comprises a hypoxia responsive element (HRE). A "hypoxia responsive element (HRE)" is a response element that responds to hypoxia-inducible factor (HIF). The HRE, in some embodiments, comprises a consensus motif NCGTG (where N is either A or G).

Activation-Conditional Control Polypeptide (ACP) Promoter Systems

In some embodiments, a synthetic promoter is a promoter system including an activation-conditional control polypeptide- (ACP-) binding domain sequence and a promoter sequence. Such a system is also referred to herein as an "ACP-responsive promoter." In general, an ACP promoter system includes a first expression cassette encoding an activation-conditional control polypeptide (ACP) and a second expression cassette encoding an ACP-responsive promoter operably linked to an exogenous polynucleotide sequence, such as the exogenous polynucleotide sequence encoding the membrane-cleavable chimeric proteins described herein or any other protein of interest (*e.g.*, a protease). In some embodiments, the first expression cassette and second expression cassette are each encoded by a separate engineered nucleic acid. In other embodiments, the first expression cassette and the second expression cassette are encoded by the same engineered nucleic acid. The ACP-responsive promoter can be operably linked to a nucleotide sequence encoding a single protein of interest or multiple proteins of interest.

The promoters of the ACP promoter system, *e.g.*, either a promoter driving expression of the ACP or the promoter sequence of the ACP-responsive promoter, can include any of the promoter sequences described herein (*see* "Promoters" above). The ACP-responsive promoter can be derived from minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF

response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, minCMV, YB_TATA, minTK, inducer molecule responsive promoters, and tandem repeats thereof. In some embodiments, the ACP-responsive promoter includes a minimal promoter.

In some embodiments, the ACP-binding domain includes one or more zinc finger binding sites. In some embodiments, the ACP-responsive promoter includes a minimal promoter and the ACP-binding domain includes one or more zinc finger binding sites. The ACP-binding domain can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more zinc finger binding sites. In some embodiments, the transcription factor is a zinc-finger-containing transcription factor. In some embodiments, the zinc-finger-containing transcription factor is a synthetic transcription factor. In some embodiments, the ACP-binding domain includes one or more zinc finger binding sites and the ACP has a DNA-binding zinc finger protein domain (ZF protein domain). In some embodiments, the ACP has a DNA-binding zinc finger protein domain (ZF protein domain) and an effector domain. In some embodiments, the ACP-binding domain includes one or more zinc finger binding sites and the ACP has a DNA-binding zinc finger protein domain (ZF protein domain) and an effector domain. In some embodiments, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). A zinc finger array comprises multiple zinc finger protein motifs that are linked together. Each zinc finger motif binds to a different nucleic acid motif. This results in a ZFA with specificity to any desired nucleic acid sequence, *e.g.*, a ZFA with desired specificity to an ACP-binding domain having a specific zinc finger binding site composition and/or configuration. The ZF motifs can be directly adjacent to each other, or separated by a flexible linker sequence. In some embodiments, a ZFA is an array, string, or chain of ZF motifs arranged in tandem. A ZFA can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,1 3, 14, or 15 zinc finger motifs. The ZFA can have from 1-10, 1-15, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5 3-6, 3-7, 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, or 5-15 zinc finger motifs. The ZF protein domain can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more ZFAs. The ZF domain can have from 1-10, 1-15, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5 3-6, 3-7, 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, or 5-15 ZFAs. In some embodiments, the ZF protein domain comprises one to ten ZFA(s). In some embodiments, the ZF protein domain comprises at least one ZFA. In some embodiments, the ZF protein domain comprises at least two ZFAs. In some embodiments, the ZF protein domain comprises at least three ZFAs. In some embodiments, the ZF protein domain comprises at least four ZFAs. In some embodiments, the ZF protein domain comprises at least five ZFAs. In some embodiments, the ZF protein domain comprises at least ten ZFAs.

In some embodiments, the ACP is a transcriptional modulator. In some embodiments, the ACP is a transcriptional repressor. In some embodiments, the ACP is a transcriptional activator. In some embodiments, the ACP is a transcription factor. In some embodiments, the ACP comprises a DNA-binding domain and a transcriptional effector domain. In some embodiments, the DNA-binding domain comprises a tetracycline (or derivative thereof) repressor (TetR) domain. In some embodiments, the ACP is an antigen recognizing receptor of the present disclosure.

The ACP can also further include an effector domain, such as a transcriptional effector domain. For instance, a transcriptional effector domain can be the effector or activator domain of a transcription factor. Transcription factor activation domains are also known as transactivation domains, and act as scaffold domains for proteins such as transcription coregulators that act to activate or repress transcription of genes. Any suitable transcriptional effector domains can be used in the ACP including, but not limited to, a Herpes Simplex Virus Protein 16 (VP16) activation domain; an activation domain consisting of four tandem copies of VP16, a VP64 activation domain; a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator (Rta) activation domain; a tripartite activator comprising the VP64, the p65, and the Rta activation domains, the tripartite activator is known as a VPR activation domain; a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300, known as a p300 HAT core activation domain; a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain, or any combination thereof.

In some embodiments, the effector domain is s transcription effector domain selected from: a Herpes Simplex Virus Protein 16 (VP16) activation domain; an activation domain consisting of four tandem copies of VP16, a VP64 activation domain; a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator (Rta) activation domain; a tripartite activator comprising the VP64, the p65, and the Rta activation domains, the tripartite activator is known as a VPR activation domain; a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300, known as a p300 HAT core activation domain; a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

In some embodiments, the ACP is a small molecule (*e.g.*, drug) inducible polypeptide. For example, in some embodiments, the ACP may be induced by tetracycline (or derivative thereof), and comprises a TetR domain and a VP16 effector domain. In some embodiments, the ACP includes an estrogen receptor variant, such as ERT2, and may be regulated by tamoxifen, or a metabolite thereof (such as 4-hydroxy-tamoxifen [4-OHT], N-desmethyltamoxifen, tamoxifen-N-oxide, or endoxifen), through tamoxifen-controlled nuclear localization.

In some embodiments, the ACP is a small molecule (e.g., drug) inducible polypeptide that includes a repressible protease and one or more cognate cleavage sites of the repressible protease. In some embodiments, a repressible protease is active (cleaves a cognate cleavage site) in the absence of the specific agent and is inactive (does not cleave a cognate cleavage site) in the presence of the specific agent. In some embodiments, the specific agent is a protease inhibitor. In some embodiments, the protease inhibitor specifically inhibits a given repressible protease of the present disclosure. The repressible protease can be any of the proteases described herein that is capable of inactivation by the presence or absence of a specific agent (*see* "Protease Cleavage Site" above for exemplary repressible proteases, cognate cleavage sites, and protease inhibitors).

In some embodiments, the ACP has a degron domain (*see* "Degron Systems and Domains" above for exemplary degron sequences). The degron domain can be in any order or position relative to the individual domains of the ACP. For example, the degron domain can be N-terminal of the repressible protease, C-terminal of the repressible protease, N-terminal of the ZF protein domain, C-terminal of the ZF protein domain, N-terminal of the effector domain, or C-terminal of the effector domain.

Multicistronic and Multiple Promoter Systems

In some embodiments, engineered nucleic acids (*e.g.*, an engineered nucleic acid comprising an expression cassette) are configured to produce multiple chimeric proteins. For example, nucleic acids may be configured to produce 2-20 different chimeric proteins. In some embodiments, nucleic acids are configured to produce 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14,

9-13, 9-12, 9-11, 9-10, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-20, 15-19, 15-18, 15-17, 15-16, 16-20, 16-19, 16-18, 16-17, 17-20, 17-19, 17-18, 18-20, 18-19, or 19-20 chimeric proteins. In some embodiments, nucleic acids are configured to produce 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chimeric proteins.

In general, the engineered nucleic acids described herein are multicistronic, *i.e.*, as described above.

Engineered nucleic acids can also use multiple promoters to express genes from multiple ORFs, *i.e.*, more than one separate mRNA transcripts can be produced from a single engineered nucleic acid. For example, a first promoter can be operably linked to a polynucleotide sequence encoding a first chimeric protein, and a second promoter can be operably linked to a polynucleotide sequence encoding a second chimeric protein. In general, any number of promoters can be used to express any number of chimeric proteins. In some embodiments, at least one of the ORFs expressed from the multiple promoters can be multicistronic.

"Linkers," as used herein can refer to polypeptides that link a first polypeptide sequence and a second polypeptide sequence, the multicistronic linkers described above, or the additional promoters that are operably linked to additional ORFs described above.

Engineered Cells

Provided herein are engineered cells, and methods of producing the engineered cells, that produce each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR.. In general, engineered cells of the present disclosure may be engineered to express the chimeric proteins provided for herein, such as each of the membrane-cleavable chimeric proteins, the bivalent aCARs, and the iCARsdescribed herein. These cells are referred to herein as "engineered cells." These cells, which typically contain engineered nucleic acid, do not occur in nature. In some embodiments, the cells are engineered to include a nucleic acid comprising a promoter operably linked to a nucleotide sequence encoding a chimeric protein, for example, a membrane-cleavable chimeric protein. An engineered cell can comprise an engineered nucleic acid integrated into the cell's genome. An engineered cell can comprise an engineered nucleic acid capable of expression without integrating into the cell's genome, for example, engineered with a transient expression system such as a plasmid or mRNA.

The present disclosure also encompasses additivity and synergy between a chimeric protein(s) and the engineered cell from which they are produced. In some embodiments, cells

are engineered to produce at least two (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) chimeric proteins, for example ate least two membrane-cleavable chimeric proteins. In other embodiments, cells are engineered to produce at least one chimeric proteins having an effector molecule that is not natively produced by the cells. Such an effector molecule may, for example, complement the function of effector molecules natively produced by the cells.

In some embodiments, cells are engineered to express membrane-tethered anti-CD3 and/or anti-CD28 agonist extracellular domains.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce multiple chimeric proteins. For example, cells may be engineered to produce 2-20 different chimeric proteins, such as 2-20 different membrane-cleavable chimeric proteins. In some embodiments, cells engineered to produce 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14, 9-13, 9-12, 9-11, 9-10, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-20, 15-19, 15-18, 15-17, 15-16, 16-20, 16-19, 16-18, 16-17, 17-20, 17-19, 17-18, 18-20, 18-19, or 19-20 chimeric proteins. In some embodiments, cells are engineered to produce 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chimeric proteins.

In some embodiments, engineered cells comprise one or more engineered nucleic acids encoding a promoter operably linked to a nucleotide sequence encoding a chimeric protein. In some embodiments, cells are engineered to include a plurality of engineered nucleic acids, *e.g.*, at least two engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (*e.g.*, 1, 2 or 3) chimeric protein. For example, cells may be engineered to comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10, engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (*e.g.*, 1, 2 or 3) chimeric protein. In some embodiments, the cells are engineered to comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, or more engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (*e.g.*, 1, 2 or 3) chimeric protein. Engineered cells can comprise an engineered nucleic acid encoding at least one of the linkers described above, such as polypeptides that link a first

polypeptide sequence and a second polypeptide sequence, one or more multicistronic linker described above, one or more additional promoters operably linked to additional ORFs, or a combination thereof.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to express a protease. In some embodiments, a cell is engineered to express a protease heterologous to a cell. In some embodiments, a cell is engineered to express a protease heterologous to a cell expressing a chimeric protein, such as a heterologous protease that cleaves the protease cleavage site of a membrane-cleavable chimeric protein. In some embodiments, engineered cells comprise one or more engineered nucleic acids encoding a promoter operably linked to a nucleotide sequence encoding a protease, such as a heterologous protease. Protease and protease cleavage sites are described in greater detail in the Section herein titled "Protease Cleavage site."

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one homing molecule. "Homing," refers to active navigation (migration) of a cell to a target site (*e.g.*, a cell, tissue (*e.g.*, tumor), or organ). A "homing molecule" refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate interaction of an engineered cell to a target site. Non-limiting examples of homing molecules include CXCR1, CCR9, CXCR2, CXCR3, CXCR4, CCR2, CCR4, FPR2, VEGFR, IL6R, CXCR1, CSCR7, and PDGFR.

In some embodiments, a homing molecule is a chemokine receptor (cell surface molecule that binds to a chemokine). Non-limiting examples of chemokine receptors that may be produced by the engineered cells of the present disclosure include: CXC chemokine receptors (*e.g.*, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7), CC chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, and CCR11), CX3C chemokine receptors (*e.g.*, CX3CR1, which binds to CX3CL1), and XC chemokine receptors (*e.g.*, XCR1). In some embodiments, a chemokine receptor is a G protein-linked transmembrane receptor, or a member of the tumor necrosis factor (TNF) receptor superfamily (including but not limited to TNFRSF1A, TNFRSF1B). In some embodiments, cells are engineered to produce CXCL8, CXCL9, and/or CXCL10 (promote T-cell recruitment), CCL3 and/or CXCL5, CCL21 (Th1 recruitment and polarization). In some embodiments, cells are engineered to produce CXCR4.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce G-protein coupled receptors (GPCRs) that detect N-formylated-containing oligopeptides (including but not limited to FPR2 and FPRL1).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce receptors that detect interleukins (including but not limited to IL6R).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce receptors that detect growth factors secreted from other cells, tissues, or tumors (including but not limited to FGFR, PDGFR, EGFR, and receptors of the VEGF family, including but not limited to VEGF-C and VEGF-D).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce one or more integrins. Cells of the present disclosure may be engineered to produce any combination of integrin α and β subunits. The α subunit of an integrin may be, without limitation: ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, IGTA7, ITGA8, ITGA9, IGTA10, IGTA11, ITGAD, ITGAE, ITGAL, ITGAM, ITGAV, ITGA2B, ITGAX. The β subunit of an integrin may be, without limitation: ITGB1, ITGB2, ITGB3, ITGB4, ITGB5, ITGB6, ITGB7, and ITGB8.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce one or more matrix metalloproteinases (MMP). Non-limiting examples of MMPs include MMP-2, MMP-9, and MMP. In some embodiments, cells are engineered to produce an inhibitor of a molecule (*e.g.*, protein) that inhibits MMPs. For example, cells may be engineered to express an inhibitor (*e.g.*, an RNAi molecule) of membrane type 1 MMP (MT1-MMP) or TIMP metallopeptidase inhibitor 1 (TIMP-1).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce a ligand that binds to selectin (*e.g.*, hematopoietic cell E-/L-selectin ligand (HCELL), Dykstran *et al.*, Stem Cells. 2016 Oct;34(10):2501-2511) on the endothelium of a target tissue, for example.

The term "homing molecule" also encompasses transcription factors that regulate the production of molecules that improve/enhance homing of cells.

Also provided herein are engineered cells that are engineered to produce multiple chimeric proteins, at least two of which include effector molecules that modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, at least one (*e.g.*, 1, 2, 3, 4, 5, or more) chimeric protein includes an effector molecule that stimulates at least one immunostimulatory mechanism in the tumor microenvironment, or inhibits at least one immunosuppressive mechanism in the tumor microenvironment. In some embodiments, at least one (*e.g.*, 1, 2, 3, 4, 5, or more) chimeric protein includes an effector molecule that inhibits at least one immunosuppressive mechanism in the tumor microenvironment, and at least one chimeric protein (*e.g.*, 1, 2, 3, 4, 5, or more) inhibits at least one immunosuppressive mechanism in the tumor microenvironment. In yet other embodiments, at least two (*e.g.*, 2, 3, 4, 5, or more) chimeric proteins includes an effector molecule that stimulate at least one immunostimulatory mechanism in the tumor microenvironment. In still other embodiments, at least two (*e.g.*, 1, 2, 3,

4, 5, or more) chimeric proteins includes an effector molecule that inhibit at least one immunosuppressive mechanism in the tumor microenvironment.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one chimeric protein including an effector molecule that stimulates T cell signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates antigen presentation and/or processing. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates dendritic cell differentiation and/or maturation. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates immune cell recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein includes an effector molecule that that stimulates M1 macrophage signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates Th1 polarization. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates stroma degradation. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates immunostimulatory metabolite production. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates Type I interferon signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits negative costimulatory signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits pro-apoptotic signaling (*e.g.*, via TRAIL) of anti-tumor immune cells. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits T regulatory (T_{reg}) cell signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits tumor checkpoint molecules. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that activates stimulator of interferon genes (STING) signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that degrades immunosuppressive factors/metabolites. In some embodiments, a cell is engineered to produce

at least one chimeric protein that includes an effector molecule that inhibits vascular endothelial growth factor signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that directly kills tumor cells (*e.g.*, granzyme, perforin, oncolytic viruses, cytolytic peptides and enzymes, anti-tumor antibodies, *e.g.*, that trigger ADCC).

In some embodiments, at least one chimeric protein including an effector molecule that: stimulates T cell signaling, activity and/or recruitment, stimulates antigen presentation and/or processing, stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, stimulates dendritic cell differentiation and/or maturation, stimulates immune cell recruitment, stimulates macrophage signaling, stimulates stroma degradation, stimulates immunostimulatory metabolite production, or stimulates Type I interferon signaling; and at least one chimeric protein including an effector molecule that inhibits negative costimulatory signaling, inhibits pro-apoptotic signaling of anti-tumor immune cells, inhibits T regulatory (Treg) cell signaling, activity and/or recruitment, inhibits tumor checkpoint molecules, activates stimulator of interferon genes (STING) signaling, inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment, degrades immunosuppressive factors/metabolites, inhibits vascular endothelial growth factor signaling, or directly kills tumor cells.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one chimeric protein including an effector molecule selected from IL-12, IFN-β, IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and CD40L; and/or at least a checkpoint inhibitor. Illustrative immune checkpoint molecules that can be targeted for blocking or inhibition include, but are not limited to, CTLA-4, 4-1BB (CD137), 4-1BBL (CD137L), PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, □□□□, and memory CD8+ (□□) T cells), CD160 (also referred to as BY55), and CGEN-15049. Immune checkpoint inhibitors include antibodies, or antigen binding fragments thereof, or other binding proteins, that bind to and block or inhibit the activity of one or more of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4, CD160, and CGEN-15049. Exemplary checkpoint inhibitors include, but are not limited to, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies. Illustrative immune checkpoint inhibitors include

pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumamb (anti-PD-1; Opdivo® - BMS), pidilizumab (anti-PD-1 antibody; CT-011 – Teva/CureTech), AMP224 (anti-PD-1; NCI), avelumab (anti-PD-L1; Bavencio® - Pfizer), durvalumab (anti-PD-L1; MEDI4736/Imfinzi® - Medimmune/AstraZeneca), atezolizumab (anti-PD-L1; Tecentriq® - Roche/Genentech), BMS-936559 (anti-PD-L1 - BMS), tremelimumab (anti-CTLA-4; Medimmune/AstraZeneca), ipilimumab (anti-CTLA-4; Yervoy ® - BMS), lirilumab (anti-KIR; BMS), monalizumab (anti-NKG2A; Innate Pharma/AstraZeneca).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one chimeric protein including an effector molecule selected from IL-12, IFN-β, IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and CD40L; and/or at least one checkpoint inhibitor selected from anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-CTLA-4 antibodies, and anti-IL-35 antibodies; and/or at least one chimeric protein including an effector molecule selected from MIP1α (CCL3), MIP1β (CCL5), and CCL21; and/or at least one chimeric protein including an effector molecule selected from CpG oligodeoxynucleotides; and/or at least one chimeric protein including an effector molecule selected from microbial peptides.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce IFN-β and at least one chimeric protein including an effector molecule selected from cytokines, antibodies, chemokines, nucleotides, peptides, enzymes, and stimulators of interferon genes (STINGs). In some embodiments, a cell is engineered to produce IFN-β and at least one cytokine or receptor/ligand (*e.g.*, IL-12, IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and/or CD40L).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, "S" in the formula **S – C – MT** or **MT – C – S**) is a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a peptide, or an enzyme. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a cytokine. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a chemokine. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a homing molecule. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a growth factor. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable

effector molecule is a co-activation molecule. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a tumor microenvironment modifier. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a ligand. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an antibody. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a peptide. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an enzyme.

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, "S" in the formula **S – C – MT** or **MT – C – S**) is IL-1-beta, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, an IL-12p70 fusion protein, IL-15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, or TNF-alpha. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, or XCL1. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is anti-integrin alpha4,beta7, anti-MAdCAM, SDF1, or MMP-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is 4-1BBL or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is adenosine deaminase, a TGFbeta inhibitor, an immune checkpoint inhibitor, a VEGF inhibitor, or HPGE2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, or combinations thereof. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-VEGF antibody, an anti-VEGF peptide, or a combination thereof. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-LAG-3 antibody, an anti-TIM-3 antibody, an anti-TIGIT antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-GAL9 antibody, an anti-A2AR antibody, an anti-

phosphatidylserine antibody, an anti-CD27 antibody, an anti-TNFa antibody, an anti-TREM1 antibody, or an anti-TREM2 antibody.

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, "S" in the formula **S – C – MT** or **MT – C – S**) comprises IL-15. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a fusion of IL-15 and the sushi domain of IL-15Rα. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector consists of IL-15. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce one or more additional effector molecules. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce one or more additional secretable effector molecules. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a polynucleotide, a peptide, or an enzyme. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-12, IFN-γ, IL-2, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-12. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IFN-γ. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-7. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-36γ. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-18. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector

molecule is IL-15 and the cell is further engineered to produce IL-1β. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce OX40-ligand. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce CD40L.

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein and the cell is further engineered to produce one or more additional membrane-cleavable chimeric proteins.

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, "S" in the formula **S – C – MT** or **MT – C – S**) is IL-15 and the cell is further engineered to produce one or more additional membrane-cleavable chimeric proteins. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is produce a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a polynucleotide, a peptide, or an enzyme. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-12, IFN-γ, IL-2, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-12. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IFN-γ. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-7. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric

protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-36γ. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-18. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-1β. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is OX40-ligand. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is CD40L.

A cell can also be further engineered to express additional proteins in addition to the chimeric proteins (*e.g.*, the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein), proteins of interest, or effector molecules described herein. A cell can be further engineered to express one or more antigen recognizing receptors. Examples of antigens that may be targeted by one or more antigen recognizing receptors include, but are not limited to, 5T4, ADAM9, AFP, AXL, B7-H3, B7-H4, B7-H6, BCMA, C4.4, CA6, Cadherin 3, Cadherin 6, CCR4, CD19, CD20, CD22, CD123, CD133, CD138, CD142, CD166, CD25, CD30, CD33, CD352, CD37, CD38, CD44, CD56, CD66e, CD70, CD71, CD74, CD79b, CD80, CEA, CEACAM5, Claudin18.2, cMet, CSPG4, CTLA, DLK1, DLL3, DR5, EGFR, ENPP3, EpCAM, EphA2, Ephrin A4, ETBR, FGFR2, FGFR3, FLT3, FRalpha, FRb, GCC, GD2, GFRa4, gpA33, GPC2, GPC3, gpNBM, GPRC5, HER2, IL-13R, IL-13Ra, IL-13Ra2, IL-8, IL-15, IL1RAP, Integrin aV, KIT, L1CAM, LAMP1, Lewis Y, LeY, LIV-1, LRRC, LY6E, MCSP, Mesothelin, MUC1, MUC16, MUC1C, NaPi2B, Nectin 4, NKG2D, NOTCH3, NY ESO 1, Ovarin, P-cadherin, pan-Erb2, PSCA, PSMA, PTK7, ROR1, S Aures, SCT, SLAMF7, SLITRK6, SSTR2, STEAP1, Survivin, TDGF1, TIM1, TROP2, and WT1.

An antigen recognizing receptor can include an antigen-binding domain, such as an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). An antigen recognizing receptors can include an scFv. An scFv can include a heavy chain variable domain

(VH) and a light chain variable domain (VL), which can be separated by a peptide linker. For example, an scFv can include the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

An antigen recognizing receptor can be a chimeric antigen receptor (CAR). A CAR can have one or more intracellular signaling domains, such as a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, fragments thereof, combinations thereof, or combinations of fragments thereof. A CAR can have a transmembrane domain, such as a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, a BTLA transmembrane domain, fragments thereof, combinations thereof, or combinations of fragments thereof. A CAR can have a spacer region between the antigen-binding domain and the transmembrane domain.

An antigen recognizing receptor can be a T cell receptor (TCR).

Engineered Cell Types

Also provided herein are engineered cells. Cells can be engineered to comprise any of the engineered nucleic acids described herein (*e.g.*, any of the multicistronic systems encoding each of the membrane-cleavable chimeric proteins, the bivalent aCARs, and the iCARsdescribed herein). Cells can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are cells engineered to produce each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR. In a particular aspect, provided herein are cells engineered to produce two or more chimeric proteins. In a particular aspect, provided herein are cells engineered to produce two or more of the chimeric proteins described herein, where each chimeric protein is a different protein of interest or effector molecule. In a particular aspect, provided herein are cells engineered to produce any

of the chimeric proteins described herein and engineered to separately produce a different effector molecule or protein of interest (*e.g.*, a homing molecule, antigen receptor, etc.).

The engineered cells can be an immune cell, including but not limited to, a T cell, a CD8+ T cell, a CD4+ T cell, a gamma-delta ($\gamma\delta$) T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, or a dendritic cell. The engineered cells can be a T cell. The engineered cells can be an NK cell. Cells engineered to express a chimeric antigen receptor (CAR) are also referred to as CAR cells. For example, T cells engineered to express a CAR are also referred to as CAR-T cells and likewise NK cells engineered to express a CAR are also referred to as CAR-NK cells.

The engineered cells can be a stem cell, including but not limited to, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, a mesenchymal stromal cell (MSC), an induced pluripotent stem cell (iPSC), or an iPSC-derived cell.

The engineered cells can be tumor-derived cells. Examples of tumor cells include, but are not limited to, a bladder tumor cell, a brain tumor cell, a breast tumor cell, a cervical tumor cell, a colorectal tumor cell, an esophageal tumor cell, a glioma cell, a kidney tumor cell, a liver tumor cell, a lung tumor cell, a melanoma cell, an ovarian tumor cell, a pancreatic tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

A cell can be engineered to produce the chimeric proteins using methods known to those skilled in the art. For example, cells can be transduced to engineer the tumor. In an embodiment, the cell is transduced using a virus.

In a particular embodiment, the cell is transduced using an oncolytic virus. Examples of oncolytic viruses include, but are not limited to, an oncolytic herpes simplex virus, an oncolytic adenovirus, an oncolytic measles virus, an oncolytic influenza virus, an oncolytic Indiana vesiculovirus, an oncolytic Newcastle disease virus, an oncolytic vaccinia virus, an oncolytic poliovirus, an oncolytic myxoma virus, an oncolytic reovirus, an oncolytic mumps virus, an oncolytic Maraba virus, an oncolytic rabies virus, an oncolytic rotavirus, an oncolytic hepatitis virus, an oncolytic rubella virus, an oncolytic dengue virus, an oncolytic chikungunya virus, an oncolytic respiratory syncytial virus, an oncolytic lymphocytic choriomeningitis virus, an oncolytic morbillivirus, an oncolytic lentivirus, an oncolytic replicating retrovirus, an oncolytic rhabdovirus, an oncolytic Seneca Valley virus, an oncolytic sindbis virus, and any variant or derivative thereof.

The virus, including any of the oncolytic viruses described herein, can be a recombinant virus that encodes one more transgenes encoding one or more chimeric proteins, such as any of

the engineered nucleic acids described herein. The virus, including any of the oncolytic viruses described herein, can be a recombinant virus that encodes one more transgenes encoding one or more of the two or more chimeric proteins, such as any of the engineered nucleic acids described herein.

Also provided herein are engineered erythrocytes. Erythrocytes can be engineered to comprise any of the engineered nucleic acids described herein. Erythrocytes can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are erythrocytes engineered to produce one or more of the chimeric proteins described herein. In a particular aspect, provided herein are erythrocytes engineered to produce two or more of the chimeric proteins described herein.

Also provided herein are engineered platelet cells. Platelet cells can be engineered to comprise any of the engineered nucleic acids described herein. Platelet cells can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are platelet cells engineered to produce one or more of the chimeric proteins described herein. In a particular aspect, provided herein are platelet cells engineered to produce two or more of the chimeric proteins described herein.

Also provided herein are engineered bacterial cells. Bacterial cells can be engineered to comprise any of the engineered nucleic acids described herein. Bacterial cells can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are bacterial cells engineered to produce two or more of the chimeric proteins described herein. Bacterial cells can be engineered to produce one or more mammalian-derived chimeric proteins. Bacterial cells can be engineered to produce two or more mammalian-derived chimeric proteins. Examples of bacterial cells include, but are not limited to, *Clostridium beijerinckii*, *Clostridium sporogenes, Clostridium novyi, Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Salmonella choleraesuis.*

An engineered cell can be a human cell. An engineered cell can be a human primary cell. An engineered primary cell can be a tumor infiltrating primary cell. An engineered primary cell can be a primary T cell. An engineered primary cell can be a hematopoietic stem cell (HSC). An engineered primary cell can be a natural killer (NK) cell. An engineered primary cell can be any somatic cell. An engineered primary cell can be a MSC. Human cells (*e.g.*, immune cells) can be engineered to comprise any of the engineered nucleic acids described herein. Human cells (*e.g.*, immune cells) can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are human cells (*e.g.*, immune cells) engineered to produce one or more of the chimeric proteins described herein. In a particular

aspect, provided herein are human cells (*e.g.*, immune cells) engineered to produce two or more of the chimeric proteins described herein.

An engineered cell can be isolated from a subject (autologous), such as a subject known or suspected to have cancer. Cell isolation methods are known to those skilled in the art and include, but are not limited to, sorting techniques based on cell-surface marker expression, such as FACS sorting, positive isolation techniques, and negative isolation, magnetic isolation, and combinations thereof. An engineered cell can be allogenic with reference to the subject being administered a treatment. Allogenic modified cells can be HLA-matched to the subject being administered a treatment. An engineered cell can be a cultured cell, such as an *ex vivo* cultured cell. An engineered cell can be an *ex vivo* cultured cell, such as a primary cell isolated from a subject. A cultured cell can be cultured with one or more cytokines.

Also provided herein are methods that include culturing the engineered cells of the present disclosure. Methods of culturing the engineered cells described herein are known. One skilled in the art will recognize that culturing conditions will depend on the particular engineered cell of interest. One skilled in the art will recognize that culturing conditions will depend on the specific downstream use of the engineered cell, for example, specific culturing conditions for subsequent administration of the engineered cell to a subject.

Methods of Engineering Cells

Also provided herein are compositions and methods for engineering cells to produce one or more proteins of interest or effector molecules (*e.g.*, the multicistronic expression systems that encode each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein).

In general, cells are engineered to produce proteins of interest or effector molecules through introduction (*i.e.*, delivery) of polynucleotides encoding the one or more proteins of interest or effector molecules, *e.g.*, the chimeric proteins described herein including the protein of interest or effector molecule, into the cell's cytosol and/or nucleus. For example, the polynucleotides encoding the one or more chimeric proteins can be any of the multicistronic expression systems that encode each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein. Delivery methods include, but are not limited to, viral-mediated delivery, lipid-mediated transfection, nanoparticle delivery, electroporation, sonication, and cell membrane deformation by physical means. One skilled in the art will appreciate the choice of delivery method can depend on the specific cell type to be engineered.

Viral-Mediated Delivery

Viral vector-based delivery platforms can be used to engineer cells. In general, a viral vector-based delivery platform engineers a cell through introducing (*i.e.,* delivering) into a host cell. For example, a viral vector-based delivery platform can engineer a cell through introducing any of the engineered nucleic acids described herein (*e.g.*, any of the multicistronic systems encoding each of each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein, and/or any of the expression cassettes described herein containing a promoter and an exogenous polynucleotide sequence encoding the chimeric proteins, oriented from N-terminal to C-terminal). A viral vector-based delivery platform can be a nucleic acid, and as such, an engineered nucleic acid can also encompass an engineered virally-derived nucleic acid. Such engineered virally-derived nucleic acids can also be referred to as recombinant viruses or engineered viruses.

A viral vector-based delivery platform can encode more than one engineered nucleic acid, gene, or transgene within the same nucleic acid. For example, an engineered virally-derived nucleic acid, *e.g.*, a recombinant virus or an engineered virus, can encode one or more transgenes, including, but not limited to, any of the engineered nucleic acids described herein that encode one or more of the chimeric proteins described herein. The one or more transgenes encoding the one or more chimeric proteins can be configured to express the one or more chimeric proteins and/or other protein of interest. A viral vector-based delivery platform can encode one or more genes in addition to the one or more transgenes (*e.g.*, transgenes encoding the one or more chimeric proteins and/or other protein of interest), such as viral genes needed for viral infectivity and/or viral production (*e.g.*, capsid proteins, envelope proteins, viral polymerases, viral transcriptases, *etc.*), referred to as cis-acting elements or genes.

A viral vector-based delivery platform can comprise more than one viral vector, such as separate viral vectors encoding the engineered nucleic acids, genes, or transgenes described herein, and referred to as trans-acting elements or genes. For example, a helper-dependent viral vector-based delivery platform can provide additional genes needed for viral infectivity and/or viral production on one or more additional separate vectors in addition to the vector encoding the one or more chimeric proteins and/or other protein of interest. One viral vector can deliver more than one engineered nucleic acids, such as one vector that delivers engineered nucleic acids that are configured to produce two or more chimeric proteins and/or other protein of interest. More than one viral vector can deliver more than one engineered nucleic acids, such as more than one vector that delivers one or more engineered nucleic acid configured to produce

one or more chimeric proteins and/or other protein of interest. The number of viral vectors used can depend on the packaging capacity of the above mentioned viral vector-based vaccine platforms, and one skilled in the art can select the appropriate number of viral vectors.

In general, any of the viral vector-based systems can be used for the *in vitro* production of molecules, such as the chimeric proteins, effector molecules, and/or other protein of interest described herein, or used *in vivo* and *ex vivo* gene therapy procedures, *e.g.*, for *in vivo* delivery of the engineered nucleic acids encoding one or more chimeric proteins and/or other protein of interest. The selection of an appropriate viral vector-based system will depend on a variety of factors, such as cargo/payload size, immunogenicity of the viral system, target cell of interest, gene expression strength and timing, and other factors appreciated by one skilled in the art.

Viral vector-based delivery platforms can be RNA-based viruses or DNA-based viruses. Exemplary viral vector-based delivery platforms include, but are not limited to, a herpes simplex virus, a adenovirus, a measles virus, an influenza virus, a Indiana vesiculovirus, a Newcastle disease virus, a vaccinia virus, a poliovirus, a myxoma virus, a reovirus, a mumps virus, a Maraba virus, a rabies virus, a rotavirus, a hepatitis virus, a rubella virus, a dengue virus, a chikungunya virus, a respiratory syncytial virus, a lymphocytic choriomeningitis virus, a morbillivirus, a lentivirus, a replicating retrovirus, a rhabdovirus, a Seneca Valley virus, a sindbis virus, and any variant or derivative thereof. Other exemplary viral vector-based delivery platforms are described in the art, such as vaccinia, fowlpox, self-replicating alphavirus, marabavirus, adenovirus (*See, e.g.,* Tatsis *et al.*, Adenoviruses, *Molecular Therapy* (2004) 10, 616—629), or lentivirus, including but not limited to second, third or hybrid second/third generation lentivirus and recombinant lentivirus of any generation designed to target specific cell types or receptors (*See, e.g.,* Hu *et al.*, Immunization Delivered by Lentiviral Vectors for Cancer and Infectious Diseases, *Immunol Rev.* (2011) 239(1): 45-61, Sakuman e*t al.*, Lentiviral vectors: basic to translational, *Biochem J.* (2012) 443(3):603-18, Cooper *et al.*, Rescue of splicing-mediated intron loss maximizes expression in lentiviral vectors containing the human ubiquitin C promoter, *Nucl. Acids Res.* (2015) 43 (1): 682-690, Zufferey *et al.*, Self-Inactivating Lentivirus Vector for Safe and Efficient *In vivo* Gene Delivery, *J. Virol.* (1998) 72 (12): 9873-9880).

The sequences may be preceded with one or more sequences targeting a subcellular compartment. Upon introduction (*i.e.* delivery) into a host cell, infected cells (*i.e.*, an engineered cell) can express the chimeric proteins and/or other protein of interest. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Pat. No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.* (Nature 351:456-460 (1991)). A wide variety of other vectors useful for the introduction (*i.e.*,

delivery) of engineered nucleic acids, *e.g.*, Salmonella typhi vectors, and the like will be apparent to those skilled in the art from the description herein.

The viral vector-based delivery platforms can be a virus that targets a cell, herein referred to as an oncolytic virus. Examples of oncolytic viruses include, but are not limited to, an oncolytic herpes simplex virus, an oncolytic adenovirus, an oncolytic measles virus, an oncolytic influenza virus, an oncolytic Indiana vesiculovirus, an oncolytic Newcastle disease virus, an oncolytic vaccinia virus, an oncolytic poliovirus, an oncolytic myxoma virus, an oncolytic reovirus, an oncolytic mumps virus, an oncolytic Maraba virus, an oncolytic rabies virus, an oncolytic rotavirus, an oncolytic hepatitis virus, an oncolytic rubella virus, an oncolytic dengue virus, an oncolytic chikungunya virus, an oncolytic respiratory syncytial virus, an oncolytic lymphocytic choriomeningitis virus, an oncolytic morbillivirus, an oncolytic lentivirus, an oncolytic replicating retrovirus, an oncolytic rhabdovirus, an oncolytic Seneca Valley virus, an oncolytic sindbis virus, and any variant or derivative thereof. Any of the oncolytic viruses described herein can be a recombinant oncolytic virus comprising one more transgenes (*e.g.*, an engineered nucleic acid) encoding one or more chimeric proteins and/or other protein of interest. The transgenes encoding the one or more chimeric proteins and/or other protein of interest can be configured to express the chimeric proteins and/or other protein of interest.

The viral vector-based delivery platform can be retrovirus-based. In general, retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the one or more engineered nucleic acids (*e.g.*, transgenes encoding the one or more chimeric proteins and/or other protein of interest) into the target cell to provide permanent transgene expression. Retroviral-based delivery systems include, but are not limited to, those based upon murine leukemia, virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency vims (SIV), human immuno deficiency vims (HIV), and combinations thereof (see, *e.g.*, Buchscher *et al.*, J. Virol. 66:2731-2739 (1992); Johann et ah, J. Virol. 66:1635-1640 (1992); Sommnerfelt *et al.*, Virol. 176:58-59 (1990); Wilson et ah, J. Virol. 63:2374-2378 (1989); Miller et al, J, Virol. 65:2220-2224 (1991); PCT/US94/05700). Other retroviral systems include the Phoenix retrovirus system.

The viral vector-based delivery platform can be lentivirus-based. In general, lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Lentiviral-based delivery platforms can be HIV-based, such as ViraPower systems (ThermoFisher) or pLenti systems (Cell Biolabs). Lentiviral-based delivery platforms can be SIV, or FIV-based. Other exemplary lentivirus-based delivery platforms are described in more detail in U.S. Pat. Nos. 7,311,907; 7,262,049; 7,250,299; 7,226,780;

7,220,578; 7,211,247; 7,160,721; 7,078,031; 7,070,993; 7,056,699; 6,955,919, each herein incorporated by reference for all purposes.

The viral vector-based delivery platform can be adenovirus-based. In general, adenoviral based vectors are capable of very high transduction efficiency in many cell types, do not require cell division, achieve high titer and levels of expression, and can be produced in large quantities in a relatively simple system. In general, adenoviruses can be used for transient expression of a transgene within an infected cell since adenoviruses do not typically integrate into a host's genome. Adenovirus-based delivery platforms are described in more detail in Li *et al.*, Invest Opthalmol Vis Sci 35:2543 2549, 1994; Borras *et al.*, Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto *et al.*, H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655, each herein incorporated by reference for all purposes. Other exemplary adenovirus-based delivery platforms are described in more detail in U.S. Pat. Nos. 5585362; 6,083,716, 7,371,570; 7,348,178; 7,323,177; 7,319,033; 7,318,919; and 7,306,793 and International Patent Application WO96/13597, each herein incorporated by reference for all purposes.

The viral vector-based delivery platform can be adeno-associated virus (AAV)-based. Adeno-associated virus ("AAV") vectors may be used to transduce cells with engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein). AAV systems can be used for the *in vitro* production of proteins of interest, such as the chimeric proteins described herein and/or effector molecules, or used *in vivo* and *ex vivo* gene therapy procedures, *e.g.*, for *in vivo* delivery of the engineered nucleic acids encoding one or more chimeric proteins and/or other protein of interest (see, *e.g.*, West *et al.*, Virology 160:38-47 (1987); U.S. Pat. Nos. 4,797,368; 5,436,146; 6,632,670; 6,642,051; 7,078,387; 7,314,912; 6,498,244; 7,906,111; US patent publications US 2003-0138772, US 2007/0036760, and US 2009/0197338; Gao, *et al.*, J. Virol, 78(12):6381-6388 (June 2004); Gao, et al, Proc Natl Acad Sci USA, 100(10):6081-6086 (May 13, 2003); and International Patent applications WO 2010/138263 and WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994), each herein incorporated by reference for all purposes). Exemplary methods for constructing recombinant AAV vectors are described in more detail in U.S. Pat. No, 5,173,414; Tratschin et ah, Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, et ah, Mol. Cell, Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:64666470 (1984); and Samuiski et ah, J. Virol. 63:03822-3828 (1989), each herein incorporated by reference for all purposes. In general, an AAV-based vector comprises a capsid protein having an amino acid sequence corresponding to any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV.Rh10, AAV11 and variants thereof. In particular examples, an AAV-based vector has a capsid protein having an amino acid

sequence corresponding to AAV2. In particular examples, an AAV-based vector has a capsid protein having an amino acid sequence corresponding to AAV8.

AAV vectors can be engineered to have any of the exogenous polynucleotide sequences encoding the membrane-cleavable chimeric proteins described herein having the formula: **S – C – MT** or **MT – C – S.**

The viral vector-based delivery platform can be a virus-like particle (VLP) platform. In general, VLPs are constructed by producing viral structural proteins and purifying resulting viral particles. Then, following purification, a cargo/payload (*e.g.*, any of the engineered nucleic acids described herein) is encapsulated within the purified particle *ex vivo*. Accordingly, production of VLPs maintains separation of the nucleic acids encoding viral structural proteins and the nucleic acids encoding the cargo/payload. The viral structural proteins used in VLP production can be produced in a variety of expression systems, including mammalian, yeast, insect, bacterial, or *in vivo* translation expression systems. The purified viral particles can be denatured and reformed in the presence of the desired cargo to produce VLPs using methods known to those skilled in the art. Production of VLPs are described in more detail in Seow *et al.* (Mol Ther. 2009 May; 17(5): 767–777), herein incorporated by reference for all purposes.

The viral vector-based delivery platform can be engineered to target (*i.e.*, infect) a range of cells, target a narrow subset of cells, or target a specific cell. In general, the envelope protein chosen for the viral vector-based delivery platform will determine the viral tropism. The virus used in the viral vector-based delivery platform can be pseudotyped to target a specific cell of interest. The viral vector-based delivery platform can be pantropic and infect a range of cells. For example, pantropic viral vector-based delivery platforms can include the VSV-G envelope. The viral vector-based delivery platform can be amphotropic and infect mammalian cells. Accordingly, one skilled in the art can select the appropriate tropism, pseudotype, and/or envelope protein for targeting a desired cell type.

Lipid Structure Delivery Systems

Engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein) can be introduced into a cell using a lipid-mediated delivery system. In general, a lipid-mediated delivery system uses a structure composed of an outer lipid membrane enveloping an internal compartment. Examples of lipid-based structures include, but are not limited to, a lipid-based nanoparticle, a liposome, a micelle, an exosome, a vesicle, an extracellular vesicle, a cell, or a tissue. Lipid structure delivery systems can deliver a cargo/payload (*e.g.*, any of the engineered nucleic acids described herein) *in vitro*, *in vivo*, or *ex vivo*.

A lipid-based nanoparticle can include, but is not limited to, a unilamellar liposome, a multilamellar liposome, and a lipid preparation. As used herein, a "liposome" is a generic term encompassing *in vitro* preparations of lipid vehicles formed by enclosing a desired cargo, *e.g.*, an engineered nucleic acid, such as any of the engineered nucleic acids described herein, within a lipid shell or a lipid aggregate. Liposomes may be characterized as having vesicular structures with a bilayer membrane, generally comprising a phospholipid, and an inner medium that generally comprises an aqueous composition. Liposomes include, but are not limited to, emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. Liposomes can be unilamellar liposomes. Liposomes can be multilamellar liposomes. Liposomes can be multivesicular liposomes. Liposomes can be positively charged, negatively charged, or neutrally charged. In certain embodiments, the liposomes are neutral in charge. Liposomes can be formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of a desired purpose, *e.g.*, criteria for *in vivo* delivery, such as liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szokan e*t al.*, Ann. Rev. Biophys. Bioeng. 9; 467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728, 4,501,728, 4,837,028, and 5,019,369, each herein incorporated by reference for all purposes.

A multilamellar liposome is generated spontaneously when lipids comprising phospholipids are suspended in an excess of aqueous solution such that multiple lipid layers are separated by an aqueous medium. Water and dissolved solutes are entrapped in closed structures between the lipid bilayers following the lipid components undergoing self-rearrangement. A desired cargo (*e.g.*, a polypeptide, a nucleic acid, a small molecule drug, an engineered nucleic acid, such as any of the engineered nucleic acids described herein, a viral vector, a viral-based delivery system, *etc.*) can be encapsulated in the aqueous interior of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the polypeptide/nucleic acid, interspersed within the lipid bilayer of a liposome, entrapped in a liposome, complexed with a liposome, or otherwise associated with the liposome such that it can be delivered to a target entity. Lipophilic molecules or molecules with lipophilic regions may also dissolve in or associate with the lipid bilayer.

A liposome used according to the present embodiments can be made by different methods, as would be known to one of ordinary skill in the art. Preparations of liposomes are described in further detail in WO 2016/201323, International Applications PCT/US85/01161

and PCT/US89/05040, and U.S. Patents 4,728,578, 4,728,575, 4,737,323, 4,533,254, 4,162,282, 4,310,505, and 4,921,706; each herein incorporated by reference for all purposes.

Liposomes can be cationic liposomes. Examples of cationic liposomes are described in more detail in U.S. Patent No. 5,962,016; 5,030,453; 6,680,068, U.S. Application 2004/0208921, and International Patent Applications WO03/015757A1, WO04029213A2, and WO02/100435A1, each hereby incorporated by reference in their entirety.

Lipid-mediated gene delivery methods are described, for instance, in WO 96/18372; WO 93/24640; Mannino & Gould-Fogerite, BioTechniques 6(7): 682-691 (1988); U.S. Pat. No. 5,279,833 Rose U.S. Pat. No. 5,279,833; WO91/06309; and Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84: 7413-7414 (1987), each herein incorporated by reference for all purposes.

Exosomes are small membrane vesicles of endocytic origin that are released into the extracellular environment following fusion of multivesicular bodies with the plasma membrane. The size of exosomes ranges between 30 and 100 nm in diameter. Their surface consists of a lipid bilayer from the donor cell's cell membrane, and they contain cytosol from the cell that produced the exosome, and exhibit membrane proteins from the parental cell on the surface. Exosomes useful for the delivery of nucleic acids are known to those skilled in the art, *e.g.*, the exosomes described in more detail in U.S. Pat. No. 9,889,210, herein incorporated by reference for all purposes.

As used herein, the term "extracellular vesicle" or "EV" refers to a cell-derived vesicle comprising a membrane that encloses an internal space. In general, extracellular vesicles comprise all membrane-bound vesicles that have a smaller diameter than the cell from which they are derived. Generally extracellular vesicles range in diameter from 20 nm to 1000 nm, and can comprise various macromolecular cargo either within the internal space, displayed on the external surface of the extracellular vesicle, and/or spanning the membrane. The cargo can comprise nucleic acids (*e.g.*, any of the engineered nucleic acids described herein), proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. By way of example and without limitation, extracellular vesicles include apoptotic bodies, fragments of cells, vesicles derived from cells by direct or indirect manipulation (*e.g.*, by serial extrusion or treatment with alkaline solutions), vesiculated organelles, and vesicles produced by living cells (*e.g.*, by direct plasma membrane budding or fusion of the late endosome with the plasma membrane). Extracellular vesicles can be derived from a living or dead organism, explanted tissues or organs, and/or cultured cells.

As used herein the term "exosome" refers to a cell-derived small (between 20-300 nm in diameter, more preferably 40-200 nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from the cell by direct plasma membrane budding or

by fusion of the late endosome with the plasma membrane. The exosome comprises lipid or fatty acid and polypeptide and optionally comprises a payload (*e.g.*, a therapeutic agent), a receiver (*e.g.*, a targeting moiety), a polynucleotide (*e.g.*, a nucleic acid, RNA, or DNA, such as any of the engineered nucleic acids described herein), a sugar (*e.g.*, a simple sugar, polysaccharide, or glycan) or other molecules. The exosome can be derived from a producer cell, and isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof. An exosome is a species of extracellular vesicle. Generally, exosome production/biogenesis does not result in the destruction of the producer cell. Exosomes and preparation of exosomes are described in further detail in WO 2016/201323, which is hereby incorporated by reference in its entirety.

As used herein, the term "nanovesicle" (also referred to as a "microvesicle") refers to a cell-derived small (between 20-250 nm in diameter, more preferably 30-150 nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from the cell by direct or indirect manipulation such that said nanovesicle would not be produced by said producer cell without said manipulation. In general, a nanovesicle is a sub-species of an extracellular vesicle. Appropriate manipulations of the producer cell include but are not limited to serial extrusion, treatment with alkaline solutions, sonication, or combinations thereof. The production of nanovesicles may, in some instances, result in the destruction of said producer cell. Preferably, populations of nanovesicles are substantially free of vesicles that are derived from producer cells by way of direct budding from the plasma membrane or fusion of the late endosome with the plasma membrane. The nanovesicle comprises lipid or fatty acid and polypeptide, and optionally comprises a payload (*e.g.*, a therapeutic agent), a receiver (*e.g.*, a targeting moiety), a polynucleotide (*e.g.*, a nucleic acid, RNA, or DNA, such as any of the engineered nucleic acids described herein), a sugar (*e.g.*, a simple sugar, polysaccharide, or glycan) or other molecules. The nanovesicle, once it is derived from a producer cell according to said manipulation, may be isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof.

Lipid nanoparticles (LNPs), in general, are synthetic lipid structures that rely on the amphiphilic nature of lipids to form membranes and vesicle like structures (Riley 2017). In general, these vesicles deliver cargo/payloads, such as any of the engineered nucleic acids or viral systems described herein, by absorbing into the membrane of target cells and releasing the cargo into the cytosol. Lipids used in LNP formation can be cationic, anionic, or neutral. The lipids can be synthetic or naturally derived, and in some instances biodegradable. Lipids can include fats, cholesterol, phospholipids, lipid conjugates including, but not limited to, polyethyleneglycol (PEG) conjugates (PEGylated lipids), waxes, oils, glycerides, and fat soluble

vitamins. Lipid compositions generally include defined mixtures of materials, such as the cationic, neutral, anionic, and amphipathic lipids. In some instances, specific lipids are included to prevent LNP aggregation, prevent lipid oxidation, or provide functional chemical groups that facilitate attachment of additional moieties. Lipid composition can influence overall LNP size and stability. In an example, the lipid composition comprises dilinoleylmethyl- 4-dimethylaminobutyrate (MC3) or MC3-like molecules. MC3 and MC3-like lipid compositions can be formulated to include one or more other lipids, such as a PEG or PEG-conjugated lipid, a sterol, or neutral lipids. In addition, LNPs can be further engineered or functionalized to facilitate targeting of specific cell types. Another consideration in LNP design is the balance between targeting efficiency and cytotoxicity.

Micelles, in general, are spherical synthetic lipid structures that are formed using single-chain lipids, where the single-chain lipid's hydrophilic head forms an outer layer or membrane and the single-chain lipid's hydrophobic tails form the micelle center. Micelles typically refer to lipid structures only containing a lipid mono-layer. Micelles are described in more detail in Quader *et al.* (Mol Ther. 2017 Jul 5; 25(7): 1501–1513), herein incorporated by reference for all purposes.

Nucleic-acid vectors, such as expression vectors, exposed directly to serum can have several undesirable consequences, including degradation of the nucleic acid by serum nucleases or off-target stimulation of the immune system by the free nucleic acids. Similarly, viral delivery systems exposed directly to serum can trigger an undesired immune response and/or neutralization of the viral delivery system. Therefore, encapsulation of an engineered nucleic acid and/or viral delivery system can be used to avoid degradation, while also avoiding potential off-target affects. In certain examples, an engineered nucleic acid and/or viral delivery system is fully encapsulated within the delivery vehicle, such as within the aqueous interior of an LNP. Encapsulation of an engineered nucleic acid and/or viral delivery system within an LNP can be carried out by techniques well-known to those skilled in the art, such as microfluidic mixing and droplet generation carried out on a microfluidic droplet generating device. Such devices include, but are not limited to, standard T-junction devices or flow-focusing devices. In an example, the desired lipid formulation, such as MC3 or MC3-like containing compositions, is provided to the droplet generating device in parallel with an engineered nucleic acid or viral delivery system and any other desired agents, such that the delivery vector and desired agents are fully encapsulated within the interior of the MC3 or MC3-like based LNP. In an example, the droplet generating device can control the size range and size distribution of the LNPs produced. For example, the LNP can have a size ranging from 1 to 1000 nanometers in diameter, *e.g.*, 1, 10, 50, 100, 500, or 1000 nanometers. Following droplet generation, the delivery vehicles encapsulating the

cargo/payload (*e.g.,* an engineered nucleic acid and/or viral delivery system) can be further treated or engineered to prepare them for administration.

Nanoparticle Delivery

Nanomaterials can be used to deliver engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein). Nanomaterial vehicles, importantly, can be made of non-immunogenic materials and generally avoid eliciting immunity to the delivery vector itself. These materials can include, but are not limited to, lipids (as previously described), inorganic nanomaterials, and other polymeric materials. Nanomaterial particles are described in more detail in Riley *et al.* (Recent Advances in Nanomaterials for Gene Delivery—A Review. Nanomaterials 2017, 7(5), 94), herein incorporated by reference for all purposes.

Genomic Editing Systems

A genomic editing systems can be used to engineer a host genome to encode an engineered nucleic acid, such as an engineered nucleic acid encoding one or more of the chimeric proteins (*e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein). In general, a "genomic editing system" refers to any system for integrating an exogenous gene into a host cell's genome. Genomic editing systems include, but are not limited to, a transposon system, a nuclease genomic editing system, and a viral vector-based delivery platform.

A transposon system can be used to integrate an engineered nucleic acid, such as an engineered nucleic acid encoding one or more of the chimeric proteins (*e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCARdescribed herein), into a host genome. Transposons generally comprise terminal inverted repeats (TIR) that flank a cargo/payload nucleic acid and a transposase. The transposon system can provide the transposon in *cis* or in *trans* with the TIR-flanked cargo. A transposon system can be a retrotransposon system or a DNA transposon system. In general, transposon systems integrate a cargo/payload (*e.g.*, an engineered nucleic acid) randomly into a host genome. Examples of transposon systems include systems using a transposon of the Tc1/mariner transposon superfamily, such as a Sleeping Beauty transposon system, described in more detail in Hudecek *et al.* (Crit Rev Biochem Mol Biol. 2017 Aug;52(4):355-380), and U.S. Patent Nos. 6,489,458, 6,613,752 and 7,985,739, each of which is herein incorporated by reference for all purposes. Another example of a transposon system

includes a PiggyBac transposon system, described in more detail in U.S. Patent Nos. 6,218,185 and 6,962,810, each of which is herein incorporated by reference for all purposes.

A nuclease genomic editing system can be used to engineer a host genome to encode an engineered nucleic acid, such as an engineered nucleic acid encoding one or more of the chimeric proteins (*e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein). Without wishing to be bound by theory, in general, the nuclease-mediated gene editing systems used to introduce an exogenous gene take advantage of a cell's natural DNA repair mechanisms, particularly homologous recombination (HR) repair pathways. Briefly, following an insult to genomic DNA (typically a double-stranded break), a cell can resolve the insult by using another DNA source that has identical, or substantially identical, sequences at both its 5' and 3' ends as a template during DNA synthesis to repair the lesion. In a natural context, HDR can use the other chromosome present in a cell as a template. In gene editing systems, exogenous polynucleotides are introduced into the cell to be used as a homologous recombination template (HRT or HR template). In general, any additional exogenous sequence not originally found in the chromosome with the lesion that is included between the 5' and 3' complimentary ends within the HRT (*e.g.*, a gene or a portion of a gene) can be incorporated (*i.e.*, "integrated") into the given genomic locus during templated HDR. Thus, a typical HR template for a given genomic locus has a nucleotide sequence identical to a first region of an endogenous genomic target locus, a nucleotide sequence identical to a second region of the endogenous genomic target locus, and a nucleotide sequence encoding a cargo/payload nucleic acid (*e.g.*, any of the engineered nucleic acids described herein, such as any of the engineered nucleic acids encoding one or more chimeric proteins (*e.g.*, any of the multicistronic systems encoding each of each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCARdescribed herein)).

In some examples, a HR template can be linear. Examples of linear HR templates include, but are not limited to, a linearized plasmid vector, a ssDNA, a synthesized DNA, and a PCR amplified DNA. In particular examples, a HR template can be circular, such as a plasmid. A circular template can include a supercoiled template.

The identical, or substantially identical, sequences found at the 5' and 3' ends of the HR template, with respect to the exogenous sequence to be introduced, are generally referred to as arms (HR arms). HR arms can be identical to regions of the endogenous genomic target locus (*i.e.*, 100% identical). HR arms in some examples can be substantially identical to regions of the endogenous genomic target locus. While substantially identical HR arms can be used, it can be

advantageous for HR arms to be identical as the efficiency of the HDR pathway may be impacted by HR arms having less than 100% identity.

Each HR arm, *i.e.*, the 5' and 3' HR arms, can be the same size or different sizes. Each HR arm can each be greater than or equal to 50, 100, 200, 300, 400, or 500 bases in length. Although HR arms can, in general, be of any length, practical considerations, such as the impact of HR arm length and overall template size on overall editing efficiency, can also be taken into account. An HR arms can be identical, or substantially identical to, regions of an endogenous genomic target locus immediately adjacent to a cleavage site. Each HR arms can be identical to, or substantially identical to, regions of an endogenous genomic target locus immediately adjacent to a cleavage site. Each HR arms can be identical, or substantially identical to, regions of an endogenous genomic target locus within a certain distance of a cleavage site, such as 1 base-pair, less than or equal to 10 base-pairs, less than or equal to 50 base-pairs, or less than or equal to 100 base-pairs of each other.

A nuclease genomic editing system can use a variety of nucleases to cut a target genomic locus, including, but not limited to, a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease or derivative thereof, a Transcription activator-like effector nuclease (TALEN) or derivative thereof, a zinc-finger nuclease (ZFN) or derivative thereof, and a homing endonuclease (HE) or derivative thereof.

A CRISPR-mediated gene editing system can be used to engineer a host genome to encode an engineered nucleic acid, such as an engineered nucleic acid encoding one or more of the chimeric proteins (*e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein). CRISPR systems are described in more detail in M. Adli ("The CRISPR tool kit for genome editing and beyond" Nature Communications; volume 9 (2018), Article number: 1911), herein incorporated by reference for all that it teaches. In general, a CRISPR-mediated gene editing system comprises a CRISPR-associated (Cas) nuclease and a RNA(s) that directs cleavage to a particular target sequence. An exemplary CRISPR-mediated gene editing system is the CRISPR/Cas9 systems comprised of a Cas9 nuclease and a RNA(s) that has a CRISPR RNA (crRNA) domain and a trans-activating CRISPR (tracrRNA) domain. The crRNA typically has two RNA domains: a guide RNA sequence (gRNA) that directs specificity through base-pair hybridization to a target sequence ("a defined nucleotide sequence"), *e.g.*, a genomic sequence; and an RNA domain that hybridizes to a tracrRNA. A tracrRNA can interact with and thereby promote recruitment of a nuclease (*e.g.*, Cas9) to a genomic locus. The crRNA and tracrRNA polynucleotides can be separate polynucleotides. The crRNA and tracrRNA polynucleotides can be a single polynucleotide, also referred to as a single guide RNA (sgRNA). While the Cas9

system is illustrated here, other CRISPR systems can be used, such as the Cpf1/Cas12 or Cas13 systems. Nucleases can include derivatives thereof, such as Cas9 functional mutants, *e.g.*, a Cas9 "nickase" mutant that in general mediates cleavage of only a single strand of a defined nucleotide sequence as opposed to a complete double-stranded break typically produced by Cas9 enzymes.

In general, the components of a CRISPR system interact with each other to form a Ribonucleoprotein (RNP) complex to mediate sequence specific cleavage. In some CRISPR systems, each component can be separately produced and used to form the RNP complex. In some CRISPR systems, each component can be separately produced *in vitro* and contacted (*i.e.*, "complexed") with each other *in vitro* to form the RNP complex. The *in vitro* produced RNP can then be introduced (*i.e.*, "delivered") into a cell's cytosol and/or nucleus, *e.g.*, a T cell's cytosol and/or nucleus. The *in vitro* produced RNP complexes can be delivered to a cell by a variety of means including, but not limited to, electroporation, lipid-mediated transfection, cell membrane deformation by physical means, lipid nanoparticles (LNP), virus like particles (VLP), and sonication. In a particular example, *in vitro* produced RNP complexes can be delivered to a cell using a Nucleofactor/Nucleofection® electroporation-based delivery system (Lonza®). Other electroporation systems include, but are not limited to, MaxCyte electroporation systems, Miltenyi CliniMACS electroporation systems, Neon electroporation systems, and BTX electroporation systems. CRISPR nucleases, *e.g.*, Cas9, can be produced *in vitro* (*i.e.*, synthesized and purified) using a variety of protein production techniques known to those skilled in the art. CRISPR system RNAs, *e.g.*, an sgRNA, can be produced *in vitro* (*i.e.*, synthesized and purified) using a variety of RNA production techniques known to those skilled in the art, such as *in vitro* transcription or chemical synthesis.

An *in vitro* produced RNP complex can be complexed at different ratios of nuclease to gRNA. An *in vitro* produced RNP complex can be also be used at different amounts in a CRISPR-mediated editing system. For example, depending on the number of cells desired to be edited, the total RNP amount added can be adjusted, such as a reduction in the amount of RNP complex added when editing a large number of cells in a reaction.

In some CRISPR systems, each component (*e.g.*, Cas9 and an sgRNA) can be separately encoded by a polynucleotide with each polynucleotide introduced into a cell together or separately. In some CRISPR systems, each component can be encoded by a single polynucleotide (*i.e.*, a multi-promoter or multicistronic vector, *see* description of exemplary multicistronic systems below) and introduced into a cell. Following expression of each polynucleotide encoded CRISPR component within a cell (*e.g.*, translation of a nuclease and

transcription of CRISPR RNAs), an RNP complex can form within the cell and can then direct site-specific cleavage.

Some RNPs can be engineered to have moieties that promote delivery of the RNP into the nucleus. For example, a Cas9 nuclease can have a nuclear localization signal (NLS) domain such that if a Cas9 RNP complex is delivered into a cell's cytosol or following translation of Cas9 and subsequent RNP formation, the NLS can promote further trafficking of a Cas9 RNP into the nucleus.

The engineered cells described herein can be engineered using non-viral methods, *e.g.*, the nuclease and/or CRISPR mediated gene editing systems described herein can be delivered to a cell using non-viral methods. The engineered cells described herein can be engineered using viral methods, *e.g.*, the nuclease and/or CRISPR mediated gene editing systems described herein can be delivered to a cell using viral methods such as adenoviral, retroviral, lentiviral, or any of the other viral-based delivery methods described herein.

In some CRISPR systems, more than one CRISPR composition can be provided such that each separately target the same gene or general genomic locus at more than target nucleotide sequence. For example, two separate CRISPR compositions can be provided to direct cleavage at two different target nucleotide sequences within a certain distance of each other. In some CRISPR systems, more than one CRISPR composition can be provided such that each separately target opposite strands of the same gene or general genomic locus. For example, two separate CRISPR "nickase" compositions can be provided to direct cleavage at the same gene or general genomic locus at opposite strands.

In general, the features of a CRISPR-mediated editing system described herein can apply to other nuclease-based genomic editing systems. TALEN is an engineered site-specific nuclease, which is composed of the DNA- binding domain of TALE (transcription activator-like effectors) and the catalytic domain of restriction endonuclease FokI. By changing the amino acids present in the highly variable residue region of the monomers of the DNA binding domain, different artificial TALENs can be created to target various nucleotides sequences. The DNA binding domain subsequently directs the nuclease to the target sequences and creates a double-stranded break. TALEN-based systems are described in more detail in U.S. Ser. No. 12/965,590; U.S. Pat. No. 8,450,471; U.S. Pat. No. 8,440,431; U.S. Pat. No. 8,440,432; U.S. Pat. No. 10,172,880; and U.S. Ser. No. 13/738,381, all of which are incorporated by reference herein in their entirety. ZFN-based editing systems are described in more detail in U.S. Patent Nos. 6,453,242; 6,534,261; 6,599,692; 6,503,717; 6,689,558; 7,030,215; 6,794,136; 7,067,317; 7,262,054; 7,070,934; 7,361,635; 7,253,273; and U.S. Patent Publication Nos. 2005/0064474;

2007/0218528; 2005/0267061, all incorporated herein by reference in their entireties for all purposes.

Other Engineering Delivery Systems

Various additional means to introduce engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein) into a cell or other target recipient entity, such as any of the lipid structures described herein.

Electroporation can used to deliver polynucleotides to recipient entities. Electroporation is a method of internalizing a cargo/payload into a target cell or entity's interior compartment through applying an electrical field to transiently permeabilize the outer membrane or shell of the target cell or entity. In general, the method involves placing cells or target entities between two electrodes in a solution containing a cargo of interest (*e.g.*, any of the engineered nucleic acids described herein). The lipid membrane of the cells is then disrupted, *i.e.*, permeabilized, by applying a transient set voltage that allows the cargo to enter the interior of the entity, such as the cytoplasm of the cell. In the example of cells, at least some, if not a majority, of the cells remain viable. Cells and other entities can be electroporated *in vitro*, *in vivo*, or *ex vivo*. Electroporation conditions (*e.g.*, number of cells, concentration of cargo, recovery conditions, voltage, time, capacitance, pulse type, pulse length, volume, cuvette length, electroporation solution composition, *etc.*) vary depending on several factors including, but not limited to, the type of cell or other recipient entity, the cargo to be delivered, the efficiency of internalization desired, and the viability desired. Optimization of such criteria are within the scope of those skilled in the art. A variety devices and protocols can be used for electroporation. Examples include, but are not limited to, Neon® Transfection System, MaxCyte® Flow Electroporation™, Lonza® Nucleofector™ systems, and Bio-Rad® electroporation systems.

Other means for introducing engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein) into a cell or other target recipient entity include, but are not limited to, sonication, gene gun, hydrodynamic injection, and cell membrane deformation by physical means.

Compositions and methods for delivering engineered mRNAs *in vivo*, such as naked plasmids or mRNA, are described in detail in Kowalski *et al.* (Mol Ther. 2019 Apr 10; 27(4): 710–728) and Kaczmarek *et al.* (Genome Med. 2017; 9: 60.), each herein incorporated by reference for all purposes.

Delivery Vehicles

Also provided herein are compositions for delivering a cargo/payload (a "delivery vehicle").

The cargo can comprise nucleic acids (*e.g.*, any of the engineered nucleic acids described herein, such as any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein), as described above. The cargo can comprise proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. The cargo can be any of the chimeric proteins provided for herein (*e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein). The cargo can be a combination of the chimeric proteins described herein, *e.g.*, two or more of the chimeric proteins described herein. The cargo can be a combination of the chimeric protein described herein and another cargo of interest, such as another protein, carbohydrate, lipid, small molecule, and/or combination thereof.

The delivery vehicle can comprise any composition suitable for delivering a cargo. The delivery vehicle can comprise any composition suitable for delivering a protein (*e.g.*, any of the chimeric proteins described herein). The delivery vehicle can be any of the lipid structure delivery systems described herein. For example, a delivery vehicle can be a lipid-based structure including, but not limited to, a lipid-based nanoparticle, a liposome, a micelle, an exosome, a vesicle, an extracellular vesicle, a cell, or a tissue. The delivery vehicle can be any of the nanoparticles described herein, such as nanoparticles comprising lipids (as previously described), inorganic nanomaterials, and other polymeric materials.

The delivery vehicle can be capable of delivering the cargo to a cell, such as delivering any of the chimeric proteins described herein to a cell. The delivery vehicle can be capable of delivering the cargo to a cell, such as delivering any of the chimeric proteins described herein to a cell. The delivery vehicle can be configured to target a specific cell, such as configured with a re-directing antibody to target a specific cell. The delivery vehicle can be capable of delivering the cargo to a cell *in vivo*.

The delivery vehicle can be capable of delivering the cargo to a tissue or tissue environment (*e.g.*, a tumor microenvironment), such as delivering any of the chimeric proteins described herein to a tissue or tissue environment *in vivo*. Delivering a cargo can include secreting the cargo, such as secreting any of the chimeric proteins described herein. Accordingly, the delivery vehicle can be capable of secreting the cargo, such as secreting any of the chimeric proteins described herein. The delivery vehicle can be capable of secreting the

cargo to a tissue or tissue environment (*e.g.*, a tumor microenvironment), such as secreting any of the chimeric proteins described herein into a tissue or tissue environment. The delivery vehicle can be configured to target a specific tissue or tissue environment (*e.g.*, a tumor microenvironment), such as configured with a re-directing antibody to target a specific tissue or tissue environment.

Methods of Treatment

Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) engineered cells as provided herein to produce *in vivo* at least one protein of interest produced by the engineered cells (*e.g.*, any of the chimeric proteins provided for herein, such as any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein, or the secreted effector molecules provided for herein following protease cleavage of the chimeric protein). Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) engineered cells as provided herein to produce *in vivo* at least two proteins of interest, *e.g.*, at least two of the chimeric proteins provided for herein, such as each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein, produced by the engineered cells.

Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) any of the delivery vehicles described herein, such as any of the delivery vehicles described herein comprising any of the proteins of interest described herein, *e.g.*, any of the chimeric proteins provided for herein, such as each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein. Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) any of the delivery vehicles described herein, such as any of the delivery vehicles described herein comprising two or more proteins of, *e.g.*, at least two of the chimeric proteins provided for herein, such as each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein.

In some embodiments, the engineered cells or delivery vehicles are administered via intravenous, intraperitoneal, intratracheal, subcutaneous, intratumoral, oral, anal, intranasal (*e.g.*, packed in a delivery particle), or arterial (*e.g.*, internal carotid artery) routes. Thus, the engineered cells or delivery vehicles may be administered systemically or locally (*e.g.*, to a TME or via intratumoral administration). An engineered cell can be isolated from a subject, such as a subject known or suspected to have cancer. An engineered cell can be allogenic with reference to the subject being administered a treatment. Allogenic modified cells can be HLA-

matched to the subject being administered a treatment. Delivery vehicles can be any of the lipid structure delivery systems described herein. Delivery vehicles can be any of the nanoparticles described herein.

Engineered cells or delivery vehicles can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. For example, engineered cells or delivery vehicles can be administered in combination with one or more IMiDs described herein. FDA-approved IMiDs can be administered in their approved fashion. In another example, engineered cells or delivery vehicles can be administered in combination with a checkpoint inhibitor therapy. Exemplary checkpoint inhibitors include, but are not limited to, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies. Illustrative immune checkpoint inhibitors include pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumamb (anti-PD-1; Opdivo® - BMS), pidilizumab (anti-PD-1 antibody; CT-011 – Teva/CureTech), AMP224 (anti-PD-1; NCI), avelumab (anti-PD-L1; Bavencio® - Pfizer), durvalumab (anti-PD-L1; MEDI4736/Imfinzi® - Medimmune/AstraZeneca), atezolizumab (anti-PD-L1; Tecentriq® - Roche/Genentech), BMS-936559 (anti-PD-L1 - BMS), tremelimumab (anti-CTLA-4; Medimmune/AstraZeneca), ipilimumab (anti-CTLA-4; Yervoy ® - BMS), lirilumab (anti-KIR; BMS), monalizumab (anti-NKG2A; Innate Pharma/AstraZeneca). In other examples, engineered cells or delivery vehicles can be administered in combination with TGFbeta inhibitors, VEGF inhibitors, or HPGE2. In another example, engineered cells or delivery vehicles can be administered in combination with an anti-CD40 antibody.

Some methods comprise selecting a subject (or patient population) having a tumor (or cancer) and treating that subject with engineered cells or delivery vehicles that modulate tumor-mediated immunosuppressive mechanisms.

The engineered cells or delivery vehicles of the present disclosure may be used, in some instances, to treat cancer, such as ovarian cancer. Other cancers are described herein. For example, the engineered cells may be used to treat bladder tumors, brain tumors, breast tumors, cervical tumors, colorectal tumors, esophageal tumors, gliomas, kidney tumors, liver tumors, lung tumors, melanomas, ovarian tumors, pancreatic tumors, prostate tumors, skin tumors, thyroid tumors, and/or uterine tumors. The engineered cells or delivery vehicles of the present disclosure can be used to treat cancers with tumors located in the peritoneal space of a subject.

The methods provided herein also include delivering a preparation of engineered cells or delivery vehicles. A preparation, in some embodiments, is a substantially pure preparation, containing, for example, less than 5% (*e.g.*, less than 4%, 3%, 2%, or 1%) of cells other than engineered cells. A preparation may comprise $1x10^5$ cells/kg to $1x10^7$ cells/kg cells. Preparation of engineered cells or delivery vehicles can include pharmaceutical compositions having one or more pharmaceutically acceptable carriers. For example, preparations of engineered cells or delivery vehicles can include any of the engineered viruses, such as an engineered AAV virus, or any of the engineered viral vectors, such as AAV vector, described herein.

***In vivo* Expression**

The methods provided herein also include delivering a composition *in vivo* capable of producing the engineered cells described herein, *e.g.*, capable of delivering any of the engineered nucleic acids described herein to a cell *in vivo*. Such compositions include any of the viral-mediated delivery platforms, any of the lipid structure delivery systems, any of the nanoparticle delivery systems, any of the genomic editing systems, or any of the other engineering delivery systems described herein capable of engineering a cell *in vivo*.

The methods provided herein also include delivering a composition *in vivo* capable of producing any of the proteins of interest described herein, *e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein. The methods provided herein also include delivering a composition *in vivo* capable of producing two or more of the proteins of interest described herein. Compositions capable of *in vivo* production of proteins of interest include, but are not limited to, any of the engineered nucleic acids described herein. Compositions capable of *in vivo* production proteins of interest can be a naked mRNA or a naked plasmid.

Enumerated Embodiments

Embodiment 1: A multicistronic expression system comprising an engineered nucleic acid comprising:

(A) an exogenous polynucleotide encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula:

1. **S – C – MT** or **MT – C – S**

ii. wherein

iii. S comprises a secretable effector molecule, wherein the secretable effector molecule comprises IL-15,

iv. C comprises a protease cleavage site, and

v. MT comprises a cell membrane tethering domain,

vi. wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide;

(B) an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising:

(i) an antigen-binding domain specific for endomucin (EMCN);

(ii) one or more intracellular inhibitory domains that inhibit an immune response; and

(iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and

(C) an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising:

(i) an antigen-binding domain specific for FLT3;

(ii) an antigen-binding domain specific for CD33;

(iii) one or more intracellular signaling domains that stimulate an immune response; and

(iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

Embodiment 2: The multicistronic expression system of embodiment 1, wherein the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are linked together by a polynucleotide linker encoding a 2A ribosome skipping element.

Embodiment 3: The multicistronic expression system of embodiment 2, wherein the 2A ribosome skipping element is selected from the group consisting of: a T2A ribosome skipping element, a E2A ribosome skipping element, a P2A ribosome skipping element, a F2A ribosome skipping element, ribosome skipping element fusions thereof, and combinations thereof.

Embodiment 4: The multicistronic expression system of embodiment 3, wherein the ribosome skipping element fusion comprises an E2A/T2A ribosome skipping element.

Embodiment 5: The multicistronic expression system of embodiment 4, wherein the E2A/T2A ribosome skipping element comprises the amino acid sequence QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221).

Embodiment 6: The multicistronic expression system of embodiment 5, wherein the E2A/T2A ribosome skipping element is encoded by a polynucleotide sequence comprising the sequence CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATC CTGGACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACG TGGAGGAAAACCCTGGACCT (SEQ ID NO: 222) or the sequence CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTCGAGAGTAACC CAGGACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGT AGAAGAAAATCCTGGACCT (SEQ ID NO: 223).

Embodiment 7: The multicistronic expression system of any one of embodiments 1-7, wherein the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR.

Embodiment 8: The multicistronic expression system of any one of embodiments 1-7, wherein the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) iCAR; and (iii) bivalent aCAR.

Embodiment 9: The multicistronic expression system of any one of embodiments 1-8, wherein the secretable effector molecule comprises a signal peptide or a signal-anchor sequence.

Embodiment 10: The multicistronic expression system of embodiment 9, wherein the signal peptide comprises a native signal peptide native to the secretable effector molecule.

Embodiment 11: The multicistronic expression system of embodiment 9, wherein the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule.

Embodiment 12: The multicistronic expression system of embodiment 11, wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-CSFRa.

Embodiment 13: The multicistronic expression system of embodiment 11, wherein the non-native signal peptide comprises an IgE signal peptide.

Embodiment 14: The multicistronic expression system of embodiment 13, wherein the IgE signal peptide comprises the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228).

Embodiment 15: The multicistronic expression system of embodiment 14, wherein the IgE signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACT CT (SEQ ID NO: 229).

Embodiment 16: The multicistronic expression system of any one of embodiments 1-15, wherein the IL-15 comprises the amino acid sequence NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS (SEQ ID NO: 224).

Embodiment 17: The multicistronic expression system of embodiment 16, wherein the IL-15 is encoded by a polynucleotide sequence comprising the sequence AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAG AGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCT GCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCT GGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAAAATCTGATCATCCT GGCCAACAACAGCCTGTCCAGCAACGGCAATGTGACCGAGAGCGGCTGCAA AGAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTT CGTCCACATCGTGCAGATGTTCATCAACACCTCA (SEQ ID NO: 225).

Embodiment 18: The multicistronic expression system of any one of embodiments 1-17, wherein the protease cleavage site further comprises the N-terminal peptide linker, optionally selected from the group consisting of: SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; and EAAAKEAAAKEAAAKEAAAK.

Embodiment 19: The multicistronic expression system of any one of embodiments 1-17, wherein the protease cleavage site further comprises the N-terminal peptide linker SGGGGSGGGGSG (SEQ ID NO: 230).

Embodiment 20: The multicistronic expression system of any one of embodiments 1-19, wherein the protease cleavage site further comprises a C-terminal peptide linker, optionally selected from the group consisting of: SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; and EAAAKEAAAKEAAAKEAAAK.

Embodiment 21: The multicistronic expression system of any one of embodiments 1-19, wherein the protease cleavage site further comprises the C-terminal linker GGGSGGGGSGGGSLQ (SEQ ID NO: 231).

Embodiment 22: The multicistronic expression system of any one of embodiments 1-21, wherein the protease cleavage site comprises a Tumor Necrosis Factor-α Converting Enzyme (TACE)-specific cleavage site.

Embodiment 23: The multicistronic expression system of embodiment 22, wherein the TACE-specific cleavage site comprises the amino acid sequence VTPEPIFSLI (SEQ ID NO: 191).

Embodiment 24: The multicistronic expression system of embodiment 22, wherein the TACE-specific cleavage site comprises the amino acid sequence SGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQ (SEQ ID NO: 250).

Embodiment 25: The multicistronic expression system of embodiment 24, wherein the TACE-specific cleavage site is encoded by a polynucleotide sequence comprising the sequence TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCT ATCTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGAT CTCTTCAA (SEQ ID NO: 251).

Embodiment 26: The multicistronic expression system of any one of embodiments 1-25, wherein the cell membrane tethering domain comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, LIR1, B7-1, and BTLA.

Embodiment 27: The multicistronic expression system of any one of embodiments 1-25, wherein the cell membrane tethering domain comprises a B7-1 transmembrane domain.

Embodiment 28: The multicistronic expression system of embodiment 27, wherein the B7-1 transmembrane domain comprises the amino acid sequence LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204).

Embodiment 29: The multicistronic expression system of embodiment 28, wherein the B7-1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGAT CTGCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAAC GAGCGGCTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252).

Embodiment 30: The multicistronic expression system of any one of embodiments 1-29, wherein the membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula **S – C – MT**.

Embodiment 31: The multicistronic expression system of embodiment 30, wherein the membrane-cleavable chimeric protein comprises the amino acid sequence MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK VTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELE EKNIKEFLQSFVHIVQMFINTSSGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGG SLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 226).

Embodiment 32: The multicistronic expression system of embodiment 31, wherein the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence comprising the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACT CTAATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCA GAGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGC TGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCC TGGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAAAATCTGATCATCCT GGCCAACAACAGCCTGTCCAGCAACGGCAATGTGACCGAGAGCGGCTGCAA AGAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTT CGTCCACATCGTGCAGATGTTCATCAACACCTCATCAGGCGGCGGTGGTAGT GGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTTCAGCCTGATCGGCG GAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAATTGCTGCCTAG CTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGCTGCCTGA CCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCTGA GAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

Embodiment 33: The multicistronic expression system of any one of embodiments 1-32, wherein the antigen-binding domain specific for EMCN comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

(A) the EMCN-VH comprises:

(B) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291),

(C) a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 296), and

(D) a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and

(E) the EMCN-VL comprises:

(F) a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 299),

(G) a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 300), and

(H) a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 301), and

(I) wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

Embodiment 34: The multicistronic expression system of embodiment 33, wherein:

(A) the EMCN-VH comprises the amino acid sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS (SEQ ID NO: 302); and

(B) the EMCN-VL comprises the amino acid sequence DVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQGTKLEIK (SEQ ID NO: 310).

Embodiment 35: The multicistronic expression system of embodiment 33 or 34, wherein the EMCN-VH and the EMCN-VL are separated by a peptide linker.

Embodiment 36: The multicistronic expression system of embodiment 35, wherein the antigen-binding domain specific for EMCN comprises the structure VH-L-VL or VL-L-VH, wherein L is the peptide linker.

Embodiment 37: The multicistronic expression system of embodiment 36, wherein the peptide linker comprises the amino acid sequence SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; or EAAAKEAAAKEAAAKEAAAK.

Embodiment 38: The multicistronic expression system of embodiment 36, wherein the peptide linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 244).

Embodiment 39: The multicistronic expression system of embodiment 38, wherein the peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253).

Embodiment 40: The multicistronic expression system of any one of embodiments 1-39, wherein the antigen-binding domain specific for EMCN comprises the structure VH-L-VL and comprises the amino acid sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIW GNGNTHYHSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQG TMVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVAS DENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCLQGIHLPWTFGQGTKLEIK (SEQ ID NO: 311).

Embodiment 41: The multicistronic expression system of embodiment 40, wherein the antigen-binding domain specific for EMCN is encoded by a polynucleotide sequence comprising the sequence GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTC TGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCA CTGGGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGG GGCAACGGCAACACACACTACCACAGCGCCCTGAAGTCCCGGTTCACCATCT CCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAG CCGAGGACACCGCCGTGTACTACTGCACCCTGAGAATCAAGGATTGGGGCCA GGGCACCATGGTCACCGTTTCTTCTGGAGGCGGAGGATCTGGTGGCGGAGGA AGTGGCGGAGGCGGTTCTGACGTGGTCATGACACAGAGCCCTCTGAGCCTGC CTGTGACACTGGGACAGCCTGCCAGCATCAGCTGCAAGTCTAGCCAGTCTCT GGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTCCAGCAGAGGCCCGG ACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGGATAGCGGCGTG CCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAAGATCA GCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGGCATCC ATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAG (SEQ ID NO: 312).

Embodiment 42: The multicistronic expression system of any one of embodiments 1-41, wherein the intracellular inhibitory domain comprises a LIR1 intracellular inhibitory domain.

Embodiment 43: The multicistronic expression system of embodiment 42, wherein the LIR1 intracellular inhibitory domain comprises the amino acid sequence LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYA AVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDT KDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPAVPSI YATLAIH (SEQ ID NO: 285).

Embodiment 44: The multicistronic expression system of embodiment 43, wherein the LIR1 intracellular inhibitory domain is encoded by a polynucleotide sequence comprising the sequence CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCC GACTTTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGAC TGCAGTGGCGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGC CGCCGTGAAGCACACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATC TCCCCACGATGAGGACCCTCAGGCCGTGACATACGCAGAAGTGAAGCACTCC AGACCTCGGAGAGAGATGGCAAGCCCTCCATCTCCTCTGAGCGGCGAGTTCC TGGACACCAAAGACAGACAGGCCGAAGAGGACAGACAGATGGATACCGAA GCCGCCGCTTCTGAAGCCCCACAGGATGTGACATATGCCCAGCTGCATAGCC TGACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAAGGCCCATC TCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTCAC (SEQ ID NO: 286).

Embodiment 45: The multicistronic expression system of any one of embodiments 1-44, wherein the signal peptide is present in the iCAR.

Embodiment 46: The multicistronic expression system of embodiment 45, wherein the signal peptide of the iCAR comprises a native signal peptide native or a non-native signal peptide, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF.

Embodiment 47: The multicistronic expression system of embodiment 45, wherein the signal peptide of the iCAR comprises a CD8 signal peptide.

Embodiment 48: The multicistronic expression system of embodiment 47, wherein the CD8 signal peptide comprises the amino acid sequence MALPVTALLLPLALLLHAARP (SEQ ID NO: 137).

Embodiment 49: The multicistronic expression system of embodiment 48, wherein the CD8 signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGC TGCTAGACCA (SEQ ID NO: 416).

Embodiment 50: The multicistronic expression system of any one of embodiments 1-49, wherein the hinge domain is present in the iCAR comprises.

Embodiment 51: The multicistronic expression system of embodiment 50, wherein the hinge domain of the iCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof.

Embodiment 52: The multicistronic expression system of embodiment 50, wherein the hinge domain of the iCAR comprises a LIR1 hinge.

Embodiment 53: The multicistronic expression system of embodiment 52, wherein the LIR1 hinge comprises the amino acid sequence HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 417).

Embodiment 54: The multicistronic expression system of embodiment 53, wherein the LIR1 hinge comprises is encoded by a polynucleotide sequence comprising the sequence CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAG CAGCCCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCT GACACCAACAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418).

Embodiment 55: The multicistronic expression system of embodiment 50, wherein the hinge domain of the iCAR comprises a CD8 hinge.

Embodiment 56: The multicistronic expression system of embodiment 55, wherein the CD8 hinge comprises the amino acid sequence TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271).

Embodiment 57: The multicistronic expression system of embodiment 56, wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGC AACCCCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGT TCACACTAGAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283).

Embodiment 58: The multicistronic expression system of any one of embodiments 1-57, wherein the transmembrane domain of the iCAR is present.

Embodiment 59: The multicistronic expression system of embodiment 58, wherein the transmembrane domain of the iCAR comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, LIR1, and BTLA.

Embodiment 60: The multicistronic expression system of embodiment 58, wherein the transmembrane domain of the iCAR comprises a LIR1 transmembrane domain.

Embodiment 61: The multicistronic expression system of embodiment 60, wherein the LIR1 transmembrane domain comprises the amino acid sequence VIGILVAVILLLLLLLLLFLI (SEQ ID NO: 259).

Embodiment 62: The multicistronic expression system of embodiment 61, wherein the LIR1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCT GTTCCTGATC (SEQ ID NO: 260).

Embodiment 63: The multicistronic expression system of any one of embodiments 1-62, wherein the iCAR comprises the amino acid sequence

(A) MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASG FTFSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRD NSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSSGGGGSG GGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLN WFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCLQGIHLPWTFGQGTKLEIKHPSDPLELVVSGPSGGPSSPTTG PTSTSGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILLLLLLLLFLI LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADA QEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRRE MASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSL TLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 430); or

(B) MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASG
FTFSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSSGGGGSG
GGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLN
WFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAED
VGVYYCLQGIHLPWTFGQGTKLEIKngaaHPSDPLELVVSGPSGGPSSP
TTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILLLLLLLLL
FLILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAA
DAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPR
REMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLH
SLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 419).

Embodiment 64: The multicistronic expression system of embodiment 63, wherein the iCAR is encoded by a polynucleotide sequence comprising the sequence ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGC TGCTAGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCT GGCGGATCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGAT ACGATATGCACTGGGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGTC CGTGATCTGGGGCAACGGCAACACACACTACCACAGCGCCCTGAAGTCCCG GTTCACCATCTCCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAAC AGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCACCCTGAGAATCAAG GATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTGGAGGCGGAGGATCTG GTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCATGACACAGAGCC CTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCTGCAAGTC TAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTCCAG CAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGG ATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCAC CCTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCT GCAAGGCATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATC AAGAATGGCGCTGCACACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGAC CTTCTGGCGGCCCTAGCAGCCCTACAACAGGACCTACAAGCACAAGCGGCCC TGAGGACCAACCTCTGACACCAACAGGCAGCGATCCTCAGTCTGGACTGGGG AGACATCTGGGCGTTGTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTT GCTCCTGCTGCTTCTGTTCCTGATCCTGCGGCACAGAAGGCAGGGCAAGCAC TGGACAAGCACCCAGAGAAAGGCCGACTTTCAGCATCCTGCTGGCGCCGTTG GACCTGAGCCTACAGATAGAGGACTGCAGTGGCGGTCTAGCCCTGCCGCTGA TGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCACACCCAGCCTGAGGAT GGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACCCTCAGGCCGTG ACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGCAAGCCCT CCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCGAA GAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGAT GTGACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAG CCTCCACCTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCAC TCTGGCCATTCAC (SEQ ID NO: 420).

Embodiment 65: The multicistronic expression system of any one of embodiments 1-62, wherein the iCAR comprises the amino acid sequence

(A) MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASG
FTFSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSSGGGGSG
GGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLN
WFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAED
VGVYYCLQGIHLPWTFGQGTKLEIKTTTPAPRPPTPAPTIALQPLSLRP
EACRPAAGGAVHTRGLDFACDVIGILVAVILLLLLLLLLFLILRHRRQG
KHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAA
VKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLS
GEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATEP
PPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 431); or

(B) MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASG
FTFSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSSGGGGSG
GGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLN
WFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAED
VGVYYCLQGIHLPWTFGQGTKLEIKngaaTTTPAPRPPTPAPTIALQPLS
LRPEACRPAAGGAVHTRGLDFACDVIGILVAVILLLLLLLLLFLILRHR
RQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENL
YAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPP
SPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRRE
ATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 421).

Embodiment 66: The multicistronic expression system of embodiment 65, wherein the iCAR is encoded by a polynucleotide sequence comprising the sequence ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGC TGCTAGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCT GGCGGATCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGAT ACGATATGCACTGGGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGTC CGTGATCTGGGGCAACGGCAACACACACTACCACAGCGCCCTGAAGTCCCG GTTCACCATCTCCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAAC AGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCACCCTGAGAATCAAG GATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTGGAGGCGGAGGATCTG GTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCATGACACAGAGCC CTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCTGCAAGTC TAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTCCAG CAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGG ATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCAC CCTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCT GCAAGGCATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATC AAGAATGGCGCTGCAACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTC CAACAATTGCACTGCAACCCCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGC AGCTGGCGGAGCTGTTCACACTAGAGGCCTGGACTTTGCCTGTGACGTGATC GGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCCTG ATCCTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAG GCCGACTTTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAG GACTGCAGTGGCGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTA CGCCGCCGTGAAGCACACCCAGCCTGAGGATGGCGTGGAAATGGACACCAG ATCTCCCCACGATGAGGACCCTCAGGCCGTGACATACGCAGAAGTGAAGCA CTCCAGACCTCGGAGAGAGATGGCAAGCCCTCCATCTCCTCTGAGCGGCGAG TTCCTGGACACCAAAGACAGACAGGCCGAAGAGGACAGACAGATGGATACC GAAGCCGCCGCTTCTGAAGCCCCACAGGATGTGACATATGCCCAGCTGCATA GCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAAGGCC CATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTCAC (SEQ ID NO: 422).

Embodiment 67: The multicistronic expression system of any one of embodiments 1-66, wherein the antigen-binding domain specific for FLT3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

(A) the FLT3-VH comprises:

(B) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GGTFSSYAIS (SEQ ID NO: 360),

(C) a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIIPIFGTANYAQKFQG (SEQ ID NO: 361), and

(D) a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQAFDI (SEQ ID NO: 362), and

(E) the FLT3-VL comprises:

(F) a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASQSISSYLN (SEQ ID NO: 363),

(G) a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and

(H) a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and

(I) wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

Embodiment 68: The multicistronic expression system of embodiment 67, wherein:

(A) the FLT3-VH comprises the amino acid sequence EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLE WMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVY YCATFALFGFREQAFDIWGQGTTVTVSS (SEQ ID NO: 315); and

(B) the FLT3-VL comprises the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTF GPGTKVDIK (SEQ ID NO: 316).

Embodiment 69: The multicistronic expression system of any one of embodiments 1-68, wherein the antigen-binding domain specific for CD33 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

(A) the CD33-VH comprises:

(B) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402),

(C) a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and

(D) a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and

(E) the CD33-VL comprises:

(F) a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405),

(G) a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and

(H) a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and

(I) wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

Embodiment 70: The multicistronic expression system of embodiment 69, wherein:

(A) the CD33-VH comprises the amino acid sequence QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSS (SEQ ID NO: 329); and

(B) the CD33-VL comprises the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIK (SEQ ID NO: 330).

Embodiment 71: The multicistronic expression system of any one of embodiments 67-70, wherein the FLT3-VH, the FLT3-VL, the CD33-VH, and the CD33-VL are separated by peptide linkers.

Embodiment 72: The multicistronic expression system of embodiment 71, wherein the aCAR antigen binding domains comprises the structure (FLT3-VH) – L1 – (CD33-VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a third peptide linker, respectively.

Embodiment 73: The multicistronic expression system of embodiment 72, wherein the L1, L2, and/or L3 are each independently selected from the group consisting of: SEQ ID Nos 230 – 248.

Embodiment 74: The multicistronic expression system of embodiment 72, wherein the L1 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or GGGGSGGGGS (SEQ ID NO: 243).

Embodiment 75: The multicistronic expression system of embodiment 73, wherein the L1 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGCGGCGGTGGCTCT (SEQ ID NO: 254) or the L1 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256).

Embodiment 76: The multicistronic expression system of any one of embodiments 72-75, wherein the L2 peptide linker is the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247).

Embodiment 77: The multicistronic expression system of embodiment 76, wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGG GC (SEQ ID NO: 249).

Embodiment 78: The multicistronic expression system of any one of embodiments 72-75, wherein the L2 peptide linker is the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243).

Embodiment 79: The multicistronic expression system of embodiment 76, wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257).

Embodiment 80: The multicistronic expression system of any one of embodiments 72-79, wherein the L3 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242)or GGGGSGGGGS (SEQ ID NO: 243).

Embodiment 81: The multicistronic expression system of embodiment 80, wherein the L3 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGCGGATCC (SEQ ID NO: 255) or the L3 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258).

Embodiment 82: The multicistronic expression system of any one of embodiments 1-81, wherein the aCAR intracellular signaling domains that stimulate an immune response is selected from the group consisting of: CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278, FcεRI, DAP10, DAP12, CD66d, CD97, CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, LFA-1, CD7, LIGHT, NKG2C, B7-H3, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a SLAM protein, an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLAl, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and combinations thereof.

Embodiment 83: The multicistronic expression system of any one of embodiments 1-81, wherein the aCAR intracellular signaling domains that stimulate an immune response comprise a CD28 co-stimulatory domain and a CD3ζ signaling domain.

Embodiment 84: The multicistronic expression system of embodiment 83, wherein the CD28 co-stimulatory domain comprises the amino acid sequence RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 287).

Embodiment 85: The multicistronic expression system of embodiment 84, wherein the CD28 co-stimulatory domain is encoded by a polynucleotide sequence comprising the sequence AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCT AGACGGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAG ATTTCGCCGCCTACCGGTCC (SEQ ID NO: 288).

Embodiment 86: The multicistronic expression system of embodiment 83, wherein the CD3ζ signaling domain comprises the amino acid sequence RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR (SEQ ID NO: 289).

Embodiment 87: The multicistronic expression system of embodiment 86, wherein the CD3ζ signaling domain is encoded by a polynucleotide sequence comprising the sequence AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAG AACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTT TGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGG AAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCG GAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGG GCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGAC GCCCTTCACATGCAGGCCCTGCCCCCTCGC (SEQ ID NO: 290).

Embodiment 88: The multicistronic expression system of any one of embodiments 1-87, wherein the hinge domain of the aCAR is present.

Embodiment 89: The multicistronic expression system of embodiment 88, wherein the hinge domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof.

Embodiment 90: The multicistronic expression system of embodiment 88, wherein the hinge domain of the aCAR comprises a CD8 hinge.

Embodiment 91: The multicistronic expression system of embodiment 90, wherein the CD8 hinge comprises the amino acid sequence ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACD (SEQ ID NO: 272).

Embodiment 92: The multicistronic expression system of embodiment 91, wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCC CGCCAAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACA ATCGCCAGCCAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAGCTGCTG GCGGAGCCGTGCATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284).

Embodiment 93: The multicistronic expression system of any one of embodiments 1-92, wherein the transmembrane domain of the aCAR is present.

Embodiment 94: The multicistronic expression system of embodiment 93, wherein the transmembrane domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof.

Embodiment 95: The multicistronic expression system of embodiment 93, wherein the transmembrane domain of the aCAR comprises a CD8 hinge.

Embodiment 96: The multicistronic expression system of embodiment 95, wherein the CD8 transmembrane comprises the amino acid sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206).

Embodiment 97: The multicistronic expression system of embodiment 96, wherein the CD8 transmembrane comprises is encoded by a polynucleotide sequence comprising the sequence ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCT GGTCATCACCCTGTACTGCAACCACAGA (SEQ ID NO: 261).

Embodiment 98: The multicistronic expression system of any one of embodiments 1-97, wherein the aCAR signal peptide of the aCAR is present.

Embodiment 99: The multicistronic expression system of embodiment 98, wherein the signal peptide of the aCAR is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-CSFRa.

Embodiment 100: The multicistronic expression system of embodiment 98, wherein the signal peptide of the aCAR comprises a GM-CSFRa signal peptide.

Embodiment 101: The multicistronic expression system of embodiment 100, wherein the GM-CSFRa signal peptide comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIP (SEQ ID NO: 423).

Embodiment 102: The multicistronic expression system of embodiment 101, wherein the GM-CSFRa signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTTTCTGCTTATTCCT (SEQ ID NO: 424).

Embodiment 103: The multicistronic expression system of any one of embodiments 1-103, wherein the aCAR comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAI SWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRS EDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGGGGSQVQLVQSGAEVKKP GSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKS KATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSSGST SGSGKPGSGEGSTKGDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWF QQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQS KEVPWTFGQGTKVEIKGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLN WYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQ QSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQ PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAY KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 414).

Embodiment 104: The multicistronic expression system of any one of embodiments 1-103, wherein the aCAR comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAI SWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRS EDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGGGGSGGGGSQVQLVQSGA EVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYN QKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTV SSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQ KPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKE VPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISS YLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATY YCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTI ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCN HRRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP AYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 415).

Embodiment 105: An expression vector comprising the multicistronic expression system of any one of embodiments 1-104.

Embodiment 106: The expression vector of embodiment 105, wherein the expression vector comprises the polynucleotide of SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 433, or SEQ ID NO: 432.

Embodiment 107: An isolated cell comprising the multicistronic expression system of any one of embodiments 1-104 or the expression vector of embodiment 105 or 106.

Embodiment 108: The isolated cell of embodiment 107, wherein the cell comprises an immune cell.

Embodiment 109: The isolated cell of embodiment 107, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

Embodiment 110: The isolated cell of embodiment 107, wherein the cell is an NK cell.

Embodiment 111: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 433, or SEQ ID NO: 432.

Embodiment 112: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 425.

Embodiment 113: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 426.

Embodiment 114: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 427.

Embodiment 115: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 428.

Embodiment 116: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 429.

Embodiment 117: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 432.

Embodiment 118: A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells of any one of embodiments 107-110 or the polynucleotide of any one of embodiments 111-117, and 124.

Embodiment 119: A method of treating a subject with cancer, the method comprising administering to the subject an immunotherapy comprising of any of the isolated cells of any one of embodiments 107-110 or the polynucleotide of any one of embodiments 111-117, and 124.

Embodiment 120: A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells of any one of embodiments 107-110 or the polynucleotide of any one of embodiments 111-117, and 124.

Embodiment 121: The method of any one of embodiments 111-120, wherein the isolated cell is derived from the subject.

Embodiment 122: The method of any one of embodiments 111-121, wherein the isolated cell is allogeneic with reference to the subject.

Embodiment 123: A method of making an engineered cell, comprising transducing an isolated cell with the multicistronic expression system of any one of embodiments 1-104, the expression vector of embodiment 105 or 106, or the polynucleotide of any one of embodiments 111-117, and 124.

Embodiment 124: A polynucleotide comprising the nucleic acid sequence of SEQ ID NO: 433.

EXAMPLES

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. For example, the experiments described and performed below demonstrate the general utility of engineering cells to secrete payloads (*e.g.*, effector molecules) and delivering those cells to induce an immunogenic response against tumors.

Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1 - FLT3 OR CD33 NOT EMCN Logic Gated CAR-NK Cell Circuit Optimization

Various components, organization, and methods for FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell therapies were assessed. The overall FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell system is illustrated in **FIG. 1**, showing the bivalent activating CAR (aCAR) including a FLT3-targeting scFv and CD33-targeting scFv, the inhibitory CAR (iCAR) including an EMCN-targeting scFv, and a conditional-release IL-15 (crIL-15). The EMCN iCAR represents the "NOT-logic gate." The bivalent FLT3/CD33 aCAR represents the "OR-logic gate."

All the components were encoded in constructs in a multicistronic system where each of the components were encoded in a single engineered nucleic acid capable of transcription as a single mRNA transcript and separated by 2A ribosome skipping elements. Following transcription, each of the encoded proteins would be translated as distinct peptides.

The various components and organizations that are assessed are shown in **FIG. 2**. Assessed were the order the EMCN iCAR and FLT3/CD33 bivalent aCAR were encoded, various inhibitory intracellular inhibitory domains, tag presence, various hinges, various linkers between antibody binding domains, and different retrovirus-based vectors. In total, the initial screen assessed 90 constructs.

Methods

Transduction For Construct Screening:

The following transduction procedure was used:

- Add 1e6 NK cells/well
- Titer: 1e10 genomic titer/1e6 cells or 1e9 genomic titer/1e6 cells
- Serum-free transduction
- NK MACS with IL-2 only
- Spinoculate for 2 hours
- Rest cells after for 2+ hours before adding NK MACS + Human Ab serum + IL-2
- **Day 2:** Transfer to 24-well G-Rex
- **Option 1 (Day 8):** Check FLT3/CD33 and EMCN expression + IL-15 expression on surface and on **Day 10** perform a cytotoxicity assay at a 1:1 E:T ratio (target cells = MV4-11 +/- EMCN in IMDM media incubated for 18-20 hours)

- **Option 2 (Day 15):** Check FLT3/CD33 and EMCN expression + IL-15 expression on surface and on **Day 17** perform a cytotoxicity assay at a 1:2 E:T ratio (target cells = MV4-11 +/- EMCN in IMDM media incubated for 18-20 hours)
- Expression and target killing were assessed by flow cytometry

Constructs:

The components and their order of the control constructs used are shown in **Table A**. The components and their order of the top constructs from the initial screen are shown in **Table B**.

Table A

Controls	Construct	Vector
SB05438	crIL15 E2A/T2A Myc bivalent loop6 aCAR E2A/T2A EMCN iCAR	Retrovec
SB05977	crIL-15_ E2A/T2A _ Myc bivalent loop6 fitzer attas CD8 hinge/TM CD28 ICD "ALT CD3z"_ E2A/T2A thermo fisher codon optimized EMCN V5 LIR1 iCAR	Retrovec
SB05439	crIL15 E2A/T2A Myc CEA aCAR E2A/T2A EMCN iCAR	Retrovec

Table B

SB#	***Vector***	***Orientation***	***Tags***	***ICD***
SB06461	***Sinvec SV40***	***crIL-15- aCAR-iCAR***	***tags + native hinge from ICD***	***LIR-1***
SB06462	***Sinvec SV40***	***crIL-15- aCAR-iCAR***	***iCAR tag only + CD8***	***LIR-1***
SB06541	*Retrovec*	*crIL-15- iCAR-aCAR*	*iCAR tag only + CD8*	*LIR-1*
SB06467	*Retrovec*	*crIL-15- aCAR-iCAR*	*tags + native hinge from ICD*	*LIR-1*
SB06433	***Sinvec SV40***	***crIL-15- aCAR-iCAR***	***iCAR tag only + CD8***	***KIR3DL1***

SB06511	*Retrovec*	*crIL-15- iCAR-aCAR*	*tags + native hinge from ICD*	*KIR3DL1*
SB06512	*Retrovec*	*crIL-15- iCAR-aCAR*	*iCAR tag only + CD8*	*KIR3DL1*
SB06447	***Sinvec SV40***	***crIL-15- aCAR-iCAR***	***tags + native hinge from ICD***	***LAIR-1***
SB06448	***Sinvec SV40***	***crIL-15- aCAR-iCAR***	***iCAR tag only + CD8***	***LAIR-1***
SB06472	*Sinvec*	*crIL-15- aCAR-iCAR*	*iCAR tag only + CD8*	*SIGLEC2*
SB06557	*Retrovec*	*crIL-15- iCAR-aCAR*	*iCAR tag only + CD8*	*SIGLEC2*
SB06417	*Sinvec SV40*	*crIL-15- aCAR-iCAR*	*tags + native hinge from ICD*	*CEACAM1*
SB06487	*Sinvec SV40*	*crIL-15- iCAR-aCAR*	*iCAR tag only + CD8*	*CEACAM1*

Component Sequences:

The component sequences of the assessed lead constructs are shown in **Table C** (**Table C1** showing amino acid sequences and **Table C2** showing nucleotide sequences).

Table C1

Component	Amino Acid Sequence
IgGE Signal Sequence	MDWTWILFLVAAATRVHS (SEQ ID NO: 228)
Il15	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV HIVQMFINTS (SEQ ID NO: 224)
LR1 split N term linker + Tace10	SGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQ (SEQ ID NO: 250)
B7-1 TM	LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204)
E2A/T2A Ribosome Skipping Element	QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221)
GM-CSFRa Signal Sequence	MLLLVTSLLLCELPHPAFLLIP (SEQ ID NO: 423)
GGGGS Linker	GGGGS (SEQ ID NO: 242)
FLT3 VH	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFAL FGFREQAFDIWGQGTTVTVSS (SEQ ID NO: 315)
CD33 VH	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWI GYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCAR GRPAMDYWGQGTLVTVSS (SEQ ID NO: 329)
6 Loop Linker	GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247)
CD33 VL	DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLL IYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFG QGTKVEIK (SEQ ID NO: 330)
FLT3 VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAAS SLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKV DIK (SEQ ID NO: 316)
CD8 Hinge #1	ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACD (SEQ ID NO: 272)
CD8 TM	IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206)
CD28 Co-Stim Domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 287)

CD3z ICD	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR (SEQ ID NO: 289)
CD8 Signal Sequence	MALPVTALLLPLALLLHAARP (SEQ ID NO: 137)
EMCN VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVS VIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTLRI KDWGQGTMVTVSS (SEQ ID NO: 302)
(GGGGS)3 linker	GGGGSGGGGSGGGGS (SEQ ID NO: 244)
EMCN VL	DVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPGQSPRR LIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWT FGQGTKLEIK (SEQ ID NO: 310)
CD8 Hinge #2	TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271)
LIR1 TM	VIGILVAVILLLLLLLLLFLI (SEQ ID NO: 259)
LIR1 ICD	LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEEN LYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPL SGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPP SQEGPSPAVPSIYATLAIH (SEQ ID NO: 285)
LIR1 Hinge	HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 417)
(GGGGS)2 Linker	GGGGSGGGGS (SEQ ID NO: 243)

Table C2

Component	Nucleic Acid Sequence
IgGE Signal Sequence	ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGC ACTCT
Il15	AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATC CAGAGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTGCAC CCTAGCTGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGCAA GTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAA AATCTGATCATCCTGGCCAACAACAGCCTGTCCAGCAACGGCAATGTG ACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAGAACAT CAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAAC ACCTCA (SEQ ID NO: 225)
LR1 split N term linker + Tace10	TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAG CCTATCTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCG GAGGATCTCTTCAA (SEQ ID NO: 251)
B7-1 TM	TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCG TGATCTGCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAG

	AAGAAACGAGCGGCTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252)
E2A/T2A Ribosome Skipping Element	CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCT AATCCTGGACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGT GGAGACGTGGAGGAAAACCCTGGACCT (SEQ ID NO: 222); or CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTCGAGAGT AACCCAGGACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGT GGTGATGTAGAAGAAAATCCTGGACCT (SEQ ID NO: 223)
GM-CSFRa Signal Sequence	ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTG CCTTTCTGCTTATTCCT (SEQ ID NO: 424)
GGGGS Linker	GGCGGCGGTGGCTCT (SEQ ID NO: 254); or GGTGGCGGCGGATCC (SEQ ID NO: 255)
FLT3 VH	GAAGTTCAGCTGGTTCAGAGCGGAGCCGAAGTGAAGAAACCTGGCAGC AGCGTGAAGGTGTCCTGCAAAGCTTCTGGCGGCACCTTCAGCAGCTAC GCCATCTCTTGGGTTCGACAGGCACCTGGACAGGGCCTCGAGTGGATG GGAGGAATCATCCCTATCTTCGGCACCGCCAATTACGCCCAGAAATTTC AGGGAAGAGTGACCATCACCGCCGACAAGAGCACCAGCACCGCCTACA TGGAACTGAGCAGCCTGAGAAGCGAGGATACCGCTGTGTATTACTGCG CCACATTCGCCCTGTTCGGCTTCAGAGAGCAGGCCTTCGATATCTGGGG CCAGGGCACAACCGTGACAGTTTCATCT
CD33 VH	CAGGTTCAGCTTGTGCAATCTGGCGCTGAAGTGAAAAAGCCCGGCTCC TCCGTGAAAGTGTCTTGTAAAGCCAGCGGCTACACCTTTACCGACTACA ATATGCATTGGGTCCGCCAGGCACCAGGCCAGGGACTTGAATGGATCG GCTACATCTACCCCTACAACGGCGGCACCGGCTACAACCAGAAGTTCA AGTCCAAGGCCACAATCACAGCCGACGAGTCCACCAATACTGCTTATA TGGAACTGTCTAGCCTTCGGAGCGAGGACACAGCCGTGTACTATTGCG CTAGAGGCAGACCCGCCATGGACTATTGGGGACAGGGAACCCTGGTCA CAGTGTCCAGC
6 Loop Linker	GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACC AAGGGC (SEQ ID NO: 249)
CD33 VL	GACATCCAGATGACACAGAGCCCTAGTAGCCTGAGCGCCAGCGTGGGA GACAGAGTGACAATTACCTGTCGGGCCAGCGAGAGCGTGGACAACTAC GGCATCAGCTTCATGAACTGGTTCCAGCAGAAGCCTGGCAAGGCCCCT AAGCTGCTGATCTATGCCGCCAGCAATCAAGGCAGCGGAGTGCCCTCA AGATTCAGCGGAAGCGGCTCCGGCACCGACTTTACCCTGACAATCAGC TCCCTGCAGCCTGACGACTTCGCCACCTACTACTGCCAGCAGAGCAAA GAGGTGCCCTGGACATTCGGACAGGGCACCAAGGTGGAAATCAAA
FLT3 VL	GATATTCAGATGACTCAGTCCCCAAGCAGCCTGTCAGCCTCCGTGGGCG ATAGAGTCACCATTACATGCAGAGCCAGCCAGTCCATCTCCAGCTACCT GAACTGGTATCAGCAAAAACCCGGAAAGGCTCCAAAGCTCCTCATCTA CGCCGCTTCTAGCCTGCAGTCTGGCGTGCCATCTAGATTCTCTGGCTCC GGAAGCGGGACCGACTTCACACTGACCATATCTTCTCTGCAGCCCGAA GATCTGGCCACGTACTATTGTCAGCAGTCCTACAGCACCCCTTTCACCT TCGGACCCGGAACAAAGGTGGACATTAAG
CD8 Hinge #1	GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTC TGCCCGCCAAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGC

	TCCTACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGA CCAGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTTGCCTGC GAC (SEQ ID NO: 284)
CD8 TM	ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCA GCCTGGTCATCACCCTGTACTGCAACCACAGA (SEQ ID NO: 261)
CD28 Co-Stim Domain	AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACC CCTAGACGGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCT CCTAGAGATTTCGCCGCCTACCGGTCC (SEQ ID NO: 288)
CD3z ICD	AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGG CCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTA CGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAA GCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGA AAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAG CGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACA GCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTC GC (SEQ ID NO: 290)
CD8 Signal Sequence	ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGC ATGCTGCTAGACCA (SEQ ID NO: 416)
EMCN VH	GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGA TCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACG ATATGCACTGGGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGT CCGTGATCTGGGGCAACGGCAACACACACTACCACAGCGCCCTGAAGT CCCGGTTCACCATCTCCAGAGACAACAGCAAGAACACCCTGTACCTGC AGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCACCC TGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCT
(GGGGS)3 linker	GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253)
EMCN VL	GACGTGGTCATGACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGA CAGCCTGCCAGCATCAGCTGCAAGTCTAGCCAGTCTCTGGTGGCCAGC GACGAGAACACCTACCTGAACTGGTTCCAGCAGAGGCCCGGACAGTCT CCTAGACGGCTGATCTACCAGGTGTCCAAGCTGGATAGCGGCGTGCCC GATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAAGATCA GCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGGCA TCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAG
CD8 Hinge	ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCA CTGCAACCCCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGC GGAGCTGTTCACACTAGAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283)
LIR1 TM	GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCT TCTGTTCCTGATC (SEQ ID NO: 260)
LIR1 ICD	CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAA GGCCGACTTTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGAT AGAGGACTGCAGTGGCGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAA AATCTTTACGCCGCCGTGAAGCACACCCAGCCTGAGGATGGCGTGGAA ATGGACACCAGATCTCCCCACGATGAGGACCCTCAGGCCGTGACATAC GCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGCAAGCCCTCCA TCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCGAA GAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAG

	GATGTGACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCC ACAGAGCCTCCACCTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCA TCTATGCCACTCTGGCCATTCAC (SEQ ID NO: 286)
LIR1 Hinge	CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCC CTAGCAGCCCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACC AACCTCTGACACCAACAGGCAGCGATCCTCAGTCTGGACTGGGGAGAC ATCTGGGCGTT (SEQ ID NO: 418)
(GGGGS)2 Linker	GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256); or GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257); or GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258)

CAR and crIL-15 Expression Assessment

The expression of the EMCN iCAR, the FLT3/CD33 bivalent aCAR, and IL-15 were assessed for various constructs by flow cytometry. Each of the constructs in the screen had tags and used the "loop 6" linker. The gating strategy for iCAR and aCAR expression is shown in **FIG. 3**. The CEA control was used as a baseline.

Representative flow cytometry plots are shown for aCAR and iCAR expression at Day 15 for a RetroVec (**FIG. 4**) and self-inactivated (SIN) γ-retroviral SINvec (**FIG. 5**) series of constructs. Representative flow cytometry plots are shown for membrane-bound IL-15 expression at Day 15 for a RetroVec (**FIG. 6**) and SINvec (**FIG. 7**) series of constructs. A first series of comparisons for expression is shown in **FIG. 8**. A second series of comparisons for expression is shown in **FIG. 9**. A third series of comparisons for expression is shown in **FIG. 10**. A summary of aCAR and iCAR as assessed by MFI is shown in **FIG. 11**. Retrovec also had a higher virus yield than SINvec SV40 (data not shown).

The results show at the same genomic titer MOI, the SINvec SV40 constructs demonstrated a similar CAR expression percentage as the Retrovec system while the MFI is lower. A different SINvec system (SINvec NFAT) generally had lower CAR expression. The results also show at the same genomic titer MOI, the SINvec SV40 constructs show lower membrane-bound IL-15 expression than the RetroVec system. Expression generally trended higher for constructs with a native hinge from the intracellular domain (ICD) LIR1. A general summary of expression considerations for the various components and organizations are shown in **FIG. 12**, including that the different hinge and vector combinations may impact expression and/or impact in vitro/in vivo efficacy.

Cytotoxicity Assessment

Cytotoxicity for the FLT3 OR CD33 NOT EMCN logic gate (*e.g.*, CD33/FLT3 target killing and EMCN protection) was assessed for multicistronic systems expressing the EMCN

iCAR, the FLT3/CD33 bivalent aCAR, and IL-15. Each of the constructs in the screen had tags and used the "loop 6" linker.

Total cytotoxicity against target cell lines expressing FLT3/CD33 (AML cell line MV4-11) and target cell lines engineered to also express the NOT gate target antigen EMCN (MV4-11 + EMCN) for constructs using a LIR1 ICD is shown in **FIG. 13**. Normalized cytotoxicity was also assessed, as shown in **FIG. 14**. LIR1 constructs demonstrated up to 55% total killing (endogenous + aCAR-mediated killing). The LIR1 constructs also demonstrated up to ~50% protection from total killing (24% absolute protection; 100% protection of aCAR-mediated killing). As shown in **FIG. 15**, cytotoxicity was also assessed at Day 17 post-transduction for different effector:target ratios and demonstrated good cytotoxicity profiles at 1:2 and 1:4 ratios. As shown in **FIG. 16**, normalized cytotoxicity was also assessed at Day 10 post-transduction at a 1:2 effector:target ratio for the various LIR1-ICD constructs and demonstrated up to 18% absolute protection. The cytotoxicity profile indicate constructs with a native hinge from the intracellular domain (ICD) LIR1 generally worked best.

Example 2 - CAR-NK Cell Assessment in Mouse Models

Assessed were bivalent FLT3 OR CD33 CAR-NK cells in comparison to monospecific CAR-NK cells in mouse models. Also assessed were aCARs using either "loop 4" or "loop 6" linkers sequences.

Methods

The components and their order of the constructs are shown in **Table D**. The organization of the CAR constructs is shown in **FIG. 19**. A total of 35e6 NK cells were administered as a single CAR-NK injection with 5 mice per group. CAR NK cells were pre-mixed with tumor cells prior to injection. All constructs were RetroVec vector systems. Target cells (tumor models) assessed were: (1) MV4-11 [FLT3 expression high; CD33 expression high]; (2) Molm13 [FLT3 expression medium; CD33 expression high]; and SEM [FLT3 expression high; CD33 expression low].

Table D

Group	SB #	Components	Target cells and potential killing level
1	None	Tumor cell only	MV4-11, No killing

2	None	Unengineered NK cells	MV4-11, Basal killing
3	SB05413	FLT3/CD33 bivalent loop6 aCAR_HER2 iCAR	MV4-11, High
4	SB05414	FLT3 aCAR_dual 2A_HER2 iCAR	MV4-11, Medium-High
5	SB05973	CD33 aCAR_dual 2A_HER2 iCAR	MV4-11, Medium-High
6	SB06019	FLT3/CD33 bivalent loop4 aCAR_HER2 iCAR	MV4-11, High
7	SB05413	Transduction titration for bivalent loop6	MV4-11, High

Component Sequences:

The component sequence of the assessed constructs are shown in **Table C** in **Example 1**.

Bivalent FLT3 OR CD33 NK CAR vs Monospecific NK CAR Assessment

Bivalent FLT3 OR CD33 CAR-NK cells and monospecific CAR-NK cells were assessed in MV4-11 murine tumor models. Survival is shown in **FIG. 20**. Representative tumor imaging is shown in **FIG. 21**. The results show that CARs with the loop 4 linker demonstrated better efficacy than CARs with the loop 4 linker. The order for efficacy (starting with the best) was determined to be: (1) Bivalent Loop4 CAR, (2) CD33 mono CAR, (3) Bivalent Loop4 CAR, (4) FLT3 mono CAR. Also noted is the NV NK group had higher basal killing observed than in previous experiments (data not shown). The results demonstrate that the bivalent loop4 CAR NK had the best anti-tumor efficacy and survival rate among all the groups and had similar/better efficacy as compared to the mono CAR approach.

Example 3 - FLT3 OR CD33 NOT EMCN CAR-NK Cell Further Optimization

Various components, organization, and methods for FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell therapies were further assessed. As above, all the components were encoded in constructs in a multicistronic system.

Assessed were the order the EMCN iCAR and FLT3/CD33 bivalent aCAR were encoded, various inhibitory intracellular inhibitory domains, tag presence, various hinges, various linkers between antibody binding domains, and different retrovirus-based vectors.

Specifically assessed were constructs that in part varied whether CARs included affinity tags or were "tagless" versions, as well as the linker "loop 4" used between the variable domains of the bivalent aCAR. **FIG. 22** illustrates the various tagged and tagless combinations assessed. The additional candidates assessed are shown in **Table E**. Also assessed were the top candidates from **Example 1**, as shown in **Table F**. Controls were the same as those in **Example 1**.

Methods

Transduction For Construct Screening:

The following transduction procedure was used (Days refer Days Post-Transduction):

- Add 1e6 NK cells/well
- Titer: 3e10 genomic titer/1e6 cells or 2e10 genomic titer/1e6 cells
- Serum-free transduction
- NK MACS with IL-2 only
- Spinoculate for 2 hours
- Rest cells after for 2+ hours before adding NK MACS + Human Ab serum + IL-2
- **Day 2:** Transfer to 24-well G-Rex
- **Day 8:** Check FLT3/CD33 and EMCN expression + IL-15 expression on surface and on **Day 9-10** perform a cytotoxicity assay at a 1:2 E:T ratio or 1:4 E:T ratio (target cells = MV4-11 +/- EMCN in IMDM media incubated for 18-20 hours)
- Expression and target killing were assessed by flow cytometry

Table E

SB #	Construct	Vector	Tags
SB07401	crIL-15_aCAR_iCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Sinvec SV40	no tags + CD8
SB07403	crIL-15_aCAR_iCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Retrovec	no tags + CD8
SB07405	crIL-15_aCAR_iCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Sinvec SV40	no tags + native hinge from ICD
SB07404	crIL-15_aCAR_iCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Sinvec SV40	iCAR tag only + CD8
SB07408	crIL-15_aCAR_iCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Retrovec	iCAR tag only + CD8

SB07409	crIL-15_aCAR_iCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Retrovec	no tags + native hinge from ICD
SB07410	crIL15_iCAR_aCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Sinvec SV40	iCAR tag only + CD8
SB07412	crIL15_iCAR_aCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Sinvec SV40	no tags + native hinge from ICD
SB07416	crIL15_iCAR_aCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Retrovec	iCAR tag only + CD8
SB07417	crIL15_iCAR_aCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Retrovec	no tags + CD8
SB07418	crIL15_iCAR_aCAR orientation:aCAR scfv - loop 4 humanized EMCN LIR1 iCAR tags/hinge	Retrovec	no tags + native hinge from ICD

Table F

SB #	Construct	Vector	Tags
SB06511	crIL-15-iCAR-aCAR, loop 6, KIR3DL1	Retrovec	tags + native hinge from ICD
SB06512	crIL-15-aCAR-iCAR, loop 6, KIR3DL1	Retrovec	iCAR tag only + CD8
SB06557	crIL-15-iCAR-aCAR loop 6, SIGLEC2	Retrovec	iCAR tag only + CD8
SB06541	crIL-15-iCAR-aCAR loop 6, LIR1	Retrovec	tags + native hinge from ICD
SB06467	crIL-15-aCAR-iCAR, loop 6, LIR1	Retrovec	tags + native hinge from ICD

Component Sequences:

The component sequence of the assessed constructs are shown in **Table C** in **Example 1**. Lead candidates and their domain organizations are:

- SB06342; SINvec SV40 (full vector sequence is SEQ ID NO: 425)
  - IgGE SS -- Il15 -- LR1 split N term linker + Tace10 -- B7-1 TM -- E2A/T2A -- GM-CSFRa SS -- FLT3 VH – GGGGS -- CD33 VH -- 6 Loop (GSTSGSGKPGSGEGSTKG) -- CD33 VL – GGGGS -- FLT3 VL -- CD8 Hinge -- CD8 TM -- CD28 -- CD3z -- E2A/T2A -- CD8 SS -- EMCN VH -- (GGGGS)3 linker -- EMCN VL -- CD8 Hinge --LIR1 TM -- LIR1 ICD
- SB06463; SINvec SV40 (full vector sequence is SEQ ID NO: 426)
  - IgGE SS -- Il15 -- LR1 split N term linker + Tace10 -- B7-1 TM -- E2A/T2A -- GM-CSFRa SS -- FLT3 VH – GGGGS -- CD33 VH -- 6 Loop

(GSTSGSGKPGSGEGSTKG) -- CD33 VL -- GGGGS -- FLT3 VL -- CD8 Hinge -- CD8 TM -- CD28 -- CD3z -- E2A/T2A -- CD8 SS -- EMCN VH -- (GGGGS)3 linker -- EMCN VL – LIR1 Hinge --LIR1 TM -- LIR1 ICD

- SB07401; SINvec SV40 (full vector sequence is SEQ ID NO: 427)
  - IgGE SS -- Il15 -- LR1 split N term linker + Tace10 -- B7-1 TM -- E2A/T2A -- GM-CSFRa SS -- FLT3 VH – (GGGGS)2 linker -- CD33 VH -- (GGGGS)2 linker -- CD33 VL -- (GGGGS)2 linker -- FLT3 VL -- CD8 Hinge -- CD8 TM -- CD28 -- CD3z -- E2A/T2A -- CD8 SS -- EMCN VH -- (GGGGS)3 linker -- EMCN VL – CD8 Hinge -- LIR1 TM -- LIR1 ICD
- SB07405; SINvec SV40 (full vector sequence is SEQ ID NO: 428)
  - IgGE SS -- Il15 -- LR1 split N term linker + Tace10 -- B7-1 TM -- E2A/T2A -- GM-CSFRa SS -- FLT3 VH – (GGGGS)2 linker -- CD33 VH -- (GGGGS)2 linker -- CD33 VL -- (GGGGS)2 linker -- FLT3 VL -- CD8 Hinge -- CD8 TM -- CD28 -- CD3z -- E2A/T2A -- CD8 SS -- EMCN VH -- (GGGGS)3 linker -- EMCN VL – LIR1 Hinge -- LIR1 TM -- LIR1 ICD
- SB07412; SINvec SV40 (full vector sequence is SEQ ID NO: 429)
  - IgGE SS -- Il15 -- LR1 split N term linker + Tace10 -- B7-1 TM -- E2A/T2A -- CD8 SS -- EMCN VH -- (GGGGS)3 linker -- EMCN VL – LIR1 Hinge -- LIR1 TM -- LIR1 ICD -- E2A/T2A -- GM-CSFRa SS -- FLT3 VH – (GGGGS)2 linker -- CD33 VH -- (GGGGS)2 linker -- CD33 VL -- (GGGGS)2 linker -- FLT3 VL -- CD8 Hinge -- CD8 TM -- CD28 -- CD3z

Flow Cytometry of Tagless Constructs:

Fluorophore/biotin-conjugated antigen for CD33, FLT3, and EMCN CARs was added to CAR NK cells. CD33 and EMCN were run in same panel and FLT3 run in separate panel.

Tagged and Tagless Expression Assessment

The expression of the tagless versions of the EMCN iCAR and the FLT3/CD33 bivalent aCAR were assessed for various constructs by flow cytometry. Staining of the "no virus" control is shown in **FIG. 23**. Staining of tagless constructs SB07401 and SB07405 is shown in **FIG. 24**. Staining of iCAR tagged only construct SB06467 is shown in **FIG. 25**. The results demonstrate "tagless" CARs could be detected using FLT3, CD33, and EMCN fluorophore-conjugated antigens expression was similar to tagged CAR versions.

Loop 4 and Loop 6 Expression Assessment

The expression of constructs including either Loop 4 or Loop 6 linkers were assessed for various constructs by flow cytometry. A summary of CD33/EMCN double positive cells is shown in **FIG. 26**, with the results demonstrating double positive populations of CD33+ and EMCN+ CAR NK cells ranged from 40-60%. A summary of expression for each of the CD33, FLT3, and EMCN CARs for constructs with Loop 4 linkers is shown in **FIG. 27**, demonstrating expression levels of CD33 and FLT3 populations were nearly identical and EMCN expression generally detectable. A summary of expression for each of the CD33, FLT3, and EMCN CARs for constructs with Loop 6 linkers is shown in **FIG. 28**, also demonstrating expression levels of CD33 and FLT3 populations were nearly identical and EMCN expression generally detectable.

Loop 4/Loop 6 and Tagless Cytotoxicity Assessment

Cytotoxicity for constructs including either Loop 4 or Loop 6 linkers, as well as tagless versions of the EMCN iCAR and the FLT3/CD33 bivalent aCAR, were assessed for various constructs. A summary of killing activity for effector:target ratio of 1:2 is shown in **FIG. 29** and normalized to no virus (NV) in **FIG. 30**. A summary of killing activity for effector:target ratio of 1:4 is shown in **FIG. 31**. The results demonstrated significant CAR-specific killing and significant EMCN-dependent protection of MV4-11 target cells. While there was lower overall killing for 1:4 E:T ratio, there was still significant killing activity and EMCN iCAR mediated protection.

Example 4 – In vitro characterization of SEM target cells

A cell cytotoxicity assay was performed using SEM cells to assess the logic gating of an aCAR/iCAR system in NK cells expressing the aCAR/iCAR. In **FIG. 32**, NK cells transduced with SB07401, SB07403, SB07412, and SB07418 were co-cultured with SEM cells expressing EMCN ("EMCN+") or not expressing EMCN ("EMCN-") for 20 hours. **FIG. 32** shows an assessment of specific cytotoxicity and iCAR protection on Day 13 post-transduction (1:2 effector-to-target ration). Specific cytotoxicity was calculated by normalization through subtracting the background cytotoxicity of untransduced NK cells. The study demonstrated that SB07403, SB07412, and SB07418 provided specific aCAR-mediated cytotoxicity of EMCN-SEM cells, as well as protection of EMCN+ SEM cells through the iCAR.

A further cytotoxicity assay was performed for a serial killing assay where multiple rounds of co-culturing with CD33+ SEM cells expressing EMCN (SEM CD33+ EMCN+) or not expressing EMCN (SEM CD33+) were performed. Serial killings were performed at 24

hours, 48 hours, and 96 hours. **FIG. 33A – FIG. 33C** shows specific killing and protections after 2 challenges. SB07412 and SB07418 demonstrated significant CAR-specific killing and iCAR protection after two re-challenges.

Example 5 – In vivo

SEM in vivo study

The aCAR/iCAR constructs were tested in an *in vivo* mouse model. JAX IL15 NSG mice were infection with a mixture of tumor cells and NK cells (1:1 ratio) at day 0. IL-2 was injected twice per week. **Table G** shows the constructs used for this study. **FIG. 34A** shows a general schematic of the experimnet. **FIG. 34B** shows the expression of the SEM population comprising a 1:1 mixture of SEM cells expressing EMCN (CD33+/EMCN+) and SEM cells not expressing EMCN (CD33+/EMCN-).

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Following injection of the SEM cell population, the animals were visualized by bioluminesence imaging (BLI) on days 11 and 19 (days after SEM injection). In addition to BLI, blood is collected from mice and SEM cells quantified. **FIG. 35** shows mice treated wth PBS or NK cells transduced with no virus (NV), SB07401, SB07779, SB07403, or SB07569. BLI measurements were performed at specified time points after SEM cell injection. On day 19, mice injected with NK cells transduced with SB07779 and SB07569 exhibited reduced BLI as compared to mice injected with untransduced NK cells (NV). **FIG. 36** shows BLI quantification of the region of interested from time points, day 11 and day 19. **Table H** summarizies the observations of the study as noted in **Table G**. SB07779, SB07569 and SB07403 groups desmonstrated lower BLI quantification at day 19 as compared to NV Group.

To assess the effects of protection of cells expressing the safety antigen (EMCN), peripheral blood was drawn from animals and quantified. Expression of the safety antigen was used as a model for healthy cells, thus EMCN+ SEM cells should be protected from cell killing in the presence of NK cells expressing the aCAR and the EMCN-specific iCAR. **FIG. 37A**

shows quantification of proportion of SEM cells in the peripheral blood after injection of the NK cells transduced with the respective constructs. Mice were injected with a 1:1 ratio of EMCN(+) to EMCN(-) SEM cells, thus an increase in the percentage of EMCN+ cells would be indicative of iCAR expression when compared to the treatment group consisting of NK cells expressing the aCAR alone (OR Gate only control). On day 13, peripheral blood was drawn and the proportion of EMCN+ cells were quantified using flow cytometry. Analysis of the peripheral blood found that NK cells transduced with SB07403 (aCAR/iCAR) signifcantly protected EMCN+ SEM cells as compared to the NK cells transduced with the OR gate control SB06569. **FIG. 37B** shows the results of peripheral blood quantification of SEM in mice at day-21. At this timepoint, NK cells trasnsduced with 7401 and 7412 showed increased protection of EMCN+ cells as compared to the NK cells transduced with 7779 OR gate only control, resulting in EMCN+ cells as the more dominant population as compared to EMCN- SEM cells. The significant protective effect of the iCAR (as observed at day 13) persists for the the Retrovec candidate when compared to its respecitve OR gate only control 7569. On day-27, the peripheral blood was again analyzed to determine whether the iCAR mediated protection persisted at longer time points. It was found that the proporation of EMCN positive cells remained high at day 27 and 7401, 7412, and 7403 showed significant protection as compared to their respecitive OR gate control constructs (**FIG. 37C**).

Table G: Experimental Groups used in SEM in vivo study

Group	**Vector**
PBS	**N/A**
NV	**N/A**
SB07401	**Bivalent aCAR + EMCN iCAR (sinvec)**
SB07412	**Bivalent aCAR + EMCN iCAR (sinvec)**
SB07779	**Bivalent aCAR Her2 KLRG1 iCAR (sinvec)**
SB07403	**Bivalent aCAR + EMCN iCAR (retrovec)**
SB07418	**Bivalent aCAR+ EMCN iCAR (retrovec)**
SB07569	**Bivalent aCAR+ Her2 KLRG1 iCAR (retrovec)**

Table H: Structure and Brief Summary of Study

Group	**Tumor cells**	**Treatment NK Cells**	**Mouse #**	**Observation (2-6 hr post treatment)**	**Summary**
1	Nalm6-FLT3 (0.25e6) + Nalm6-EMCN-FLT3 (0.25e6) 1:1 mix	PBS	4		
2		NK NV	4		
3		NK SB07401	4		
4		NK SB07412	4		
5		NK SB07779	4	Low body temperature, less active	Recovered
6		NK SB07403	4	Low body temperature, less active	Recovered
7		NK SB07418	4	Low body temperature, less active	2 out of 4 dead
8		NK SB07569	4	Low body temperature, less active	3 out of 4 dead
9		PBS	6		
10		NK NV	5		

11	SEM-CD33 (1.5e6) + SEM-EMCN-CD33 (1.5e6) 1:1 mix	NK SB07401	5		
12		NK SB07412	6	Low body temperature, less active	Recovered
13		NK SB07779	6	Low body temperature, less active	Recovered
14		NK SB07403	6		
15		NK SB07418	6	Low body temperature, less active	5 out of 6 dead. 1 euthanized
16		NK SB07569	6	Low body temperature, less active	1 out of 6 dead

MV4-11 in vivo study

An *in vivo* study was conducted to assess the functionality of NK cells expressing the aCAR/iCAR, by using MV4-11 target cells in a mouse model. **Table I** summarizes the experimental groups used in this study and **Table J** summarizes the results of the study. 50 µL of MV4-11 cells and 150 µL of NK cells were injected into the JAX IL15 NSG mouse on day 0. IL2 was also administered to the animal two times per week. On day 21, IL2 administration was ended. BLI measurements were performed on days 7, 21, 28, and 34. **FIG. 38A-B** shows representative images and quantification, respectively, of the BLI measurements at the indicated time points. Injection of NK cells transduced with constructs SB7401, SB7412, SB07403, and SB07418 showed reduced tumor growth, as quantified through BLI as compared to the PBS or untransduced NK cell controls. This observation persisted for SB07412 and SB04128 when the study was continued to ~ 55 days (**FIG. 39A**). **FIG. 39B** shows a representative BLI image of animals injected with PBS, untransduced NK cells, and NK cells transduced with SB07412 and SB07418 at day 55. The image shows that mice receiving NK cells trasnduced with constructs SB07412 and SB07418 exhibited decreased signal at day 55, indicating killing of the injected MV4-11 cells compared to the PBS and transduced NK cell controls. In **FIG. 39C**, median survival for mice receiving PBS or untransduced NK cells were calculated to be 49 and 55 days respectively, whereas the mice receiving the transduced NK cells (SB07412 and SB07418) have undefined median survival. The differences in the median survival of mice treated with NK cells transduced with SB07412 or SB07418 were found to be significant when compared to mice treated with PBS or untransduced NK cells, showing that the constructs encoded by SB07412 or SB07418 increase survival in an MV4-11 tumor model. In **FIG. 39D**, the same study of **FIG.**

39C has been carried out until day 120, in which the median survival (days) was found for mice injected with PBS (49 days), non-engineered NK cells (NV) (55 days), engineered NK cells transduced with SB07412 Sinvec (81.5 days) or SB07418 Retrovec (108 days).

Table I

Group	**Vector**
PBS	N/A
NV	N/A
SB07401	Bivalent aCAR + EMCN iCAR (Sinvec)
SB07412	Bivalent aCAR + EMCN iCAR (Sinvec)
SB07403	Bivalent aCAR + EMCN iCAR (Retrovec)
SB07418	Bivalent aCAR + EMCN iCAR (Retrovec)

Table J shows the quantification of the NK cells after transduction with the respective viruses having the specific construct.

Table J

SB#	**Expression CD33+ %**	**Expression EMCN+ %**	**Copy#**
SB07401(SinVec)	79.3	58.8	38.1
SB07412(SinVec)	86.3	65.8	47.7
SB07403 (RetroVec)	59.70	71.90	27.6
SB07418 (RetroVec)	80.1	58.4	25.7

Example 6: Validation of other iCAR Architectures

In this study, additional ICDs were tested for use in an EMCN iCAR. **Table 9** provides the sequences of the full-length anti-EMCN iCARs assessed, which include the various ICDs indicated with all iCARs including the same anti-EMCN binder sequence: iCARs with the various ICDs were tested in a cytotoxicity assay as previously described in the present disclosure.

Cells were engineered to express an aCAR and an iCAR with different ICDs to determine the inhibitory effect of various iCAR architectures on the activity of an aCAR. FLT3 NOT EMCN CAR-NK cells expressing iCARs having different ICDs (primary NK cells engineered to co-express the same FLT3 aCAR with different EMCN iCARs) were used in an in

vitro cytotoxicity protection assay screen using target cells that expressed only FLT3 or target cells that expressed both FLT3 and EMCN (**FIG. 40**). The target cell population expressing FLT3 and EMCN should be protected from aCAR-mediated killing if the tested iCAR is functional and able to inhibit the aCAR activity. Multiple functional iCAR architectures were identified that enabled robust target cell killing in the absence of EMCN expression and provided significant antigen-dependent target cell protection in the presence of EMCN.

Table 9: List of exemplary EMCN iCARs having different intracellular signaling domains

ICD	SEQ ID NO:	EMCN iCAR + ICD AA Sequence
LIR1	489	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVIGILVAVILLLLLLLLLFLILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTD RGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEV KHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLH SLTLRREATEPPPSQEGPSPAVPSIYATLAIH
LIR2	490	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVIGILVAVVLLLLLLLLFLILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTD RGLQWRSSPAADAQEENLYAAVKDTQPEDGVEMDTRAAASEAPQDVTYAQL HSLTLRRKATEPPPSQEREPPAEPSIYATLAIH
LIR3	491	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVLIGVSVAFVLLLFLLLFLLLRRQRHSKHRTSDQRKTDFQRPAGAAETEPKDR GLLRRSSPAADVQEENLYAAVKDTQSEDRVELDSQSPHDEDPQAVTYAPVKHS SPRREMASPPSSLSGEFLDTKDRQVEEDRQMDTEAAASEASQDVTYAQLHSLT LRRKATEPPPSQEGEPPAEPSIYATLAIH

LIR5	492	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVLIGVLVVSILLLSLLLFLLLQHWRQGKHRTLAQRQADFQRPPGAAEPEPKDG GLQRRSSPAADVQGENFCAAVKNTQPEDGVEMDTRQSPHDEDPQAVTYAKV KHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLH SFTLRQKATEPPPSQEGASPAEPSVYATLAIH
KIR2DL1	493	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DILIGTSVVIILFILLFFLLHRWCSNKKNAAVMDQESAGNRTANSEDSDEQDPQE VTYTQLNHCVFTQRKITRPSQRPKTPPTDIIVYTELPNAESRSKVVSCP
KIR3DL1	494	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DILIGTSVVIILFILLLFFLLHLWCSNKKNAAVMDQEPAGNRTANSEDSDEQDPE EVTYAQLDHCVFTQRKITRPSQRPKTPPTDTILYTELPNAKPRSKVVSCP
LIR1- KIR3DL1	495	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVIGILVAVILLLLLLLLLFLILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTD RGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEV KHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLH SLTLRREATEPPPSQEGPSPAVPSIYATLAIHHLWCSNKKNAAVMDQEPAGNRT ANSEDSDEQDPEEVTYAQLDHCVFTQRKITRPSQRPKTPPTDTILYTELPNAKPR SKVVSCP

KIR3DL1-KIR3DL1	496	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DILIGTSVVIILFILLLFFLLHLWCSNKKNAAVMDQEPAGNRTANSEDSDEQDPE EVTYAQLDHCVFTQRKITRPSQRPKTPPTDTILYTELPNAKPRSKVVSCP
LIR1-LIR1	497	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVIGILVAVILLLLLLLLLFLILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTD RGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEV KHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLH SLTLRREATEPPPSQEGPSPAVPSIYATLAIH
BTLA	498	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DLLPLGGLPLLITTCFCLFCCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEAS TRQNSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKPGIVYASLNHSVI GPNSRLARNVKEAPTEYASICVRS
SIGLEC2	499	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVAVGLGSCLAILILAICGLKLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRR APLSEGPHSLGCYNPMMEDGISYTTLRFPEMNIPRTGDAESSEMQRPPPDCDDT VTYSALHKRQVGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENVDYVILKH

SIGLEC 10	500	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DGAFLGIGITALLFLCLALIIMKILPKRRTQTETPRPRFSRHSTILDYINVVPTAGP LAQKRNQKATPNSPRTPLPPGAPSPESKKNQKKQYQLPSFPEPKSSTQAPESQES QEELHYATLNFPGVRPRPEARMPKGTQADYAEVKFQ
KLRG1	501	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVAIALGLLTAVLLSVLLYQWIMTDSVIYSMLELPTATQAQNDYGPQQKSSSSR PSCSCLGSG
LAIR1	502	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DILIGVSVVFLFCLLLLVLFCLHRQNQIKQGPPRSKDEEQKPQQRPDLAVDVLER TADKATVNGLPEKDRETDTSALAAGSSQEVTYAQLDHWALTQRTARAVSPQS TKPMAESITYAAVARH
NKG2A	503	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSLTCTVSGFSLSRYD MHWVRQPPGQGLEWMGVIWGNGNTHYHSALKSRLSISRDTSKSQVFLKMNSL QTEDTAIYFCTLRIKDWGPGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPPSL SVALGQSVSISCKSSQSLVASDENTYLNWLLQSPGRSPKRLIYQVSKLDSGVPD RFSGSGSEKDFTLKISRVEAEDLGVYYCLQGIHLPWTFGGGTKLELKGKPIPNPL LGLDSTNGAATTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFAC DVIGILVAVILLLLLLLLLFLIMDNQGVIYSDLNLPPNPKRQQRKPKGNKNSILA TEQEITYAELNLQKASQDFQGNDKTYHCKDLPSAPEK

Example 7: Characterization of FLT3/CD33 NOT EMCN expressing NK cells derived from different donors

In this study, NK cells obtained from different donors were characterized to assess donor-to-donor variability. These cells were non-engineered or engineered with the FLT3/CD33

NOT EMCN Circuit (SB07412). NK cells derived from different donors were characterized for their ability to express different components within SB07412 (FLT3/CD33 bivalent aCAR, EMCN iCAR, and crIL15). **FIGs 45A-45D** show expression of crIL15-positive NK cells (**FIG. 45A**), secreted crIL15 (**FIG. 45B**), the CD33/FLT3 bivalent aCAR (**FIG. 45C**), and the EMCN iCAR (**FIG. 45D**).

NK Cell Cytotoxicity

In **FIGs. 41A-41F**, non-engineered or engineered NK cells were tested for cell-mediated cytotoxicity against AML cell lines line MOLM-13 or MV4-11, and SEM (FLT3+/CD33+/EMCN-). A total of 16 lots of NK cells, produced from 11 different NK donors, were used in these assays. When cytotoxicity data was compared across all tested lots of NK cells, the average cytotoxicity induced by the transduced cells was significantly higher than that observed from non-engineered NK cells in all cell lines (**FIGs. 41A-41C**). For both the engineered NK cells and non-engineered NK cells, the amount of cytotoxicity observed was dependent on the E:T ratio used in the experiment (**FIG. 41D** and **FIG. 41E**), indicating that the cytotoxic effect correlates with E:T ratio. In the case of cell-mediated cytotoxicity against SEM (CD33+/FLT3+/EMCN-) target cells, the observed cytotoxicity was further observed to correlate with activating CAR expression on engineered NK cells (**FIG. 41F**).

Cytotoxicity of engineered NK cells and non-engineered NK cells from 16 different lots was tested against MOLM-13, MV4-11, and SEM-CD33 cell lines. NK cytotoxicity against MOLM-13 (**FIG. 41A**), MV4-11 (**FIG. 41B**), and SEM-CD33 (**FIG. 41C**) cells across multiple NK lots (each line represents a different NK lot), at an E:T ratio of 2:1 (**FIGs. 41A-41C**). Each point represents the mean cytotoxicity value from one experiment performed in triplicate, and each line connects a paired set of data points (non-engineered and engineered NK cells) from the same lot of cells tested. Statistics performed using paired t-tests; ** $p < 0.01$, **** $p < 0.0001$. **FIGs. 41D-41E** demonstrate NK cytotoxicity against MOLM-13 (**FIG. 41D**) and MV4-11 (**FIG. 41E**) cells at multiple E:T ratios. Data is shown from a single NK cell donor. Error bars represent sample standard deviation. **FIG. 41F** shows cytotoxicity of engineered NK cells and non-engineered NK cells from multiple experiments plotted against CD33 CAR expression. Error bars represent standard deviation across samples in triplicate, and data was fit using linear regression.

Cytokine Profile of Engineered NK cells

In **FIG. 42**, the cytokine profiles were obtained from the cell supernatant of non-engineered or engineered NK cells following the first round of serial killing of MOLM-13 or

MV4-11. In **FIG. 42**, a heat map was generated by analysis of the specified cytokine profiles, comparing the cytokine levels between the engineered and unengineered NK cells for the respective cell lines that the NK cells were co-cultutred with. Fold expression was calculated using the values generated using the formula: fold expression = $\log_2$ $((engineered\ NK\ cell\ cytokine\ level)/(nonengineered\ NK\ cell\ cytokined\ level))$.

Higher levels of cytokines in the supernatant were measured in the engineered NK cells compared to non-engineered NK cells from the same donor (**FIG. 42**). Values were calculated by determining the fold change of cytokines measured in engineered NK cell samples compared to non-engineered NK samples from the same lot. The cytokines with increased production from co-culturing with engineered NK cells were IFNγ, GM-CSF, IL10, TNFα, and perforin which all mediate inflammatory or cytotoxic functions in NK cells. Cytokines were not detectable when NK cells were not added to the AML cell line culture, suggesting that cytokine production was due to presence of NK cells rather than expressed inherently by the target cell lines.

Cytokines assayed from supernatants of the engineered NK cells and non-engineered NK cells from 5 different lots after co-culture with MOLM-13 and MV4-11 AML target cells at an E:T ratio of 2:1.

Engineered NK Cell Cytotoxicity against Primary AML Samples

Primary AML patient samples from different subtypes were purchased from Discovery Life Sciences. Cryopreserved samples were thawed and cultured overnight for assays. The day after, thawed and rested NK cells (engineered NK cells and donor-matched non-engineered NK cell controls) were co-cultured with primary AML cells at 2:1 E:T ratio overnight. Viability of AML target cells was determined via flow cytometry using a viability dye (Sytox Red). To distinguish between AML blasts and LSCs, CD45low target cells were further profiled using CD34 and CD38. AML blasts = CD56-CD45lowSSClow; LSC = CD56-CD45lowCD34highCD38low.

Engineered cells were further tested for cytotoxicity against primary tumor-derived AML samples. AML patient samples from M0, M1, M4 and M5 subtypes were co-cultured with engineered NK cells or donor-matched non-engineered NK cells. Cytotoxicity was determined after an overnight co-culture in at 37ºC/5% CO2 incubator via flow cytometry gating on AML blasts and leukemic stem cells (LSCs).

In both lots of engineered NK cells tested, engineered NK cells demonstrated high target cell killing of primary tumor-derived AML samples. Cytotoxicity was demonstrated against each primary AML sample tested, including against both AML blasts and LSC cell types (**FIGs.**

43A - 43B). Notably, engineered NK cells demonstrated significantly greater cytotoxicity than non-engineered NK cells against primary AML blasts and LSCs in all samples tested.

iCAR Mediated Protection of Human Stem Cells

Using the engineered NK cells (SB07412), the previously disclosed examples describe protection of cells that express the protective antigen EMCN as well as the target antigens, CD33 and/or FLT3. EMCN was chosen as a protective antigen for healthy hematopoietic stem cells (HSCs) and other progenitor cells since it is specifically expressed in these healthy cell subsets (See **FIG. 47** for representative EMCN expression in HSCs, multipotent progenitors (MPPs), lympho-myeloid primed progenitor (LMPP), and hematopoietic progenitor cell (HPC)). In this study, protection of human hematopoietic stem cells (HSCs) (defined as Lineage- CD34+ CD38- CD90+ CD45RA as shown in flow cytometry gating strategy in **FIG. 48**) was also investigated. NK cells engineered to express only the FLT3/CD33 bivalent aCAR and NK cells engineered to express both the FLT3/CD33 bivalent aCAR and an EMCN iCAR (SB07412) were tested for their ability to kill leukemia cells, while sparing primary healthy HSCs (in CD34-enriched healthy bone marrow) within an in vitro cytotoxicity assay. For killing of FLT3/CD33-expression leukemia cells, CAR NK cells were co-cultured with the leukemia cells at at an effector:target ratio of 1:2 for approximately 18 hours, then cells were collected, stained, and analyzed by flow cytometry, revealing that the EMCN iCAR did not significantly inhibit the killing of the tumor target cells in comparison to the CAR NK cells without an EMCN iCAR (**FIG. 46B**). However, when the same CAR NK cells were co-cultured with primary healthy HSCs (within a CD34-enriched healthy bone marrow mixture) at an effector:target ratio of 3:1 for approximately 18 hours, then collected, stained, and analyzed by flow cytometry, the NK cells expressing the FLT3/CD33 bivalent aCAR and an EMCN iCAR demonstrated significantly reduced cytotoxicity against EMCN+ HSCs (**FIG. 46A**).

Importantly, both OR gate alone (FLT3/CD33 bivalent aCAR) control CAR NK cells and CAR-NK cells that expressed both the FLT3/CD33 bivalent aCAR and an EMCN iCAR (SB07412) killed tumor cells equally, suggesting that when not engaged, the iCAR does not tonically inhibit cytotoxicity. In summary, the killing of healthy EMCN+ HSCs but not the leukemia cells were significantly decreased with those CAR NK cells expressing both the aCAR and the protective iCAR.

In **FIG. 46C**, a colony forming cell assay was performed to further demonstrate that hematopoietic stem and progenitor cells (HSPCs) populations are maintained in the presence of engineered NK cells transduced with SB07412, which express both the FLT3/CD33 bivalent aCAR and an EMCN iCAR. First, primary healthy HSPCs (CD34-enriched bone marrow) were

co-cultured with (or without, as control) CAR-NK cells that were transduced to express the FLT3/CD33 bivalent aCAR and an EMCN iCAR (SB07412) at an effector:target ratio of 1:2 for approximately 18 hours (in triplicate), then cells from each well were collected and 1/10 of the cells were re-plated methylcellulose optimized for the growth and enumeration of hematopoietic stem and progenitor cell colonies, where each colony is a functional readout for a single parental stem/progenitor cell. By counting and scoring colonies from the methylcellulose assay, the co-culture of HSPCs with CAR NK cells possessing the EMCN iCAR did not significantly reduce the number of stem/progenitors (with colony-forming potential) compared to the HSPC-alone control.

As a part of the HSC experiment detailed in **FIGs. 46A-46B**, the ability of the EMCN iCAR to also reduce killing of progenitor cells was also assessed, specifically multipotent progenitor (MPP) cells (defined as Lineage- CD34+ CD38- CD90- CD45RA- as shown in the flow cytometry gating strategy in **FIG. 48**). NK cells engineered to express only the FLT3/CD33 bivalent aCAR and NK cells engineered to express both the FLT3/CD33 bivalent aCAR and an EMCN iCAR (SB07412) were tested for their ability to spare primary healthy MPPs (in CD34-enriched healthy bone marrow) within an in vitro cytotoxicity assay. CAR NK cells were co-cultured with primary healthy MPPs (within a CD34-enriched healthy bone marrow mixture) at an effector:target ratio of 3:1 for approximately 18 hours, then collected, stained, and analyzed by flow cytometry, revealing that the NK cells expressing the FLT3/CD33 bivalent aCAR and an EMCN iCAR demonstrated significantly reduced cytotoxicity against both the whole healthy MPP population (**FIG. 46D**) and healthy EMCN+ MPPs (**FIG. 46E**).

NK Cell Phenotyping

Three different lots of engineered NK cells from separate donors and non-engineered NK cells were analyzed for the expression of phenotypic markers related to NK cell activation, proliferation, and function. Fold change analysis indicated that several markers had elevated expression in engineered NK cells compared to non-engineered NK cells. (**FIG. 44**). The top seven markers upregulated in engineered NK cells were identified as the cytotoxicity markers TRAIL, NKG2D, NKp30, NKp44, CD69, Ki-67, and Tim-3. Fold expression was calculated using the formula: $log2(gMFI\ engineered\ NK\ cells/gMFI\ non-engineered\ NK\ cells)$.

Analysis of NK phenotype after co-culture with MV4-11 target cells. Heat map analysis of NK activation marker expression analyzed by flow cytometry (**FIG. 44**). Each row represents a different phenotypic marker. Markers are rank ordered based on the mean fold change value across all samples.

Other Sequences

HIV-1 protease (SEQ ID NO: 144):

PQVTLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWKPKMIGGIGGFIKVRQY
DQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF

Angiotensin converting enzyme (ACE) (SEQ ID NO: 156):

MGAASGRRGPGLLLPLPLLLLLPPQPALALDPGLQPGNFSADEAGAQLFAQSYNSSAEQ
VLFQSVAASWAHDTNITAENARRQEEAALLSQEFAEAWGQKAKELYEPIWQNFTDPQL
RRIIGAVRTLGSANLPLAKRQQYNALLSNMSRIYSTAKVCLPNKTATCWSLDPDLTNIL
ASSRSYAMLLFAWEGWHNAAGIPLKPLYEDFTALSNEAYKQDGFTDTGAYWRSWYNS
PTFEDDLEHLYQQLEPLYLNLHAFVRRALHRRYGDRYINLRGPIPAHLLGDMWAQSWE
NIYDMVVPFPDKPNLDVTSTMLQQGWNATHMFRVAEEFFTSLELSPMPPEFWEGSMLE
KPADGREVVCHASAWDFYNRKDFRIKQCTRVTMDQLSTVHHEMGHIQYYLQYKDLPV
SLRRGANPGFHEAIGDVLALSVSTPEHLHKIGLLDRVTNDTESDINYLLKMALEKIAFLP
FGYLVDQWRWGVFSGRTPPSRYNFDWWYLRTKYQGICPPVTRNETHFDAGAKFHVPN
VTPYIRYFVSFVLQFQFHEALCKEAGYEGPLHQCDIYRSTKAGAKLRKVLQAGSSRPWQ
EVLKDMVGLDALDAQPLLKYFQPVTQWLQEQNQQNGEVLGWPEYQWHPPLPDNYPE
GIDLVTDEAEASKFVEEYDRTSQVVWNEYAEANWNYNTNITTETSKILLQKNMQIANH
TLKYGTQARKFDVNQLQNTTIKRIIKKVQDLERAALPAQELEEYNKILLDMETTYSVAT
VCHPNGSCLQLEPDLTNVMATSRKYEDLLWAWEGWRDKAGRAILQFYPKYVELINQA
ARLNGYVDAGDSWRSMYETPSLEQDLERLFQELQPLYLNLHAYVRRALHRHYGAQHI
NLEGPIPAHLLGNMWAQTWSNIYDLVVPFPSAPSMDTTEAMLKQGWTPRRMFKEADD
FFTSLGLLPVPPEFWNKSMLEKPTDGREVVCHASAWDFYNGKDFRIKQCTTVNLEDLV
VAHHEMGHIQYFMQYKDLPVALREGANPGFHEAIGDVLALSVSTPKHLHSLNLLSSEG
GSDEHDINFLMKMALDKIAFIPFSYLVDQWRWRVFDGSITKENYNQEWWSLRLKYQGL
CPPVPRTQGDFDPGAKFHIPSSVPYIRYFVSFIIQFQFHEALCQAAGHTGPLHKCDIYQSK
EAGQRLATAMKLGFSRPWPEAMQLITGQPNMSASAMLSYFKPLLDWLRTENELHGEK
LGWPQYNWTPNSARSEGPLPDSGRVSFLGLDLDAQQARVGQWLLLFLGIALLVATLGL
SQRLFSIRHRSLHRHSHGPQFGSEVELRHS

DENV NS3pro (NS2B/NS3) >sp|P33478|1475-2093 (SEQ ID NO: 157):

SGVLWDTPSPPEVERAVLDDGIYRIMQRGLLGRSQVGVGVFQDGVFHTMWHVTRGAV
LMYQGKRLEPSWASVKKDLISYGGGWRFQGSWNTGEEVQVIAVEPGKNPKNVQTAPG
TFKTPEGEVGAIALDFKPGTSGSPIVNREGKIVGLYGNGVVTTSGTYVSAIAQAKASQEG
PLPEIEDEVFRKRNLTIMDLHPGSGKTRRYLPAIVREAIRRNVRTLILAPTRVVASEMAE

ALKGMPIRYQTTAVKSEHTGKEIVDLMCHATFTMRLLSPVRVPNYNMIIMDEAHFTDP
ASIARRGYISTRVGMGEAAAIFMTATPPGSVEAFPQSNAVIQDEERDIPERSWNSGYEWI
TDFPGKTVWFVPSIKSGNDIANCLRKNGKRVIQLSRKTFDTEYQKTKNNDWDYVVTTD
ISEMGANFRADRVIDPRRCLKPVILKDGPERVILAGPMPVTVASAAQRRGRIGRNQNKE
GDQYVYMGQPLNNDEDHAHWTEAKMLLDNINTPEGIIPALFEPEREKSAAIDGEYRLR
GEARKTFVELMRRGDLPVWLSYKVASEGFQYSDRRWCFDGERNNQVLEENMDVEMW
TKEGERKKLRPRWLDARTYSDPLALREFKEFAAGRR

DENV NS3pro (NS2B/NS3) >sp|P14340|1476-2093 (SEQ ID NO: 158):
AGVLWDVPSPPPVGKAELEDGAYRIKQKGILGYSQIGAGVYKEGTFHTMWHVTRGAV
LMHKGKRIEPSWADVKKDLISYGGGWKLEGEWKEGEEVQVLALEPGKNPRAVQTKPG
LFKTNAGTIGAVSLDFSPGTSGSPIIDKKGKVVGLYGNGVVTRSGAYVSAIAQTEKSIED
NPEIEDDIFRKRKLTIMDLHPGAGKTKRYLPAIVREAIKRGLRTLILAPTRVVAAEMEEA
LRGLPIRYQTPAIRAEHTGREIVDLMCHATFTMRLLSPVRVPNYNLIIMDEAHFTDPASIA
ARGYISTRVEMGEAAGIFMTATPPGSRDPFPQSNAPIMDEEREIPERSWSSGHEWVTDFK
GKTVWFVPSIKAGNDIAACLRKNGKKVIQLSRKTFDSEYVKTRTNDWDFVVTTDISEM
GANFKAERVIDPRRCMKPVILTDGEERVILAGPMPVTHSSAAQRRGRIGRNPKNENDQY
IYMGEPLENDEDCAHWKEAKMLLDNINTPEGIIPSMFEPEREKVDAIDGEYRLRGEARK
TFVDLMRRGDLPVWLAYRVAAEGINYADRRWCFDGIKNNQILEENVEVEIWTKEGER
KKLKPRWLDAKIYSDPLALKEFKEFAAGRK

DENV NS3pro (NS2B/NS3) >sp|Q99D35|1474-2092 (SEQ ID NO: 159):
SGVLWDVPSPPETQKAELEEGVYRIKQQGIFGKTQVGVGVQKEGVFHTMWHVTRGAV
LTHNGKRLEPNWASVKKDLISYGGGWRLSAQWQKGEEVQVIAVEPGKNPKNFQTMPG
IFQTTTGEIGAIALDFKPGTSGSPIINREGKVVGLYGNGVVTKNGGYVSGIAQTNAEPDG
PTPELEEEMFKKRNLTIMDLHPGSGKTRKYLPAIVREAIKRRLRTLILAPTRVVAAEMEE
ALKGLPIRYQTTATKSEHTGREIVDLMCHATFTMRLLSPVRVPNYNLIIMDEAHFTDPAS
IAARGYISTRVGMGEAAAIFMTATPPGTADAFPQSNAPIQDEERDIPERSWNSGNEWITD
FVGKTVWFVPSIKAGNDIANCLRKNGKKVIQLSRKTFDTEYQKTKLNDWDFVVTTDISE
MGANFKADRVIDPRRCLKPVILTDGPERVILAGPMPVTVASAAQRRGRVGRNPQKEND
QYIFMGQPLNKDEDHAHWTEAKMLLDNINTPEGIIPALFEPEREKSAAIDGEYRLKGESR
KTFVELMRRGDLPVWLAHKVASEGIKYTDRKWCFDGERNNQILEENMDVEIWTKEGE
KKKLRPRWLDARTYSDPLALKEFKDFAAGRK

DENV NS3pro (NS2B/NS3) >sp|Q5UCB8|1475-2092 (SEQ ID NO: 160):
SGALWDVPSPAATQKAALSEGVYRIMQRGLFGKTQVGVGIHIEGVFHTMWHVTRGSVI
CHETGRLEPSWADVRNDMISYGGGWRLGDKWDKEEDVQVLAIEPGKNPKHVQTKPGL
FKTLTGEIGAVTLDFKPGTSGSPIINRKGKVIGLYGNGVVTKSGDYVSAITQAERIGEPDY
EVDEDIFRKKRLTIMDLHPGAGKTKRILPSIVREALKRRLRTLILAPTRVVAAEMEEALR
GLPIRYQTPAVKSEHTGREIVDLMCHATFTTRLLSSTRVPNYNLIVMDEAHFTDPSSVAA
RGYISTRVEMGEAAAIFMTATPPGTTDPFPQSNSPIEDIEREIPERSWNTGFDWITDYQGK
TVWFVPSIKAGNDIANCLRKSGKKVIQLSRKTFDTEYPKTKLTDWDFVVTTDISEMGAN
FRAGRVIDPRRCLKPVILPDGPERVILAGPIPVTPASAAQRRGRIGRNPAQEDDQYVFSG
DPLKNDEDHAHWTEAKMLLDNIYTPEGIIPTLFGPEREKTQAIDGEFRLRGEQRKTFVEL
MRRGDLPVWLSYKVASAGISYKDREWCFTGERNNQILEENMEVEIWTREGEKKKLRPK
WLDARVYADPMALKDFKEFASGRK

PEST (two copies of residues 277-307 of human IκBα; SEQ ID NO: 161):
LQMLPESEDEESYDTESEFTEFTEDELPYDDGSLQMLPESEDEESYDTESEFTEFTEDELP
YDD

GRR (residues 352-408 of human p105; SEQ ID NO: 162):
EIKDKEEVQRKRQKLMPNFSDSFGGGSGAGAGGGGMFGSGGGGGGTGSTGPGYSFPH

DRR (residues 210-295 of yeast Cdc34; SEQ ID NO: 163):
IDDENGSVILQDDDYDDGNNHIPFEDDDVYNYNDNDDDDERIEFEDDDDDDDDSIDND
SVMDRKQPHKAEDESEDVEDVERVSKKD

SNS (tandem repeat of SP2 and NB (SP2-NB-SP2 of influenza A or influenza B; e.g., SEQ ID NO: 164):
PESMREEYRKEGSKRIKCPDCEPFCNKRGSPESMREEYRKE

RPB (four copies of residues 1688-1702 of yeast RPB; SEQ ID NO: 165):
RSYSPTSPNYSPTSPSGSYSPTSPNYSPTSPSGGSRSYSPTSPNYSPTSPSGSYSPTSPNYSP
TSPSG

SPmix (tandem repeat of SP1 and SP2 (SP2-SP1-SP2-SP1-SP2 of influenza A virus M2 protein; SEQ ID NO: 166):
PESMREEYRKEGSSLLTEVETPGSPESMREEYRKEGSSLLTEVETPGSPESMREEYRKE

NS2 (three copies of residues 79-93 of influenza A virus NS protein; SEQ ID NO: 167):
LIEEVRHRLKTTENSGSLIEEVRHRLKTTENSGSLIEEVRHRLKTTENSGS

ODC (residues 106-142 of ornithine decarboxylase; SEQ ID NO: 168):
FPPEVEEQDDGTLPMSCAQESGMDRHPAACASARINV

mouse ODC (residues 422–461; SEQ ID NO: 169):
SHGFPPEVEEQAAGTLPMSCAQESGMDRHPAACASARINV

SB03513 (SEQ ID NO: 210)
AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSDEPHYSQRRLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNER
LRRESVRPV

SB03514 (SEQ ID NO: 211)
AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSVTPEPIFSLILLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNER
LRRESVRPV

SB03515 (SEQ ID NO: 212)
AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSDEPHYSQRRSGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSWAI
TLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03516 (SEQ ID NO: 213)
AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSVTPEPIFSLISGGGGSGGGGSGGGGSGGGGSGGGSLQLLPSWAIT
LISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03517 (SEQ ID NO: 214)
AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSSGGGGSGGGGSGDEPHYSQRRGGGSGGGGSGGGSLQLLPSWAI
TLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03518 (SEQ ID NO: 215)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSSGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQLLPSWAIT
LISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03519 (SEQ ID NO: 216)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSSGGGGSGGGGSGGGGSGGGGSGGGSLQDEPHYSQRRLLPSWAI
TLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03520 (SEQ ID NO: 217)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE
FLQSFVHIVQMFINTSSGGGGSGGGGSGGGGSGGGGSGGGSLQVTPEPIFSLILLPSWAIT
LISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

5X NFAT minADEp promoter (SEQ ID NO: 218)

GGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGG
GGACTTTCCACTCCTGCAGGagctGGCGCGCCAGACGCTAGCGGGGGGCTATAAAAG
GGGGTGGGGGCGTTCGTCCTCACTCT

SB06342; SINvec SV40 (SEQ ID NO: 425)

aagcttgaattcgagcttgcatgcctgcaggtcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccatt
gacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttg
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CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAG
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GCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATA
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CCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCC
TCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAA
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GACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAATTGG
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CGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCGCCA
TGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCCTGGAAAGCGGCGACGCCAGC
ATCCACGATACCGTGGAAAATCTGATCATCCTGGCCAACAACAGCCTGTCCAGCAA
CGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAGAAC
ATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTCATCAACACCTCA
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CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAAT
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CCAATTACGCCCAGAAATTTCAGGGAAGAGTGACCATCACCGCCGACAAGAGCACC
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GGGCACAACCGTGACAGTTTCATCTGGCGGCGGTGGCTCTCAGGTTCAGCTTGTGCA
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TACCTGTCGGGCCAGCGAGAGCGTGGACAACTACGGCATCAGCTTCATGAACTGGT
TCCAGCAGAAGCCTGGCAAGGCCCCTAAGCTGCTGATCTATGCCGCCAGCAATCAA
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TATCAGCAAAAACCCGGAAAGGCTCCAAAGCTCCTCATCTACGCCGCTTCTAGCCT
GCAGTCTGGCGTGCCATCTAGATTCTCTGGCTCCGGAAGCGGGACCGACTTCACACT
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CAGCACCCCTTTCACCTTCGGACCCGGAACAAAGGTGGACATTAAGGCCCTGAGCA
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SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
SB05438	Retrovec	DNA	ATGGA	AATTGG	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	CAGGT	GGAGG	GATATG	AACAC	GTTATA	TTGCG
			CTGGA	GTCAAC	CGGCG	CCTAG	TACCAA	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CAGAT	CGGCG	CAGAT	GAGCA	ATCTG	CAAGC	GAAGTT	CACAAA	CTTACC	GCAGC	CGGAG	GTGAT	GAGGC	GGGAT	CCACA
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			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA		AAAAGC	CGGCG	GAGCG		GTCAG	CTTCGT	GCGGA	GACTAC	CCGCC	GGGGA	GCTGG	GGTGC	GCGGA	GTCTGT	CGGCG	TTGCTC	ACTAG
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				CCCTA	GGTTC	GAAAA	ACGTG		GCTAC		ATATGC		CAACTA		GCTAC	ACCAG	GAGCT	AGGCT	CCTGAG	GTGTG		CAGATA		GGCCT	GCGGC		AACCC
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA	CTGCT	CTGAAG	GCACG	GTGAT		CCGAC		GCGAC	GGGGG		ACTGA
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				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC	CGCCT	GACAAA	GAAAAT			GGGTC		CACCTA	GTGTCA		CAATG
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				TGGAAA		ATCTGA			GCCCA		AGTTCA		AGTGC		CTCTCTG	GATTTT	ATGAG	ATGAG	AGCTA			AGCTA		AGCTG			ACACC
				TCATCC		TGGCC			GAAATT		AGTCCA		CCTCAA		GCTCC	GCCTG	CTGCA	CTGCA	CAGAG			CGGCG		GACAG			CAACC
				TGGCC		AACAAC			TCAAGG		ACAATC		GATTCA		GGAAG	CGAC	GAAAG	GAAAG	ACAAGA			TGAAGT		CGGCG			GGAGG
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				GGCAA		TGTCAA			ACAAGA		GCACC		GCACC		CATATC		CGAAG	CGAAG	GCAAG			GCAAG		GCGGC			CCCTC
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				CGAGA		GCGGC			AGCAC		ATATGG		GACAAT		AACTGT		GCAGC	ATGAAG	ATGAAG			GGTGTT		GGTGTT			GAAGA
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				GCAAC		GAGTG			GAACT		CTTCG		CAGCC		GCCAC		GCCAC	GCCAC	GGAGA			CAGCC		CAGCC			GTCAC
				GGCAA		GAGTG			GAGCA		TGACG		TGACG		GTACTA		GTACTA	GTACTA	GGCAA			TGCAG		TCCAG			TTACG
				CGAGG		AAGAG			GCCTG		AGGAC		ACTTCG		TTGTCA		TTGTCA	TTGTCA	GGGAC			ACCGA		AGCCG			CGGAA
				AACTG		ACAGC			AGAAG		CCAGT		CCACCT		CCAGT		ACAGT	ACAGT	ACGAT			GGACA		GAGGA			GTAAA
				GAAGA		GAAGA			CGAGG		CGTGTA		CCTACT		CCTACA		CCTACA	CCTACA	GGATA			CCGCC		CCTGG			GCATA
				GAAGA		GAAGA			ATACCG		CTATTG		GCCAG		GCACC		CCTTTC	CCTTTC	CCTAC			GCTAC		GCGTG			GCCGC
				ACATCA		ACATCA			CTGTGT		CGCTA		CAGAG		GGACA		GTGGA	GTGGA	GGGCC			ATCTAT		TACTAC			CCCAG
				AAGAGT		CACATC			GAGGC		AGACC		CAAAGA		GGTGC		ACCTTC	ACCTTC	CTGGC			ACCCT		TGTCTG			ACGGG
				TTCTGC		GTGCA			AGACC		CGCCC		GGTGC		GGACC		GGACC	GGACC	ACCGC			GCGGA		CAAGG			AAATG
				AGAGC					ACATTC		CGCCA		CGTGG		CGGAA		CGGAA		CACCAA			TCAAG		CATCCA			CATAG
				TTCGTC					GCCCT		TGGACT		ACATTC		CAAAG		GGATA		GGATA			GATTG		TCTGC			CCGCC
				CACATC					GTTCG		GGACA		GGACA		GTGGA		CCTAC		CCTAC			GGGCC		CTTGG			CGTCC
				GTGCA					GCTTCA		GGACA		GGGCA				GATGC		GATGC			CTGGC		ACCTTT			CCCCT

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
									GAGAG CAGGC CTTCGA TATCTG GGGCC AGGGC ACAACC GTGAC AGTTTC ATCT		GGGAA CCCTG GTCACA GTGTC CAGC		CGAAG GTGGA AATCAA A		CATTAA G										GGAGG CGGCA CAAAG CTGGA ACTGAA A		
		AA	MDWTW ILFLVAA ATRVHS	GATGTT CATCAA CACCTG A NWVNVI SDLKKI EDLIQS MHIDAT LYTESD VHPSCK VTAMKC FLLELQ VISLES GDASIH DTVENL IILANNS LSSNGN VTESGC KECEEL EEKNIK EFLQSF VHIVQM FINTS	SGGGG SGGGG SGVTPE PIFSLIG GGSGG GGSGG GSLQ	LLPSWA ITLISVN GIFWCC LTYCFA PRCRE RRRNE RLRRES VRPV	QCTNY ALLKLA GDVES NPGPG SGEGR GSLLTC GDVEE NPGP	MLLLVT SLLLCE LPHFAF LLIP	EVQLVQ SGAEVK KPGSSV KVSCKA SGGTFS SYAISW VRQAP GQGLE WMGGII PIFGTA NYAQKF QGRVTI TADKST STAYME LSSLRS EDTAVY YCATFA LFGFRE GAFDIW GQGTT VTVSS	GGGGS QVQLV QSGAE VKKPGS SVKVSC KASGYT FTDYNM HWVRQ APGQG LEWIGY YPYNG GTGYN QKFKSK ATITAD ESTNTA YMELSS LRSEDT AYYYCA RGRPA MDYWG QGTLVT VSS	GSTSGS GKPGS GEGSTK G	DIQMTQ SPSSLS ASVGD RVTITC RASQSI SSYLN WYQQIK PGKAPK LLIYAAS SLQSGV PSRFSG SGSGT DFTLTIS SLQPED QPDDFA TYYCQ QSKEVP WTFGQ GTKVEI K	GGGGS	DIQMTQ SPSSLS ASVGD RVTITC RASQSI SSYLN WYQQIK PGKAPK LLIYAAS SLQSGV PSRFSG SGSGT DFTLTIS SLQPED LATYYC QQSYST PFTFGP GTKVDI K	ALSNSI MYFSHF VPVFLP AKPTTT PAPRPP TFAPTIA SQFLSL RPEACR PAAGG AVHTRG LDFACD	IYIWAPL AGTCG VLLLSL VITLYC NHR	RSKRSR LLHSDY MNMTP RRPGPT RKHYQ PYAPPR DFAAYR S	RVKFSR SADAPA YKQGQ NQLYNE LNLGRR EEYDVL DKFRRG RDPEM GGKFPR RKNPQ EGLYNE LQKDK MAEAYS EIGMKG ERRRG KGHDG LYQGLS TATKDT YDALHM QALPPR	QCTNY ALLKLA GDVES NPGPG SGEGR GSLLTC GDVEE NPGP	MALPVT ALLLPL ALLLHA ARP	QVQLKE SGPGLV QPSQTL SLTCTV SGFSLS RYDMH WVRQP PGQGL EWMGVI WGNGN THYHSA LKSRLSI SRDTSK SQVFLK MNSLQT EDTAIY FCTLRIK DWGPG TMVTVS S	GGGGS GGGGS GGGGS	DIVMTQ TPPSLS VALGQS VSISCK SSQSLV ASDENT YLNWLL QSPGR SPKRLI YQVSKL DSGVP DRFSG SGSEK DFTLKIS RVEAED LGVYYC LQGIHL PWTFG GGTKLE LK	ITTPAP RPFTPA PTIALQ PLSLRP EAGRPA AGGAV HTRGLD FACD	VGILVA VILLLLL LLLFLI	LRHRR QGKHW TSTQR KADFQ HPAGA VGPFP TDRGL QWRSS PAADA QEENL YAAVK HTQPE DGVEM DTRSP HDEDP QAVTY AEVKH SRPRR EMASP PSPLSG EFLDTK DRQAE

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders								Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
SB05977			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
	Retrovec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	CAGGT	GGCGG	GATATT	ACAACA	GTGATC	CTGCG
			CTGGA	GTCAAC	CGCGG	CCTAG	TACCAA	ATGCT	CAGCT	CGGTG	CAGCTT	TACATC	CACAT	CGGCG	CAGAT	GAGCA	ATCTG	CAAGC	GAAGTT	CACAAA	TCTGC	CCAGC	AGGCG	GTGAT	ACACC	GGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTGGT	CTACG	GGTTAC	GGTTCA	AGCTCT	GTGCA	TGGCT	GACAC	GATCC	GACTCA	AACAGC	GGCCC	CAGCA	TTATGC	CCGTG	TGAAAG	GTTCTG	GACAC	CGCAC	CTGGT	GAAGG
			TTACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	GAGCG		GTCCC	ATCATG	CTCTG		AGACT	GGAGC	ACTGCT	ACAGC	AAAGC	GCGGA	AAACC	CTCGG	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAAA	GCTGC	GAGCC		CGCTG	AAACCT	CCTAGT		CAAGC	GCTGC	GCTGG	GCAGA	GAAGC	TTTGCT	GGCCC	GGCGG	CCTCC	CCTCCA	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT		AGCCT	AGCCA	CACAT		AGCCT	ACAGC	CGCCC	TCGCC	GCTGC	TGGACT	TTAGCG	AAGCC	ACTCCA	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA		GTCAG	CTTCGT	GCGGA		GACTAC	CCGCG	GGGGA	CTTTGG	GGTGC	GCGGA	TGAGC	GCTCC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG		CCGGC	AGGGA	CCAGC		CCTCC	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	AGCCT	AACAAT	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC		TCCTCC	TCTACC	GTGGG		TGTGGG	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCCAA	CTCT	ACTGG	TGCACT	TGCTTC	AAGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCCT	CCTTTC	AGCGT		GTGAAA	AAGGG	AGACA		CGATA	TGCCC	CAGCC	CCTAGA	CCCAG	GCATG	ACACTG	GACAG	GCAAC	TGTTCC	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG		GTGTCTC		GAGTG		GAGTC	GCCAA	TGGTC	CGGCC	ACCAG	GACCT	CTGCTA	AGCCT	TCTGTG	GCCCTG	TGATC	CGACT
				GAGGC	CAGCC	GACCTA	TGAAT	TTGCT	TGTCCT		ACAATT	GCCTA	ATCACC		CTCTAT	GGAAG	CGGAC	CTCTAT	GGAAG	GACCA	GACCT	TCCATC	AGTCTG	TCGAG		TTCAG
				CACACT	TGATCG	CTGCTT	CTGGC		GCCAG		ACCTGT		CGGGC		ACATGC	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG	GTACC	GTGTC	CAAGA	AGGCC	CATCC	
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT		CGGGT		CAGCG		AGAGC	CCCCT	TGCAA	GAAAG	GCTCAAAAGGT		GATCAT	CTAGCC	GCAGC	CGAGG	TGCTG	
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG		ACACCT		CAGCG		CAGCC	GCTCCT	CCACA	CACTAC	TCTAGG	GGTGG	CGGCT	CAGTC	GCAGC	CCTGTA	GCGAG
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT		TTACCG		AGAGC		AGTCCA	AGAGC	GA	CAGCC	ACGAA	TAGTCT	TTAGCC	CAGTC	GGCCA	GTTGG	GCAGG
				GTGCA	GAGCG	CTACTG			CAGCA		ACTACA		GTGGA		TCTCCA	GAGAG	TTACGC	GAGTA	ACTAAC		TGTCCA	ACTGG	GCAGC	ACCTG	AGCCT
				CCCTA	GGTTG	GAAGA	ACGTG		GCTAC		ATATGC		CAACTA		GCTAC	ACCAG	TCCTCC	GAGTA	GTGTG		GATAC	TGGCC	TGGCG	AGCCT	GCGGT
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA	TAGAGA	CGATGT	GTGAT		GATATG	AGCGA	GAGCT	ACAGA	CTAGC
				AAAGTG	GAGGA	GAGCG	ACGTG		CTCTTG		GTCGG		TCAGCT		TGGTAT	CAATCG	TTTCGC	TTTGGA	GTAGAA		CATTGG	CGAGA	GTTCAC	CGAGA	TAGAG
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC	CGCCT	CAAGA	GAAAAT		GTCCG	ACACAT	ACTAGA	GACTG	GAGTG
				CATGAA	CAA	GAAGA	AAACC		GACAG		CACCA		ACTGGT		AAAACC	CCAGC	ACCGG	GACGT	CCTGG		CCAGC	ACCTG	GGCCT	CAGTG	CAGTG
				GTGCTT		GAAAG	CTGGA		GCACC		GGCCA		TCCAG		CGGAA	TCTGTC		CGCGG	ACCT		CACCA	AATTGG	GGACTT	GCGGT	GCGGT
				TCTGCT		CGTGC	CCT		TGGAC		GGGAC		CAGAA		AGGCT	TCTGAG		GGACC			GGACA	CTGCT	TGCCT	CTAGC	CTAGC
				AGGAAC		GAGCG			AGGGC		TTGAAT		GCCTG		CCAAA	GCCCG		CTGAG			GCAGA	GCAGA	GTGAC	CCTGC	CCTGC
				TGCAA		GAGCA			GCGCC		GGATC		GCAAG		GCTCCT	AAGCTT		ATGGG			GGCCT	GCCCA		CGCTG	CGCTG
				GTGATC		GGCTA			GCCCC		CACCA		GCCCC		CATCTA	GTAGA		GGGAA			GGATG	GGCAG		ATGCC	ATGCC
				AGCCT		GAGCG			CATCTA		CCCCTA		TAAGCT		CGCCG	CCAGC		AGCCG			GGAGT	GGCAG		AAGCC	AAGCC
				GGAAA		GAGCA			GGAAT		CATCCC		GCTGAT		CTTCTA	TGCTG		AGAAG			GATCTG	CAAAAC		CAAAA	GGAAA
				GCGGC		GACGC			CATCCC		CAAGG		CTATGC		GCCTG	GCGGA		GAAGA			GGGCA	GGCTG		ATCTTT	ATCTTT
				GACGC		GCGGC			TATCTT		GCGCA		CGCCA		CAGTCT	GCCGT		ACCCTC			ACGGC	ATCTAT		ACGCC	GCGCT
				CAGCAT		CCGCC			CGGCA		ACGGG		AAGGC		GGCGT	GCATAC		AGGAA			AACACC	CAGGT		GCGGT	GCGGT
				CCACG		ATACCG			CTACAA		CCAGA		AAGAG		GCCAT	GACTG		GGCCT			CACTAC	CACAG		GAAGC	GAAGC
				TGGAAA		TGGAAA			AATTAC		GCCCA		AGCGG		CTAGAT	GACTG		GTACAA			CACAG	CGCCC		ACACC	ACACC
				ATCTGA		TCATCC			GAAATT		AGTTCA		AGTGC		GCTGCG	GATTTT		TGAACT			CGCCC	CAGCC		CAGCC	CAGCC
				TCATCC		TGGCC			TCAGG		AGGCC		CCTGAA		TCTCTG	GCTCC		GCAGA			TGAGC	TGAAGT		TGAGG	TGAGG
				TGGCC		AACAAC			GAAGA		ACAATC		GATTCA		GGAAG	GCCTG		AAGATA			IGCCC	ATGGC		ATGGC	ATGGC
				AACAAC		AGCCT			GTGAC		ACAGC		GCGGA		CGGGA	CGGAA		AGATG			GATAG	CTGAG		GTGGA	GTGGA
				AGCCT		GTCCA			CATCAC		CGACG		AGCGG		CCGAC	GCGGG		GCGGA			ATTCAG	CATCTC		AATGG	AATGG
				GTCCA		GCAAC			CTCAAA		CTCCG		CTCCG		TTCACA	CCGCC		GGCCT			CAGAG	CAGAG		ACACC	ACACC
				GCAAC		GGCAA			AGTCCA		GCACC		GCACC		CTGAC	GCCAC		ACAGT			ACAGT	ACAGCA		CCGGC	AGATC	AGATC
				GGCAA		GAGTG			ACAAGA		CCAATA		GACTTT		CATATC	GACTT		GAGATT			GAGATT	GCAAG		TCTCC	TCCCC	TCCCC
				GGCAA		GAGTG			GCACC		CTGCTT		ACCCT		TTCTCT	ACCCT		GGGAT			GGGAT	TCCCA		ACGAT	ACGAT	ACGAT
				TGTGAC		CGAGA			AGCAC		ATATGG		CGCCT		CCGAA	CCGAA		GAAAG			GAAAG	GGTGTT		GAGGA	GAGGA	GAGGA
				CGAGA		GGGGC			CGCCT		AACTGT		CAGCT		GATCTG	CGGCG		GCGAG			GCGAG	CCTGAA		CCCTC	CCCTC	CCCTC
				GGGGC		GCAAC			ACATG		CTAGC		CCCTG		GCCAC	CGCGG		CGCGG			GAGGG	GATGAA		AAGATC	AGGCC	AGGCC
				GAGTG		GAGCA			GAACT		CTTCG		CAGCC		GCACC	GAGGG		GAGGG			CAGCC	AGCCA		AGTCG	AGTCG	AGTCG
				CGAGG		AACTG			GAGCA		AGGAC		GCCTG		ACTTCG	GGTCA		GCAAG			TGCAG	CGTGG		GTGAC	ATACG	ATACG
				AAGCT		GAAGC			AGAAG		CGAGG		GAACT		CCTGG	GCCAC		GGGCA			ACCGA	AAGCC		CAGAA	CAGAA	CAGAA
				GAAGA		ACATCA			ACATTC		CGAGG		ATACCG		CTATTG	GTACC		ACCTTC			AGCCA	AGATAC		GTGAA	GTGAA	GTGAA
				ACATCA		AAGAGT			ATTACT		CTGTGT		CTGTAG		CGCTA	GTCTA		CCTTTC			CAGTAC	CGGAT		GCACT	GCACT	GCACT
				AAGAGT		TTCTGC			GCGCC		GCGTGC		GCGTGC		GAGGC	ACTTCG		ACCTTC			GGACC	AGCCA		CATCCA	ACTCC	ACTCC
				TTCTGC		AGAGC			ACATTC		CGCCA		CGCCA		CGCCA	ACTTCA		GGACC			CGGAA	CCAAG		CAAGG	CAAGG	CAAGG
				AGAGC		TTCGTC			TGCCCT		TGGACT		TGGACT		ACATTC	GCCCT		CGAAA			GACAC	GACAC		CATTG	CCCTC	CCCTC
				TTCGTC		CACATC			GTTCG		ATTGG		ATTGG		GGACA	GTCGA		GTGGA			CTACGA	GGCCC		TCTGC	CATCT	CATCT

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				GTGCA					GCTTCA					CATTAA							AGGCA				CCTCT
				GATGTT					GAGAG					G							CAATG				GAGCG
				CATCAA					CAGGC												GTCACA				GCGAG
				CACCTG					CTTCGA												GTTTCT				TTCCT
				A					TATCTG												TCC				GGACA
									GGGCC													ACTGAA			CCAAA
									AGGGC													G			GACAG
									ACAACC																ACAGG
									GTGAC																CCGAA
									AGTTTC																GAGGA
									ATCT																CAGAC
										GGGCA															AGATG
										CCAAG															GATAC
										GTGGA															CGAAG
										AATCAA															CCGCC
										A															GCTTC
																									TGAAG
											GGGCA														CCCCA
											CCAAG														CAGGA
											GTGGA														TGTGA
											AATCAA														CATAT
											A														GCCCA
																									GCTGC
																									ATAGC
																									CTGAC
																									ACTGC
																									GGAGA
																									GAAGC
																									CACAG
																									AGCCT
																									CCACC
																									TTCTCA
																									AGAAG
																									GCCCA
																									TCTCCT
																									GCCGT
																									GCCTT
																									CCATC
																									TATGC
																									CACTC
																									TGGCC
																									ATTCAC
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGGS	QVQLV	GSTSGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	QVQLKE	GGGGS	ITTPAP	VGILVA
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLQE	SGAEVK		QSGAE	GKPGS	SPSSLS		SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLLPL	SGFGLV	GGGGS	TPPSLS	RPFTPA	VILLLLL
			ATRVHS	EDLIQS	SGVTPE	GIFWCC	GDVES	LPHFAF	KPGSSV		KVSCKA	GEGSTK	ASVGD		RVTITC	VPVFLP	VLLLSL	MNMTP	YKGQG	GDVES	ALLLHA	QPSQTL	GGGGS	VALGQS	PTIALQ	LLLFLI
				MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP	SVKVSC		SGGTFS		RASESV		RASQSI	PAPRPP	NHR	RRPGPT	NQLYNE	NPGPG	ARP	SLTCTV		VSISCK	PLSLRP	TSTQR
				LYTESD	GGSGG	PRCRE	SGEGR		KASGYT		SYAISW		DIVYGIS		SSYLN	TFAPTIA		RKHYQ	LNLGRR	SGEGR		SGFSLS		SSQSLV	EAQRPA	KADFQ
				VHPSCK	GGSGG	RRRNE	GSLLTC		FTDYNM		HWVRQ		FMNWF		WYGQIK	SQFLSL		PYAPPR	EEYDVL	GSLLTC		RYDMH		ASDENT	AGGAV	HPAGA
				VTAMKC	GSLQ	RLRRES	GDVEE		HWVRQ		APGQG		PGKAPK		PGKAPK	RPEACR		DFAAYR	DKFRG	GDVEE		WVRQP		YLNWLL	HTRGLD	VGPEP
				FLLELQ		VRPV	NPGP		GGGLE		WMGGII		LLIYAAS		LLIYAAS	PAAGG		S	RDFEM	NPGP		PGQGL		QSPGR	FACD	TDRGL
				VISLES					LEWIGVI		PIFGTA		KAPKLLI		SLQSGV	AVHTRG			GGKFR			EWMGVI		SPKRLI		QWRSS
				GDASIH					YPYNG		NYAQKF		YAASNQ		PSRFSG	LDFACD			RKNPQ			WGNGN		YQVSKL		PAADA
				DTVENL					GTGYN		QGRVTI		GSGVP		SGSGT				EGLYNE			THYHSA		DSGVP		QEENL
				IILANNS					QKFKSK		TADKST		SRFSGS		DFTLTIS				LQKDK			LKSRLSI		DRFSG		YAAVK
				LSSNGN					ATITAD		STAYME		GSGTDF		SLQPED				MAEAYS			SRDTSK		SGSEK		HTQPE
				VTESGC					ESTNTA		LSSLRS		TLTISSL		LATYYC				EIGIMKG			SQVFLK		DFTLKIS		DGVEM
				KECEEL					YMELSS		EDTAVY		QPDDFA		QQSYST				ERFRG			MNSLQI		RVEAED		DTRSP
				EEKNIK					LRSEDT		YCATFA		TYYCQ		PFTFGP				KGHDG			EDTAIY		LGVYYC		HDEDP
				EFLQSF					AYYYCA		LFGFRE		QSKEVP		GTKVDI				LYQGLS			FCTLRIK		LQGIHL		QAVTY
				VHIVQM					RGRPA		QAFDIW		WTFGQ		K				TATKDT			DWGPG		PWTFG		AEVKH
				FINTS					MDYWG		GGGTT		GTKVEI						YDALHM			TMVTVS		GGTKLE		SRPRR
									QGTLVT		VTVSS		K						QALPPR			S		LK		EMASP
									VSS																	PSPLSG
																										EFLDTK
																										DRQAE

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders								Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

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SB06337			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
	Retroviral	DNA	ATGGA	AATTGGA	TCAGG	TTGCTG	CAGTGT	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGCT	GAGGT	GGAGG	GACGT	ACAACA	GTGATG	CTGCG
			CTGGA	CTCAAC	CGCGCG	CCTAG	ACCAAC	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CAGAT	CGGCG	CAGCA	GAGCA	GAAGT	CAAGC	GAAGTT	CACAAA	CTGCC	GCAGC	CGGAG	GGTCAT	ACAGC	GGCATT	GCACA
			CTTGGA	GTGATC	GTGGT	CTGGG	TACGC	GGTTAC	GGCTCT	AGCTCT	GTGCA	TGGCT	GACAC	GATCC	GACTCA	AACAGC	GCCCC	GGAGC	CAGCA	TTATGC	CGTGA	TGGTTG	GATCTG	GAACAC	CGCAC	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCTGCT	ATCTCT	GAGCG		ATCTGG	CTGGC	GTCCC		GTCCC	ATCATG	TCTGG	AGACT	GGAGC	ACTGCT	CAGCTT	AATCTG	GTGGC	AGAGC	CTCGG	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	GAAACT	GCTGC	GAGCC		CGCTG	AAACCT	CCTAGT		CAAGC	TACTTC	CTGGC	GCTGC	GCAGA	GAAGC	TGCTG	GCGGA	GGAGG	CCTCTG	CCTCCA	ATGATC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	GGCCG	TGTGC	GAAGT		AAGTGA	GGAAG	AGCCT		AGCCT	AGCCA	ACATG	GCTGC	CTGCC	TCGCC	CTGCCT	GGACT	AAGTG	AGCCT	ACTCCA	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	GCGAC	GAGCT	GAAGA		AAAGCC	GGCGG	GTCAG		GTCAG	CTTGGT	GGAGT	GACTAC	CCGCG	GGGGA	TTGGCA	GGTTCA	GCGGA	GGCTG	GCTCC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	GTGGA	GCCCC	AACCTG		CCGGC	AGGGA	CCAGC		CCTCC	GCCCG	TCTGCT	ATGAAC	TACAAG	TGTCGA	CTGCT	GCCTG	GGCGG	TGACAC	AACAAT	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	ATCTAA	ATCCTG	TCCTCC	CTACC	GTGGG		GTGGG		GTGGG	TGTTTC	GCTCA	ATGACC	CAGGG	GAGTAA	GCTGC	GCGGA	TTCT		TGGGA	TGCACT	TTGCTT	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	TCTGCT	CCTTTC	AGCGT		GTGAAA	AAGGG	AGACA		CGATA	TGCCC	GCCTG	CCTAGA	CCAGA	CCCAG	ATGCTG			CAGCC	GCAAC	TGTTCC	AAGGC
				CACATC	TATCTT	CTGCCT	GACCT	TGCTTA	GAAGG		TGTCTC		GAGTG		ACCATT	GCCTA	ACCCT	CGGAC	CTCTAT	GGAAG	CA			TGCCA	CCCTG	TGATC	CGACT
				GAGGC	CAGCC	GACTA	GGATCT	TTGCT	TGTCCT		GCCAA		ACAATT		ACATGC	CAACAA	GTACTG	CTACCA	AACGA	CGGAG				GCTGC	AGGCC		CATCC
				CACACT	TGATCG	CTGCTT	GGCGA		GCCAG		ACCTGT		CAATT		ACATGC	CAACAA	GTACTG	CTACCA	AACGA	CGGAG				GCTGC	AGGCC		CATCC
				GTACAC	GCGGA	CGCCC	GGGAC		GCTTCT		ACACCT		CGGCT		AGAGC	CCCCT	CAACCA	GAAAG	GCTCAC	AAGGT				AAGTCT	CGAGG		TGCTG
				CGAGT	GGTTC	CTAGAT	GCGGG		GGCGG		ACACCT		CGGCT		AGAGC	CCCCT	CAACCA	GAAAG	GCTCAC	AAGGT				AAGTCT	CGAGG		TGCTG
				CCGAT	CGGAG	GCAGA	AGTCTA		CAGCTT		TTACCG		AGAGC		AGTCCA	AAGACC		CAGCC	ACGAA	TAGTCT				CAGCTT	GGTGG		GTTGG
				GTGCA	GTGGC	GAGCG	CTGAC		CAGCA		ACTACA		CAACTA		TCTCCA	ACCTAC		TTACGC	GAGAG	ACTAAC				GGTGG	GCAGC		ACCTG
				CCCTA	GGTTC	GAGAA	GTGTG		GCTAC		ATATGC		CAACTA		GCTAC	ACCAG		TCCTCC	GAGTA	GTGTG				CCAGC	TGGCG		AGCCT
				GCTGC	CGGCG	GAAAC	GAGAC		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA		TAGAGA	CGATGT	GTGAT				GACGA	GAGCT		ACAGA
				AAAGTG	GAGGA	GAGCG	GTGGA		GTCCG		CTCTTG		TCAGCT		TGGTAT	CAATCG		TTTGGC	TTTGGA	GTAGAA				GAACA	GTTCAC		TAGAG
				ACCGC	TCTCTT	GCTGA	GGAAA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC		CGCCT	CAAGA	GAAAAT				GGTCC	CCTACC		GACTG
				CATGAA	CAA	GAAGA	ACCCT		GACAG		CACCA		ACTGGT		AAAACC	CCAGCC		ACCGG	GACGT	CCTGG				GACAG	TGAACT	GGCCT	CACTG
				ATCTGA		GAAAG	GGACC		GCACC		GGCCA		TCCAG		CGGAA	TCTGTC		GGCCG	GGCCC	ACCT				GCCCC	GGTTC	GGACTT	GCGGT
				TCTGCT		CGTGC	T		TGGAC		GGGAC		AGGCT		AGGCT	TCTGAG		TCC	GGACC					TGGCA	CAGCA	TGCCT	CTAGC
				GGAAC		GAGCG			AGGGC		TTGAAT		GCCTG		CCAAA	GCCCG			TCAGG					AAGGA	GAGGC	GTGAC	CCTGC
				TGCAA		GGCGT			CTCGA		GTGGA		GCAAG		GCTCCT	AAGCTT			ATGGG					CTTGAA	CCGGA		CGCTG
				GTGATG		GTG			GTGGA		GGCTA		GCCCC		CATCTA	GTAGA			GGGAA					TGGGT	CAGTCT		ATGCC
				AGCCT					TGGGA		CATCTA		TAAGCT		CGCCG	CCAGC			AGCCG					GTCCG	CCTAGA		CAAGA
				GGAAA					GGAAT		CCCCTA		GCTGAT		CTTCTA	TGCTG			AGAAG					TGATCT	CGGCT		GGAAA
				GCGGC					CATCCC		CAACG		CTATGC		GCCTG	GCGGA			GAAGA					GGGGC	GATCTA		ATCTTT
				GACGC					TATCTT		GCGGC		CGCCA		CAGTCT	GCCGT			ACCCTC					AACGG	CCAGG		ACCGC
				CAGCAT					CGGCA		ACGGG		GCAATC		GGCGT	GCATAC			AGGAA					CAACAC	TGTGCA		GCGGT
				CCACG					CTACAA		CTACAA		AAGGC		GCCAT	AAGAG			GGCCT					ACACTA	AGCTG		GAAGC
				ATACCG					AATTAC		CCAGA		AGCGG		CTAGAT	GACTG			GTACAA					CCAGA	GATAG		ACACC
				TGGAAA					GCCCA		AGTTCA		AGTGC		TCTCTG	GATTTT			TGAACT					GCGCC	CGGCG		CAGCC
				ATCTGA					GAAATT		TCAGG		AGGCC		GGAAG	CGAGA			GCAGA					CTGAA	TGCCC		TGAGG
				TCATCC					TCAGG		GAAGA		ACAATC		CGGGA				AAGATA					GTCCC	GATAGA		ATGGC
				TGGCC					GAAGA		GTGAC		ACAGC		CCGAC				AGATG					GGTTCA	TTTTCT		GTGGA
				AACAAC					GTGAC		CATCAC		CGACG		TTCAC				GCGGA					CCATCT	GGCAG		AATGG
				AGCCT					CATCAC		CTCCG		CGACG		TTCAC				GCGCT					CCAGA	CGGCT		ACACC
				GTCCA					CGCCG		GCACC		CCAGC		CTGAC				AGCGT					GACAA	CTGGC		AGATC
				GCAAC					ACAAGA		CCAATA		GACTTT		CATATC				GACTT					CAGCA	CCAGA		TCCCC
				GGCAA					GCACC		CTGCTT		ACCCT		TTCTCT				GGGAT					AGAACA	CTTCAC		ACGAT
				TGTGAC					AGCAC		ATATGG		GACAAT		TTGCAT				GGAAG					CCGTGT	CCTGAA		GAGGA
				CGAGA					CGCCT		AACTGT		CAGCT		CCGAA				GCGAG					ACCTG	GATCA		CCCTC
				GCGGC					ACATG		CTAGC		CCCTG		GATCTG				CGCGG					CAGAT	GCAGA		AGGCC
				TGCAAA					GAACT		CTTCG		CAGCC		GCCAC				GAGGG					GAACA	GTGGA		GTGAC
				GAGTG					GAGCA		AGGCG		TGACG		GTACTA				GCAAG					GCCTG	AGCGG		ATACG
				CGAGG					GCCTG		AGGAC		ACTTCG		TTGTCA				GGGCA					AGGAC	AGGAC		CAGAA
				AACTG					AGAAG		ACAGC		CACCT		GCAGT				CGATG					CGAGG	GTGGG		GTGAA
				GAAGA					CGAGG		CGTGTA		ACTACT		CCTACA				GCCTT					ACACC	CGTGTA		GCACT
				GAAGA					ATACGG		CTATTG		GCCAG		GCACC				ACCAG					GCCGT	CTACTG		CCAGA
				ACATCA					CTGTGT		CGCTA		CAGAG		CCTTTC				GGTCT					GTACTA	TCTGCA		CCTCG
				AAGAGT					ATTACT		GAGGC		CAAAGA		ACCTTC				CAGTAC					CTGCA	AGGCA		GAGAG
				TTCTGC					GCGCC		GGTGC		AGACC		GGACC				AGCCA					TCCCTG	TCCATC		AGATG
				AGAGC					ACATTC		CGCCA		CGCCA		CGGAA				CCAAG					AGAATC	TGCCTT		GCAAG
				TTCGTC					GCCCT		TGGACT		ACATTC		CAAAG				GACAC					AAGGAT	GGACC		CCCTC
				CACATC					GTTCG		ATTGG		GGACA		GTGGA				CTACGA					TGGGG	TTTGGC		CATCT

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders					Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
				GTGCA					GCTTCA	EVQLVQ	GGGGS	QVQLV	GSTSGSG	DIQMTQ	GGGGS	CATTAA									CCTCT
				GATGTT					GAGAG			GGACA		GGGCA		G									GAGCG
				CATCAA					CAGGC	QSGAE	GKPGS	GGAAA		CCAAG							CAGGAG				GCGAG
				CACCTC					CTTCGA	GKPGS	GEGSTK	CCCTG		GTGGA							GCTGG				TTCCT
				A					TATCTG	KPGSSV	ASVGD	GTCACA		AATCAA							AAATCA				GGACA
									GGGCC	KVSCKA	RVTITC	GTGTC		A							AG				CGAAA
									AGGGC	SGGTFS	RASESV	CAGC													GACAG
									ACAACC	SYAISW	DNYGIS														ACAGG
									GTGAC	VRQAP	FMNWF														CCGAA
									AGTTTC	GQGLE	QQKPG														GAGGA
									ATCT	WMGGII	KAPKLLI														CAGAC
										PIFGTA	YAASNQ														AGATG
										NYAQKF	GSGVP														GATAC
										QGRVTI	SRFSGS														CGAAG
										TADKST	GSGTDF														CCGCC
										ATITAD	TLTISSL														GCTTC
										ESTNTA	QPDDFA														TGAAG
										YMELSS	TYYCQ														CCCCA
										LRSEDT	QSKEVP														CAGGA
										AYYYCA	WTFGQ														TGTGA
										RGRPA	GTKVEI														CATAT
										MDYWG	K														GCCCA
										QGTLVT															GCTGC
										VSS															ATAGC
																									CTGAC
																									ACTGC
																									GGAGA
																									GAAGC
																									CAGAG
																									AGCCT
																									CCACC
																									TTCTC
																									AAGAA
																									GGCCC
																									ATCTC
																									CTGCC
																									GTGCC
																									TTCCA
																									TCTAT
																									GCCAC
																									TCTGG
																									CCATT
																									CAC
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGS	QVQLV	GSTSGSG	DIQMTQ	GGGGS	ALSNSI	IYWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	EVQLVE	GGGGS	TTTPAP	VGILVA	LRHRR
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLCE	SGAEYK	QSGAE	GKPGS	SPSSLS	SPSSLS		MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLPL	SGGGL	GGGGS	QSPLSL	RPPTPA	VILLLLL	QGKH
			ATRVHS	EDLIQS	SGVTPE	GIFVICC	GDVES	LPHPAF	KPGSSV			ASVGD			VPVFLP	VLLLSL	MNMTP	YKQGQ	GDVES	ALLLHA	VQPGG	GGGGS	PVTLGQ	PTIALQ	LLLLFLI	WTSTQ
				MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP	KVSCKA			RVTITC	AKFTTT	VITLYC	RRPGPT	NQLYNE	NPGPG	SLRLSC	PASISC	PLSLRP	RKADF
				LYTESD	GGSGG	PRCRE	SGEGR		SGGTFS			RASESV	RASQSI	PAPRPP	NHR	PYAPPR	EEYDVL	GSLLTC	AASGFT	KSSQSL	EACRPA	QHFAG
				VHFSCK	GGSGG	RRRNE	GSLLTC		SYAISW			DNYGIS	SSYLN	TPAPTIA			DKRRG	GDVEE	FSRYD	VASDEN	AGGAV	AVGPE
				VTAMKC	GSLQ	RLRRES	GDVEE		VRQAP			FMNWF	WYQQK	SQPLSL			RDPEM	NPGP	MHWVR	QAPGK	GLEWV	SVIWGN	GNTHY	HSALKS	RFTISR	DNSKNT	TYLNWF	HTRGLD	PTDRG
				FLLELQ		VRPV	NPGP		GGGLE			APGQG	FGKAPK	RPEACR			GGKFR		QAPGK	GLEWV	SVIWGN	GNTHY	HSALKS	RFTISR	DNSKNT	TYLNWF	HTRGLD	QQRPG	FACD	LQWFS
				VISLES					WMGGII			LEWIGYI	KAPKLLI	RPEACR			RKNPQ		QAPGK	GLEWV	SVIWGN	GNTHY	HSALKS	RFTISR	DNSKNT	TYLNWF	HTRGLD	QSPRRL		SPAAD
				GDASIH					PIFGTA			YPYNG	YAASNQ				EGLYNE		LQKDK	MAEAYS	EIGMKG	ERRRG	KGHDG	LYQGLS	TATKDT	YDALHM	QALPPR	SLRAED	VYYCLQ	GIHLFW	TFGQGT	KLEIK	QAEEN
				DTVENL					NYAQKF			GTGYN	GSGVP																VYYCLQ	GIHLFW	TFGQGT	KLEIK	LYAAV
				ILANNS					QGRVTI			QKFKSK	SRFSGS																				KHTQP
				LSGNGN					TADKST			ATITAD	GSGTDF																				EDGVE
				VTESGC					STAYME			ESTNTA	TLTISSL																				MDTRS
				KECEEL					LSSLRS			YMELSS	QPDDFA																				PHDED
				EEKNIK					EDTAYY			LRSEDT	TYYCQ																				PQAVT
				EFLQSF					YCATFA			AYYYCA	QSKEVP																				YAEVK
				VHIVQM					LFGFRE			RGRPA	WTFGQ																				HSRPR
				FINTS					QAFDIW			MDYWG	GTKVEI																				REMAS
									GQGTT			QGTLVT	K																				PPSPL
									VTVSS			VSS																				SGEFL

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders	Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder	Hinge	TM domain	LIR1 ICD
																			DTKDR QAEED RQMDT EAAAS EAPQD VTYAQ LHSLTL RREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
SB06340			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
	Sinvec	DNA	ATGGA CTGGA CTTGGA TACTCT TTCTGG TCGCT GCCGC CACAC GGGTG CACTCT	AATTGG GTCAAC GTGATC AGCGA GCCTGAA GAAGAT CGAGG TGCAGA TGCAAC GCATG CACATC	TCAGG CGGCG TGGAT AGTGG AGGCG GAGGC TCAGG CCCCT GAGCC CTATCTT CTGCCT	TTGCTG CCTAG CTGGG CCATCA CACTGA GAGCG TCAGG GGCAT CTTCGT GATCTG CTGCCT	CAGTG TACCAA CTACG CCCTG ACTGAA TCTCG TGAAC ACGTG GAATCT AATCCT	ATGCTG CTGCT GGTTAC ATCTCT GCTGC GAGCT CGGCG GCCCC ATCCTG GCTTTC	GAAGTT CGGTG GGTTCA GAGCG GAGCC GAAGT GAAGA GCCCC AACCTG AGCAGC CCTTTC	GGCGG CAGTTC GCTCT TGTGC GAGCT AAGGA AACCTG GAAGT GGAAGT CTTCGT AGCGT TGCTA	GAGTTGGCCTC CAGCTTTACATC GTGCA TGGCT GACAC ATCTGGCTGGC CGCTG AAACCT AAGTGAGGAAG AAAAGCCGGCG CCGGC AGGGA TCCTCCTCTACC GTGAAAAAGGG GAGTG ACCTGT TCCAG GCCAG CGGCT ACACCT CAGCG AGAGC ACTACA ATATGC ATTGG GTCCG CCAGG CACCA GCCAC TGGAC GGCCA CAAGA GCCTG GCAAG CATCTA CCCCTA CAACG GCGGC CGCCA CTACAA CCAGA AGTGC AGTCCA AGGCC ACAATC ACAGC CGACG AGTCCA CCAATA ACGCT ATATGG AACTGT CCCTG CAGCC TGACG ACTTCG CCACCT ACTACT GCCAC TGGACT	GACATC CAGAT GACTCA GTCCC CAAGC GTCAG CCTCC GTGGG CGTGA ACCATT ACATGC AGAGC CAGCC AGAGC TCTCCA GCTAC CTGAAG ITGGTA CAGCA AAAACC CTTCTA GCCTG CCAAA GCTCCT CATCTA CGCCG CTTCTA GCCTG CAGTCT GGCGT CTAGAT TCTCTG GCTCC GGAAG CGGGA CCGAC TTCACA CTGAC CATATC TTCTGT GCAGT GTACTA TTGTGA GCAGT CCTACA GGTGC CAAAG	GGCGT GAGCA GGCCC ATCATG TACTTC CTTCGT AAGCC CCAGC GCTCCT AAGCC GCTGAG TCTGAC CCAGC CCAGC CATCTA TTGCTG GCCAG GCCGG CTTGTG GCTGAT TCTGAG GCCAG GCCGT AAGAG CGAC	ATCTAT ATCTG GGAGC CTCTG AGCGC GCTGC GACTAC CACAT GTTCTG ATGAAC TACAAG GA CTGCT CAGCC TGGTC ATCACC CTGTAC TGCAA TGCAC CAGCC ACGAA TTAGTC GAGAG ACTAAC GATGA GTGTG ACCAG GAAAG CTGAAG CTGCTG GCGGG CCAGC CCAGC ACCTAC GCGCA GCGGT CTGAAG GCGGC GCGAT CCTGC GCGGC GCGAT GCGGC GCGAA GCGAT GCGAT GCGAT

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR	EMCN binder	Hinge	TM domain	LIR1 ICD
									GTTCG		ATTGG		GGACA	GTGGA				CTACGA			TGGGG				CATCT
				CACATC					GCTTCA		GGACA		GGGCA	CATTAA				CGCCC			CCAGG				CCTCT
				GTGCA					GAGAG		GGAAA		CCAAG	G				TTCACA			GCACC				GAGCG
				GATGTT					CAGGC		CCCTG		GTGGA					TGCAG			ATGGTC				GCGAG
				CATCAA					CTTCGA		GTCACA		AATCAA					GCCCT			ACCGTT				TTCCT
				CACCTC					TATCTG		GTGTC		A					GCCCC			TCTTCT				GGACA
				A					GGGCC		CAGC							CTCGC							CCAAA
									AGGGCC																GACAG
									ACAACC																ACAGG
									GTGAC																CCGAA
									AGTTTC																GAGGA
									ATCT																CAGAC
																									AGATG
																									GATAC
																									CGAAG
																									CCGCC
																									GCTTC
																									TGAAG
																									CCCCA
																									CAGGA
																									TGTGA
																									CATAT
																									GCCCA
																									GCTGC
																									ATAGC
																									CTGAC
																									ACTGC
																									GGAGA
																									GAAGC
																									CACAG
																									AGCCT
																									CCACC
																									TTCTCA
																									AGAAG
																									GCCCA
																									TCTCCT
																									GCCGT
																									GCCTT
																									CCATC
																									TATGC
																									CACTC
																									TGGCC
																									ATTCAC
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGGS	QVQLV	GSTSGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	EVQLVE	GGGGS	DVVMT	TTTPAP	VIGILVA
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLCE	SGAEYK		QSGAE	GKPGS	SPSSLS		SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLLPL	SGGGL	GGGGS	QSPLSL	RPFTPA	VILLLLL
			ATRVHS	EDLIQS	SGVTPE	GIFVICC	GDVES	LPHPAF	KPGSSV		KVSCKA	GEGSTK	ASVGD		RVTITC	AKPTTT	VTLYC	VLLLSL	MNMTP	YKQGQ	GDVES	ALLLHA	VQPGG	GGGGS	PVTLGQ	PTIALQ	LLLFLI
				MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP	KVSCKA		SVKVSCG		RVTITC		RASQSI	PAPRPP	NHR	RRPGPT	NQLYNE	NPGPG	ARP			PASISC	PLSLRP	TSTQR
				LYTESD	GGSGG	PRCRE	SGEGR		SGGTFS		KASGYT		RASESV		SSYLN	TFAPTIA		RKHYQ	LNLGRR	SGEGR				KSSQSL	EACRPA	KADFQ
				VHPSCK	GGSGG	RRRNE	GSLLTC		SYAISW		FTDYNM		DNYGIS		WYGQK	SQPLSL		PYAPPR	EEYDVL	GSLLTC				VASDEN	AGGAV	HPAGA
				VTAMKC	GSLQ	RLRRES	GDVEE		VRQAP		HWVRQ		FMNWF		SQPLSL	DFKHRG		DFAAYR	DKHRG	GDVEE				MHWVR	VGPEP
				FLLELQ		VRPV	NPGP		GQGLE		APGQG		QQKPG		RPEACR	RDPEM		S	GKFPR	NPGP				QAPGK	TDRGL
				VISLES					WMGGII		LEWIGYI		KAPKLLI		PAAGG	GGKFR			RKNPQ					GLEWV	QWRSS
				GDASIH					PIFGTA		YPYNG		YAASNQ		LLIYAAS	RKNPQ			EGLYNE					SVIWGN	QSPRRL	FAADA
				DTVENL					NYAQKF		GTGYN		GSGVP		SLQSGV	EGLYNE			LQKDK					GNTHY	IYQVSK	QEENL
				IILANNS					GGRVTI		QKFKSK		SRFSGS		AVHTRG	LQKDK			MAEAYS					HSALKS	LDSGVP	YAAYK
				LSGNGN					TADKST		ATITAD		GSGTDF		PSRFSG	MAEAYS			EIGMKG					RFTISR	DRFSG	HTQPE
				VTESGC					STAYME		ESTNTA		TLTISSL		LDFACD	EIGMKG			ERRRG					DNSKNT	SGSGT	DGVEM
				KECEEL					LSSLRS		YMELSS		QPDDFA			ERRRG			KGHDG					LYLQMN	DFTLKIS	DTRSP
				EEKNIK					EDTAVY		LRSEDT		TYYCQ			KGHDG			LYQGLS					SLRAED	RVEAED	HDEDP
				EFLQSF					YCATFA		AYYYCA		QSKEVP			LYQGLS			TATKDT					TAVYYC	VGVYY	QAVTY
				VHIVQM					LFGFRE		RGRPA		WTFGQ			TATKDT			YDALHM					TLRIKD	CLQGIH	AEVKH
				FINTS					QAFDIW		MDYWG		GTKVEI			YDALHM			QALPPR					LPWTF	LPWTF	SRPRR
									GQGTT		QGTLVT		K			QALPPR								WGQGT	GQGTK	EMASP
									VTVSS		VSS													MVTVSS	LEIK	PSPLSG
																										EFLDTK

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																											DRQAE EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
									FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL						EMCN VH	(GGGGS)3 linker	EMCN VL			
SB06342																									
	Sinvec	DNA	ATGGA CTGGA CTTGGA TACTCT GCGAT TTCTGG TCGCT GCGGC CAGAC GGGTG CACTCT	IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa		CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL		CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
			AATTGGT GTCAAC CGGCGG TTGCTG CCTAG CGGCG TCAGG CCTGG CTGGA AGCGA AGTGG CCATCA CCCTG ATCTCT GAGAG TCAGG TGAAC GGCAT ACGTG GCCCC AACCTG CTCGT GAATCT ATCCTG GCAGC CTTCGT GAATCT CCTTTC AGCGT TGTCGT GAATCCT TGCTT GACGC CGGCG GAGGG ACGTG TGGAAG GCAGA GGAGT CTACTG GAGCG ACGTG GAGGA AAACC CTGGA CGTTC GAAAC TGGAAG CTTCTT GGCGG GAGCT GCCCC GTGAA GAGGA CTCTT GCTGA GAAGA CTGGA CCT GTG	CAGGTT GAAGTT CAGCT CGGTG CTACAA CTGCT GGTTAC GGTTCA TGTCGT GAAGA GCCCC GGCAGA TTACCG GAAGG GCACC TGGAC GTGAA CATCTA GTGGA GCCCA AGTTCA AGTCCA CCAATA CTGCTT ATATGG CGTGTA CTATTG CTGTGT ATTACT	GGCGG CGGTG AGCTCT GAGCAC GAGCC GAAGA GCCCC AACCTG GGCGG GAGGC GCAGA AGGGA GGTTC CAGCT GAGCG GAAGG AAACC CTGGA GCCCC GCCTG GTGAC GGCCA GCACC AGCAC CCTGC GCCTG AGAAG CGAGG GCCTG AGGAC GCCCC TTACCG GGAAT CATCCC CGGCA CCGCC GTGAC GCCCA ACAAGA GCACC AGCAC CGCCG ACATG CGAGG GAACT GCCAG GAAGA CTTCG GAGCA GGCCC AGAAG CTGCC GAGCT GAAGA GGCGG GAGCG ACGTG GAGGA ACGTG GAGGG GCAGG GCAGG GCA

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	**QCTNY**	MLLLVT	EVQLVQ	GGGGS	QVQLV	GSTSGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	**QCTNY**	MALPVT	EVQLVE	GGGGS	DVVMT	TTTPAP	VGILVA
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	**ALLKLA**	SLLLQF	SGAEVK		QSGAE	GKPGS	SPSSLS		SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	**ALLKLA**	ALLLPL	SGGGL	GGGGS	QSPLSL	RPFTPA	VILLLLL
			ATRVHS	EDLIQS	SGVTPE	GIFWCC	**GDVES**	LPHFAF	KPGSSV		VKKPGS	GEGSTK	ASVGD		ASVGD	VPVFLP	YKQGQ	MNMTP	YKQGQ	**GDVES**	ALLLHA	VQPGG	GGGGS	PVTLGQ	PTIALQ	LLLFLI
				MHIDAT	**PIFSLIG**	LTYCFA	**NPGPG**	LLIP	KVSCKA		SVKVSC	G	RVTITC		RVTITC	AKPTTT	VITLYC	RRPGPT	NQLYNE	**NPGPG**	ARP	SLRLSC		PASISC	PLSLRP	KADFQ
				LYTESD	GGSGG	PRCRE	**SGEGR**		SGGTFS		SYAISW		RASESV		RASQSI	PAPRPP	NHR	RKHYQ	LNLGRR	**SGEGR**		AASGFT		KSSQSL	EACRPA	HPAGA
				VHPSCK	GGSGG	RRRNE	**GSLLTC**		SYAISW		VRQAP		FTDYNM		SSYLN	TFAPTIA		PYAPPR	EEYDVL	**GSLLTC**		FSRYD		VASDEN	AGGAV	VGPEP
				VTAMKC	GGSLQ	RLRRES	**GDVEE**		HWVRQ		APGQG		FMNWF		WYQQIK	SQPLSL		DFAAYR	DKRRG	**GDVEE**		MHWVR		TYLNWF	HTRGLD	TDRGL
				FLLELQ		VRPV	**NPGP**		GGGLE		LEWIGY	I	QQKPG		PGKAPK	RPEACR		S	RDPEM	**NPGP**		QAPGK		QQRPG	FACD	QWRSS
				VISLES					WMGGII		PIFGTA		KAPKLLI		LIYAAS	PAAGG			GGKPR			GLEWV		QSPRRL		PAADA
				GDASIH					PIFGTA		NYAQKF		YAASNQ		SLQSGV	AVHTRG			RKNPQ			SVIWGN		IYQVSK		QEENL
				DTVENL					NYAQKF		QGRVTI		YPYNG		PSRFSG	LDFACD			EGLYNE			GNTHY		LDSGVP		YAAVK
				IILANNS					QGRVTI		TADKST		GTGYN		SGSGT				LQKDK			HSALKS		DRFSG		HTQPE
				LSSNGN					TADKST		ATITAD		QKFKSK		DFTLTIS				MAEAYS			RFTISR		SGSGT		DGVEM
				VTESGC					STAYME		ESTNTA		SRFSGS		SLQPED				EIGMKG			DNSKNT		DFTLKIS		DTRSP
				KECEEL					LSSLRS		YMELSS		GSGTDF		LATYYC				ERFRG			LYLQMN		RVEAED		HDEDP
				EEKNIK					EDTAVY		LRSEDT		TLTISSL		QGSYST				KGHDG			VGVYY		VGVYY		QAVTY
				EFLQSF					YCATFA		AYYYCA		QPDDFA		PFTFGP				LYQGLS			CLQGIH		CLQGIH		AEVKH
									LFGFRE		RGRPA		TTYCQ						TATKDT			TLRIKD		LPWTF		SRPRR
													QSKEVP													
													WTFGQ													

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders					Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
				VHIVQMFINTS					QAFDIWGGGTTVTVSS		MDYWGQGTLVTVSS		GTKVEIK		GTKVDIK					WGGQGTMVTVSS				EMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
SB06343			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
	Sinvec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	ACAACA	GTGATC	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CACAT	CGGCG	CACAT	GAGCA	ATCTG	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	ACACC	GGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTGGT	CTACG	GGTTAC	GGTTCA	AGCTCT	GTGCA	TGGCT	GACAC	GATCC	GACTCA	AACAGC	GGCCC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	CGCAC	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	GAGCG		ATCTGG	CTGGC	AGAGC		GTCCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CTCGG	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAC	GCTGC	GAGCC		CGCTG	AAACCT	CCTAGT		CAAGC	TACTTC	GCTGG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	CCTCCA	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT		AAGTGA	GGAAG	AGCCT		AGCCT	AGCCA	ACAGC	CGCCC	TCGCC	GCTGC	GGACT	AAGTG	GAGCC	ACTCCA	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA		AAAGCC	GGCG	GAAGCG		GTCAG	CTTCGT	GCGGA	GACTAC	CCGCG	GGGGA	CTTTGG	GTTCA	GCGGA	TGCCT	GCTCC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG		CGGC	AGGGA	CCAGC		CCTCC	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	AACAAT	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC		TCCTCC	TCTACC	GTGGG		TGTGG	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT	ACTGG	TGCACT	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCC	CCTTCA	AGCGT		GTGAAA	AAGGG	AGACA		CGATA	TGCCC	CAGCC	CCTAGA	CCCAG	GCATG	CTGCTA	AGACT		GACAG	GCAAC	TGTTCC	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG		GTGTCTC		GAGTG		GAGTC	GCCAA	TGGTC	CGGCC	ACCAG	GACCT	CTGCTA	AGACT		CCTGC	CCCTG	TGATC	CGACT
				GAGGC	CAGCC	GACTAT	TGAAT	TTGCT	TGTCCT		TGTAAA		ACAATT		AGCCTA	ATCACC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG	CAGCA	AGTCTG				
				CACACT	TGATCG	CTGCTT	CTGGC		GCCAA		GCCAG		ACCTGT		ACATGC	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG	TGCCG	TCAGCT	AGGCC				
				GTACAC	GCGGA	CGCCC	GAGGG		GCTTCT		CGGGT		CGGGC		AGAGC	CCCCT	TGCAA	GAAAG	GCTCAA	AAAGGT	CCAGC	GCAAG	CGAGG				
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG		ACACCT		CAGCG		CAGCC	GCTCCT	CCACA	CACTAC	TCTAGG	GGTGG	GGACTT	TCTAGC	CCTGTA				
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT		TTACCG		AGAGC		AGTCCA	AGAGCC	GA	CAGCC	AGGAA	TAGTCT	CACCTT	CAGTCT	GGCCA				
				GTGCA	GAGCG	CTACTG			CAGCA		ACTACA		GTGGA		TCTCCA	ACCTAC		TTACGC	GAGAG	ACTAAC	CAGCA	CTGGT	GCAGC				
				CCCTA	GGTTG	GAAGA	ACGTG		GCTAC		ATATGC		CAACTA		GCTAC	ACCAG		TCCTCC	GAGTA	GTGTG	GATAC	GGCCA	TGGCG				
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA		TAGAGA	CGATGT	GTGAT	GATATG	GCGAC	GAGCT				
				AAAGTG	GAGGA	GAGCG	AGGTG		CTCTTG		GTCGG		TCAGCT		TGGTAT	CAATCG		TTTCGC	TTTGGA	GTAGAA	CACTG	GAGAA	GTTCAC				
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC		CGCCT	CAAGA	GAAAAT	GGTCC	CACCTA	AACTAG				
				CATGAA	CAA	GAAGA	AAACC		GACAG		CACCA		ACTGGT		AAAACC	CAGCC		ACCGG	GACGT	CCTGG	GACAG	CCTGA	GGCCT				
				GTGCTT		GAAAG	CTGGA		GCACC		GGCCA		TCCAG		CGGAA	TCTGTC			GAGCG	ACCT	GCCCC	ACTGG	GGAGT				
				TCTGCT		CGTGC	CCT		TGGAC		GGGAC		CAGAA		AGGCT	TCTGAG			GGACC		TTCCAG	TGCCT				
				AGGGC		GGCCT			AGGGC		GCCTG		GCCAA		CCAAA	GCCCG			ATGGG		AAGGA	CTAGC				
				TGCAA					CTCGA		TTGAAT		GCAAG		GCTCCT	AAGCTT			GGCCT		CTTGAA	TCCAA				
				GTGATC					GTGGA		GGCTA		GCCCC		CATCTA	GTAGA			AGCCG		TGGGT	GACAG				
				AGCCT					TGGGA		CATCTA		TAAGCT		CGCCG	CCAGC			AGAAG		GTCCG	CCTGA				
				GGAAA					GGAAT		CCCCTA		GCTGAT		CTTCTA	TGCTG			AGAAG		TGATCT	AGACG				
				GCGGC					CATCCC		CAAGG		CTATGC		GCCTG	GCGGA			GAAGA		GGGGC	GCTGA				
				GACGC					TATCTT		GCGCC		CGCCA		CAGTCT	GCCGT			ACCCTC		AACGG	TCTACC				
				CAGCAT					CGGCA		ACCGG		GCAATC		GGCGT	GCATAC			AGGAA		CAACAC	AGGTG				
				GCCAC					CCACC		CTACAA		AAGGC		GCCAT	AAGAG			GGCCT		ACACTA	TCCAA				
				ATACCG					AATTAC		CCAGA		AGCGG		CTAGAT	GACTG			GTACAA		CCACA	GCTGG				
				TGGAAA					GCCCA		AGTTCA		AGTGC		TCTCTG	GATTTT			TGAACT		GCGCC	ATAGC				
				ATCTGA					GAAATT		CCTGAA		CCTGC		GAAAG	GCCTG			GCAGA		CTGAA	GGGGT				
				TCATCC					TCAGG		AGGCC		GATTCA		GGAAG	CGAGA			AAGATA		GTCCC	GCCCG				
				TGGCC					GAAGA		ACAATC		GCGGA		CGGGA	CGAG			AGATG		GGTTCA	ATAGAT				
				AACAAC					GTGAC		ACAGC		AGCGG		CCGAAC				GCGGA		CCATCT	TTTCTG				
				AGCCT					CATCAC		CGACG		CTCCG		TTCACA				GGCCT		CCAGA	GCAGC				
				GTCCA					CGCCG		AGTCCA		GCACC		CTGAC				ACAGT		GACAA	GGCTC				
				GCAAC					ACAAGA		CCAATA		GACTTT		CATATC				GAGATT		CAGCA	TGGCA				
				GGCAA					GCACC		CTGCTT		ACCCT		TTCTCT				GGGAT		AGAACA	CCGAC				
				TGTGAC					AGCAC		ATATGG		AGCAAT		CCGAA				GAAAG		GCCTGT	ACCTG				
				CGAGA					CGCCT		AACTGT		CAGCT		GATCTG				GCGAG		ACCTG	CTGAA				
				GCGGC					ACATG		CTAGC		CCCTG		GCCAC				CGCGG		CAGAT	GATCA				
				GCAGC					CTTCG		CAGCC		TGACG		GTACTA				GAGGG		GAACA	GCAGA				
				GAGTG					GAGCA		TGACG		CAAAGA		ACCTTC				GCAAG		GCCTG	GTGGA				
				CGAGG					GCCTG		AGGAC		ACTTCG		GGTGC				GGGCA		AGAGC	AGCCG				
				AACTG					AGAAG		ACAGC		CCACCT		GGTCG				CGATG		CGAGG	AGGAC				
				GAAGA					CGAGG		CGTGTA		ACTACT		CGGAA				GCCTT		ACACC	GTGGG				
				GAAGA					ATACCG		GCCAG		GCCAC		CAAAG				AGCAG		GACAC	CGTGT				
				ACATCA					CTGTGT		CGCTA		CAGAG		CCTTTC				GGTCT		GTACTA	ACTACT				
				AAGAGT					ATTACT		GAGGC		CAAAGA		ACCTTC				CAGTAC		CTGCA	GTCTG				
				TTCTGC					GCGCC		AGACC		GGTGC		GGACC				AGCCA		CCCTG	CAAGG				
				AGAGC					ACATTC		CGCCA		CCTGG		CGGAA				CCAAG		AGAATC	CATCCA				
				TTCGTC					GCCCT		TGGACT		ACATTC		CAAAG				GACAC		AAGGAT	TCTGC				
				CACATC					GTTCG		ATTGG		GGACA		GTGGA				CTACGA		TGGGG	CTTGG				

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
														CATTAAG											
									GCTTCA	GGACA		GGGCA													
									GAGAG	GGGAA		CCAAG													
									CAGGC	CCCTG		GTGGA													
									CTTCGA	GTCACA		AATCAA													
									TATCTG	GTGTC		A													
									GGGCC	CAGC															
									AGGGC																
									ACAACC																
									GTGAC																
									AGTTTC																
									ATCT																

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders								Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

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SB06461	Sinvec	DNA	IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	LIR1
			ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	CACCC	GTGATC	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CACAT	CGGCG	CAGCA	GAGCA	AATCTG	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	ATCGCA	GGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTACG	GGTTAC	GGTTCA	GCTCT	GTGCA	TGGCT	GACAC	GATCC	GTCCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	TGGTTG	GATCTG	TGACA	TCCTCT	CTGGT	GAAGG
			TTCTGG	CCTGAAA	AGGCG	CACTGA	CTGAAA	GCTGC	GAGCC	CGCTG	AAACCT	CCTAGT	CAAGC	TACTTC	GCTGG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	TGGTG	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT	AAGTGA	GGAAG	AGCCT	AGCCT	AGCCA	ACAGC	CGCCC	TCGCC	GCTGC	GGACT	AAGTG	GAGCC	GTTTCT	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA	AAAGCC	GCGGCG	GAAGCA	GTCAG	CTTCGT	GCGGA	GACTAC	CCGCG	GGGGA	CTTTGG	GTTCA	GCGGA	TGGCT	GGACC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG	CCGGC	AGGGA	CCAGC	CCTCC	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	TTCTGG	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC	GTGGG	TCCTCC	TCTACC	GTGGG	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT	ACTGG	CGGCC	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCT	CCTTTC	AGCGT	GTGAAA	AAGGG	AGACA	GAGTC	CGATA	TGCCC	CAGCC	CCTAGA	CCCAG	GCATG	CTCTCTG	GACAG	CCTGC	GCCCT	TGATC	CGACT
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG	GTGTCTC	GAGTG	GCCAA	GAGTC	CGGCC	ACCAG	GAAGA	CGGAC	GACCT	CTGCTA	AGACT	CAGCA	ACAGCA	CCTGC	GCCCT	TGATC	CGACT
				GAGGC	CAGCC	GACGTA	TGTCCT	TTCGT	TGTCCT	TGTAAA	ACAATT	GCCTA	ACACCC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG	CAGCA	ACAACA						
				CACACT	TGATCG	CTGCTT	CTGGC		GCCAA	GCCAG	ACCTGT	CGGGC	CCCCT	TGCAA	CTGTAC	CTACCA	AACGA	CGGAG	TGCCG	TCAGCT	GGACC					
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT	CGGGT	CAGCC	GCTCCT	CCACA	CACTAC	TCTAGG	GGTGG	CCAGC	GCAAG	TACAAG							
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG	ACACCT	CAGCG	GCTCCT	AGAGC	AGTCCA	AGAGC	GA	CAGCC	AGGAA	TAGTCT	CCTAGC	CACCTT					
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT	TTACCG	AGAGC	AGAGC	GTGGA	TCTCCA	ACCTAC		TTACGC	GAGAG	ACTAAC	CAGCA	CAGCA					
				GTGCA	GAGCG	CTACTG	GCTAC		CAGCA	ACTACA	ATATGC	CAACTA	GTGGA	GCTAC	ACCAG		TCCTCC	GAGTA	GTGTG	GATAC	GATAC					
				CCCTA	GGTTC	GAGAA	ACGTG		ATATGC	ATTGG	CGGCA	CAGCA	CTGAAC	CTGGA	CTCCTA		TTAGAG	ACGATG	TGTGAT	GATATG	GCGAC					
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT	GTCGG	TCAGCT	TCATGA	TGGTAT	CAATCG	TTTCGC		TTTCGC	TTTGGA	GTAGAA	CACTG	GAGAA					
				AAAGTG	GAGGA	GAGCG	ACGTG		CTCTTG	TCAGCT	TCATGA	ACACCT	CAGCA	ACTGGT	CGGAAA		CAGCA	GAAAAT	CACTG	GGTCC	CACCTA					
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC	GTGGA	CACCA	GCCCC	AAAACC	CAGCC	CCGCG		CGCCT	CAAGA	GAAAAT	GGTCC	CACCTA					
				CATGAA	CAA	GAAGA	AAACC		GACAG	GTGGA	GACCA	TAAGCT	CAGCC	CAGCC	AGCCG		ACCGG	GACGT	CCTGG	GACAG	CCTGA					
				GTGCTT		GAAAG	CTGGA		GCACC	TGGAA	GGCCA	TCCAG	CGGAA	AGCCG	GCAGC			GAGCG	ACCT	GGCCC	GGCGA					
				TCTGCT		CGTGC	CCT		TGGAC	GGGAC	CAGAA	GCCTG	TCTGAG	AGGCT	TCTGAG			GGACC		TGGCA	ACTGG					
				AAAGTG	GAGGA	GAGCG	AGGTG		CTCTTG	TTGAAT	GCCTG	GCAAG	TCAGCT	CCAAA	GCCCG			ATGGG		CTTGAA	AAGGA					
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC	GTGGA	CTCGA	GCCCC	TCATGA	GCTCT	CAGCA			GGAAA		AACAC	CTTGAA					
				CATGAA	CAA	GAAGA	AAACC		GACAG	GTGGA	CACCA	TAAGCT	ACTGGT	CGGAAA	AAAACC			AGCCG		TGGGT	GACAG					
				GTGCTT		GAAAG	CTGGA		GCACC	TGGAA	GGCCA	TCCAG	CGGAA	AGCCG	GCAGC			GAGCG		GCCCC	GGCGA					
				TCTGCT		CGTGC	CCT		TGGAC	GGGAC	CAGAA	GCCTG	TCTGAG	AGGCT	TCTGAG			GGACC		TGGCA	ACTGG					
				AAAGTG	GAGGA	GAGCG	AGGTG		CTCTTG	TTGAAT	GCCTG	GCAAG	TCAGCT	CCAAA	GCCCG			ATGGG		CTTGAA	AAGGA					
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC	GTGGA	CACCA	GCCCC	AAAACC	CAGCC	CCGCG			GAAAA		AACAC	CTTGAA					
				CATGAA	CAA	GAAGA	AAACC		GACAG	GTGGA	G

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
				GTGCA					GCTTCA		GGACA			GGGCA	CATTAA							CCAGG				CCTCT
				GATGTT					GAGAG		GGGAA			CCAAG	G							GGCCA				GAGCG
				CATCAA					CAGGC		CCCTG			GTGGA								GGGCA				GCGAG
				CACCTC					CTTCGA		GTCACA			AATCAA								CAAAG				TTCCT
				A					TATCTG		GTGTC			A								CTGGA				GGACA
									GGGCC		CAGC											AATCAA				CCAAA
									AGGGC													G				GACAG
									ACAACC																	ACAGG
									GTGAC																	CCGAA
									AGTTTC																	GAGGA
									ATCT																	CAGAC
																										AGATG
																										GATAC
																										CGAAG
																										CCGCC
																										GCTTC
																										TGAAG
																										CCCCA
																										CAGGA
																										TGTGA
																										CATAT
																										GCCCA
																										GCTGC
																										ATAGC
																										CTGAC
																										ACTGC
																										GGAGA
																										GAAGC
																										CACAG
																										AGCCT
																										CCACC
																										TTCTCA
																										AGAAG
																										GCCCA
																										TCTCCT
																										GCCGT
																										GCCTT
																										CCATC
																										TATGC
																										CACTC
																										TGGCC
																										ATTCAC
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGS	QVQLV	GSTSGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	EVQLVE	GGGGS	DVVMT	HPSDPL	VGILVA
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLQE	SGAEVK		QSGAE		SPSSLS		SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLPL	SGGGL	GGGGS	QSPLSL	ELVVSG	VILLLLL
			ATRVHS	EDLIQS	SGVTPE	GIFWCC	GDVES	LPHFAF	KPGSSV		VKKPGS	GEGSTK	ASVGD		ASVGD	VPVFLP	VLLLSL	MNMTP	YKQGQ	GDVES	ALLLHA	VQPGG	GGGGS	PVTLGQ	PSGGP	GKHW
				MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP	KVSCKA		SVKVSC	G	RVTITC		RVTITC	AKPTTT	VITLYC	RRPGPT	NQLYNE	NPGPG	ARP	SLRLSC		PASISC	SSPTTG	TSTQR
				LYTESD	GGSGG	PRCRE	SGEGR		SGGTFS		KASGYT		RASESV		RASQSI	PAPRPP	NHR	RKHYQ	LNLGRR	SGEGR	AASGFT			KSSQSL	PTSTSG	KADFQ
				VHPSCK	GGSGG	RRRNE	GSLLTC		SYAISW		FTDYNM		DIVYGIS		SSYLN	TFAPTIA		PYAPPR	EEYDVL	GSLLTC		FSRYD		VASDEN	PEDQPL	HPAGA
				VTAMKC	GGSLQ	RLRRES	GDVEE		VRQAP		HWVRQ		FMNWF		WYQQIK	SQFLSL		DFAAYR	DKFRRG	GDVEE		MHWVR		TYLNWF	TPTGSD	VGPEP
				FLLELQ		VRPV	NPGP		GGGLE		APGQG		QQKPG		PGKAPK	RPEACR		S	RDPEM	NPGP		QAPGK		GQRPG	PQSGL	TDRGL
				VISLES					WMGGII		LEWIGYI		KAPKLLI		LIYAAS	PAAGG			GGKPR			GLEWV		QSPRRL	GRHLG	QWRSS
				GDASIH					PIFGTA		YPYNG		YAASNQ		SLQSGV	AVHTRG			RKNPQ			SVIWGN		YQVSK	V	PAADA
				DTVENL					NYAQKF		GTGYN		GSGVP		PSRFSG	LDFACD			EGLYNE			GNTHY		LDSGVP		QEENL
				IILANNS					QGRVTI		QKFKSK		SRFSGS		SGSGT				LQKDK			HSALKS		DRFSG		YAAVK
				LSSNGN					TADKST		ATITAD		GSGTDF		DFTLTIS				MAEAYS			RFTISR		SGSGT		HTQPE
				VTESGC					STAYME		ESTNTA		TLTISSL		SLQPED				EIGMKG			DNSKNT		DFTLKIS		DGVEM
				KECEEL					LSSLRS		YMELSS		QPDDFA		LATYYC				ERRRG			LYLQMN		RVEAED		DTRSP
				EEKNIK					EDTAVY		LRSEDT		TYYCQ		QQSYST				KGHDG			SLRAED		VGVYY		HDEDP
				EFLQSF					YCATFA		AYYYCA		QSKEVP		PFTFGP				LYQGLS			TAVYYC		CLQGIH		QAVTY
				VHIVQM					LFGFRE		RGRPA		WTFGQ		GTKVDI				TATKDT			TLRIKD		LPWTF		AEVKH
				FINTS					QAFDIW		MDYWG		GTKVEI		K				YDALHM			WGQGT		GQGTK		SRPRR
									GQGTT		QGTLVT		K						QALPPR			MVTVSS		LEIK		EMASP
									VTVSS		VSS															PSPLSG
																										EFLDTK
																										DRQAE

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																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

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SB06462			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
	Sinvec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	ACAACA	GTGATC	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CACAT	CGGCG	CAGAT	GAGCA	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	AGACC	GGCATT	GCACA
			CTTGGA	GTGATC	GTGGT	CTGGG	CTACG	GGTTAC	GGTTCA	AGCTCT	GTGCA	TGGCT	GACAC	GATCC	GACTCA	AACAGC	GGCCC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	CGCAC	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	GAGCG		ATCTGG	CTGGC	AGAGC		GTCCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CTCGG	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAAA	GCTGC	GAGCC		CGCTG	AAACCT	CCTAGT		CAAGC	TACTTC	GCTGG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	CCTCCA	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT		AAGTGA	GGAAG	AGCCT		AGCCT	AGCCA	ACAGC	CGCCC	TCGCC	GCTGC	GGACT	AAGTG	GAGCC	ACTCCA	TGCTCC	ACACTG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA		AAAGCC	GGCG	GAAGCA		GTCAG	CTTCGT	GCGGA	GACTAC	CCGCG	CTTTGG	GTTCA	GCGGA	TGCCT	GCTCC	TGTTGC	ACAAG
			GCACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG		CCGGC	AGGGA	CCAGC		CCTCC	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	AACAAT	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC		TCCTCC	TCTACC	GTGGG		GTGGG	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT	ACTGG	TGCACT	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCCT	CCTTTC	AGCGT		GTGAAA	AAGGG	AGACA		CGATA	TGCCC	CAGCC	CCTAGA	CCCAG	GCATG	CTGCTA	AGACT		GACAG	GCAAC	TGTTCC	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG		GTGTCTC		GAGTG		GAGTC	GCCAA	TGGTC	CGGCC	ACCAG	GACCT	CTGCTA	AGACT		CCTGC	CCCTG	TGATC	CGACT
				GAGGC	CAGCC	GACTAT	TGAAT	TTGCT	TGTCGT		TGTAAA		ACAATT		GCCTA	ATCACC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG			CAGCA	AGTCTG		TTCAG
				CACACT	TGATCG	CTGCTT	CTGGC		GCCAA		GCCAG		ACCTGT		ACATGC	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG	TGCCG			TCAGCT	AGGCC		CATCC
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT		CGGGT		CGGGC		AGAGC	CCCCT	TGCAA	GAAAG	GCTCAA	AAGGT	CCAGC			GCAAG	CGAGG		TGCTG
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG		ACACCT		CAGCG		CAGCC	GCTCCT	CCACA	CACTAC	TCTAGA	GGTGG	GGCTT			TCTAG	CCTGTA		GCGCC
				CCGAT	CGGAG	GCAGA	GGAGT		CACTTT		TTACCG		AGAGC		AGTCCA	AGAAGC	GA	CAGCC	ACGAA	TAGTCT	CACCTT			CAGTC	TGGCCA		GTTGG
				GTGCA	GAGCG	CTACTG			ACTACA		CAGCA		GTGGA		TCTCCA	ACCTAC		TTACGC	GAGAG	ACTAAC	CAGCA			CTGGT	GCAGC		AGCCT
				CCCTA	GGTTG	GAAGA	ACGTG		GCTAC		ATATGC		CAACTA		GCTAC	ACCAG		TCCTCC	GAGTA	GTGTG	GATAC			GGCCA	TGGCG		AGCCT
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA		TTAGAG	ACGATG	TGTGAT	GATATG			GCGAC	GAGCT		ACAGA
				AAAGTG	GAGGA	GAGCG	ACGTG		CTCTTG		GTCGG		TCAGCT		TGGTAT	CAATCG		TTTCGC	TTTGGA	GTAGAA	CACTG			GAGAA	GTTCAC		TAGAG
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC		CGCCT	CAAGA	GAAAAT	GGTCC			CACCT	AACTAG		GACTG
				CATGAA	CAA	GAAGA	AAACC		GACAG		CACCA		ACTGGT		AAAACC	CAGCC		ACCGG	GACGT	CCTGG	CCTGA			CCTGA	GGCCT		CAGTG
				GTGCTT		GAAAG	CTGGA		GCACC		GGCCA		TCCAG		CGGAA	TCTGTC			GACGT		GGCCC			ACTGG	GGACT		GCGGT
				TCTGCT		CGTGC	CCT		TGGAC		GGGAC		CAGAA		AGGCT	TCTGAG			GAGCC		TGGCA			TTCCAG	TGCCT		CTAGC
				AGGAAC		GGCGT			AGGGC		GCCTG		GCAAG		CCAAA	GCCCG			CTGAG		AAGGA			GCCCG			CCTGC
				TGCAA		GTG			CTCGA		GGCTA		GCCCC		GCTCTA	TAAGCT			ATGGG		CTTGAA			GCCCG			CGCTG
				GTGATC					GTGGA		CATCTA		TAAGCT		CATCTA	GTAGA			GGGAA		TGGGT			GACAG			ATGCC
				AGCCT					GGAAT		CCCCTA		GCTGAT		CGCCG	CCAGC			AGCCG		GTCCG			AGACG			CAGCC
				GGAAA					CATCCG		CAAGG		CTATGC		CTTCTA	TGCTG			AGAAG		TGATCT			GCTGA			GGAAA
				GCGGC					TATCTT		GCGGC		CGCCA		GCCTG	GCGGA			GAAGA		GTCCC			GGGGC			ATCTTT
				GACGC					CGGCA		ACGGG		CTCAGT		CAGTCT	GCCGT			ACCCTC		GGTTCA			AACGG			ACGCC
				CAGCAT					CCAGC		GCAAT		GCAATC		GGCGT	GCATAC			AGGAA		CAACAC			AGGTG			GCGGT
				CCACG					CTACAA		AAGGC		GCAAT		GCCAT	AAGAG			GGCCT		ACACTA			TCCAA			GAAGC
				ATACCG					AATTAC		CCAGA		AGCGG		CTAGAT	GACTG			GTACAA		CCACA			GCTGG			ACACC
				TGGAAA					GCCCA		AGTTCA		AGTGC		TCTCTG	GATTTT			TGAACT		GCGCC			ATAGC			CAGCC
				ATCTGA					GAAATT		TCAGG		AGGCC		GGAAG	CGAGA			GCAGA		CTGAA			GGGGT			TGAGG
				TCATCC					TCAGG		GAAGA		ACAATC		CGGGA	GATTCA			AAGATG		GTCCC			GCCCG			ATGGC
				TGGCC					GAAGA		GTGAC		ACAGC		CCGAC	GCGGA			AGATG		GGTTCA			ATAGAT			GTGGA
				AACAAC					AGCCT		CATCAC		CGACG		TTCACA	CCGAC			GCGGA		CCATCT			ITTCTG			AATGG
				AGCCT					GTCCA		CGCCG		CTCCG		TTCACA	GCCGT			GGCCT		CCAGA			GCAGC			ACACC
				GTCCA					AGTCCA		CCAGC		GCACC		CTGAC	CCGAC			ACAGT		GACAA			GGCTC			AGATC
				GCAAC					ACAAGA		CCAATA		GACTTT		CATATC	GACTTT			GAGATT		CAGCA			TGGCA			TCCCC
				GGCAA					GCACC		CTGCTT		ACCCT		TTCTCT	CTGCAT			GGGAT		AGAACA			CCGAC			ACGAT
				TGTGAC					AGCAC		ATATGG		CCGAA		CCGAA	GACAAT			GAAAG		CCGTGT			CCTGA			GAGGA
				CGAGA					CGCCT		AACTGT		CAGCT		GATCTG	CGCCG			GCGAG		ACCTG			CTGAA			CCCTC
				GCGGC					ACATG		CTAGC		CCCTG		GCCAC	CGCCG			GAGGG		CAGAT			GATCA			AGGCC
				GTCCA					GAACT		CTTCG		CAGCC		GCACC	GAGGG			GCAAG		GAACA			GCAGA			GTGAC
				GAGTG					GAGCA		GAGCG		TGACG		GTACTA	GCAAG			GCCTG		GCCTG			GTGGA			ATACG
				CGAGG					GCCTG		AGGAC		ACTTCG		TTGTCA	GGGCA			CGATG		CGAGG			AGCCG			AGAAA
				AACTG					AGAAG		ACAGC		CCACCT		GCAGT	GCAAG			GCTTT		ACACC			AGGAC			GTGAA
				GAAGA					CGAGG		CGTGTA		CCTACT		CCTACA	CCTTTC			GCACC		AGGAAT			GTGGG			GCACT
				GAAGA					ATACCG		CTATTG		GCCAG		ACCTTC	GGAAA			CCAAG		GACAC			CGTGT			CCAGA
				ACATCA					CTGTGT		CGCTA		CAGAG		CAAAGA	ACCTTC			CAGTAC		CTGCA			ACTACT			CCTCG
				AAGAGT					ATTACT		GAGGC		CAAAG		GGAAG	GGTCC			CAGTAC		CTGCA			GTCTG			GAGAG
				TTCTGC					GCGCC		AGACC		GGTGC		GGACC	GGTCC			AGCCA		CCCTG			CAAGG			AGATG
				AGAGC					ACATTC		CGCCA		CCTGG		CGGAA	CCAAG			CCAAG		CATCCA			CATCCA			GCAAG
				TTCGTC					TGGACT		GCCCT		TGGACT		ACATTC	CAAAG			GACAC		AAGGAT			TCTGC			CCCTC
				CACATC					GTTCG		ATTGG		GGACA		GTGGA	CTACGA			CTACGA		TGGGG			CTTGG			CATCT

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				GTGCA					GCTTCA		GGACA			GGGCA												
				GATGTT					GAGAG		GGGAA			CCAAG												
				CATCAA					CAGGC		CCCTG			GTGGA												
				CACCTG					CTTCGA		GTCACA			AATCAA												
				A					TATCTG		GTGTC			A												
									GGGCC		CAGC															
									AGGGC																	
									ACAACC																	
									GTGAC																	
									AGTTTC																	
									ATCT																	
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGS	QVQLV	GSTSGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	EVQLVE	GGGGS	DVVMT	ITTPAP	VGILVA
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLQE	SGAEVK		QSGAE	GKPGS	SPSSLS		SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLLPL	SGGGL	GGGGS	QSPLSL	RPFTPA	VILLLLL
			EDLIQS	SGVTPE	EGIFWCC	GDVES	NPGPG	KPGSSV	KVSCKA		VKKPGS	GEGSTK	ASVGD		ASVGD	VPVFLP	VLLLSL	MNMTP	YKQGQ	GDVES	ALLLHA	VQPGG	GGGGS	PVTLGQ	PTIALQ	GKKHW
			ATRVHS	MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP	SGGTFS		KASGYT		RVTITC		RVTITC	AKPTTT	VITLYC	RRPGPT	NQLYNE	NPGPG	ARP	SLRLSC		PASISC	PLSLRP	TSTQR
				LYTESD	GGSGG	PRCRE	SGEGR		SYAISW		FTDYNM		RASESV		RASQSI	PAPRPP	NHR	RKHYQ	LNLGRR	SGEGR		AASGFT		KSSQSL	EAQRPA	KADFQ
				VHPSCK	GGSGG	RRRNE	GSLLTC		QGRVTI		QKFKSK		SSYLN		DNYGIS	TFAPTIA		PYAPPR	EEYDVL	GSLLTC		FSRYD		VASDEN	AGGAV	HPAGA
				VTAMKC	GGSLQ	RLRRES	GDVEE		TADKST		ATITAD		WYQQIK		FMNWF	SQFLSL		DFAAYR	DKFRRG	GDVEE		MHWVR		TYLNWF	HTRGLD	VGPEP
				FLLELQ		VRPV	NPGP		STAYME		ESTNTA		PGKAPK		QQKPG	RPEACR		S	RDPEM	NPGP		QAPGK		QGRPG	FACD	TDRGL
				VISLES					ESTNTA		APGQG		LIYAAS		KAPKLLI	PAAGG			GGKPR			GLEWV		QSPRRL		QWRSS
				GDASIH					LSSLRS		LEWIGYI		SLQSGV		YAASNQ	AVHTRG			RKNPQ			SVIWGN		YQVSK		PAADA
				DTVENL					WMEGII		YPYNG		PSRFSG		GSGVP	LDFACD			EGLYNE			GNTHY		LDSGVP		QEENL
				IILANNS					EDTAVY		GTGYN		SGSGT		SRFSGS				LQKDK			HSALKS		DRFSG		YAAVK
				LSSNGN					NYAQKF		QKFKSK		DFTLTIS		GSGTDF				MAEAYS			RFTISR		SGSGT		HTQPE
				VTESGC					YCATFA		ATITAD		SLQPED		TLTISSL				EIGMKG			DNSKNT		DFTLKIS		DGVEM
				KECEEL					RGRPA		ESTNTA		LATYYC		QPDDFA				ERRRG			LYLQMN		RVEAED		DTRSP
				EEKNIK					LFGFRE		YMELSS		QGSYST		TYYCQ				KGHDG			SLRAED		VGVYY		HDEDP
				EFLQSF					QAFDIW		LRSEDT		PFTFGP		QSKEVP				LYQGLS			TAVYYC		CLQGIH		QAVTY
				VHIVQM					GQGTT		AYYYCA		GTKVDI		WTFGQ				TATKDT			TLRIKD		LPWTF		AEVKH
				FINTS					VTVSS		RGRPA		K		GTKVEI				YDALHM			WGQGT		GQGTK		SRPRR
											MDYWG				K				QALPPR			MVTVSS		LEIK		EMASP
											QGTLVT															PSPLSG
											VSS															EFLDTK
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																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
SB06463			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	LIR1
	Sinvec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	CACCC	GTGATC	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CACAT	CGGCG	CAGAT	GAGCA	ATCTG	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	ATCGCG	GGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTGGT	CTACG	GGTTAC	GGTTCA	AGCTCT	GTGCA	TGGCT	GACAC	GATCC	GACTCA	ACAGC	GGCCC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	TCCTCT	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	GAGCG		ATCTGG	CTGGC	AGAGC		GTCCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CGAGC	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAAA	GCTGC	GAGCC		CGCTG	AAACCT	CCTAGT		CAAGC	TACTTC	GCTGG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	TGGTG	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT		AAGTGA	GGAAG	AGCCT		AGCCT	AGCCA	ACAGC	TCGCC	TCGCC	GCTGC	GCTGC	GGACT	AAGTG	GAGCC	GTTTCT	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA		AAAAGC	CGGCG	GAAGCA		GTCAG	CTTCGT	GCGGA	GACTAC	CCGCG	GGGGA	CTTTGG	GTTCA	GCGGA	TGGCT	GGACC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG		CCGGC	AGGGA	CCAGC		CCTCC	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	TTCTGG	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	TCCTCC	TCTACC	GTGGG		GTGGG		TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT		ACTGG	CGGCC	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCT	CCTTTC	AGCGT		GTGAAA	AAGGG	AGACA		CGATA	TGCCC	CAGCC	CCTAGA	CCCAG	GCATG		GACAG		CCTGC	GCCCT	CTAGCA	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG		GTGTCTC		GAGTG		GAGTC	GCCAA	TGGTC	CGGCC	ACCAG	GACCT	CTGCTA	AGACT		CCTGC	GCCCT	TGATC	CGACT
				GAGGC	CAGCC	GACTAT	TGAAT	TTGCT	TGTCCT		ACAATT		ACAATT		GCCTA	ATCACC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG	CAGCA	ACAACA			TTCAG
				CACACT	TGATCG	CTGCTT	CTGGC		GCCAG		ACCTGT		ACCTGT		ACATGC	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG	TGCCG	TCAGCT	GGACC			CATCC
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT		ACACCT		CGGGC		AGAGC	CCCCT	TGCAA	GAAAG	GCTCAA	AAAGGT	CCAGC	GCAAG	TACAAG			TGCTG
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG		TTACCG		AGAGC		AGTCCA	AGAGC	GCTCCT	CACTAC	CTAGG	GGTGG	GGACT	TCTAGC	CACAA			TGAGC
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT		CAGCA		GTGGA		TCTCCA	ACCTAC	GAGAG	ACTAAC	TAGTCT	CAGCA	CACCTT	CAGTCT	GGCGC			GTTGG
				GTGCA	GAGCG	CTACTG	GAAGT		ACTACA		ACTACA		CTGGT		GCTAC	ACCAG	TTACGC	TCCTCC	GAGTA	GTGTG	CAGCA	CTGGT	CCTGA			AGCCT
				CCCTA	GGTTG	GAAGA	ACGTG		GCTAC		ATATGC		CAACTA		CAACTA	ACCAG	TCCTCC	GAGTA	GTGTG	GATAC	GATAC	GGCCA	GGACC			AGCCT
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA	TAGAGA	CGATGT	GTGAT	GATATG	GCGAC	AACCTC			ACAGA
				AAAGTG	GAGGA	GAGCG	ACGTG		CTCTTG		GTCGG		TCAGCT		TGGTAT	CAATCG	TTTCGC	TTTGGA	GTAGAA	CACTG	GAGAA	TGACAC			TAGAG
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC	CGCCT	CAAGA	GAAAAT	GGTCC	CACCTA	CAACAA			GACTG
				CATGAA	CAA	GAAGA	AAACC		GACAG		CACCA		ACTGGT		AAAACC	CAAGC	ACCGG	GACGT	CCTGG	GGCCC	CCTGA	GGCAG			CAGTG
				GTGCTT		GAAAG	CTGGA		GCACC		GGCCA		TCCAG		CGGAA	TCTGTC		GAGCG	ACCT	GGCCC	ACTGG	CGATC			GCGGT
				TCTGCT		CGTGC	CCT		TGGAC		GGGAC		CAGAA		AGGCT	TCTGAG				TGGCA	TTCCAG	CTAGC			CCTGC
				TTGAAC		AGGGC			CTCGA		GCCTG		GCCTG		CCAAA	GCCCG				AAGGA	CTGGA	CCTGC			CGCTG
				TGCAA		GTGAT			GTGGA		GCAAG		GCAAG		GCTCCT	AAGCTT				CTTGAA	GACAG	GAGAC			ATGCC
				GTGATC		GAGCG			CACCA		GCCCC		ACTGGT		CATCTA	GTAGA				TGGGT	GACAG	GAGAC			CAGTG
				AGCCT		GAGCA			GACAG		CACCA		TCCAG		CGGAA	AGCCG				GTCCG	GACAG	CAGTG			GCGGT
				GGAAA		GAAGC			GGCCA		TAAGCT		TAAGCT		CTTCTA	AGCAG				AGAAG	AGACG	GCGTT			GGAAA
				GCGGC		GACGC			GAAAT		CCCCTA		GCTGAT		CTTCTA	TGCTG				GAAGA	GCTGA				ATCTTT
				GACGC		GCTGC			GAAAT		CCTGCA		GCTGAT		GCCTG	GCGGA				GAAGA	AACGG				ACGCC
				CAGCAT		CCACC			TCATCC		AGTTCA		CTATGC		GCCTG	GCGGA				ACCCTC	TCTACC				GCCGT
				CAGCAC		CTACAA			GCCAT		CTACAA		GCAATC		CAGTCT	GCCGT				AGGAA	CAACAC				GCCGT
				ATACCG		AATTAC			GCCCA		CCAGA		AAGAG		GGCGT	GCATAC				GGCCT	ACACTA				GAAGC
				TGGAAA		GCCCA			AGTTCA		AAGCG		AGTGC		CTAGAT	GACTG				CTACAA	CCACA				ACACC
				ATCTGA		GAAATT			AGTGCA		AGTGC		CCTCAA		TCTCTG	GATTTT				TGAACT	GCGCC				CAGCC
				TCATCC		TCAGG			AGGCC		CCTCAA		GATTCA		GGAAG	CGAC				GCAGA	CTCCC				TGAGG
				TGGCC		GAAGA			ACAATC		GCGGA		GCGGA		CGGGA					AAGATG	GCCCG				ATGGC
				AACAAC		ACAGC			AGCGG		ACAGC		ACGGG		CCGAC					GCGGA	GGTTCA				GTGGA
				AGCCT		CATCAC			CTCCG		CGACG		CTCCG		TTCACA					GGCCT	CCATCT				AATGG
				GTCCA		CGCCG			CATCAC		GCACC		GCACC		CTGAC					ACAGT	CCAGA				ACACC
				GCAAC		ACAAGA			CCAATA		GACTTT		GACTTT		CATATC					GAGATT	GACAA				AGATC
				GGCAA		GCACC			CTGCTT		ACCCT		ACCCT		TTCTCT					GGGAT	AGAACA				TCCCC
				TGTGAC		AGCAC			AIATGG		CGAAT		CGAAT		CCGAA					GAAAG	CCATCT				ACGAT
				CGAGA		CGCCT			AACTGT		CAGCT		CAGCT		GATCTG					GCGAG	GCGAG				GAGGA
				GCGGC		ACATG			CTAGC		CCCTG		CCCTG		GATCTG					CGCGG	CCATCT				CCCTC
				TGCAAA		GAACT			CTTCG		CAGCC		CAGCC		GCCAC					GAGGG	GAACA				AGGCC
				GAGTG		GAGCA			GAGCG		TGACG		CAAAGA		GTACTA					GCAAG	GCCTG				GTGAC
				CGAGG		AGGAC			GCCTG		ACTTCG		ACTTCG		TTGTCA					GGGCA	CGATG				ATACG
				AACTG		AGAAG			CGAGG		ACAGC		CCACCT		GCAGT					GCAGT	CGAGG				CAGAA
				GAAGA		CGAGG			ATACGG		CGTGTA		ACTACT		CCTACA					GCCTT	ACACC				GTGAA
				GAAGA		ATACCG			CTATTG		GCCAG		GCCAG		GCACC					AGCAG	GCCGT				GCACT
				ACATCA		AAGAGT			CTGTGT		CGCTA		CAGAG		CCTTTC					GGTCT	GTACTA				CCTCG
				AAGAGT		TTCTGC			ATTACT		GAGGC		CAAAGA		ACCTTC					CAGTAC	CTGCA				GAGAG
				TTCTGC		AGAGC			GCGCC		GGTGC		GGTGC		GGACC					AGCCA	CCCTG				AGATG
				AGAGC		ACATTC			ACATTC		CGCCA		TGGACT		CGGAA					CCAAG	AGAATC				GCAAG
				TTCGTC		TGGACT			GCCCT		TGGACT		ACATTC		CAAAG					GACAC	AAGGAT				CCCTC
				CACATC					GTTCG		ATTGG		GGACA		GTGGA					CTACGA	TGGGG				CATCT

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															CATTAA G											
									GGACA			GGGCA														
									GGGAA			CCAAG														
									CCCTG			GTGGA														
									GTCACA			AATCAA														
									GTGTC			A														
									CAGC																	

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SB06467			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	LIR1
	Retrovec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	CACCC	GTGATC	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CACAT	CGGCG	CAGAT	GAGCA	ATCTG	CAACG	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	ATCGCG	AGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTGGG	CTACG	GGTTAC	GGTTCA	AGCTCT	GTGCA	TGGCT	GACAC	GATCC	GACTCA	AACAGC	GGCCC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	TCCTCT	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	GAGCG		ATCTGG	CTGGC	AGAGC		GTCCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CGAGC	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAAA	GCTGC	GAGCC		CGCTG	AAACCT	CCTAGT		CAAGC	TACTTC	GCTGG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	TGGTG	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT		AAGTGA	GGAAG	AGCCT		AGCCT	AGCCA	ACAGC	CGCCC	TCGCC	GCTGC	GGACT	AAGTG	GAGCC	GTTTCT	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA		AAAGCC	CGGCG	GAAGCG		GTCAG	CTTCGT	GCGGA	GACTAC	CCGCG	GGGGA	CTTTGG	GTTCA	GCGGA	TGCCT	GGACC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG		CCGGC	AGGGA	CCAGC		CCTCC	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	TTCTGG	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC		TCCTCC	TCTACC	GTGGG		GTGGG	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT	ACTGG	CGGCC	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCT	CCTTTC	AGCGT		GTGAAA	AAGGG	AGACA		CGATA	TGCCC	CAGCC	CCTAGA	CCCAG	GCATG	CTGCTA	GACAG	CTAGCA	TGTTCC	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG		GTGTCTC		GAGTG		GAGTC	GCCAA	TGGTC	CGGCC	ACCAG	GACCT	CTGCTA	AGACT		CCTGC	GCCCT	TGATC	CGACT
				GAGGC	CAGCC	GACTAT	TGAAT	TTGCT	TGTCGT		ACAATT	GCCTA	ATCACC		ACATCC	GCAAC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG	CAGCA	ACAACA			
				CACACT	TGATCG	CTGCTT	CTGGC		GCCAG		ACCTGT		CGGCA		ACATGC	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG		TGCCG		TCAGCT	GGACC		
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT		CGGGT		CGGCA		AGAGC	CCCCT	TGCAA	GAAAG	GCTCAA	AAGGT		CCAGC		GCAAG	TACAAG		
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG		ACACCT		CAGCG		CAGCC	GCTCCT	CCACA	CACTAC	TCTAGG	GGTGG		GGCTT		TCTAGC	CACAA		
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT		TTACCG		AGAGC		AGTCCA	AGAGC	GA	CAGCC	AGGAA	TAGTCT		CACCTT		CAGTCT	GGGGC		GTTGG
				GTGCA	GAGCG	CTACTG			CAGCA		ACTACA		GTGGA		TCTCCA	ACCTAC		TTACGC	GAGAG	ACTAAC		CAGCA		CTGGT	CCTGA		ACCTG
				CCCTA	GGTTG	GAAGA	ACGTG		GCTAC		ATATGC		CAACTA		GCTAC	ACCAG		TCCTCC	GAGTA	GTGTG		GATAC		GGCCA	GGACC		AGCCT
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA		TAGAGA	CGATGT	GTGAT		GATATG		GCGAC	AACCTC		ACAGA
				AAAGTG	GAGGA	GAGCG	ACGTG		CTCTTG		GTCGG		TCAGCT		TGGTAT	CAATCG		TTTCGC	TTTGGA	GTAGAA		CACTG		GAGAA	TGACAC		TAGAG
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC		CGCCT	CAAGA	GAAAAT		GGTCC		CACCTA	CAACA		GACTG
				CATGAA	CAA	GAAGA	AAACC		GACAG		CACCA		ACTGGT		AAAACC	CAAGC		ACCGG	GACGT	CCTGG		GACAG		CCTGA	GGCAG		CAGTG
				GTGCTT		GAAAG	CTGGA		GCACC		GGCCA		TCCAG		CGGAA	TCTGTC			GGCCG	ACCT		GCCCC		ACTGG	CGATC		GCGGT
				TCTGCT		CGTGC	CCT		TGGAC		GGGAC		CAGAA		AGGCT	TCTGAG			GGACC			TGGCA		TTCCAG	GCTCAG		CTAGC
				AAGGC		GGCCT			AGGGC		GCCTG		GCCTG		CCAAA	GCCCG			ATGGG			AAGGA		CTGGA	CTGGG		CCTGC
				TGCAA		GTG			CTCGA		GCAAG		GCAAG		GCTCCT	AAGCTT			GGGAA			CTTGAA		GCCCG	CTGGG		CGCTG
				GTGATC					GTGGA		GGCCC		GCCCC		CATCTA	GTAGA			AGCCG			TGGGT		CACCTA	GGCAG		ATGCC
				AGCCT					TGGGA		CATCTA		TAAGCT		CGCCG	GCAGC			AGAAG			GTCCG		CCTGA	GAGAC		CAGAA
				GGAAA					GGAAT		CCCCTA		GCTGAT		CTTCTA	TGCTG			CTTAGT			TGATCT		AGACG	GCGTT		GGAAA
				GCGGC					CATCCC		CAAGG		CTATGC		GCCTG	GCGGA			GAAGA			GCGGC		GCTGA			ATCTTT
				GACGC					TATCTT		GCGGC		CGCCA		CAGTCT	GCCGT			ACCCTC			AACGG		TCTACC			ACGCC
				CAGCAT					CGGCA		ACGGG		GCAATC		GGCGT	GCATAC			AGGAA			CAACAC		AGGTG			GCCGT
				CCACG					CCACC		CTACAA		AAGGC		GCCAT	AAGAG			GGCCT			ACACTA		TCCAA			GAAGC
				ATACCG					AATTAC		CCAGA		AGCGG		CTAGAT	GACTG			GTACAA			CCACA		GCTGG			ACACC
				TGGAAA					GCCCA		AGTTCA		AGTGC		TCTCTG	GATTTT			TGAACT			GCGCC		ATAGC			CAGCC
				ATCTGA					GAAATT		AGTCCA		CCTCAA		GGAAG	CGAGA			GCAGA			GCCAG		GGCGT			TGAGG
				TCATCC					TCAGG		AGGCC		GATTCA		GGAAG	CGAGA			AAGATA			GTCCC		GCCCG			ATGGC
				TGGCC					GAAGA		ACAATC		GCGGA		CGGGA				AGATG			GGTTCA		ATAGAT			GTGGA
				AACAAC					GTGAC		ACAGC		AGCGG		CCGAC				GCGGA			CCATCT		TTTCTG			AATGG
				AGCCT					CATCAC		CGACG		CTCCG		TTCACA				GGCCT			CCAGA		GCAGC			ACACC
				GTCCA					CGCCG		AGTCCA		GCACC		CTGAC				ACAGT			GACAA		GGCTC			AGATC
				GCAAC					ACAAGA		CCAATA		GACTTT		CATATC				GAGATT			CAGCA		TCGCA			TCCCC
				GGCAA					GCACC		CTGCTT		ACCCT		TTCTCT				GGGAT			AGAACA		CCGAC			ACGAT
				TGTGAC					AGCAC		ATATGG		CCGAA		CCGAC				GAAAG			CCGTGT		ACCTG			GAGGA
				CGAGA					CGCCT		AACTGT		CAGCT		CCGAA				GCGAG			ACCTG		CTGAA			CCCTC
				GCGGC					ACATG		CTAGC		CCCTG		GATCTG				CGCGG			CAGAT		GATCA			AGGCC
				GCAAA					GAACT		CTTCG		CAGCC		GCCAC				GAGGG			GAACA		GCAGA			GTGAC
				GAGTG					GAGCA		GAGCG		TGACG		GTACTA				GCAAG			GCCTG		GTGGA			ATACG
				CGAGG					GCCTG		AGGAC		ACTTCG		TTGTCA				GGGCA			AGAGC		AGCCG			CAGAA
				AACTG					AGAAG		ACAGC		CCACCT		GCAGT				CGATG			CGAGG		AGGAC			GTGAA
				GAAGA					CGAGG		CGTGTA		ACTACT		CCTACA				GCCTT			ACACC		GTGGG			GCACT
				GAAGA					ATACCG		CTATTG		GCCAG		GACAC				AGCAG			GCCGT		CGTGT			CCAGA
				ACATCA					CTGTGT		CGCTA		CAGAG		CCTTTC				GGTCT			GTACTA		ACTACT			CCTCG
				AAGAGT					ATTACT		GAGGC		CAAAGA		ACCTTC				CAGTAC			CTGCA		GTCTG			GAGAG
				TTCTGC					GCGCC		AGACC		GGTGC		GGACC				AGCCA			CCCTG		CAAGG			AGATG
				AGAGC					ACATTC		CGCCA		CCTGG		CGGAA				CCAAG			AGAATC		CATCCA			GCAAG
				TTCGTC					TGGACT		GCCCT		TGGACT		CAAAG				GACAC			AAGGAT		TCTGC			CCCTC
				CACATC					GTTCG		ATTGG		GGACA		GTGGA				CTACGA			TGGGG		CTTGG			CATCT

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										GGGCA CCAAG GTGGA AATCAA A															
									GGACA GGGAA CCCTG GTCACA GTGTC CAGC															
			MDWTW ILFLVAA ATRVHS	GTGCA GATGTT CATCAA CACCTC A	SGGGG SGGGG SGVTPE PIFSLIG GGSGG GGSGG	LLPSWA ITLISVN GIFWCC LTYCFA PRCRE RRRNE RLRRES VRPV	QCTNY ALLKLA GDVES NPGPG SGEGR GSLLTC GDVEE NPGP	MLLLVT SLLLCE LPHFAF LIP	EVQLVQ QSGAE KPGSSV KVSCKA SGGTFS SYAISW VRQAP GGGLE WMGGII PIFGTA NYAQKF QGRVTI TADKST STAYME LSSLRS EDTAVY YCATFA LFGFRE GAFDIW GGGTT VTVSS	GGGGS	QVQLV GSTSGS QSGAE GKPGS VKKPGS GEGSTK SVKVSC G KASGYT FTDYNM HWVRQ APGQG LEWIGYI YPYNG GTGYN QKFKSK ATITAD STAYME YMELSS LRSEDT AYYYCA RGRPA MDYWG QGTLVT VSS	DIQMTQ SPSSLS ASVGD RVTITC RASQSI SSYLN WYQQIK PGKAPK LIYAAS SLQSGV PSRFSG SGSGT DFTLTIS SLQPED LATYYC QQSYST PFTFGP GTKVDI K	GGGGS	DIQMTQ SPSSLS ASVGD RVTITC RASQSI DIVGIS FMNWF QQKPG KAPKLLI YAASNQ GSGVP SRFSGS GSGTDF TLTISSL QPDDFA TYYCQ QSKEVP WTFGQ GTKVEI K	ALSNSI MYFSHF VPVFLP AKPTTT PAPRPP TFAPTIA SQFLSL RPEACR PAAGG AVHTRG LDFACD	IYIWAPL AGTCG VLLLSL VITLYC NHR	RSKRSR LLHSDY MNMTP RRPGPT YKQRD DFAAYR S	RVKFSR SADAPA YKQGQ NQLYNE DKFRRG RDPEM GGKFR RKNPQ EGLYNE LQKDK MAEAYS EIGMKG ERRRG KGHDG LYQGLS TATKDT YDALHM QALPPR	QCTNY ALLKLA GDVES NPGPG SGEGR GSLLTC GDVEE NPGP	MALPVT ALLLPL ALLLHA ARP	EVQLVE SGGGL VQPGG SLRLSC AASGFT FSRYD MHWVR QAPGK GLEWV SVIWGN GNTHY HSALKS RFTISR DNSKNT LYLQMN SLRAED TAVYYC TLRIKD WGQGT MVTVSS	GGGGS GGGGS GGGGS	DVVMT QSPLSL PVTLGQ PASISC KSSQSL VASDEN TYLNWF QQRPG QSPRRL YQVSK LDSGVP DRFSG SGSGT DFTLKIS RVEAED VGVYY CLQGIH LPWTF GQGTK LEIK	HPSDPL ELVVSG PSGGP SSPTTG PTSTSG PEDQPL TPTGSD PQSGL GRHLG V	VGILVA VILLLLL LLLFLI	CCTCT GAGCG GCGAG TTCCT GGACA CCAAA GACAG ACAGG CCGAA GAGGA CAGAC AGATG GATAC CGAAG CCGCC GCTTC TGAAG CCCCA CAGGA TGTGA CATAT GCCCA GCTGC ATAGC CTGAC ACTGC GGAGA GAAGC CACAG AGCCT CCACC TTCTCA AGAAG GCCCA TCTCCT GCCGT GCCTT CCATC TATGC CACTC TGGCC ATTCAC LRHRR QGKHW TSTQR KADFQ HPAGA VGPEP TDRGL QWRSS PAADA QEENL YAAVK HTQPE DGVEM DTRSP HDEDP QAVTY AEVKH SRPRR EMASP PSPLSG EFLDTK DRQAE

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders								Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
SB06469			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH	6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	LIR1
	Retrovec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTC	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	CACCC	GTGATC	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	CAGCT	CGGTG	CAGCTT	TACATC	CACAT	CGGCG	CAGCA	GAGCA	AATCTG	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	ATCGCG	AGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTAGG	GTTACG	GGTTAC	AGCTCT	GACAC	GAACAC	GGCGCC	GACTCA	AACAGC	GGAGC	GGCGCC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	TCCTCT	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	GAGCG		ATCTGG	CTGGC	AGAGC		GTCCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CGAGC	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAAA	GCTGC	GAGCC		CGCTG	AAACCT	CCTAGT		CAAGC	TACTTC	GCTGG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	TGGTG	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT		AAGTGA	GGAAG	AGCCT		AGCCT	AGCCA	ACAGC	CGCCC	TCGCC	GCTGC	GGACT	AAGTG	GAGCC	GTTTCT	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA		AAAGCC	CGGCG	GAAGCG		GTCAG	CTTCGT	GCGGA	GAACTA	CCGCG	GGGGA	CTTTGG	GTTCA	GCGGA	TGCCT	GGACC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG		CCGGC	AGGGA	CCAGC		CCTCC	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	TTCTGG	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC		TCCTCC	TCTACC	GTGGG		GTGGG	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT	ACTGG	CGGCC	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	CTGCCT	AGCGT	CCTTTC		AGACA	GTGAAA	AGGGG		CGATA	TGCCC	CAGCC	CCTAGA	CCCAG	GCATG	CTGCTA	GACAG	CTAGCA	TCTGCC	GCCCT	TGATC	CGACT
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG		GTGTCTC		GAGTG		GCCAA	GAGTC	CGGCC	ACCAG	GACCT	CTGCTA	AGACT	CCTGC	GCCCT	TGATC	CGACT
				GAGGC	CAGCC	GACGTA	TGTCGT	TTGCT	ACAATT		ACAATT		GCCTA		ATCACC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG	CAGCA	ACAACA			TTCAG
				CACACT	TGATCG	CTGCTT	CTGGC		GCCAG		ACCTGT		CGGCA		ACATGC	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG	TGCCG	TCAGCT	GGACC		CATCC
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT		CGGGT		CGGGC		AGAGC	CCCCT	TGCAA	GAAAG	GCTCAA	AAAGGT	CCAGC	GCAAG	TACAAG		TGCTG
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG		ACACCT		CAGCG		CAGCC	GCTCCT	CCACA	CACTAC	TCTAGG	GGTGG	GGACTT	TCTAGC	CACAA		GCGCC
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT		TTACCG		AGAGC		AGTCCA	AGAGCC	GA	CAGCC	AGGAA	TAGTCT	CACCTT	CAGTCT	GGCGC		GTTGG
				GTGCA	GAGCG	CTACTG			CAGCA		ACTACA		GTGGA		TCTCCA	ACCTAC		TTACGC	GAGAG	ACTAAC	CAGCA	CTGGT	CCTGA		ACCTG
				CCCTA	GGTTG	GAAGA	ACGTG		GCTAC		ATATGC		CAACTA		GCTAC	ACCAG		TCCTCC	GAGTA	GTGTG	GATAC	GGCCA	GGACC		AGCCT
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATTGG		CGGCA		CTGAAC	CTCCTA		TTTCGC	TTTGGA	GTATGA	CACTG	GCGAC	AACCTC		ACAGA
				AAAGTG	GAGGA	GAGCG	ACGTG		CTCTTG		GTCGG		TCAGCT		TGGTAT	CAATCG		TTTCGC	TTTGGA	GTAGAA	CACTG	GAGAA	TGACAC		TAGAG
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		CCAGG		TCATGA		CAGCA	CCAGC		CGCCT	CAAGA	GAAAAT	GGTCC	CACCTA	CAACA		GACTG
				CATGAA	CAA	GAAGA	AAACC		GACAG		CACCA		ACTGGT		AAAACC	CAAGC		ACCGG	GACGT	CCTGG	GACAG	CCTGA	GGCAG		CAGTG
				GTGCTT		GAAAG	CTGGA		GCACC		GGCCA		TCCAG		CGGAA	TCTGTC			GAGCG	ACCT	GCCCC	CCTGG	CGATC		GCGGT
				TCTGCT		CGTGC	CCT		TGGAC		GGGAC		CAGAA		AGGCT	TCTGAG			GGACC		TTCCAG	ACTCAGT	CTAGC		CCTGC
				AAGGC		AGGGC			AGGGC		GCCTG		GCCTG		CCAAA	GCCCG			ATGGG		AAGGA	CTGGA	CCTGC		CGCTG
				TGCAA		GGCCT			CTCGA		GCAAG		GCCCC		GCTCCT	AAGCTT			GGGAA		CTTGAA	GCCCG	CTGGG		ATGCC
				GTGATC		GTGCA			GTGGA		GCCCC		TAAGCT		CATCTA	GTAGA			AGCCG		TGGGT	CACCTA	GAGAC		CAGTG
				AGCCT		GAGCG			CATCTA		CCCCTA		GCTGAT		CTTCTA	TGCTG			AGAAG		GTCCG	CCTGA	AGACG		GGAAA
				GGAAA		GAAAA			GGAAT		CCCCTA		GCTGAT		CGCCG	GCAGC			AGAAG		TGATCT	GCCCC	GCGTT		GGAAA
				GCGGC		GAAGC			CATCCC		CAAGG		CTATGC		GCCTG	GCGGA			GAAGA		GGGGC	GCTGA			ATCTTT
				GACGC		GAGCG			TATCTT		GCGGC		CGCCA		CAGTCT	GCCGT			ACCCTC		AACGG	TCTACC			ACGCC
				CAGCAT		CCACC			CGGCA		ACGGG		CTACAA		GGCGT	GCATAC			AGGAA		CAACAC	AGGTG			GCGGT
				ATACCG		CCGCC			AATTAC		CCAGA		AAGGC		GCCAT	AAGAG			GGCCT		ACACTA	TCCAA			GAAGC
				TGGAAA		ATCGAA			GCCCA		AGTTCA		AGCGG		CTAGAT	GACTG			GTACAA		CCACA	GCTGG			ACACC
				ATCTGA		TCATCC			AAATT		TCAGG		AGTGCA		TCTCTG	GATTTT			TGAACT		GCGCC	ATAGC			CAGCC
				TCATCC		TGGCC			TCAGG		AGGCC		CCTCAA		GGAAG	GCTGG			GCAGA		GTCCC	GGCCG			TGAGG
				TGGCC		AACAAC			GAAGA		ACAATC		GATTCA		CGGGA	CGAC			AAGATA		GTCCC	ATAGAT			ATGGC
				AACAAC		AGCCT			GTGAC		ACAGC		GCGGA		CCGAAC				AGATG		GGTTCA	CCATCT			GTGGA
				AGCCT		GTCCA			CATCAC		CGACG		AGCGG		TTCACA				GCGGA		CCATCT	GCAGC			AATGG
				GTCCA		GCAAC			CGCCG		CTCCG		CTCGG		TTCACA				GGCCT		CCAGA	GGCTC			ACACC
				GCAAC		ACAAGA			GCCAA		GCACC		GCACC		GCCAAC				ACAGT		GACAA	GACAA			AGATC
				GGCAA		CCTGA			GCACC		CCAATA		GACTTT		CATATC				GAGATT		CAGCA	CAGCA			TCCCC
				TGTGAC		GCGCC			CTGCTT		GACCT		ACCCT		TTCTCT				GGGAT		AGAACA	AGAACA			ACGAT
				CGAGA		GGGCC			AGCAC		AATGTG		GACAAT		CCGAA				GAAAG		CCGTGT	CCTGA			GAGGA
				GGGGC		CGAGA			CGCCT		AACTGT		CAGCT		GATCTG				GCGAG		ACCTG	ACCTG			CCCTC
				GCGGC		GAACT			ACATG		CTAGC		CCCTG		GATCTG				GCGCG		CAGAT	GATCA			AGGCC
				TGCAAA		GAGTG			CTTCG		CAGCC		GCCAC		GCCAC				GAGGG		GAACA	GCAGA			GTGAC
				GAGTG		CGAGG			GAGCA		GAGCG		TGACG		GTACTA				GCAAG		GCCTG	GTGGA			ATACG
				CGAGG		AACTG			GCCTG		AGGAC		ACTTCG		TTGTCA				GGGCA		AGAGC	AGCCG			CAGAA
				AACTG		GAAGA			AGAAG		ACAGC		CCACCT		GCAGT				CGATG		CGAGG	AGGAC			GTGAA
				GAAGA		GAAGA			CGAGG		CGTGTA		CTATTG		CCTACA				GCCTT		ACACC	GTGGG			GCACT
				GAAGA		ATACGA			ATACCG		GCCAG		CTATTG		GCCAA				AGCAG		GCCGT	CGTGT			CCAGA
				ACATCA		AAGAGT			ATTACT		CTGTGT		CGCTA		CAGAG				GGTCT		GTACTA	ACTACT			CCTCG
				AAGAGT		TTCTGC			GCGCC		AGACC		GAGGC		CAAAGA				CAGTAC		CTGCA	GTCTG			GAGAG
				TTCTGC		AGAGC			ACATTC		GCCCT		TGGACT		ACCTTC				AGCCA		CCCTG	CAAGG			AGATG
				AGAGC		TTCGTC			GCCCT		TGGACT		ACATTC		GGACC				GACAC		AGGAT	CATCCA			GCAAG
				TTCGTC		TGGACT			GTTCG		ATTGG		GGACA		GTGGA				CTACGA		TGGGG	CTTGG			CCCTC
				CACATC																					CATCT

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
														CATTAA G											
									GGACA			GGGCA													
									GGGAA			CCAAG													
									CCCTG			GTGGA													
									GTCACA			AATCAA													
									GTGTC			A													
									CAGC																

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders								Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
SB07401	Sinvec	DNA	IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	CD33 VL	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
			ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GGTGG	GATATT	GGCGG	GATATG	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	ACAACA	GTGATG	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	GCAGC	CGGAG	CAGCT	CGGAG	CACAT	TGGCG	CAAATG	GAGCA	ATCTG	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	ACACC	GGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTACG	GGTTAC	GTGGTTC	GGTGC	GAAGT	GACTCA	GATCT	ACCCA	ACAGC	GGCCC	GAGCG	AGACT	GGAGC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	CGCAC	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	AGTCTG	GGTGG	AGAGC	GGCGG	GTCTCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CTCGG	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGCTG	GCGCC	TGGTG	GAAGT	GGAGC	CGGAG	ATCCAG	AGGTG	CCTGA	AGCCA	GCTGC	GCAGA	GAAGC	TCGCC	GCTGC	GCATC	GGAGG	CCCTC	CTCCA	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT	GAAGT	GGAGC	GGAGG	CCTGA	AGCCA	CACAT	CGCCC	CTGCA	ACACG	TCGCC	TGCGC	GCTGC	GGACT	AAGTG	GAGCC	ACTCCA	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA	GCCAG	GTCTG	GAAAAA	AGCGTT	GCCGC	CTTCGT	GCGGA	GACTAC	CCGCG	CTTGG	GGTGA	CTTGG	GGCGG	TTCT	TGCCT	GCTCC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG	GCCAG	CCTCTG	TGGGA	AGCGTT	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	AACAAT	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC	TGGGA	GCTGCT	GGCGA	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT	ACTGG	TGCACT	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCCT	CCTTTC	AGCGT	CCGTG	GACAG	TAGAGT	TGCCC	CAGCC	CCTAGA	CCAGA	CCCAG	GCATG	CTGCTA	AGACT	GACAG	GCAAC	TGTTCC	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG	AAAGTC	AGTGA	CACAAT	GCCAA	TGGTC	CGGCC	ACCAG	GACTC	CTGCTA	AGACT	CCTGC	CCCTG	TGATC	CGACT
				GAGGC	CAGCC	GACTA	TGAAT	TTCGT	TGTCCT	CAATTA	CACCT	GCCTA	ATCACC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG	CAGCA	AGTCTG	TTCAG
				CACACT	TGATCG	CTGCTT	CTGGC		GCAAA	AAGGC	CCTGC	CGGGC	CTGTAC	CTACCA	AACGA	CGGAG		TGCCG	TCAGCT	AGGCC	CATCC
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT	CAGCG	CGGGC	CCAGA	GCCCT	TGCAA	GAAAG	GCTCAA	AAGGT	CCAGC	GCAAG	CGAGG	TGCTG
				CGAGT	GGTTC	CTAGAT	ACGCG		GCTACA	GGCGG	CAGCG	GCTCCT	CCACA	CACTAC	TCTAGG	GGTGG	GGCTT	TCTAGC	CCTGTA	GTTGG
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT	CCTTTA	AGAGC	GCATCA	AGAGCC	GA	CAGCC	AGGAA	TAGTCT	CACCTT	CAGTCT	GGCCA	GTTGG
				GTGCA	GTGGC	GAGCG	CTACTG		CAGCA	CCGAC	GTGGA	GCTCCT	ACCTAC		TTACGC	GAGAG	ACTAAC	CAGCA	CTGGT	GCAGC	AGCCT
				CCCTA	GGTTG	GAGAA	ACGTG		GCTAC	TACAAC	CAATTA	ACCTGA	AACCAG		TCCTCG	GAGTA	GTGTG	GATAC	GGCCA	TGGCG	AGCCT
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT	ATGCAC	CGGCA	ATTGGT	CTCCTA		TAGAGA	CGATGT	GTGAT	GATATG	GCGAC	GAGCT	ACAGA
				AAAGTG	GAGGA	GAGCG	ACGTG		CTCTTG	TGGGT	TCAGCT	ATCAGC	CAATCG		TTTCGC	TTTGGA	GTAGAA	CACTG	GAGAA	GTTCAC	TAGAG
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC	CCGAC	TCATGA	AGAAAC	CCAGC		CGCCT	CAAGA	GAAAAT	GGTCC	CACCTA	AACTAG	GACTG
				CATGAA	CAA	GAAGA	AAACC		GACAG	AAGCTC	ACTGGT	CAGGC	CAGCC		ACCGG	GACGT	CCTGG	GACAG	CCTGA	GGCCT	CAGTG
				GTGCTT		GAAAG	CTGGA		GCCCC	CAGGC	TCCAG	AAAGCT	CTCCA			CCTGT		GGCCC	ACCT	CTGGT	GGACTT	GCGGT
				TCTGCT		CGTGC	CCT		TGGAC	CAGGG	CCAGAA	CCCAAA	TCTGAG			GGACC		TGGCA		TTCCAG	TGGCT	CTAGC
				GGAAC		GAGCC			AAGGC	GCCCG	CTCCTG	TCAGCT	GCCCG			CTGAG		AAGGA		TCTCAC	CCTGC
				TGCAA		GGCCT			CTGGA	GTGGA	GCAAG	ATCTAT	AAGCTT			ATGGG		CTTGAA		GCCCG	CGCTG
				GTGATC		GTG			ATGAAT	TCGGC	GCCCC	TAAGCT	GCTGC	GTAGA		GGGAA		TGGGT		GACAG	ATGCC
				AGCCT					GGGAG	TACATC	CTCCA	CCAGC	AGCCG			AGCCG		GTCCG		ATAGC	CAGCC
				GGAAA					GCATCA	TACCCC	GCCTG	TGCTG	CAGAG	GCGGA		AGAAG		TGATCT		AGACG	GGAAA
				GCGGC					TCCCCA	TACAAC	CTACG	CCTAG	TGGCG	GCCGT		GAAGA		GGGGC		GCTGA	ATCTTT
				GACGC					TCTTCG	CCGCC	CCGCC	GCCAT	ACCCTC			AACGG		AACGG		TCTACC	ACGCC
				CAGCAT					GCACC	CACGG	GCAAT	AGCAAT	TGCCAT	GCATAC		AGGAA		CAACAC		AGGTG	GCCGT
				CCACG					GCCAAT	CAAGG	CTAGAT	AAGAAG	GGCCT			ACACTA		AGAAG		GAAGC		
				ATACCG					TACGC	ACCAG	CAGCG	GACTG	TCAGC	GAGTG		CCACA		ACACC		GCTGG	ACACC	
				TGGAAA					CCAGA	AAGTTC	GAGTG	CCTAG	CGGAA	GATTTT		GCAGA		CAGCC		ATAGC	CAGCC	
				ATCTGA					AATTCC	AAGAG	CCTAG	CGGAG	GCGGC	GCCTG		GCGAT		GCGGT		GGCCG	TGAGG	
				TCATCC					AGGGC	CAAGG	CAGATT	CGAGA	CGGGC	CGAC		AAGATA		GTCCC		GCCCG	ATGGC	
				TGGCC					AGAGT	CCACAA	TTCCG	CTTTAC	ACAGA			AGATG		GGTTCA		ATAGAT	GTGGA	
				AACAAC					GACCAT	TCACAG	GCTCT	CTTTAC	CTTTAC			GCGGA		CCATCT		ITTCTG	AATGG	
				AGCCT					CACCG	CCGAC	GGCAG	ACTGAC	GGCCT			GGCCT		CCAGA		GCAGC	ACACC	
				GTCCA					CCGCA	CGGCA	CGGCG	AATATC	GGCCA			ACAGT		GACAA		GGCTC	AGATC	
				GCAAC					AAGAG	CACAA	CCGATT	TTCCCT	CATTTC			GAGATT		CAGCA		TGGCA	TCCCC	
				GGCAA					CACAA	GCACC	TCACCC	GCAGC	ATTGCC			GGGAT		AGAACA		CCGAC	ACGAT	
				TGTGAC					GCACC	CTTATA	TGACCA	CAGAG	CAGAAG			GAAAG		CCGTGT		CTTCACC	GAGGA	
				CGAGA					GCCTA	TGGAA	TCAGTA	GACCT	GCGAG			GCGAG		ACCTG		CTGAA	CCCTC	
				GGGGC					CATGG	CTGTCT	GCCTG	GGCCA	CGCGG			CGCGG		CAGAT		GATCA	AGGCC	
				TGCAAA					AACGG	CCGAC	CAGCC	CATATT	GAGGG			GAGGG		GAACA		GCAGA	GTGAC	
				GAGTG					GCAGC	CGAGT	TGACG	ATTGCC	GCAAG			GCAAG		GCCTG		GTGGA	ATACG	
				CGAGG					CTGAG	ACTTCG	AGCAG	AGCGA	GGGCA			GGGCA		AGAGC		AGCCG	CAGAA	
				AACTG					AAGCG	GATACA	CGTGT	ACCACT	CGATG			CGATG		CGAGG		AGGAC	GTGAA	
				GAAGA					AGGAC	GCTAC	GCCAG	ACTACT	TCCTTT	TCCTAC		GCTTT		ACCAC		GTGGG	GCACT	
				GAAGA					ACCGC	GTACTA	GCCAG	GCCAG	CACCTT	AGCAC		AGCAG		GCCGT		CGTGT	CCAGA	
				ACATCA					CCCTGT	TTGTGC	CAGAG	CAAAGA	CGGAC	CGGAC		GGTCT		GTACTA		ACTACT	CCTCG	
				AAGAGT					TCGGC	CAGGG	ACTATT	GGACA	GTGGA	CGGAC		CAGTAC		CTGCA		GTCTG	GAGAG	
				TTCTGC						GCAGA				CCGGA		ACAAAG		AGCCA		CTGTG	AGATG	
				AGAGC						CATTTG				GGTGC		GTGGA		CCAAG		AGAATC	GCAAG	
				TTCGTC						CCGCC				GCTGG		ACAAAG		GACAC		CATCCA	CCCTC	
				CACATC						CATGG				ACCTTT		GTGGA		AAGGAT		TCTGC	CCCTC	
										CCCTGT				CACCTT				CTACGA		CTTGG	CATCT	
										TCGGC				CGGAC								
										ACTATT				CAGTAC								

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
				GTGCA					TTCAGA						CATTAA							CCAGG				ACCTCT
				GATGTT					GAGCA						G							GGCCA				GAGCG
				CATCAA					GGCCT													GGGCA				GCGAG
				CACCTC					TCGATA													CAAAG				TTCCT
				A					TCTGG													CTGGA				GGACA
									GGCCA													AATCAA				CCAAA
									GGGCA													G				GACAG
									CAACC																	ACAGG
									GTGAC																	CCGAA
									CGTTTC																	GAGGA
									TAGC																	CAGAC
										GGGGA																AGATG
										CAGGG																GATAC
										AACCCT																CGAAG
										GGTCA																CCGCC
										CAGTGT																GCTTC
										CATCT																TGAAG
											GGGCA															CCCCA
											CCAAG															CAGGA
											GTGGA															TGTGA
											AATCAA															CATAT
											A															GCCCA
												GGGGS														GCTGC
												CCAAG														ATAGC
												GTGGA														CTGAC
												AATCAA														ACTGC
												A														GGAGA
													GGGGS													GAAGC
													CCAAG													CACAG
													GTGGA													AGCCT
													AATCAA													CCACC
													A													TTCTCA
														GGGGS												AGAAG
														CCAAG												GCCCA
														GTGGA												TCTCCT
														AATCAA												GCCGT
														A												GCCTT
															GGGGS											CCATC
															CCAAG											TATGC
															GTGGA											CACTC
															AATCAA											TGGCC
															A											ATTCAC
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	EVQLVE	GGGGS	DVVMT	ITTPAP	VGILVA	LRHRR
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLQE	SGAEVK	GGGGS	SPSSLS	GGGGS	SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLPL	SGGGL	GGGGS	QSPLSL	RPFTPA	VILLLLL	QGKHW
			ATRVHS	EDLIQS	SGVTPE	GIFWCC	GDVES	LPHFAF	KPGSSV		ASVGD		RVTITC	VPVFLP	AGTCG	MNMTP	YKQGQ	GDVES	ALLLHA	VQPGG	GGGGS	PVTLGQ	PTIALQ	LLLFLI	TSTQR
				MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP	KVSCKA		RVTITC		AKPTTT	VLLLSL	VITLYC	MNMTP	RRPGPT	NPGPG	ARP			PASISC	PLSLRP		KADFQ
				LYTESD	GGSGG	PRCRE	SGEGR		SGGTFS		RASESV		RASQSI	PAPRPP	NHR	RKHYQ	LNLGRR	SGEGR				KSSQSL	EAQRPA		HPAGA
				VHPSCK	GGSGG	RRRNE	GSLLTC		SYAISW		DIVYGIS		SSYLN	TFAPTIA		PYAPPR	EEYDVL	GSLLTC				VASDEN	AGGAV		VGPEP
				VTAMKC	GSLQ	RLRRES	GDVEE		VRQAP		FMNWF		WYGQIK	SQFLSL		DFAAYR	DKFRRG	GDVEE				MHWVR	HTRGLD		TDRGL
				FLLELQ		VRPV	NPGP		GGGLE		QGKPG		PGKAPK	RPEACR		S	RDPEM	NPGP				QAPGK	FACD		QWRSS
				VISLES					WMGGII		KAPKLLI		LIYAAS	PAAGG			GGKPR					GLEWV			PAADA
				GDASIH					PIFGTA		YAASNQ		SLQSGV	AVHTRG			RKNPQ					SVIWGN			QEENL
				DTVENL					NYAQKF		GSGVP		PSRFSG	LDFACD			EGLYNE					GNTHY			YAAVK
				IILANNS					QGRVTI		SRFSGS		SGSGT				LQKDK					HSALKS			HTQPE
				LSSNGN					TADKST		GSGTDF		DFTLTIS				MAEAYS					RFTISR			DGVEM
				VTESGC					STAYME		TLTISSL		SLQPED				EIGMKG					DNSKNT			DTRSP
				KECEEL					LSSLRS		QPDDFA		LATYYC				ERRRG					LYLQMN			HDEDP
				EEKNIK					YMELSS		TYYCQ		QGSYST				KGHDG					SLRAED			QAVTY
				EFLQSF					EDTAVY		LRSEDT		PFTFGP				LYQGLS					TAVYYC			AEVKH
				VHIVQM					YCATFA		AYYYCA		GTKVDI				TATKDT					TLRIKD			SRPRR
				FINTS					LFGFRE		RGRPA		K				YDALHM					WGQGT			EMASP
									QAFDIW		MDYWG						QALPPR					MVTVSS			PSPLSG
									GQGTT		QGTLVT															EFLDTK
									VTVSS		VSS															DRQAE

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																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

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SB07403			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	CD33 VL	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1
	Retrovec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GATATT	GGCGG	GATATG	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	ACAACA	GTGATG	CTGCG
			CTGGA	CGTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	GCAGC	CGGAG	CAGCT	CGGAG	GTGGCG	CAAATG	GAGCA	ATCTG	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	AGACC	GGCATT	GCACA
			CTTGGA	GTGATC	GTGGT	CTGGG	CTACG	GGTTAC	TGGTTC	GATCT	GGTGC	GAAGT	GATCT	ACCCA	ACAGC	GGCCC	GGAGC	TTATGC	CCGTG	TGATCT	GGATCT	CGCAC	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	AGTCTG	GGTGG	AGAGC	GGCGG	GAGCC	GTCTCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CTCGG	CGCCG	CAGGG
			TTCTGG	CCTGAAA	AGGCG	CACTGA	CTGAAA	GCTGC	GCGCC	TGGTG	GGAGC	CGGAG	CCAGC	ATCCAG	TACTTC	GCTGG	ATCCTG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	CCTCCA	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT	GAAGT	AGCCT	GTCTG	AGCCT	CCTGA	AGCCA	CCCCT	TGCAA	ACAGC	CGCCC	TCGCC	GCTGC	GGACT	AAGTG	GAGCC	ACTCCA	TGCTCC	ACTGG
			CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAAAA	GTCTG		GCCAG	AGCGC	TGGGA	GCCAG	GCGCC	CTTCGT	GCGGA	GAGTAC	CCGCG	GGGGA	CTTTGG	GCGGA	TTCT	TGCCT	GCTCC	TGTTGC	ACAAG
			GCCGC	CCTGAC	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG		GCCAG	CCTCTG	TGGGA	AGCGT	CTTGGT	GCGGG	GTTCTG	ATGAAC	TACAAG	GTGTGA	CACTG	GCCTG	GGCGG	GTGAC	AACAAT	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC		CCGTG	TGGGA	GACAG	GGCGA	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	ACTGCT	GCGGA	TTCT	ACTGG	TGCACT	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GAATCCT	CCTTTC	AGCGT		AAAGTC	AGTGA	TAGAGT	TGCCC	CAGCC	CCTAGA	ACCAG	CCCAG	GACCT	CTGCTA	AGACT		GACAG	GCAAC	TGTTCC	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG		AAAGTC	AGTGA	CACAAT	GCCAA	TGGTC	CGGCC	ACCAG	GCATG	CTGCTA	AGACT			CCTGC	CCCTG	TGATC	CGACT
				GAGGC	CAGCC	GAACTA	TGAAT	TTGCT	TGTCCI		CAATTA	CAATTA	CACCT	GCCTA	AICACC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG			CAGCA	AGTCTG		TTCAG
				CACACT	TGATCG	CTGCTT	CTGGC		GCAAA		AAGGC	CCTGC	GTAGA	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG		GATATG			TCAGCT	AGGCC		CATCC
				GTACAC	GCGGA	CGGCC	GAGGG		GCTTCT		CAGCG	CGGGC	GCCAG	CCCCT	TGCAA	GAAAG	GCTCAA	AAAGGT		CACTG			CCAGC			TGCTG
				CGAGT	GGTTC	CTAGAT	ACGCG		GGCGG		GCTACA	CCAGA	GCTCCT	CCACA	CACTAC	TCTAGG	CGTGG			GGTCC			GGCTT	TCTAGC	CCTGTA	
				CCGAT	CGGAG	GCAGA	GGAGT		CACCTT		CCTTTA	AGAGC	GCATCA	AGAOC	GA		CAGCC	ACGAA	TAGTCT				CACCTT	CAGTCT	GGCCA	GTTGG
				GTGCA	GTGGC	GAGCG	CTACTG		CAGCA		CCGAC	GTGGA	GCTCCT	ACCTAC			TTACGC	GAGAG	ACTAAC				CAGCA	CTGGT	GCAGC	AGCCT
				CCCTA	GGTTC	GAAGA	ACGTG		GCTAC		TACAAC	CAATTA	ACCTGA	AACCAG			TCCTCC	GAGTA	GTGTG				GATAC	GGCCA	TGGCG	AGCCT
				GCTGC	CGGCG	GAAAC	TGGAG		GCCAT		ATGCAC	CGGCA	ATTGGT	CTCCTA			TTTCGC	GATGT	GTGAT				GCGAC	GCGAC	GAGCT	ACAGA
				AAAGTG	GAGCA	GAGCG	ACGTG		CTGTTG		TCAGCT	TGGGT	ATCAGC	CAATCG			TTTCGC	TTTGGA	GTAGAA				CACTG	GAGAA	GTTCAC	TAGAG
				ACCGC	TCTCTT	GCTGA	GAGGA		GGTTC		TCATGA	CCGAC	AGAAAC	CCAGC			CGCCT	CAAGA	GAAAAT				GGTCC	CACCTA	ACTAGA	GACTG
				CATGAA	CAA		AAACC		GACAG		AAGCTC	ACTGGT	CAGGC	CAGCC			ACCGG	GACGT	CCTGG				GACAG	CCTGA	GGCCT	CAGTG
				GTGCTT		GAAAG	CTGGA		GCCCC		CAGGC	TCCAG	CTCCA	AAAGCT	TCTGTC			GGCCG					CCTGG	ACTTG	GGAGT	GCGGT
				TCTGCT		CGTGC	CCT		TGGAC		CAGGG	CAGAA	CCCAAA	TCTGAG				GGACC					TTCCAG	TTCCAG	TGGCT	CTAGC
				GGAAC		GAGCC			AAGGC		GCCCG	CTGAA	CTCCTG	GCCCG				CTGAG					AAGGA	CTTGAC	CCTGC	CCTGC
				TGCAA		GGCGT			CTGGA		GCAAG	GTGGA	ATCTAT	AAGCTT				ATGGG					CTTGAA	CCCCG	CGCTG	CGCTG
				GTGATC		GTG			ATGGAT		TCGGC	GCCCC	GCTGC	GTAGA				GGGAA					TGGGT	GACAG	ATGCC	ATGCC
				AGCCT			GAAGA		TCGGC		TAAGCT	TAAGCT	GCTCA	CCAGC				AGCCG					GTCCG	CCTGA	CAAGA	CAAGA
				GGAAA			AAACC		GGGAG		TACCCC	ACTGGT	CTCCA	CCAGC				AGAAG					GTCCT	AGACG	GGAAA	GGAAA
				GCGGC			CTGGA		GCATCA		TCCCCA	GCTGAT	GCCTG	TGCTG				GAAGA					TGAGT	GCTGA	ATCTTT	ATCTTT
				GACGC			CCT		TCCCCA		TCTTCG	TACAAC	CAGAG	GCGGA				GGAAGA					GGGGC	TCTACC	ACGCC	ACGCC
				CAGCAT					GCACC		CACCG	CTACG	TGGCG	GCCGT				ACCCTC					AACGG	CAACAC	GAAGC	GAAGC
				ATACCG					GCCAAT		GCCAAT	AGCAAT	CTAGAT	TAAGAG				AGGAA					GGCCT	ACACTA	GCCGT	GCCGT
				TGGAAA					TACGC		TACGC	CAAGG	TCAGCA	AAGCAT				GGCCT					CCACA	AGGTG	ACACC	ACACC
				ATCTGA					CCAGA		AAGTTC	GAGTG	GAGTCA	GCTAGC				GTACAA					GCGCC	GCTGG	CAGCC	CAGCC
				TCATCC					AATTCC		AAGAG	CCTAG	GGCAG	GATTTT				TGAACT					GCGCC	ATAGC	TGAGG	TGAGG
				TGGCC					AGGGC		CAAGG	CCACAA	CGGAC	GCGGC				GCAGA					GTCCC	GCCCG	ATGGC	ATGGC
				AACAAC					AGAGT		TCACAG	GCTCT	CGGAG	ACAGA				AGATG					GGTTCA	ATAGAT	GTGGA	GTGGA
				AGCCT					GAACCAT		TCACAG	GGCAG	CTTTAC	CTTTAC				GCGGA					CCATCT	TTTCTG	AATGG	AATGG
				GTCCA					CACCG		CCGAC	CGGCA	ACTGAC	ACTGAC				GGCCT					CCAGA	GCAGC	ACACC	ACACC
				GCAAC					CCGAC		GAGTC	CGGCA	AATATC	AATATC				ACAGT					GACAA	GGCTC	AGATC	AGATC
				GGCAA					AAGAG		CACAA	CACCAA	CCGATT	TTCCCT				GAGATT					CAGCA	TGGCA	TCCCC	TCCCC
				TGTGAC					CACAA		GCACC	CACAG	TCACCC	GCAGC				GGGAT					AGAACA	CCGAC	ACGAT	ACGAT
				CGAGA					GCACC		GCCTA	TTATA	IGACCA	CAGAG				GAAAG					CCGTGT	CTGAA	GAGGA	GAGGA
				GCGGC					CATGG		TGGAA	TCAGTA	TCAGTA	GACCT				GCGAG					ACCTG	GATCA	CCCTC	CCCTC
				AGCCT					AACTGA		CATGTCT	GCCTG	GGCCA	GGCCA				CGGCG					CAGAT	GCAGA	AGGCC	AGGCC
				GAGTG					GCAGCT		AGCCT	TGACG	CATTAT	ATTGCC				GAGGG					GAACA	GTGGA	GTGAC	GTGAC
				CGAGG					CTGAG		ACTTCG	AGCAG	AGCAG	AGCAG				GGGCA					AGCCG	AGCCG	ATACG	ATACG
				AACTG					AAGCG		GATACA	GCTGT	CCACCT	TCCTAC				CGATG					CGAGG	AGGAC	CAGAA	CAGAA
				GAAGA					AGGAC		GTACTA	GCCAG	ACTACT	AGCAC				GCCTTT					ACACC	GTGGG	GTGAA	GTGAA
				GAAGA					ACCGC		GTACTA	GTGGC	GCCAG	CCCTTT				ACCAG					GCCGT	CGTGT	GCACT	GCACT
				ACATCA					CGTGTA		TTGTGC	CCTGT	CAGAG	CACCTT				GGTCT					GTACTA	ACTACT	CCAGA	CCAGA
				AAGAGT					CTACTG		CAGGG	CATTG	CAAAGA	CGGAC				CAGTAC					CTGCA	GTCTG	CCTCG	CCTCG
				TTCTGC					CGCCA		GCAGA	CATTTG	CGGGA	ACAAAG				AGCCA					CTGCA	CAAGG	GAGAG	GAGAG
				AGAGC					CATTTG		GGTGC	CCGGC	CCTGG	CCTGG				CCAAG					CATCCA	CCCTG	AGATG	AGATG
				TTCGTC					CCCTGT		CATGG	CATTG	ACCTTT	ACCTTT				GACAC					AGGAT	TCTGC	GCAAG	GCAAG
				CACATC					TCGGC		ACTATT	GGACA	CAGAG	CAAAGA	CGGAC	GTGGA		CTACGA					TGGGG	CTTGG	CCCTC	CCCTC
																									CATCT	CATCT

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															CATTAAG												
									TTCAGA			GGGGA		GGGCA													CCTCT
									GAGCA			CAGGG		CCAAG													GAGCG
									GGCCT			AACCCT		GTGGA													GCGAG
									TCGATA			GGTCA		AATCAA													TTCCT
									TCTGG			CAGTGT		A													GGACA
									GGCCA			CATCT															CCAAA
									GGGCA																		GACAG
									CAACC																		ACAGG
									GTGAC																		CCGAA
									CGTTTC																		GAGGA
									TAGC																		CAGAC
																											AGATG
																											GATAC
																											CGAAG
																											CCGCC
																											GCTTC
																											TGAAG
																											CCCCA
																											CAGGA
																											TGTGA
																											CATAT
																											GCCCA
																											GCTGC
																											ATAGC
																											CTGAC
																											ACTGC
																											GGAGA
																											GAAGC
																											CACAG
																											AGCCT
																											CCACC
																											TTCTCA
																											AGAAG
																											GCCCA
																											TCTCCT
																											GCCGT
																											GCCTT
																											CCATC
																											TATGC
																											CACTC
																											TGGCC
																											ATTCAC
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGS	QVQLV	GGGGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	EVQLVE	GGGGS	DVVMT	ITTPAP	VGILVA	LRHRR
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLQE	SGAEVK	GGGGS	QSGAE		SPSSLS		SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLLPL	SGGGL	QSPLSL	RPFTPA	VILLLLL	QGKHW
			ATRVHS	EDLIQS	SGVTPE	GIFWCC	GDVES	LPHFAF	KPGSSV		KVSCKA		ASVGD		ASVGD	VPVFLP	VLLLSL	MNMTP	YKQGQ	GDVES	ALLLHA	VQPGG	PVTLGQ	PTIALQ	LLLFLI	TSTQR
				MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP	SGGTFS		SGVSCK		RVTITC		RVTITC	AKPTTT	VITLYC	RRPGPT	NQLYNE	NPGPG	ARP		SLRLSC	PASISC	PLSLRP	KADFQ
				LYTESD	GGSGG	PRCRE	SGEGR		SYAISW		KASGYT		RASESV		RASQSI	PAPRPP	NHR	RKHYQ	LNLGRR	SGEGR		AASGFT	KSSQSL	EAQRPA	HPAGA
				VHPSCK	GGSGG	RRRNE	GSLLTC		VRQAP		FTDYNM		DIVYGIS		SSYLN	TFAPTIA		PYAPPR	EEYDVL	GSLLTC		FSRYD	VASDEN	AGGAV	VGPEP
				VTAMKC	GSLQ	RLRRES	GDVEE		GGGLE		HWVRQ		FMNWF		WYGQIK	SQFLSL		DFAAYR	DKFRRG	GDVEE		MHWVR	TYLNWF	HTRGLD	TDRGL
				FLLELQ		VRPV	NPGP		WMGGII		APGQG		QQIKPG		PGKAPK	RPEACR		S	RDPEM	NPGP		QAPGK	QGRPG	FACD	QWRSS
				VISLES					PIFGTA		LEWIGYI		KAPKLLI		LIYAAS	PAAGG			GGKPR			GLEWV	QSPRRL		PAADA
				GDASIH					NYAQKF		YPYNG		YAASNQ		SLQSGV	AVHTRG			RKNPQ			SVIWGN	YQVSK		QEENL
				DTVENL					QGRVTI		GTGYN		GSGVP		PSRFSG	LDFACD			EGLYNE			GNTHY	LDSGVP		YAAVK
				IILANNS					TADKST		QKFKSK		SRFSGS		GSGSGT				LQKDK			HSALKS	DRFSG		HTQPE
				LSSNGN					STAYME		ATITAD		GSGTDF		DFTLTIS				MAEAYS			RFTISR	SGSGT		DGVEM
				VTESGC					LSSLRS		ESTNTA		TLTISSL		SLQPED				EIGMKG			DNSKNT	DFTLKIS		DTRSP
				KECEEL					EDTAVY		YMELSS		QPDDFA		LATYYC				ERRRG			LYLQMN	RVEAED		HDEDP
				EEKNIK					YCATFA		LRSEDT		TYYCQ		QQSYST				KGHDG			SLRAED	VGVYY		QAVTY
				EFLQSF					LFGFRE		AYYYCA		QSKEVP		PFTFGP				LYQGLS			TAVYYC	CLQGIH		AEVKH
				VHIVQM					QAFDIW		RGRPA		WTFGQ		GTKVDI				TATKDT			TLRIKD	LPWTF		SRPRR
				FINTS					GQGTT		MDYWG		GTKVEI		K				YDALHM			WGQGT	GQGTK		EMASP
									VTVSS		QGTLVT		K						QALPPR			MVTVSS	LEIK		PSPLSG
											VSS																EFLDTK
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SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders								Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders						Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
SB07405			IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	CD33 VL	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1
	Sinvec	DNA	ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GGTGG	GATATT	GGCGG	GATATG	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	CACCC	GTGATG	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	GCAGC	CGGAG	CAGCT	TGGCG	CACAT	TGGCG	CAAATG	GAGCA	CAAGC	GAAGTT	CACAAA	TCTGC	CAGCC	CGGAG	GCTCA	ATCGCA	GGCATT	GCACA
			CTTGGA	GTGATC	GTGGT	CTGGG	CTACG	GGTTAC	TGGTTC	GATCT	GAACTCA	GATCT	GACTCA	GATCT	ACCCA	ACAGC	GGCCC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	TCCTCT	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	AGTCTG	GGTGG	AGAGC	GGCGG	GAGCC	GGCGG	GTCTCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CGAGC	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAC	GCGCC	TGGTG	GAAGT	GGAGC	CGGAG	CCAGC	AGGTG	ATCCAG	GCTGG	GCTGC	ACAGC	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	TGGTG	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT	GATCT	TGAAGT	GCTCT	AGCCT	GCAGT	CCTGA	AGCCA	ACAGC	CGCCC	TCGCC	GCTGC	GGACT	AAGTG	GAGCC	GTTTCT	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA	GCCAG	GTCTG	GGAAAA	GCGCC	CTCTG	GCGCG	CTTGT	GCGGA	GACTAC	CCGCG	CTTTGG	GCGGA	TTCT	ACTGG	CGGCC	TGCTTC	AGAGA
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG	GCCAG	CCTCTG	TGGGA	AGCGTT	GCCCG	GTTCTG	ATGAAC	TACAAG	TGTGGA	CACTG	GCCTG	GGCGG	GTGAC	GACAG	TTCTGG	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC	CCGTG	GACAG	GGCGA	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	CTGCT	GCGGA	ITCT	GACAG	CTAGCA	TCTTCA	AGAGA		
			CACTCT	GCATG	GAGCC	GATCTG	GATCTG	CCTTTC	AGCGT	AAAGTC	AGTGA	CAATTA	CACAAT	GCCAA	TAGAGT	TGCCC	CCTAGA	CCAGA	CCCAG	GCATG	CTGCTA	AGACT	CCTGC	GCCCT	TGATC	AAGGC
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG	TCTTGC	CAATTA	CAACAA	ACCTGA	AACCAG	CACCT	GCCTA	ATCACC	CGGAC	CTCTAT	GGAAG	GACCA	GTCTTG	CAGCA	ACAACA	CATCC	TTCAG
				CACACT	TGATCG	CTGCTT	CTGGC	GCTTCT	GCAAA	AAGGC	CCTGC	CGGGC	CCCTT	TGCAA	GTAGA	CAACAA	CTGTAC	CTACCA	AACGA	CGGAG	TGCCG	GCAAG	TACAAG	TGCTC		
				GTACAC	GCGGA	CGGCC	GAGGG	GCTACA	CGAGT	GGCGG	GCTACA	CAGCG	CCAGA	GCTCCT	GCCAC	CACTAC	TCTAGG	GGTGG	GAAGG	GCTCAA	AAGGT	GGACTT	CAGTCT	GGCGC	GTTGG	
				CCGAT	CGGAG	GCAGA	GGAGT	CACCTT	CAGCA	CCGAC	AGAGC	GTGGA	AGCGC	CAGGC	GCATCA	AGAGC	AGAAA	TAGTCT	CAGCA	CACCTT	CAGTCT	GGCGC	GTTGG		
				CCCTA	GGTTG	GAGAA	ACGTG	GCTAC	TACAAC	CAGGG	CAATTA	CGGCA	ATTGGT	CTCCTA	ACCTGA	AACCAG	ATTGGT	GTGTG	TAGAGA	CGATGT	GTGAT	GATATG	GCGAC	AACCTC	ACAGA	
				GCTGC	CGGCG	GAAAC	TGGAG	GCCAT	ATGCAC	AGGTG	CTCTTG	TGGGT	TCAGCT	ATCAGC	ATTGGT	CTCCTA	TTTCGC	TTTGGA	GTATGAA	CACTG	GAGAA	AAGGA	TGACAC	TAGAG	
				AAAGTG	GAGGA	AGGTG	CTCTTG	TGGGT	TCAGCT	ATCGTG	TCAGAC	GCCCG	CGCCT	CAAGA	CGCCT	CAAGA	GAAAAT	CCTGG	CACTG	GGTCC	CACCTA	CAACA	GACAG	GAGAC	GACTG	
				ACCGC	TCTCTT	GCTGA	GAGGA	GGTTC	CCGAC	AAGCTC	ACTGGT	TCCAG	AGAAAC	CAGGC	CAGCC	ACCGG	GACGT	CCTGG	GGTCC	CACCTA	CCACA	CACCTA	CAACA	CTGGT	GACAG	GAGAC	CAGTG
				CATGAA	CAA	GAAGA	AAACC	GACAG	CAGGC	CAGGC	TAAGCT	CTCCA	AAAGCT	CCAGC	ACCGG	TCC	GGCCG	ACCT	GGCCC	TGGGT	GTCCG	TGATCT	GGGGC	GCTGA	GCGTT	ATGCC	CAGTG
				GTGCTT	GAAAG	CGTGC	GCCCC	CAGCA	GCCCC	CAGGC	TACATC	GCCTG	CCCAAA	TCTGTC	CAGAG	GCGGA	TGGCG	GCGGC	TGGTC	GGAGC	GGAGC	GCTGA	GCGGC	GCTGA	GCGGC	GGAAA	GCAGG
				TCTGCT	CGTGC	GAAAG	TGGAC	TGGAC	CAGGG	GCCCG	CTACG	CTCCTG	ATCGCT	GCCGA	CAGAG	GCGGA	CTCCTG	GCGAC	CAGAG	GCGGA	GCGAC	CTGGA	GCGGC	CTAGC	ATCTTT	CTAGC	
				AAAGTG	GAGCG	AAGGC	CCGTG	AAGGC	ACTTGA	GCAAG	GCAAT	TCAGCT	ATCTAT	AAGCTT	ATCTAT	AAGCTT	ATGGG	ATGGG	ACCCTC	AGGAA	ATGGG	AACGG	CAGGA	CCTGC	ACGCC	CCTGC	
				TGCAA	CCGCC	GTGAT	GGCCC	GTGGA	GCAAG	TAGCC	GCCCC	TAAGCT	GCTGC	GTAGA	AGAAAC	CCAGC	AAGAA	GGAAA	GCGCG	GAAGA	GGCCT	ACACTA	GCCCG	CTGGG	GCGCT	GCGCC	
				GTGATC	GGAGC	ATGAT	ATGAT	ATGGAT	ATGGAT	GGGAG	TAAGCT	CTCCA	CTCCA	CCAGC	CTCCA	CCAGC	GTCCG	AGCCG	AGCCG	AGCCG	GTCCG	GTCCG	GCCCG	GCTGG	GAAGG	GAAGG	GAAGG
				GAAAA	GGGGC	GGAAA	GGAAA	GCATCA	GCATCA	TACCCC	GCTGAT	GCCTG	GCCTG	TGCTG	GCCTG	TGCTG	GCCTG	TGCTG	TGCTG	TGCTG	TGCTG	TGCTG	TGCTG	TGCTG	TGCTG	TGCTG	TGCTG
				GGGGC	GACGC	GGCGC	GACGC	TCCCCA	GACGC	TACAAC	CTACG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG	CAGAG
				GACGC	TGCAT	CAGCAT	CAGCAT	TCTTCG	CAGCAT	GGCGG	CCGCC	TGCCAT	GCTACG	GCTACG	TGCCAT	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG	GCTACG
				ATACCG	TGGAAA	ATCTGA	TCATCC	TGGCC	AACAAC	AGCCT	ATACCG	TACGC	AGCTTC	AAGAG	CAGATT	TTCCG	CTTTAC	ACTGAC	AATATC	TTCCCT	GCAGC	GGATT	GCAGC	GGATT	GCAGC	GGATT	GCAGC	GGATT
				TCATCC	TGGCC	AACAAC	AGCCT	GTCCA	GCCAA	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC	CCAGC
				TGGCC	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT
				AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA
				AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC
				GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT
				GCAAC	GGCAA	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA
				GGCAA	CTGTGAC	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC	AGCCT	GTCCA	AACAAC
				TGTGA

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				GTGCA					TTCAGA					CATTAA							CCAGG					CCTCT
				GATGTT					GAGCA					G							GGCCA					GAGCG
				CATCAA					GGCCT												GGGCA					GCGAG
				CACCTC					TCGATA												CAAAG					TTCCT
				A					TCTGG												CTGGA					GGACA
									GGCCA												AATCAA					CCAAA
									GGGCA												G					GACAG
									CAACC																	ACAGG
									GTGAC																	CCGAA
									CGTTTC																	GAGGA
									TAGC																	CAGAC
										GGGGA																AGATG
										CAGGG																GATAC
										AACCCT																CGAAG
										GGTCA																CCGCC
										CAGTGT																GCTTC
										CATCT																TGAAG
											GGGCA															CCCCA
											CCAAG															CAGGA
											GTGGA															TGTGA
											AATCAA															CATAT
											A															GCCCA
												GGGCA														GCTGC
												CCAAG														ATAGC
												GTGGA														CTGAC
												AATCAA														ACTGC
												A														GGAGA
													GGGCA													GAAGC
													CCAAG													CACAG
													GTGGA													AGCCT
													AATCAA													CCACC
													A													TTCTCA
														GGGCA												AGAAG
														CCAAG												GCCCA
														GTGGA												TCTCCT
														AATCAA												GCCGT
														A												GCCTT
															GGGCA											CCATC
															CCAAG											TATGC
															GTGGA											CACTC
															AATCAA											TGGCC
															A											ATTCAC
		AA	MDWTW	NWVNVI	SGGGG	LLPSWA	QCTNY	MLLLVT	EVQLVQ	GGGGS	QVQLV	GGGGS	DIQMTQ	GGGGS	DIQMTQ	ALSNSI	IYIWAPL	RSKRSR	RVKFSR	QCTNY	MALPVT	EVQLVE	GGGGS	DVVMT	HPSDPL	VGILVA	LRHRR
			ILFLVAA	SDLKKI	SGGGG	ITLISVN	ALLKLA	SLLLQE	SGAEVK	GGGGS	QSGAE	GGGGS	SPSSLS	GGGGS	SPSSLS	MYFSHF	AGTCG	LLHSDY	SADAPA	ALLKLA	ALLPL	SGGGL	GGGGS	QSPLSL	ELVVSG	VILLLLL	QGKHW
			ATRVHS	EDLIQS	SGVTPE	GIFWCC	GDVES	LPHFAF	KPGSSV		KVSCKA		ASVGD		ASVGD	VPVFLP	VLLLSL	MNMTP	YKQGQ	GDVES	ALLLHA	VQPGG	GGGGS	PVTLGQ	PSGGP	LLLFLI	TSTQR
				MHIDAT	PIFSLIG	LTYCFA	NPGPG	LLIP			SVKVSC		RVTITC		RVTITC	AKPTTT	VITLYC	RRPGPT	NQLYNE	NPGPG	ARP			PASISC	SSPTTG		KADFQ
				LYTESD	GGSGG	PRCRE	SGEGR		SGGTFS		KASGYT		RASESV		RASQSI	PAPRPP	NHR	RKHYQ	LNLGRR	SGEGR		SLRLSC		KSSQSL	PTSTSG		HPAGA
				VHPSCK	GGSGG	RRRNE	GSLLTC		SYAISW		FTDYNM		DIVYGIS		SSYLN	TFAPTIA		PYAPPR	EEYDVL	GSLLTC		AASGFT		VASDEN	PEDQPL		VGPEP
				VTAMKC	GSLQ	RLRRES	GDVEE		VRQAP		HWVRQ		FMNWF		WYQQIK	SQFLSL		DFAAYR	DKFRRG	GDVEE		FSRYD		MHWVR	TTPTGSD		TDRGL
				FLLELQ		VRPV	NPGP		GGGLE		APGQG		QQKPG		PGKAPK	RPEACR		S	RDPEM	NPGP		MHWVR		QAPGK	QGRPG	PQSGL	QWRSS
				VISLES					WMGGII		LEWIGYI		KAPKLLI		LIYAAS	PAAGG			GGKPR			GLEWV		QSPRRL	GRHLG		PAADA
				GDASIH					PIFGTA		YPYNG		YAASNQ		SLQSGV	AVHTRG			RKNPQ			SVIWGN		YQVSK	V		QEENL
				DTVENL					NYAQKF		GTGYN		GSGVP		PSRFSG	LDFACD			EGLYNE			GNTHY		LDSGVP			YAAVK
				IILANNS					QGRVTI		QKFKSK		SRFSGS		SGSGT				LQKDK			HSALKS		DRFSG			HTQPE
				LSSNGN					TADKST		ATITAD		GSGTDF		DFTLTIS				MAEAYS			RFTISR		SGSGT			DGVEM
				VTESGC					STAYME		ESTNTA		TLTISSL		SLQPED				EIGMKG			DNSKNT		DFTLKIS			DTRSP
				KECEEL					LSSLRS		YMELSS		QPDDFA		LATYYC				ERRRG			LYLQMN		RVEAED			HDEDP
				EEKNIK					EDTAVY		LRSEDT		TYYCQ		QQSYST				KGHDG			SLRAED		VGVYY			QAVTY
				EFLQSF					YCATFA		AYYYCA		QSKEVP		PFTFGP				LYQGLS			TAVYYC		CLQGIH			AEVKH
				VHIVQM					LFGFRE		RGRPA		WTFGQ		GTKVDI				TATKDT			TLRIKD		LPWTF			SRPRR
				FINTS					QAFDIW		MDYWG		GTKVEI		K				YDALHM			WGQGT		GQGTK			EMASP
									GQGTT		QGTLVT		K						QALPPR			MVTVSS		LEIK			PSPLSG
									VTVSS		VSS																	EFLDTK
																												DRQAE

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																												EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

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SB07409	Retrovec	DNA	IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	CD33 VL	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	LIR1
			ATGGA	AATTGGT	TCAGG	TTGCTG	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GGTGG	GATATT	GGCGG	GATATG	GCCCT	ATCTAT	AGAAG	AGAGT	CAGTG	ATGGC	GAGGT	GGAGG	GACGT	CACCC	GTGATG	CTGCG
			CTGGA	GTCAAC	CGCGCG	CCTAG	TACCAA	CTGCT	GCAGC	CGGAG	CAGCT	TGGCG	CACAT	TGGCG	CAAATG	GAGCA	CAAGC	GAAGTT	CACAAA	TCTGC	GCAGC	CGGAG	GCTCA	ATCGCA	GGCATT	GCACA
			CTTGGA	GTGATC	CTGGG	CTACG	GGTTAG	TGGTTC	GGATCT	GAAGT	GACTCA	GATCT	ACCCA	ACAGC	GGCCC	GTCTCC	ATCATG	GCTGC	CAGCA	TTATGC	CCGTG	TGGTTG	GATCTG	TGACA	TCCTCT	CTGGT	GAAGG
			TACTCT	AGCGA	AGTGG	CCATCA	CCCTG	ATCTCT	AGTCTG	GGTGG	AGAGC	GGCGG	GAGCC	GGCGG	GTCTCC	ATCATG	CTCTG	AGACT	GGAGC	ACTGCT	ACAGC	AATCTG	GTGGC	CAGAG	CGAGC	CGCCG	CAGGG
			TTCTGG	CCTGAA	AGGCG	CACTGA	CTGAC	GCGCC	TGGTG	GAAGT	GCAGC	CGGAG	ATCCAG	AGGTG	CCTGA	AGCCA	GTACTT	GCTGG	GCTGC	GCAGA	GAAGC	TTTGCT	GCGGA	GGAGG	CCCTCT	TGGTG	TGATCC	CAAGC
			TCGCT	GAAGAT	GAGGC	TCTCCG	CTGGC	TGTGC	GAAGT	GAAGT	AGCCT	GTCTG	CCTGA	AGCCA	CACAT	CGCCC	CTTCGT	GCGGA	GACTAC	CCGCC	GCTGC	GCTGC	GGACT	AAGTG	GAGCC	GTTTCT	TGCTCC	ACTGG
			GCCGC	CGAGG	TCAGG	TGAAC	CGGCG	GAGCT	GAAGA	GAAAAA	GTCTG	TGGGA	AGCGT	GCGCC	CTTCGT	GCGGA	GACTAC	CCGCC	CTGCA	GGGGA	CTTTGG	TTCGA	GCGGA	TTCT	TGCCT	GGACC	TGTTGC	ACAAG
			CACAC	ACCTGA	CGTGA	GGCAT	ACGTG	GCCCC	AACCTG	GCCAG	CCTCTG	TGGGA	AGCGTT	GCCCG	GTTCTG	CTGCT	ATGACC	CAGGG	GAGTAA	CTGCT	GCGGA	GCCTG	GGCGG	GTGAC	TTCTGG	TCCTGC	CACCC
			GGGTG	TCCAGA	CCCCT	CTTCGT	GAATCT	ATCCTG	GCAGC	TGGGA	GCTGCT	GGCGA	TGTTTC	CTGCT	ATGACC	CAGGG	GAGTAA	CTGCT	GCGGA	ITCT	ACTGG	CGGCC	TGCTTC	AGAGA
			CACTCT	GCATG	GAGCC	GATCTG	GATCTG	CCTTTC	AGCGT	CCGTG	GACAG	TAGAGT	TGCCC	CCTAGA	CCCAG	GCATG	GACAG	CTACAA	TCTCTG	CCTGC	GCCCT	TGATC	CGACT
				CACATC	TATCTT	CTGCCT	GGACC	TGCTTA	GAAGG	AAAGTC	AGTGA	CACCT	CAATTA	GCCTA	CACAAT	GCCAA	TGGTC	CGGCC	ACCAG	GACCT	CTGCTA	AGACT	CAGCA	ACAGCA	TGATC	TTCAG
				GAGGC	CAGCC	GAGCTA	TGATGT	TTGCT	TCTTGC	CAATTA	GTAGA	CCTGC	GCCAG	CCCCT	CCAGA	GCTCCT	CCACA	TTACGC	GAGAG	ACTAAC	GTGTG	GTCTTG	GGAGA	ACAACA	CATCC
				CACACT	TGATCG	CTGCTT	CTGGC	GCTTCT	GCAAA	AAGGC	CCTGC	CGGGC	CCAGA	GCTCCT	CCACA	TGCAA	CTACCA	AACGA	CGGAG	ATCGTG	CCAGC	TCAGCT	GCAAG	TACAAG	TGCTG
				GTACAC	GCGGA	CGGCC	GAGGG	CTAGAT	ACGCG	CGGCG	GCTACA	CAGCG	GCATCA	AGAGC	GTGGA	AAGCC	TGCAA	GAAAG	GCTCAT	CTAGAG	GGTGG	CTATAC	TCTAGC	CAGTCT	GTTGG
				CGAGT	GGTTC	CTAGAT	ACGCG	GGCGG	GAGCT	GGCTAC	CCTTTA	AGAGC	CCGAC	CCTGA	CAGCA	TCCAG	CAGCG	GAAAT	CACTAC	CAGCA	CCTGG	GGCGG	GGCCA	GGACC	GCCTG
				CCGAT	CGGAG	GCAGA	GGAGT	CACCTT	CAGCA	TACAAC	CAATTA	CGGCA	ATTGGT	CTCCTA	ATCGAC	CAGCG	ATGCAC	TGGGT	TCAGCT	AGAGAA	CGGAA	GCTCAT	CAGCA	GGCCA	AACCTC	ACAGA
				GCTGC	CGGCG	GAAAC	TGGAG	GCCAT	CTCTTG	AAGGC	GCTAC	ATGCAC	ATCGGT	TCAGCT	ATCAGC	TCAGG	ATCGAC	TTTCGC	TTTGGA	CGCCT	CAAGA	GAAAAT	GGTCC	GACAG	GAAGA	TGACAC
				AAAGTG	GAGGA	AGGTG	CTGAT	GGTTC	CTGGA	GGCAG	ACTTGA	CTGGA	GCAAG	GCCCC	TCATGA	AGAAAC	CCAGC	CAAGA	GAAAAT	CCTGG	GACGT	CCTGG	CACCT	GACAG	GAGAC	GACTG
				ACCGC	TCTCTT	GCTGA	GAGGA	GACAG	GACCC	ATGGAT	ACCAG	TCGGC	GCTGC	TAAGCT	CAGGC	CAGGC	ACCGG	CGCCT	CAAGA	GAAAAT	CCTGG	GGTCC	CACCT	CAGAG	GCTGG	ATGCC
				CATGAA	CAA	GAAGA	AAACC	GCCCC	CAGCA	AAGCTC	CAGGC	TCCAG	ACTGGT	TCCAG	CAGGC	AAAGCT	CCTCA	ACCGG	GACGT	CCTGG	GACAG	GAAAAT	GGTCC	CCTGA	GGCAG	GAGAC	CAGTG
				GTGCTT	GAAAG	GCGCA	CTACTG	GCTAC	GAGAA	TACAAC	CAATTA	CGGCA	ATTGGT	CTCCTA	ACCTGA	AACCAG	CTAGAG	GCGGA	GCAGC	GAAGA	GCAGC	GCCGC	GGCCA	ACTCAG	GGCCA	GGACC	GCGGT	AGCGT
				TGTGCT	GAAAG	GGAAA	ACGTG	TGGAC	GAAAC	CAGGG	CAGAA	AGCCG	CAGAA	GCCCC	CCCAA	ATCCTG	CTACG	TGGCA	GCGGA	GCAGC	GCCGC	GCCGC	GGCCA	GGACC	GCCTG	GCCGT	GCCTT
				TCTGCT	CGTGC	CGGCG	GAACC	CCT	TGGAC	AAGGC	GCCAT	ACTTGA	GCAAG	GCCCC	CCAAA	ATCTGA	CTACG	GCAGA	GCGGA	GCAGC	GCCGC	GCCGC	GGCCA	GGACC	GCCTG	GCCGT	GCCTT
				AAAGTG	GAGGA	AGGTG	CTGGA	GCCAT	CTCTTG	TGGGT	TCAGCT	TCAGG	ACTTGA	GCAAG	ATCAGT	GCTGC	CACGG	AGCAA	GCGGA	GCAGC	GCCGC	GCCGC	GGCCA	GGACC	GCCTG	GCCGT	GCCTT
				ACCGC	TCTCTT	GCTGA	GAGGA	GACAG	GACCC	CCGAC	TCGGC	CCGAC	AAGCTC	TAAGCT	CAGGC	CAGGC	ACCGG	CGCCT	CAAGA	GAAAAT	CCTGG	GGTCC	CACCT	CAGAG	GCTGG	ATGCC
				CATGAA	CAA	GAAGA	AAACC	GCCCC	CAGCA	AAGCTC	CAGGC	TCCAG	ACTGGT	TCCAG	CAGGC	AAAGCT	CCTCA	ACCGG	GACGT	CCTGG	GACAG	GAAAAT	GGTCC	CCTGA	GGCAG	GAGAC	CAGTG
				GTGCTT	GAAAG	GCGCA	CTACTG	GCTAC	GAGAA	TACAAC	CAATTA	CGGCA	ATTGGT	CTCCTA	ACCTGA	AACCAG	CTAGAG	GCGGA	GCAGC	GAAGA	GCAGC	GCCGC	GGCCA	GGACC	GCCTG	GCCGT	GCCTT
				TGTGCT	GAAAG	GGAAA	ACGTG	TGGAC	GAAAC	CAGGG	CAGAA	AGCCG	CAGAA	GCCCC	CCCAA	ATCCTG	CTACG	TGGCA	GCGGA	GCAGC	GCCGC	GCCGC	GGCCA	GGACC	GCCTG	GCCGT	GCCTT
				AAAGTG	GAGGA	AGGTG	CTGAT	GGTTC	CTGGA	GGCAG	ACTTGA	CTGGA	GCAAG	GCCCC	TCATGA	AGAAAC	CCAGC	CAAGA	GAAAAT	CCTGG	GACGT	CCTGG	CACCT	GACAG	GAGAC	GACTG
				ACCGC	TCTCTT	GCTGA	GAGGA	GACAG	GACCC	ATGGAT	ACCAG	TCGGC	GCTG

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders					Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD
													CATTAA G							ACCTTT				
																					GGCCA			
																					GGGCA			
																					CAAAG			
																					CTGGA			
																					AATCAA G			
		AA	MDWTW ILFLVAA ATRVHS	NWVNVI SDLKKI EDLIQS MHIDAT LYTESD VHPSCK VTAMKQ FLLELQ VISLES GDASIH DTVENL IILANNS LSSNGN VTESGC KECEEL EEKNIK EFLQSF VHIVQM FINTS	SGGGG SGGGG SGVTPE PIFSLIG GGSGG GGSGG GSLQ	LLPSWA ITLISVN GIFWCC PRCRE RRRNE RLRRES VRPV	QCTNY ALLKLA GDVES NPGPG SGEGR GSLLTC GDVEE NPGP	MLLLVT SLLLQE LPHPAF LLIP	EVQLVQ QSGAE KPGSSV KVSCKA SGGTFS SYAISW VRQAP GQGLE WMGGII PIFGTA NYAQKF QGRVTI TADKST STAYME LSSLRS EDTAVY YCATFA LFGFRE QAFDIW GQGTT VTVSS	GGGGS QSGAE KPKPGS SVKVSC KASGYT FTDYNM HWVRQ APGQG LEWIGYI YPYNG GTGYN QKFKSK ATITAD ESTNTA YMELSS LRSEDT AYYYCA RGRPA MDYWG QGTLVT VSS	GGGGS GGGGS	DIQMTQ SPSSLS ASVGD RVTITC RASQSI DINYGIS FMNWF QQKPG KAPKLLI YAASNQ GSGVP SRFSGS GSGTDF TLTISSL QPDDFA TYYCQ QSKEVP WTFGQ GTKVEI K	GGGGS GGGGS	ALSNSI MYFSHF VPVFLP AKPTTT AKPTTP PAPRPP TFAPTIA SQFLSL RPEACR PAAGG AVHTRG LDFACD	IYIWAPL AGTCG VLLLSL VITLYC NHR	RSKRSR LLHSDY MNMTP RRPGPT NQLYNE RKHYQ LNLGRR PYAPPR DFAAYR S	RVKFSR SADAPA YKQGQ NQLYNE LNLGRR EEYDVL DKFRRG RDFEM GGKFR RKNPQ EGLYNE LQKDK MAEAYS EIGMKG ERRRG KGHDG LYQGLS TATKDT YDALHM QALPPR	QCTNY ALLKLA GDVES NPGPG SGEGR GSLLTC GDVEE NPGP	MALPVT ALLLPL ALLLHA ARP	EVQLVE SGGGL VQPGG SLRLSC AASGFT FSRYD MHWVR QAPGK GLEWV SVIWGN GNTHY HSALKS RFTISR DNSKNT LYLQMN SLRAED TAVYYC TLRIKD WGQGT MVTVSS	GGGGS GGGGS GGGGS	DVVMT QSPLSL PVTLGQ PASISC KSSQSL VASDEN TYLNWF QQRPG QSPRRL VGVYY YQVSK LDSGVP DRFSG SGSGT DFTLKIS RVEAED VGVYY CLQGIH LPWTF GQGTK LEIK	HPSDPL ELVVSG PSGGP SSPTTG PTSTSG PEDQPL TPTGSD PQSGL GRHLG V	VGILVA VILLLLL LLLFLI	CCTCT GAGCG GCGAG TTCCT GGACA CCAAA GACAG ACAGG CCGAA GAGGA CAGAC AGATG GATAC CGAAG CCGCC GCTTC TGAAG CCCCA CAGGA TGTGA CATAT GCCCA GCTGC ATAGC CTGAC ACTGC GGAGA GAAGC CACAG AGCCT CCACC TTCTCA AGAAG GCCCA TCTCCT GCCGT GCCTT CCATC TATGC CACTC TGGCC ATTCAC LRHRR QGKHW TSTQR KADFQ HPAGA VGPEP TDRGL QWRSS PAADA QEENL YAAVK HTQPE DGVEM DTRSP HDEDP QAVTY AEVKH SRPRR EMASP PSPLSG EFLDTK DRQAE

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD
																											EDRQM DTEAAA SEAPQ DVTYA QLHSLT LRREAT EPPPS QEGPS PAVPSI YATLAI H

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD	E2A	SS	aCAR bivalent binders					Hinge	TM domain	Co-stim ICD	CD3z ICD
									EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	E2AT2A	GM-CSFRa	FLT3 VH	GGGGS	CD33 VH6 Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD28	CD3z
SB06541																								
	Retrovec	DNA	ATGGAC	AATTGG	TCAGG	TTGCTG	CAGTGT	ATGGCT	GAGGT	GGAGG	GAAGT	CACCCA	GTGATC	CTGCG	CAGTG	ATGCTG	GAAGTT	GGCGG	CAGGTT	GGCTCT	GACATC	GGTGG	GATATT	GCCCT	ATCTAT	AGAAG	AGAGT
			TGGACT	GTCAAC	CGGCG	CCTAGC	ACCAAC	CTGCC	GCAGC	CGGAG	GGTCAT	TCCGAT	GGCATT	GCACA	CACAAA	CTGCTG	CAGCT	CGGTG	CAGCTT	ACATCT	CAGATG	CGGCG	CAGATG	GGAGCA	ATCTGG	CAAGC	GAAGT
			TGGATA	GTGATC	GTGGTA	TGGGC	TACGCC	CGTGA	TGGTTG	GATCTG	GACACA	CCTCTC	CTGGTC	GAAGG	TTATGC	GTTACA	GGTTCA	GCTCT	GTGCAA	GGCTCT	ACACAG	GATCC	ACTCAG	ACAGCA	GCCCC	GGAGC	TCAGC
			CTCTTT	AGCGA	GTGGA	CATCAC	CTGCTG	CAGCTT	AATCTG	GTGGC	GAGCC	GAGCT	GCGGT	CAGGG	ACTGCT	TCTCTG	GAGCG		GCTGGC	GGCAA	AGCCCT		TCGCCA	TCATGT	TCTGGC	AGACTG	AGGAG
			CTGGTC	CCTGAA	GGCGG	ACTGAT	AAACTG	TGCTGC	GCGGA	GGAGG	CTCTGA	GGTGG	GATCCT	CAAGCA	GAAGCT	CTGCTG	GAGCC		GCTGAA	ACCTG	AGTAGC		AGCAG	ACTTCA	TGGCA	CTGCAC	CGCAG
			GCTGC	GAAGAT	AGGCT	CTCCGT	GCGGG	TGCCTT	GGACT	AAGTG	GCCTG	GCTCCT	CTGGA	CGCCG	TGCGA	GAAGT			GTGAAA	GAAGC	CTGAG		CCGTGT	GCCACT	CATGC	AGCGA	ACGCC
			CGCCA	CAGGC	CAGAG	GAACG	CGACG	TGGCA	GGTTCA	GCGGA	CCTGTG	GACCTT	GTTGCT	CAAGCA	GGGAT	GCTGC	GAAGAA		AAGCC	GGCGA	CGCCA		AGCCTC	TCGTGC	GGAGTT	CTACAT	CCCGC
			CACGG	ACCTGA	GTGAC	GCATCT	TGGAAT	CTGCTG	GGCCTG	GGCGG	ACACTG	CCTGCT	CCCAG	CCGATC	ACCTG				CGGCT	GGGAT	GCGTG		CGTGG	CCGTGT	CTGCTG	GAACAT	GTACA
			GTGCA	TCCAGA	CCCTGA	ATCGTG	ACTAAT	CTGCAT	GCGACA	GCCCC	GCTTCT	AGAAAG	GAGTAA	CTGCGT	GCAGC				CCTCC	CTACCA	GGAGA		GCGATA	TTCTGC	CTGAGC	GACCC	AGCAG
			CTCT	GCATG	GCCTAT	TCTCGT	CTGAA	GCTGCT	TCTCTG	TTCT	GCCTG	TAGCAG	GGTTCC	GCCGA	CCCAG	TTCTGC	AGCGT		GTGAAA	AGGGC	CAGAGT		GAGTCA	CCGCC	CTGGTC	CTAGAC	GGCCA
				CACATC	CTTCAG	GGCTG	CCTGG	AGACCA	AGACTG		CCAGC	CCCTAC	GATC	CTTTCA	GACCT	TTATTC	GAAGG		GTGTCT		GACAAT		CCATTA	AAGCCT	ATCACC	CTGCCC	GAACC
				GACGC	CCTGAT	ACCTAC	ATCTGG				ATCAGC	AACAAG		TGCTCG	GGAAAG	CT	TGTCCT		TGTAAA		TACCTG		CATGCA	ACAACA	CTGTAC	GGACC	AGCTC
				CACACT	CGGCG	TGCTTC	CGAGG		GCCGC		TGCAAG	GACCTA		TGCTG	CGGAG		GCCAG		CGGCT		TCGGG		GAGCC	ACCCCT	TGCAAC	TACCAG	TATAAC
				GTACAC	GAGGTT	GCCCC	GACGC		CAGCG		TCTAGC	CAAGCA		GCGCC	AAGGTC		GCTTCT		ACACCT		CCAGC		AGCCA	GCTCCT	CACAGA	AAAGCA	GAGCT
				CGAGT	CCGGA	TAGATG	GGGAG		GCTTCA		CAGTCT	CAAGC		GTTGGA	GTGGTA		GGCGG		GAGAG		CGTGG		GTCCAT	AGACCA		CTACCA	CAATC
				CCGAT	GGTGG	CAGAG	TCTACT		CCTTCA		CTGGT	GGCCC		CCTGA	GTCTAC		CACCTT		TTACCG		CGTGG		CTGCAG	CCTACA		GCCTTA	TAGGA
				GTGCA	CGGTTG	AGCGGG	GACGT		GCAGAT		GGCGA	TGAGG		GCCTAC	TAAGGT		CAGCA		ACTACA		ACAGT		CTACGT	CCAGCT		CGGTC	CGAAG
				CCCTAG	CGGCG	AGAAGA	GTGGA		ACGATA		TGCACT	ACCAAC		AGATAG	GTGGT		GCTAC		ATATGC		ACGGC		GAACTG	CCTACA		CTCCTA	AGAGG
				CTGCAA	GAGGA	AACGA	GACGT		TGCACT		GAGAA	CTCTGA		AGGACT	GATGTA		GCCATC		ATTGGG		ATCAGC		GTATCA	ATCGCC		GAGATT	AGTAC
				AGTGAC	TCTCTT	GCGGC	GGAGG		CACCTA	CACCAA	GGGTC	CACTGA		GCAGT	GAAGAA		TCTTGG		TCCGC		TTCATG		GCAAAA	AGCCA		TCGCC	GATGT
				CGCCAT	CAA	TGAGAA	AAAACC		CGACA	CAGGC	CCTGAA	CAGGC		GGCGG	AATCCT		GTTCGA		CAGGC		AACCAG		ACCCG	GCCTCT		GCCTAC	TTGG
				GAAGT		GAGAAA	CTGGA		GGCCC		CTGGTT	AGCGAT		TCTAGC	GGACC		CAGGC		ACCAG		TTCCAG		GAAAG	GTCTCT		CGGTC	ACAAG
				GCTTTC		GCGTTG	AGCGG		CCAGC	CCTCAG	CTGGC	GCAGAT		CCTGC	T		ACCTG		GCCAG		CAGAA		GCTCCA	GAGGC		C	AGACG
				TGCTG		CGGCC			CTGGC	TGAGG	ACGAG	TCTGGA		CGCTG			GACAG		ATATGC		AAGCT		AAGCTC	CCGAA			AGCCG
				CGACG		TGTG			AGAGG	ACCAG	AACAC	CTGGG		GCACA			GCACAC		GGCAC		ATCAGC		ACGGC	CAAGA			GGAAA
				CCAGC					ACCAG		GTGTCC	GAGAC		GCACA			CACCG		CGGCT		AAGGC		GTGCC	CAAGA			GGAAG
				ATCCAC					AACACA		CACTAC	CAGCTG		CCCAG			CACCG		CGGCT		AAGGC		CTGGC	CAAGA			GGAAG
				GATACC					CACAG		GATAGC	GAATGA		CCTGA			CCAATT		ACAACC		AGTGC		ATCTCT	GGATTT			AACCC
				GTGGA					GGCCG		CGCCC	TGAAGT		GCGTG			ACGCC		AGAAGT		TCAAGT		TGGCTC	TGCCTG			TCAGG
				AAATCT					CCCGG		TGAAGT	GCCCG		GCGTG			CAGAAA		TCAAGT		CCTCAA		CGGAA	CGAC			AAGGC
				GATCAT					ATCCAG		GCCCG	ATAGAT		GAAATG			TTTCAG		CCAAG		GATTCA		CGGGG				CTGTA
				CCTGG					TTCACC		TTCACC	TTTCTG		GACAC			GGAAG		GCCAC		GCGGA		ACCGA				CAATG
				CCAACA					ATCTCC		GCAGC	GCTCTT		CAGATC			AGTGAC		AATCAC		AGCCG		CTTCAC				AGAAA
				ACAGC					AGAGA		GGCTCT	GGCAC		TCCCCA			CATCAC		CATCAC		CTCCG		ACTGAC				AGATA
				CTGTCC					CAACAG		GGCAC	CGACTT		CGATGA			CGCCG		AGCAGT		GCACC		CATATC				AGATG
				AGCAAC					CAAGAA		CGACTT	CACCCT		GGACC			ACAAGA		CCACCA		GACTTT		ATCTCT				GCGGA
				GGCAAT					CACCCT		CACCCT	GAAGAT		CTCAG			ATACTG		GCACC		ACCCTG		TTCTCT				GGCCT
				GTGAC					GTACCT		GTACCT	GCAGAT		GCCGT			AGCAC		AGCAC		GCAGC		GCAGC				ACAGT
				CGAGA					GCAGCA		GCAGCA	CAGCA		GACATA			CTATTA		CGCCTA		ACAATC		CCGAA				GAGAT
				GCGGC					GAACA		GAACA	GAGTG		CGCAG			CATGGA		CATGGA		AGCTCC		GATCTG				TGGGA
				TGCAAA					GCCTG		GCCTG	GAAGC		AAGTGA			ACTGAG		ACTGAG		CTGCA		GCCAC				TGAAA
				GAGTG					AGAGC		AGAGC	CGAGG		AGCAGT			AGCAGT		AGCAGT		ACGACT		GCCTG				GGCGA
				CGAGG					CGAGG		CGAGG	ACGTG		CCAGA			GAGAA		CAGCCT		TCGCCA		TTGTCA				GCGCC
				AACTGG					ACACC		ACACC	GGGGT		CCTCG			CADAG		GCGAG		CCTACT		CCTACA				GGAGG
				AAGAGA					GCCGT		GCCGT	GTACTA		GAGAG			CGGTGT		GATACC		ACTGCC		GCACC				GGCAA
				AGAACA					GTACTA		GTACTA	CTGTCT		AGATG			ACTATT		GCTGT		ACTGG		CCTTTC				GGGGC
				TCAAAG					CTGCAC		GCAAG	GCAAG		GCAAG			GTATTA		GTATTA		AGCAG		CCTTTC				ACGAT
				AGTTTC					CCTGA		GCATCC	CCTGA		CCCTCC			CTGCG		GCAGCT		AGCAAA		ACGTTC				GGCCT
				TGCAGA					GAATCA		ATCTGC	GAATCA		ATCTGC			CCACAT		CCGCC		GAGGT		GCCCT				TTACC
				GCTTCG					AGGATT		CTTGGA	AGGATT		TCTGAG			TCGCC		CCGCC		GCCCT		CAAAG				AGGGT
				TCCACA					GGGGC		CCTTTG	GGGGC		CGGCG			CTGTTG		ATGGAC		GGACAT		GTGGA				CTCAG
				TCGTGC					CAGGG		GCCAG	CAGGG		AGTTCC			GGCTTC		TATTGG		CAGGG		CATTAA				TACAG
				AGATGT					CACCAT		GCCAC	CACCAT		TGGACA			AGAGA		GGAGA		CACCAA		G				CCACC
				TCATCA					GGTCA		GGCAC	AAAGCT		CCAAAG			GCAGG		GGGAA		GGTGG						AAGGA
														ACAGAC			CCTTG		CCTG								CACCT

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	iCAR EMCN binder										aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD
									EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	TM domain	LIR1	LIR1 ICD	E2A	SS		FLT3 VH	GGGGS	CD33 VH	Loop	CD33 VL	GGGGS	FLT3 VL	CD8	CD8	CD28	CD3z
SB06544																													
	Retrovec	DNA	IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	CD8																					
			ATGGACTGGAGCATCCTTTTCCTGGTCGCTGTGGCTACAGGTGCACTCCCAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCTTCTGGATACACCTTCACCGGCTACTATATGCACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATCAACCCTAACAGTGGTGGCACAAACTATGCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGGGACACGTCCATAAGCACAGCCTACATGGAGCTGAGCAGGCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGATCGCCCTTACTATGATTTTTGGAGTGGTTATTATAACCCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGTGAGTGGAGGCGGTGGCTCTGGCGGTGGCGGATCCGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGGACATTAGCAATTATTTAAACTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTACTATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTACACTCTCACCATCAGCAGCCTGCAGCCTGAAGATATTGCAACTTACTATTGTCAACAGGGTAATACGCTTCCGTACGTTCGGACAAGGGACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTCGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG																													

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B ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	iCAR EMCN binder										aCAR bivalent binders							Hinge	TM domain	Co-stim ICD	CD3z ICD
									EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	TM domain	LIR1	LIR1 ICD	E2A	SS	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z
SB07412																													
	Sinvec	DNA	IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	LIR1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z
			ATGGAC	AATTGG	TCAGG	TTGCTG	CAGTGT	ATGGCT	GAGGT	GGAGG	GAGGT	CADCCA	GTGATC	CTGCG	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GGTGG	GATATC	GGGGG	GATATC	GCCCT	ATGTAT	AGAAG	AGAGT
			TGGACT	GTCAAC	CGGCG	CCTAGC	ACCAAC	CTGCC	GCAGC	CGGAG	GGTCAT	TCCGAT	GGCATT	GCACA	CACAAA	CTGCTG	GGCAGC	CGGAG	CAGCT	CGGAG	CAAATG	GGAGCA	CAAATG	GAGCA	ATCTGG	CAAGC	GAAGT
			TGGATA	GTGATC	GTGGTA	TGGGC	TACGCC	CGTGA	TGGTTG	GATCTG	GACACA	CCTCTC	CTGGTC	GAAGG	TTATGC	GTTACA	TGGTTC	GATCTG	GGTGC	GAAGT	ACCCAG	GATCTG	ACCCAG	ACAGCA	GCCCC	GGAGC	TCAGC
			CTCTTT	AGCGA	GTGGA	CATCAC	CTGCTG	CAGCTT	AATCTG	GTGGC	GAGCC	GAGCT	GCGGT	CAGGG	ACTGCT	TCTCTG	AGTCTG	GTGGT	AGAGC	GGCGG	AGCCC	GCGGA	TCTCCA	TCATGT	TCTGGC	AGACTG	AGGAG
			CTGGTC	CCTGAA	GGCGG	ACTGAT	AAACTG	TGCTGC	GCGGA	GGAGG	CTCTGA	GGTGG	GATCCT	CAAGCA	GAAGCT	CTGCTG	GCGCCC	GGTGG	GGAGC	CGGAG	CAGCA	GGTGG	TCCAGC	ACTTCA	TGGCA	CTGCAC	CGCAG
			GCTGC	GAAGAT	AGGCT	CTCCGT	GCCGG	TGCCTT	GGACT	AAGTG	GCCTG	TTCTG	GCTCCT	CTGGA	CGCCG	TGCGA	GAAGT	ATCT	GCTCT	TGAAGT	GCCTGT	CAGT	CTGAGG	GCCACT	CATGC	AGCGA	ACGCC
			CGCCA	CGAGG	CAGGC	GAACG	CGACG	TGGCA	TGGTCA	GCGGA	CCTGTG	GACCTT	GTTGCT	CAAGCA	GGGAT	GCTGC	GAAGAA		GCCAG		GAAAAA		GCCAG	TCGTGC	GGAGTT	CTACAT	CCCGC
			CACGG	ACCTGA	GTGAC	GCATCT	TGGAAT	CTGCTG	GCCTG	GGCGG	ACACTG	CCTGCT	CCCAG	GTCGA	CCCATC	ACCTG			GCCAG		CTGTG		CGTTGG	GCGTGT	CTGCTG	GAACAT	GTACA
			GTGCA	TCCAGA	CCCTGA	ATCGTG	ACTAAT	CTGCAT	GGACA	TTCT	GGCCC	GCTTCT	AGAAAG	GAGTAA	CTGCCT	GCAGC			CCGTG		GGAGA		CGATAG	TTCTGC	CTAGCG	GACCC	AGAAG
			CTCT	GCATG	GCCTAT	TTCTGT	CTGGA	GCTGCT	GCCTG	TAGCAG	GTTCCT	GCCGA	CCCAG	TTCTGC	AGCGT				AGTCAC	CCGCC	CTGGTC	CTAGAC	GGCCA	
				CACATC	CTTCAG	GGCTG	CCTGG	AGACCA	AGAGCA	CTGCTG	CCAGC	CCCTAC	GATC	CTTTCA	GACCT	TTATTC	TGTCCT	GAAGG	AAAGTC	AATCAC	AAGCCT	ATCACC	GAACC	
				GACGC	CCTGAT	ACCTAC	ATCTGG		ATCAGC	AACAG		TCTTGT		GCATCC	GGAAG	CT	TACCTG		CTGTAG	ACAACA	CTGTAC	GGACC	AGCTC	
				CACACT	CGGCG	TGCTTC	CGAGG		TGCGC		TGCAAG	GACCTA		TGCTG	CGGAG		GCAAA		AAGGC		CCGGG		AGCCA	ACCCCT	TGCAAC	TACCAG	TATAA
				GTACAC	GAGGTT	GCCCC	GACGC		CAGCG		TCTAGC	CAAGCA		GCGCC	AAGGTC		GCTTCT		CAGCG		CCAGC		GCCAG	GCTCCT	CACAGA	AAAGCA	CGAGC
				CGAGT	CCGGA	TAGATG	GGGAG		GCTTCA		CAGTCT	CAAGC		GTTGGA	GTGGTA		GGCGG		GCTACA		GAGAG		AGCATC	AGACCA		CTACCA	TCAAT
				CCGAT	GGTGG	CAGAG	TCTACT		CCTTCA		CTGGT	GGCCC		CCTGA	GTCTAC		CACTT		CCTTTA		CGTGG		AGCTCC	CCTACA		GCCTTA	CTAGG
				GTGCA	CGGTTC	AGCGG	GACGT		GCAGAT		GGCCA	TGAGG		GCCTAC	TAACGT		CAGCA		CCGACT		ACAATT		TACCTG	CCAGCT		CGCTC	ACGAA
				CCCTAG	CGGCG	AGAAGA	GTGGA		ACGATA		TGCACT	CTGTGA		GCGAC	GATGTA		GTGGT		GCTAC		ACGGC		CTCTGA	GAGAG		C	GAGAC
				CTGCAA	GAGGA	AACGA	GACGT		TGCACT		GAGAA	CACCAA		AGGACT	GATGTA		GCCATC		TGCACT		ATCAGC		CTATCA	ATCGCC			GTGGC
				AGTGAC	TCTCTT	GCGGC	GGAGG		GGGTC		CACCTA	CACCAA		GCAGT	GAAGAA		TCTTGG		GGGTC		TTCATG		GATCTA	GCTTGT			CGGGA
				CGCCAT	CAA	TGAGAA	AAAACC		CGACA		CCTGAA	CAGGC		GGCGG	AATDCT		GTTCGA		CGACAA		AACTGG		TTGCTG	AGACGG		GCCTAC	CCCTG
				ATCAGC		GAGAAA	CTGGA		GGCCC		CTGGTT	AGCGAT		TCTAGC	GGACC		GCTCCA		GCTCCA		TTCCAG		TCTGCA	GGCTCT		CGGTC	GACAA
				CTGGAA		GAAGGG			ACGGC		ACGGC	TCTGGA		CTTTAC	T		CAAGCA		GGCCA		CAGAA		CAGCGA	GGCGG		C	GAAGC
				AGCGG		GGGCA			AAGGA		TGATCT	CGTGAA		GCCGC			CATCAT		CCCCTA		CTACGC		GAGTG	AGCCG			GAAAG
				CGACG		ACGGC			ACCAG		ACCAG	GCACA		GCACA			CAACG		CCCCAT		CGCCA		GCGTG	TGCATA			CCGAG
				CCAGC		AACACA			AACAG		GTGTCC	GTGTAA		GCACA			CTTCGG		CTTCGG		GCAATC		CCATCT	CAACGA			AAGGA
				ATCCAC		CACTAC			AAGCTG		AAGCTG	CCAAG		CCAAG			CACCG		CACCG		AAGGC		AGATTC	GGACT			AGAAC
				GATAGC		GATAGC			GATAGC		GATAGC	CCTGA		CCTGA			CCAATT		CCAATT		AGCGG		AGCGG	GGATTT			CCTCA
				GTGGA		GTGGA			GGCGC		GGCGT	GGATG		GCGTG			ACGCC		CAGAAA		AGTGC		CAGCG	TGCCTG			GGAAG
				AAATCT		CGCGC			CGCCC		TGAAGT	GCGTG		GCGTG			CAGAAA		GTTCAA		CTAGCA		GAAGC	CGAC			GCCTG
				GATCAT		CCCGG			TTCACC		TTCACC	GAAATG		GAAATG			TTCCAG		TTCCAG		GAGCA		GAATTT				TAGAA
				CCTGG		TTCACC			AGAGA		AGAGA	GACAC		GACAC			GGCAG		GGCAG		CCGGC		AGACTT				TGAAC
				CCAACA		ATCTCC			CAACAG		CAACAG	CAGATC		CAGATC			AGTGAC		AGTGAC		TCTGGC		TCTGGC	TACACT			TGCAG
				ACAGC		GCAGC			GGAAC		GGAAC	TCCCCA		TCCCCA			ACATAC		ACATAC		ACAATC		TACAAT				AAAGA
				CTGTCC		GGCTCT			CGACTT		CGACTT	CGATGA		CGATGA			CATCAC		CATCAC		AGCGG		GACAAT				TAAGA
				AGCAAC		GCAAC			CACCCT		CACCCT	GGACC		GGACC			ACAAGA		ACAAGA		CACCG		ATCTTC				TGGCG
				GGCAAT		GCACCT			GAAGAT		GAAGAT	CTCAG		CTCAG			GCACAA		GCACAA		ATTTCA		CCTGCA				GAGGC
				GTGAC		GTACCT			CAGCA		CAGCA	GCCGT		GCCGT			GCACC		GCACC		CCATCA		GCCAG				CTACA
				CGAGA		GCAGAT			GAACA		GAGTG	GACATA		GACATA			CAGCTT		CAGCTT		GTAGC		AGGAC				GTGAG
				GCGGC		GCCTG			GAAGC		GAGTG	CGCAG		CGCAG			ATATGG		ATATGG		CTGCA		CACATA				ATTGG
				TGCAAA		GAAGTG			AGAGC		CGAGG	AAGTGA		AAGTGA			AACTGT		AACTGT		GCCTG		TTATTG				GATGA
				GAGTG		CGAGG			AGAGC		CGAGG	AGCACT		AGCACT			CTAGCC		CTAGCC		GCCTG		CCAGCA				AAGGC
				CGAGG		AACTGG			ACACC		ACGTG	CCAGA		CCAGA			CAGCCT		CAGCCT		ACGAAC		GTCCTA				GAGCG
				AACTGG		AAGAGA			GCCGT		GCGGT	CCTCG		CCTCG			GCGAG		GAGAA		TCGCCA		CCTACT				CGGGA
				AAGAGA		AGAACA			GTACTA		GCCGI	GAGAG		GAGAG			GATACA		GCGAG		CCTACT		CAGCAC				GGGGC
				AGAACA		TCAAAG			CTGCAC		GTACTA	AGATG		AGATG			GCTGI		GATACA		ACTGCC		CCCTTT				GTCTC
				TCAAAG		AGTTTC			CCTGA		CCTGA	GCAAG		GCAAG			GTACTA		GCTGI		AGCAG		CACCTT				AAGGG
				AGTTTC		TGCAGA			GCATCC		GCATCC	CCCTCC		CCCTCC			TGTACT		GTACTA		CGGAC		CGGAC				GCACG
				TGCAGA		GCTTCG			AATCTC		AATCTC	ATCTGC		ATCTGC			TTGTGC		TTGTGC		AGGGT		CCGGA				ATGGC
				GCTTCG		TCCACA			AGGATT		AGGATT	GCCAC		GCCAC			CAGGG		CAGGG		GAGGT		ACAAAG				CTTTA
				TCCACA		TCGTGC			GGGGC		GGGGC	CTTGGA		CTTGGA			GCAAG		GCAAG		GCCCT		GTGGA				CCAGG
				AGATGT		AGATGT			CACGT		CACGT	CCTTTG		CCTTTG			AGTTGC		AGTTGC		GGACC		CATTAA				GTCTC
				TCATCA		TCATCA			GGTCA		GGTCA	GCCAG		GCCAG			CGGCG		CGGCG		CAGGG		G				AGTAC
												GGCAC		GGCAC			TGGACA		TGGACA		CACCAA						AGCCA
												AAAGCT		AAAGCT			CCAAAG		AGGGA		GGTGG						CCAAG
																	ACAGAC		ACCCTG								GACAC

| B ID | Backbone | Seq Type | SS | IL15 | Cleavage Site | TM domain | E2A | SS | iCAR EMCN binder | | |

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	iCAR EMCN binder			Hinge	TM domain	LIR1 ICD	E2A	SS	aCAR bivalent binders					Hinge	TM domain	Co-stim ICD	CD3z ICD
									EMCN VH	(GGGGS)3 linker	EMCN VL	CD8	LIR1	LIR1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z
SB07417																									
	Retrovec	DNA	IgGE	Il15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	CD8																	
			ATGGAC	AATTGG	TCAGG	TTGCTG	CAGTGT	ATGGCT	GAGGT	GGAGG	GAGGT	ACAACA	GTGATC	CTGCG	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GGTGG	GATATC	GCGCT	ATCTAT	AGAAG	AGAGT
			TGGACT	GTCAAC	CGGCG	CCTAGC	ACCAAC	CTGCC	GCAGC	CGGAG	GGTCAT	ACACCC	GGCATT	GCACA	CACAAA	CTGCTG	GCAGC	CGGAG	CAGCT	CGGAG	CAGATG	TGGCG	CAAATG	GAGCA	CAAGC	GAAGT
			TGGATA	GTGATC	GTGGTA	TGGGC	TACGCC	CGTGA	TGGTTG	GATCTG	GACACA	GCACCT	CTGGTC	GAAGG	TTATGC	GTTACA	TGGTTC	GATCTG	GGTGC	GAAGT	ACTCAG	GATCTG	ACCCAG	ACACAG	GCCCC	TCAGC
			CTCTTT	AGCGA	GTGGA	CATCAC	CTGCTG	CAGCTT	AATCTG	GTGGC	GAGCC	CGGCC	GCCGT	CAGGG	ACTGCT	TCTCTG	AGTCTG	GTGGT	AGAGC	GGCGG	AGCCC	GCGGA	TCTCCA	TCATGT	TCTGGC	AGACTG	AGGAG
			CTGGTC	CCTGAA	GGCGG	ACTGAT	AAACTG	TGCTGC	GCGGA	GGAGG	CTCTGA	TCCAAC	GATCCT	CAAGCA	GAAGCT	CTGCTG	GCGCCC	GGTGG	GGAGC	CGGAG	CAGCA	GGTGG	TCCAGC	ACTTCA	TGGCA	CTGCAC	CGCAG
			GCTGC	GAAGAT	AGGCT	CTCCGT	GCCGG	TGCCTT	GGACT	AAGTG	GCCTG	TCCAGC	GCTCCT	CTGGA	CGCCG	TGCGA	GAAGT	ATCT	GCTCT	TGAAGT	GCTCT	CTGAGC	GCCACT	CATGC	AGCGA	ACGCC
			CGCCA	CGAGG	CAGGC	GAACG	CGACG	TGGCA	GGTTCA	GCGGA	CCTGTG	TCCAAC	GTTGCT	CAAGCA	GGGAT	GCTGC	GAAGAA		GCCAG		GAAAAA		GCCAG	TCGTGC	GGAGTT	CTACAT	CCGGC
			CACGG	ACCTGA	GTGAC	GCATCT	TGAAT	CTGCTG	GCCTG	GGCGG	ACACTG	AATTGC	CCTGCT	CCAG	CCTTGA	CCATGA	ACCTG		GCCAG		CTGTG		CGTTGG	CGCTGT	CTGCTG	GAACAT	GTACA
			GTGCA	TCCAGA	CCCTGA	ATCGTG	ACTAAT	CTGGAT	GCGGA	TTCT	GGACA	ACTGCA	GGCTCT	AGAAAG	GAGTAA	CTGCCT	GCAGC		CCGTG		GCTCCT		CGATAG	TTCTGC	CTCAGC	GACCC	AGCAG
			CTCT	GCATC	GCCTAT	TCTCGT	CTGAA	GCTGCT	TCTCTG		GCCTG	ACCCCT	GTTCCT	GCCGA	CCCAG	TTCTGC	AGCGT		AAAGTC		CCGTG		AGTCAC	CCGCC	CTGGTC	CTAGAC	GGCCA
				CACATC	CTTCAG	GGCTG	CCTGG	AGACCA	AGACTG		TCTTGT	ATCAGC	GAAGC	CTTTCA	GACCT	TTATTC	GAAGG		TCTTGC		TACCTG		AATCAC	AAGCCT	ATCACC	GGCCC	GAACC
				GACGC	CCTGAT	ACCTAC	ATCTGG		TCTTGT		ATCAGC	GAAGC	TGCTTC	CCAAA	GGAAG	CT	TGTCCT		TCTTGC		CTGTAG		ACAACA	CTGTAC	GGACC	AGCTC
				CACACT	CGGCG	TGCTTC	CGAGG		GCCGC		TGCAAG	GCCAGC	TGCTC	GCAAA	GCTTCT		AAGGC		CCGGG		AGCCA		ACCCCT	TGCAAC	TACCAG	ITATAA
				GTACAC	GAGGTT	GCCCC	GACGC		CAGCG		TCTAGC	GCCTGT	GCCAGC	GCGCC	AAGGTC		CAGCG		CCAGC		GCCAG		GCTCCT	CACAGA	AAAGCA	DGAGC
				CCGAT	GGTGG	CAGAG	TCTACT		GCTTCA		CCTTCA	AGCAG	GTTGGA	GCTTAC	GTGTGA		GGCGG		GCTACA		AGCAG		AGACCA		CTACCA	TCAAT
				GTGCA	GGTTCA	GCAGAT	GACGT		GGCCA		GCCAGC	CTGGC	GCCTAG	CCTTCA	CACCTT		GTCGT		CCTTTA		CGTGG		AGCTCC	CCTACA	GCCTTA	CTAGG
				CCGAT	CGGTTC	AGCGG	GACGT		GGCCA		GGCTAG	CTGGC	GCCTAG	AGCAG	CCGACT		CACCTT		CCGACT		ACAATT		TACGTG	CCAGCT	CGCTC	AGAAA
				CCCTAG	CGGCG	AGAAGA	GTGGA		ACGAT		GCGAC	GGAGC	AGATAG	GTGGT	GCTAC		ACAACA		GCCGG		ACGGC		AATTGG	CCTACA	CTCCTA	GAGAG
				CTGCAA	GAGGA	AACGA	GACGT		TGCACT		GAGAA	TGTTCA	AGGACT	GATGTA	GCCATC		TGCACT		ATCAGC		TATCAG		TATCAG	ATCGCC	GAGATT	GAGTA
				AGTGAC	TCTCTT	GCGGC	GGAGG		CACCTA	CACTAG	CGAGA	TGAAT	GCAGTT	GAAGAA	TCTTGG		GGGTC		GTTCGA		CCAGG		CCAGG	GCCTCT	TCGCC	CGATG
				CGCCAT	CAA	TGAGAA	AAAACC		CGACA	AGGCC	CCTGAA	AATCCT	GGCGG	AATCCT	GTTCGA		CGACAA		CCAGC		AACTGG		CCAGG	GCCTAC	GCCTAC	TTTTG
				CAGCAC		GAGAAA	ACTGGA		GGCCC		CTGGTT	TGGACT	TCTAGC	GGACC	GCTCCA		CAGGC		GCTCCA		TTCCAG		CAAAGG	GTCTCT	CGGTC	GACAA
				GAAGT		GAGCC			GGCCC		CCAGC	CTGGC	CCTGC	T	GGCCA		CCCTG		GGCCA		CAGAA		TCCCAA	GAGGC	C	GAGAC
				GCTTTC		GCGTG	GACGT		CTGGC		CTGGC	TTGCCT	CCTGC		GCTAC		CCCTG		ACAAC		ACGGC		ACTCCT	CCGAA		GTGGC
				TGCTG		CGGCC	CCT		ACGAT	GTGGA	AGAGG	GTGAC	CGCTG		AGATAG		GACAA		GGCAC		GCCCG		ACTCCT	CCGAA		GTGGC
				CTGCAG		AACGA			TGCACT		AGAGG		AGGACT		GCCATC		GCCAG		GGCAC		GCCCC		GCCCG			CGGGA
				CAAGTG		TGTG			TGGAC		CCGGG		GCAGT		TCTTGG		GGGTC		GTTCGA		CCAGG		GATCTA	GCTTGT		CCCTG
				ATCAGC					TGGGT		ACAGTC		GCAGT		GGATCT		GGATG		GGCTA		GCTCCA		GCTGCA	GGCTGCT		AGATG
				CTGGAA					GTCCGT		TCCTAG		GAAAAT		GGATG		GGAGG		CATCTA		CAGGC		CTGCAG	GGCGG		GGGG
				AGCGG					GATCTG		ACCGC		CTTTAC		GAAGG		CATCAT		CCCCTA		CCTCG		CCTGAT	GAGCG		GAAAG
				CGACG					GGGCA		TGATCT		GCCGC		CATCAT		CCCCAT		CTACGC		GCGTG		GAGTG	AGCCG		CCGAG
				CCAGC					ACCAG		ACCAG		CGTGAA		CCCCAT		CAACG		CGCCA		CGCCA		CCATCT	CAAGA		AAGGA
				ATCCAC					AACACA		GTGTCC		GCACA		GCACAC		GCGGC		GCAATC		GCAAGC		CCATCT	GGACT		AGAAC
				GATACC					CACTAC		AAGCTG		CCAG		CTTGGG		ACCGG		ACCGG		AAGGC		AGAATC	GGACT		AGAAC
				GTGGA					GATAGC		GCGTG		CCTGA		CACCA		CCAATT		CTACAA		AGCGG		AGCGG	GGATTT		CCTCA
				AAATCT					GGCCC		GGATG		GGATG		ACGCC		CCAGAA		ACGCC		AGTGC		CAGCG	TGCCTG		GGAAG
				GATCAT					CGGCC		GCGTG		GCGTG		CAGAAA		GTTCAA		CAGAAA		CTAGCA		GAAGC	CGAC		GCCTG
				CCTGG					TGAAGT		GCCCG		GCCCG		GGCAG		GGCAG		GAGCA		TTCCAG		GAATTT			TACAA
				CCAACA					TTCACC		ATAGAT		TTTCTG		GACAC		AGGCC		AGGCC		CGGAC		AGACTT			TGAAC
				ACAGC					AGAGA		ATCTCC		GCTCTC		TCCCCA		CAGATC		CATCAC		TCCGG		TACACT			TGCAG
				CTGTCC					CAACAG		AGCAC		GGCAC		CGATGA		GCTCTC		ACAGC		AGCGG		GACAAT			AAAGA
				AGCAAC					CAAGAA		CGACTT		CGACC		GGACC		AGTCCA		CGCCG		CACCG		ATCTTC			TAAGA
				GGCAAT					CACCCT		CACCCT		CACCC		CTCAG		ACAAGA		ACAAGA		ATTTCA		CCTGCA			TGGCG
				GTGAC					GTACCT		GAAGAT		GCCGT		GCCGT		GCACAA		GCACAA		CCCTGA		GCCAG			GAGGC
				CGAGA					GCAGAT		CAGCA		GCAGAT		GCCTAC		GCACAA		GCACC		CCATCA		AGGAC			CTACA
				GCGGC					GAACA		GAGTG		GAGTG		CGCAG		GCCTAC		CAGCTT		ATATGG		CTGGC			GTGAG
				TGCAAG					GAAGC		CGAGC		AAGTGA		AAGTGA		ATGGAA		AACTGT		GTACGC		CACATA			ATTGG
				GAGTG					AGAGC		CGAGG		AGCAGT		CTGAG		CAGCCT		CTAGCC		CTGCA		CTGCA			GATGA
				CGAGG					CGAGG		ACGTG		CCAGA		CCAGA		GAGAA		CGAGAG		TCGCCA		ACAGCT			AAGGC
				AACTGG					ACACC		GGCGT		CCTCG		GCGAG		GATACA		GATACA		CCTACT		TCGCCA			GAGCG
				AAGAGA					GCCGT		GTACTA		CTGTCT		GAGAG		GACAC		GACAC		CCTTTT		CACCTT			CCGGA
				AGAACA					GTACTA		CTGTCT		GCAAG		AGATG		CGCCG		CGCCG		ACTGC		CGGAC			GGGG
				TCAAAG					CTGCAC		GCAAG		GCATCC		GCATCC		TGTACT		TTGTGC		AGCAAA		CAGGG			CAAGG
				AGTTTC					CCTGA		GCATCC		ATCTCC		CCCTCC		TGTGC		CAGGG		GAGGT		ACTGC			GGCAC
				TGCAGA					GAATCA		ATCTGC		GCCAC		ATCTCC		GCCAC		GCAGA		GCCCT		GCCAC			GATGG
				GCTTCG					AGGATT		AGGATT		CTTGGA		TCTGAG		CCCGC		ATTTGC		GCCCT		GGACC			CCTTT
				TCCACA					GGGGC		CCTTTG		CGGCG		CGGCG		CATGGA		CCTGTT		TTTGGA		CATTAA			ACCAG
				TCGTGC					CAGGG		GCCAG		AGTTCC		AGTTCC		CGGCTT		CTATTG		CAGGG		G			GGTCT
				AGATGT					CACCAT		GCCAC		TGGACA		TGGACA		TGTACT		GGGAC		CACCAA					CAGTA
				TCATCA					GGTCA		AAAGCT		CCAAAG		CCAAAG		CAGAG		AGGGA		GGTGG					CAGCC
													ACAGAC		ACAGAC		AGCAG		ACCCTG							ACCAA
																	GCCTTG									GGACA

SB ID	Backbone	Seq Type	SS	IL15	Cleavage Site	TM domain	E2A	SS	iCAR EMCN binder		Hinge	TM domain	LIR1 ICD	E2A	SS	aCAR bivalent binders					Hinge	TM domain	Co-stim ICD	CD3z ICD
									EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z
SB07418																								
	Retrovec	DNA	IgGE	Il15	LR1 split N term linker + face10	B7-1	E2A/T2A	CD8				LIR1												
			ATGGAC	AATTGG	TCAGG	TTGCTG	CAGTG	TATGGC	GAGGT	GGAGG	GAGGT	CACCCA	GTGATC	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GGTGG	GATATC	GCCCT	ATCTAT	AGAAG	AGAGT
			TGGACT	GTCAAC	CGGCG	CCTAGC	ACCAAC	CTGCC	GCAGC	CGGAG	GGTCAT	TCCGAT	GGCATT	GCACA	CACAAA	CGGAG	CAGCT	CGGAG	CAGATG	TGGCG	CAAATG	GAGCA	ATCTGG	CAAGC	GAAGT
			TGGATA	GTGATC	GTGGTA	TGGGC	TACGCC	CGTGA	TGGTTG	GATCTG	GACACA	CCTCTC	CTGGTC	GAAGG	TTATGC	GTTACA	TGGTTC	GATCTG	GGTGC	GAAGT	ACTCAG	GATCTG	ACCCAG	ACAGCA	GCCCC	GGAGC	TCAGC
			CTCTTT	AGCGA	GTGGA	CATCAC	CTGCTG	CAGCTT	AATCTG	GTGGC	GAGCC	GCCGT	CAGGG	ACTGCT	TCTCTG	AGTCTG	GTGGT	AGAGC	GGCGG	AGCCC	GCGGA	TCTCCA	TCATGT	TCTGGC	AGACTG	AGGAG
			CTGGTC	CCTGAA	GGCGG	ACTGAT	AAACTG	TGCTGC	GCGGA	GGAGG	CTCTGA	GGTGG	GATCCT	CAAGCA	GAAGCT	CTGCTG	GCGCCC	GGTGG	GGAGC	CGGAG	TCCAGC	ACTTCA	TGGCA	CTGCAC	CGCAG
			GCTGC	GAAGAT	AGGCT	CTCCGT	GCCGG	TGCCTT	GGACT	AAGTG	GCCTG	TTTCTG	GCTCCT	CTGGA	CGCCG	TGCGA	GAAGT	ATCT	TGAAGT	GCTCT	GCCTGT	GCCACT	CATGC	AGCGA	ACGCC
			CGCCA	CAGGC	GAACG	TGGCA	CGACG	GGTTCA	GCGGA	CCTGTG	GACCTT	GTTGCT	CAAGCA	GGCAT	GCTGC	GAAGAA			GAAAAA		GCCAGC	TCGTGT	GGAGTT	CTACAT	CCCGC
			CACGG	ACCTGA	GTGAC	GCATCT	TGGAAT	CTGCTG	GCCTG	GGCGG	ACACTG	CTGGC	CCTGCT	CCCAG	GTCGA	CCCATC	ACCTG		GCCAG		CTGTG	GTTGGC	CCGTGT	CTGCTG	GAACAT	GTACA
			GTGCA	TCCAGA	CCCTGA	TCATCT	GCGGA	CTGCAT	CTGCAT	TTCT	GGACA	GGCCC	GCTTCT	AGAAAG	GAGTAA	CTGCCT	GCAAGC		CCGTG		GCTCCT	GATAGA	TTCTGC	CTCAGC	GACCC	AGCAG
			CTCT	GCATG	GCCTAT	TCTGCT	CCTGGA	GCTGCT	TCTCTG		GCCTG	TAGCAG	GTTCCT	GCCGA	CCCAG	TTCTGC	AGCGT		AAAGTC		CGAAT	GTCACA	CCGCC	CTGGTC	CTAGAC	GGCCA
				CACATC	CTTCAG	GGCCTG	CCTGG	AGACCA	AGACTG		CCAGC	CCCTAC	GATC	CTTTCA	GACCT	TTATTC	GAAGG		TCTTGC		TACCTG	AAGCCT	ATCACC	GGCCC	GAACC
				GACGAC	CCTGAT	AACTAC	ATCTGG				ATCAGC	AACAG		GCATCC	GGAAG	CT	TGTCCT		AAGGC		CCGGG	ACAACA	CTGTAC	GGAOC	AGCTC
				CACACT	CGGCG	TGCTTC	CGAGG		GCCGC		TGCAAG	GACCTA		TGCTG	CGGAG		GCAAA		CAGCG		CCAGC	TGTAGA	CTGTAC	GGAOC	AGCTC
				GTACAC	GAGGTT	GCCCC	GACGC		GCTTCA		TCTAGC	CAAGCA		GCGCC	AAGGTC		GCTTCT		GCTACA		ATCAGC	ACAACA	TGCAAC	ATACAG	TATAA
				CGAGT	CCGGA	TAGATG	GGGAG		CCTTCA		CAGTCT	CAAGC		GTTGGA	GTGGTA		CACCTT		GCTACA		ATCAGC	AGACCA	ATACAG	TATAA	CGAGC
				CCGAT	GGTGG	CAGAG	TCTACT		CCTTCA		CTGGT	GGCCC		CCTGA	GTCTAC		CACCTT		CCTTTA		TCCTAC	CCTACA	TACAG	TATAA	CCCGC
				GTGCA	CGGTTG	AGCGG	GACGT		GCACAT		GGCCA	TGAGG		GCCTAC	TAACGT		CAGCA		CGTGG		CGTGG	CCTTA	CTAGC	TATAA	CGAAT
				CCCTAG	CGGCG	AGAAGA	GTGGA		ACGATA		GCGAC	ACCAAC		AGATAG	GTGGT		GCTAC		ACAACA		CTGAAT	GGAGC	CTCCTA	GAGAG	GAGTA
				CTGCAA	GAGGA	AACGA	GACGT		TGCACT		GAGAA	CTCTGA		AGGACT	GATGTA		GCCATC		TGCACT		CTGAAT	GCCTCC	GAGATT	TCGCC	CGATG
				AGTGAC	TCTCTT	GCGGC	GGAGG		CACCTA	CACCAA	CACAGT	GAAGAA		GCAGT	GAAGAA		TCTTGG		CGAGCA		GGCAAA	GCCTCT	GCCTAC	TTTTG	CGATG
				CGCCAT	CAA	TGAGAA	AAAACC		CCTGAA	CAGGC	GCTGGA	ATCTGG		GGCGC	AATCCT		GTTCGA		CGACAA		AACTGG	GCCTCT	GCCTAC	TTTTG	CGATG
				GAAGT		GAGAAA	ACTGGA		GGCCC		CTGGTT	AGCGAT		CTGAC	GGACC		CGAGC		CGAGC		GCTCCG	GTCTCT	CGGTTA	CTTTG	CGATG
				GCTTTC		GCTGTG	CCT		CTGGC		CCAGC	CCTCAG		CCCTG	T		CCCTG		GACAA		AACTCT	GAGGC	CGGTTA	CTTTG	CGATG
				TGCTG		CGGCC			AGAGG		AGAGG	TCTGGA		CGCTG			GACAA		GGGAC		CTGATC	CCGAA	CGGTTA	CTTTG	CGATG
				GAACTG		GCGGC			CCGGG		CCGGG	CTGGG		ATGCCC			TTGAGT		GCAGA		TATGCT	GCTTGT	CGGTTA	CTTTG	CGATG
				CAAGTG		TGGGT			ACAGTC	GAGAC	ACAGTC	GAGAC		AAGAG			GGAAT		GCCCC		GCCTCC	AGACCA	CGGTTA	CTTTG	CGATG
				ATCAGC		GTCCGT			TCCTAG	ATCTGG	TCCTAG	GCGTT		GAAAAT			GGATG		GCCTA		TAAGCT	AGCCTG	GCTGCT	AGATG	CGATG
				CTGGAA		GATCTG			ACGGC		ACGGC			CTTTAC			GGAGG		CATCTA		GCTGAT	GGCGG	GGGG	GAAAG	CGATG
				AGCGG		GGGCA			TGATCT		TGATCT			GCCGC			CATCAT		CCCCTA		CTACGC	GGCGT	AGCCG	GAAAG	CGATG
				CGACG		ACGGC			ACCAG		ACCAG			CGTGAA			CCCCAT		CAACG		CGCCA	GCCATC	TGCATA	CCGAG	CGATG
				CCAGC		AACACA			GTGTCC		GTGTCC			GCACA			CTTGGG		GCGGC		GCAATC	TAGATT	CAAGA	AAGGA	CGATG
				ATCCAC		CACTAC			AAGCTG		AAGCTG			CCCAG			CACCG		ACCGG		CAGCG	GGACT		AGAAC	CGATG
				GATACC		GATCTG			GAATAG		GAATAG			CCTGA			CCAATT		CCAGAA		AAGGC	GGAAT		CCTCA	CGATG
				GTGGA		CAGAG			GCGCT		GCGCT			GGATG			ACGCC		GTTCAA		AGTGC	GGAAGC	TGCCTG	GGAAG	CGATG
				AAATCT		GGAAGT			CGCCG		GCCCG			GCGTG			CAGAAA		GAGCA		CTAGCA	GGCACA	CGAC	GCCTG	CGATG
				GATCAT		CCGGG			ATAGAT		ATAGAT			GAAATG			GTTCCA		GAAGCA		GATTTT	CGAC		TACAA	CGATG
				CCTGG		TTCACC			TTTCTG		TTTCTG			GACAC			GGCAG		AGGCC		ACGCTG			TGAAC	CGATG
				CCAACA		AGATCC			GCAGC		GCAGC			CAGATC			AGTGAC		ACAATC		TCTTGC			AAAGA	CGATG
				ACAGC		AGAGA			GGCTCT		GGCTCT			TCCCCA			CATCAC		ACAGC		AGCGG			AAAGA	CGATG
				CTGTCC		CAACAG			GGCAC		GGCAC			CGATGA			CGCCG		CGACG		CACCG			TAAGA	CGATG
				AGCAAC		CAAGAA			CGACTT		CGACTT			GGACC			ACAAGA		AGTCCA		ATTTCA			TGGCG	CGATG
				GGCAAT		CACCCT			CACCCT		CACCCT			CTCAG			GCACAA		CCAACA		CCCTGA			GAGGC	CGATG
				GTGAC		GTACGT			GAAGAT		GAAGAT			GCCGT			CAGCTT		CAGCTT		CCATCA			CTACA	CGATG
				CGAGA		GCAGAT			CAGCA		CAGCA			GACATA			GCCTAC		GCCTAC		GTAGC			GTGAG	CGATG
				GCGGC		GAACA			GAGTG		GAGTG			CGCAG			ATGGAA		AACTGT		GCCAGC			ATTGG	CGATG
				TGCAAA		GAAGC			GAAGC		GAAGC			AAGTGA			CTAGCC		GCCTG		GCCTG			GATGA	CGATG
				GAGTG		AGAGC			CGAGG		CGAGG			AGCACT			CAGCCT		TGCGCT		ACGACT			AAGGC	CGATG
				CGAGG		CGAGG			ACGTG		ACGTG			CCAGA			GAGAA		CCGAG		TCGCCA			GAGCG	CGATG
				AACTGG		ACACC			GCGGT		GCGGT			CCTCG			GCGAG		GATACA		CCTACT			CCGGA	CGATG
				AAGAGA		GCCGT			GTACTA		GTACTA			GAGAG			GACAC		GCTGT		ACTGCC			GGGGC	CGATG
				AGAACA		GTACTA			CTGTCT		CTGTCT			AGATG			CGCCG		GTACTA		AGCAG			AAGGG	CGATG
				TCAAAG		CTGCAC			GCAAG		GCAAG			GCAAG			TGTACT		TTGTGC		CAAGG			GCACG	CGATG
				AGTTTC		CCTGA			GCATCC		GCATCC			CCCTCC			ACTGC		CAGGG		GAGGT			ATGGC	CGATG
				TGCAGA		GAATCA			ATCTGC		ATCTGC			ATCTCC			GCCAC		GCAGA		GCCCT			CTTTA	CGATG
				GCTTCG		AGGATT			CTTGGA		CTTGGA			TCTGAG			ATTTGC		CCCGC		GGACC			CCAGG	CGATG
				TCCACA		GGGGC			CCTTTG		CCTTTG			CGGCG			CCTGTT		CATGGA		TTTGGA			GTCTC	CGATG
				TCGTGC		CAGGG			GCCAG		GCCAG			AGTTCC			CGGCTT		CTATTG		CAGGG			AGTAC	CGATG
				AGATGT		CACCAT			GGCAC		GGCAC			TGGACA			CAGAG		GGGAC		CACCAA			AGCCA	CGATG
				TCATCA		GGTCA			AAAGCT		AAAGCT			CCAAAG			AGCAG		AGGGA		GGTGG			CCAAG	CGATG
														ACAGAC			GCCTTC		ACCCTG					GACAC	CGATG

| | | |

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	Sinvec		IgGE	Il-15	LR1 split N term linker + Tace10	B7-1	E2A/T2A	CD8	EMCN VH	(GGGGS)3 linker	EMCN VL	LIR1	LIR1	LIR1	E2A/T2A	GM-CSFRa	FLT3 VH	(GGGGS)2 linker	CD33 VH	(GGGGS)2 linker	CD33 VL	(GGGGS)2 linker	FLT3 VL	CD8	CD8	CD28	CD3z
		DNA	ATGGAC	AATTGG	TCAGG	TTGCTG	CAGTGT	ATGGCT	GAGGT	GGAGG	GACGT	CACCAG	GTGATC	CTGCG	CAGTG	ATGCTG	GAAGT	GGAGG	CAGGTT	GGTGG	GATATT	GGCGG	GATATC	GCCCT	ATCTAT	AGAAG	AGAGT
			TGGACT	GTCAAC	CGGCG	CCTAGC	ACCAAC	CTGCC	GCAGC	CGGAG	GGTCAT	TCCGAT	GGCATT	GCACA	CACAAA	CTGCTG	GCAGC	CGGAG	CAGCT	CGGAG	CAGATG	TGGCG	CAAATG	GAGCA	ATCTGG	CAAGC	GAAGT
			TGGATA	GTGATC	GTGGTA	TGGCTG	TACGCC	CGTGA	TGGTTG	GATCTG	GACACA	CCTCTC	CTGGTC	GAAGG	TTATGC	GTTACA	TGGTTC	GATCTG	GGTGC	GAAGT	ACTCAG	GATCTG	ACCCAG	ACAGCA	GCCCC	GGAGC	TCAGC
			CTCTTT	AGCGA	GTGGA	CATCAC	CTGCTG	CAGCTT	AATCTG	GTGGC	GAGCC	GCCGT	CAGGG	ACTGCT	TCTCTG	AGTCTG	GCGCC	GGTGG	AGAGC	GGCGG	AGCCC	GCGGA	TCTCCA	TCATGT	TCTGGC	AGACTG	AGGAG
			CTGGTG	CGTAAC	GGCGG	ACTGAT	AAACTG	TGCTGC	GCGGA	GGAGG	CTCTGA	GGTGG	GATCCT	CAAGCA	GAAGCT	CTGCTG	GCGCC	GGTGG	GGAGC	CGGAG	CAGCA	GGTGG	TGCAGC	ACTTGA	TGGCA	CTGCAC	CGACAG
			GCTGC	GAAGAT	AGGCT	CTCCGT	GCCGG	TGCCTT	GGACT	AAGTG	GCCTG	TTTCTG	GCTCCT	CTGGA	CGCCG	TGCGA	GAAGT	ATCT	TGAAGT	GCTCT	CTGAGC	GCCACT	CATGC	AGCGA	AGCGC
			CGCCA	CGAGG	CAGGC	GAACG	CGACG	TGGCA	GGTTCA	GCGGA	CCTGTG	GACCTT	GTTGCT	CAAGCA	GGGAT	GCTGC	GAAGAA		GAAAAA		CTGCCT		GCCAGC	TCGTGC	GGAGTT	CTACAT	CCGGC
			CACGG	ACCTGA	GTGAC	GCATCT	TGAATT	CTGCTG	GGCCTG	GGCGG	ACACTG	CTGGC	CCTGCT	CCCAG	GTCGA	CCCATG	ACCTTG		GCCAG		CTGTG		GCTTGC	CCGTGT	CTGCTG	GAACAT	GTACA
			GTGCA	TCCAGA	CCCTGA	ATCGTG	ACTAAT	CTGCAT	GCGGA	TTCT	GGACA	GGCCC	GCTTCT	AGAAAG	GAGTAA	CTGCCT	GCAGC		GCTCCT		GATAGA		TTCTGC	CTCAGC	GACCC	AGCAG
			CTCT	GCATG	GCCTAT	TCTGCT	CTGGA	GCTGCT	TCTCTG	GCCTG	TAGCAG	GTTCCT	GCCGA	CCCAG	TTCTGC	AGCGT	CCGTG		CAGAGT		GTCACA		CCGCC	CTGGTC	CTAGAC	GGCCA
				CACATC	CTTCAG	GCCTG	CCTGG	AGACCA	AGAACT	GACTG	CCAGC	CCCTAC	GATC	CTTTCA	GACCT	TTATTC	GAAGG		ACAATC		ATCACC		AAGCCT	ATCACC	GGCCC	GAACC
				GACGC	CCTGAT	ACCTAC	ATCTGG		TCTTGT		ATCAGC	AACAG		GCATCC	GGAAG	CT	TGTCCT		AAGGC		TCTTGC		TACCTG	TGTAGA	ACAADA	CTGTAC	GGACC	AGCTC
				CACACT	CGGCG	TGCTTC	CGAGG		GCCGC		TGCAAG	GACCTA		TGCTG	CGGAG		GCAAA		CGGGG		CCGGG		GCCAGC	ACCCCT	TGCAAC	TACCAG	TATAA
				GTACAC	GAGGTT	GCCCC	GACGC		CAGCG		TCTAGC	CAAGCA		GCGCC	AAGGTC		GCTTCT		CAGCG		CCAGC		CAGAGG	GCTCCT	CACAGA	AAAGCA	GGAGC
				CGAGT	CCGGA	TAGATG	GGGAG		GCTTCA		CAGTCT	CAAGC		GTTGGA	GTGGTA		GGCGG		GCTACA		GAGAG		ATCAGC	AGACCA		CTACCA	ITCAAT
				CCGAT	GGTGG	CAGAG	TCTACT		CCTTCA		CTGGT	GGCCC		CCTGA	GTCTAC		CACCTT		CCTTTA		CGTGG		TCCTAC	CCTACA		GCCTTA	TATAGG
				GTGCA	CGGTTC	AGCGG	GACGT		GCAGAT		GCAGCA	TGAGG		GCCTAC	TAACGT		CAGCA		ACAATT		TGGTAT		CTGAAT	CCAGCT		CGCTC	ACGAA
				CCCTAG	CGGCG	AGAAGA	GTGGA		ACGATA		GCGAC	ACCAAC		AGATAG	GTGGT		GCTAC		ACAACA		TGCACT		CAGCAG	ATCGCC		CTCCTA	GAGAG
				CTGCAA	GAGGA	AACGA	GACGT		TGCACT		GAGAA	CTCTGA		AGGACT	GATGTA		GCCATC		TGCACT		CAGCAG		ATCAGC	TTGTGT		GAGATT	GAGTA
				AGTGAC	TCTCTT	GCGGC	GGAGG		GGGTC		CACCTA	CACCAA		GCAGT	GAAGAA		TCTTGG		GGGTC		TTCATG		AACCA	AGCCA		TCGCC	CGATG
				CGCCAT	CAA	TGAGAA	AAAACC		CGACA		CCTGAA	CAGGC		GGCGG	AATCCT		GTTGGA		CGACAA		AACTGG		GGCAAA	GCCTCT		GCCTAC	TTTTG
				GAAGT		GAGAAA	CTGGA		GGCCC		CTGGTT	AGCGAT		TCTAGC	GGACC		CAGGC		GCTCCA		TTCCAG		GCTCCC	GTCTCT		CGGTC	GCAAA
				GCTTTC		GCGTG	CCT		CTGGC		CCAGC	CCTCAG		CCTGC	T		CCCTG		GGCCA		CAGAA		AAACTC	GAGGC		GAGAC	
				TGCTG		CGGCC			AAAGGA		AGAGG	TCTGGA		CGCTG			GACAA		GGGAC		GCCCG		CTGATC	CCGAA		C	GTGGC
				CAAGTG		TGTG			CTTGAA		CCCGG	CTGGG		ATGCCC			GGCCT		TTGAGT		GCCCC		TATGCT	GCTTGT			CGGGA
				ATCAGC					TGGGT		ACAGTC	GAGAC		AAGAG			GGAAT		GGATC		TAAGCT		GCCTCC	AGACCA			CCCTG
				CTGGAA					GTCCGT		TCCTAG	ATCTGG		GAAAAT			GGATG		GGCTA		GCAGCA		AGCCTG	GCTGCT			AGATG
				AAATCT					GATCTG		ACGGC	GCGTT		CTTTAC			GGAGG		CATCTA		CAGAGT		CAGAGT	GGCGG			GGGG
				GATCAT					GGGCA		TGATCT			GCCGC			CATCAT		CCCCTA		CTACGC		GGCGT	AGCCG			GAAAG
				CCTGG					ACGGC		ACCAG			CGTGAA			CCCCAT		CAACG		CGCCA		GCCATC	TGCATA			CCGAG
				CCAGC					AACAC		GTGTCC			GCACA			CTTCGG		GCGGC		GCAATC		ITAGAT	CAAGA			AAGGA
				ATCCAC					CACTAC		AACCTG			CCCAG			CACCG		ACCGG		AAGGC		CAGCG	GGACT			AGAAC
				GATACC					CACAG		GATAGC			CCTGA			CCAATT		CTACAA		AAGGC		GCAGC	GGATTT			CCTCA
				GTGGA					CGCCC		GGCGT			GGATG			ACGCC		CCAGAA		AGTGC		GGAAGC	TGCCTG			GGAAG
				AAATCT					CCCGG		GCGTG			GCGTG			GCAGC		GTTCAA		CTAGCA		GGCACA	CGAC			GCCTG
				GATCAT					TTCACC		ATAGAT			GAAATG			TTCCAG		GAGCA		GATTTT		GACTTT				TACAA
				CCTGG					ATCTCC		TTTCTG			GACAC			GGCAG		AGGCC		CGGGC		ACACTG				TGAAC
				CCAACA					GCAGC		GCAGC			CAGATC			AGTGAC		ACAATC		TCTGGC		ACAATA				TGCAG
				ACAGC					AGAGA		GGCTCT			TCCCCA			CATCAC		ACAGC		AGCGG		TCTTCC				AAAGA
				CTGTCC					CAACAG		GGCAC			CGATGA			CGCCG		CGACG		CACCG		CTGCAG				TAAGA
				AGCAAC					CAAGAA		CGACTT			GGACC			ACAAGA		AGTCCA		ATTTCA		CCAGAG				TGGCG
				GGCAAT					CACCCT		CACCCT			CTCAG			GCACAA		CCAACA		GACCTG		GAGGC				GAGGC
				GTGAC					GTACCT		GAAGAT			GCCGT			GCACAC		CAGCTT		GCACCA		CCATCA				CTACA
				CGAGA					GCAGAT		CAGCA			GACATA			GCCTAC		ATATGG		GTAGC		TATTATT				GTGAG
				GCGGC					GAACA		GAGTG			CGCAG			ATGGAA		AACTGT		CTGCA		GCCAGC				ATTGG
				TGCAAA					GCCTG		GAAGC			AAGTGA			CTGAG		CTAGCC		GCCTG		AGTCCT				GATGA
				GAGTG					AGAGC		CGAGG			AGCAC			CAGCT		TGCGCT		ACGAC		ACAGCA				AAGGC
				CGAGG					CGAGG		ACGTG			CCAGA			GAGAA		CCGAG		TCGCCA		CCCCTT				GAGCG
				AACTGG					ACACC		GGCGT			CCTCG			GCGAG		GAACA		CCTACT		TCACCT				CCGGA
				AAGAGA					GCCGT		GTACTA			AGATG			GCACAC		GCTGT		ACTGCC		TCGGAC				GGGGC
				AGAACA					GTACTA		CTGTCT			GCAAG			CGGCG		GTACTA		AGCAA		CGGGAA				AAGGG
				TCAAAG					CTGCAC		GCAAG			GCAAG			TGTACT		TTGTGC		AGCAAA		CAAAGG				GCACG
				AGTTTC					CCTGA		GCATCC			CCCTGC			ACTGC		CAGGG		GAGGT		TGGACA				ATGGC
				TGCAGA					GAATCA		ATCTGC			ATCTGC			GCCAC		CCGAG		GCCCT		TTAAG				CTTTA
				GCTTCG					AGGATT		CTTGGA			TCTGAG			ATTTGC		GCAGA		GGACC						CCAGG
				TCCACA					GGGGC		CCTTTG			CGGCG			CCTGTT		CATGGA		TTTGGA						GTCTC
				TCGTGC					CAGGG		GCCAG			AGTTCC			CGGCTT		CTATTG		CAGGG						AGTAC

Interpretations

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, *i.e.*, to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03

CLAIMS

What is claimed is:

1. A multicistronic expression system comprising an engineered nucleic acid comprising:

(A) an exogenous polynucleotide encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula:

S – C – MT or **MT – C – S**

wherein

S comprises a secretable effector molecule, wherein the secretable effector molecule comprises IL-15,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide;

(B) an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising:
(i) an antigen-binding domain specific for endomucin (EMCN);
(ii) one or more intracellular inhibitory domains that inhibit an immune response; and
(iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and

(C) an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising:
(i) an antigen-binding domain specific for FLT3;
(ii) an antigen-binding domain specific for CD33;
(iii) one or more intracellular signaling domains that stimulate an immune response; and
(iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

2. The multicistronic expression system of claim 1, wherein the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are linked together by a polynucleotide linker encoding a 2A ribosome skipping element, optionally wherein the 2A ribosome skipping element is selected from the group consisting of: a T2A ribosome skipping element, a E2A ribosome skipping element, a P2A ribosome skipping element, a F2A ribosome skipping element, ribosome skipping element fusions thereof, and combinations thereof, optionally wherein the ribosome skipping element fusion comprises an E2A/T2A ribosome skipping element, optionally wherein the E2A/T2A ribosome skipping element comprises the amino acid sequence QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221), optionally wherein the E2A/T2A ribosome skipping element is encoded by a polynucleotide sequence comprising the sequence CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATC CTGGACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACG TGGAGGAAAACCCTGGACCT (SEQ ID NO: 222) or the sequence CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTCGAGAGTAACC CAGGACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGT AGAAGAAAATCCTGGACCT (SEQ ID NO: 223).

3. The multicistronic expression system of claim 1 or 2, wherein the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR; or from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) iCAR; and (iii) bivalent aCAR.

4. The multicistronic expression system of any one of claims 1-3, wherein the secretable effector molecule comprises a signal peptide or a signal-anchor sequence, optionally wherein the signal peptide comprises a native signal peptide native to the secretable effector molecule, optionally wherein the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-

CSFRa, optionally wherein the non-native signal peptide comprises an IgE signal peptide, optionally wherein the IgE signal peptide comprises the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228), optionally wherein the IgE signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACT CT (SEQ ID NO: 229).

5. The multicistronic expression system of any one of claims 1-4, wherein the protease cleavage site further comprises the N-terminal peptide linker, optionally selected from the group consisting of: SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; and EAAAKEAAAKEAAAKEAAAK, optionally wherein the protease cleavage site further comprises the N-terminal peptide linker SGGGGSGGGGSG (SEQ ID NO: 230), optionally wherein the protease cleavage site further comprises a C-terminal peptide linker, optionally selected from the group consisting of: SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; and EAAAKEAAAKEAAAKEAAAK, optionally wherein the protease cleavage site further comprises the C-terminal linker GGGSGGGGSGGGSLQ (SEQ ID NO: 231), optionally wherein the protease cleavage site comprises a Tumor Necrosis Factor-α Converting Enzyme (TACE)-specific cleavage site, optionally wherein the TACE-specific cleavage site comprises the amino acid sequence VTPEPIFSLI (SEQ ID NO: 191), optionally, wherein the TACE-specific cleavage site comprises the amino acid sequence SGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQ (SEQ ID NO: 250), optionally wherein the TACE-specific cleavage site is encoded by a polynucleotide sequence comprising the sequence TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCT

ATCTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGAT CTCTTCAA (SEQ ID NO: 251).

6. The multicistronic expression system of any one of claims 1-5, wherein the cell membrane tethering domain comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, LIR1, B7-1, and BTLA, optionally wherein the cell membrane tethering domain comprises a B7-1 transmembrane domain, optionally wherein the B7-1 transmembrane domain comprises the amino acid sequence LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204), optionally wherein the B7-1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGAT CTGCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAAC GAGCGGCTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252), optionally wherein the membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula **S – C – MT**, optionally wherein the membrane-cleavable chimeric protein comprises the amino acid sequence MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK VTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELE EKNIKEFLQSFVHIVQMFINTSSGGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGG SLQLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 226), optionally wherein the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence comprising the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACT CTAATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCA GAGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGC TGCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGCAAGTGATCAGCC TGGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAAAATCTGATCATCCT GGCCAACAACAGCCTGTCCAGCAACGGCAATGTGACCGAGAGCGGCTGCAA AGAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTT CGTCCACATCGTGCAGATGTTCATCAACACCTCATCAGGCGGCGGTGGTAGT GGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTTCAGCCTGATCGGCG

GAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAATTGCTGCCTAG CTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGCTGCCTGA CCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCTGA GAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

7. The multicistronic expression system of any one of claims 1-6, wherein the antigen-binding domain specific for EMCN comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

the EMCN-VH comprises:
a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291),
a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 296), and
a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and
the EMCN-VL comprises:
a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 299),
a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 300), and
a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 301), and
wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein the EMCN-VH and the EMCN-VL are separated by a peptide linker, optionally wherein the antigen-binding domain specific for EMCN comprises the structure VH-L-VL or VL-L-VH, wherein L is the peptide linker, optionally wherein the peptide linker comprises the amino acid sequence SGGGGSGGGGSG; GGGSGGGGSGGGSLQ; GGS; GGSGGS; GGSGGSGGS; GGSGGSGGSGGS; GGSGGSGGSGGSGGS; GGGS ; GGGSGGGS; GGGSGGGSGGGS; GGGSGGGSGGGSGGGS; GGGSGGGSGGGSGGGSGGGS; GGGGS; GGGGSGGGGS; GGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGS; GGGGSGGGGSGGGGSGGGGSGGGGS; GSTSGSGKPGSGEGSTKG; or EAAAKEAAAKEAAAKEAAAK, optionally wherein

the peptide linker comprises the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 244), optionally wherein the peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253).

8. The multicistronic expression system of any one of claims 1-7, wherein the intracellular inhibitory domain comprises a LIR1 intracellular inhibitory domain, optionally wherein the LIR1 intracellular inhibitory domain comprises the amino acid sequence LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYA AVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDT KDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPAVPSI YATLAIH (SEQ ID NO: 285), optionally wherein the LIR1 intracellular inhibitory domain is encoded by a polynucleotide sequence comprising the sequence CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCC GACTTTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGAC TGCAGTGGCGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGC CGCCGTGAAGCACACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATC TCCCCACGATGAGGACCCTCAGGCCGTGACATACGCAGAAGTGAAGCACTCC AGACCTCGGAGAGAGATGGCAAGCCCTCCATCTCCTCTGAGCGGCGAGTTCC TGGACACCAAAGACAGACAGGCCGAAGAGGACAGACAGATGGATACCGAA GCCGCCGCTTCTGAAGCCCCACAGGATGTGACATATGCCCAGCTGCATAGCC TGACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAAGGCCCATC TCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTCAC (SEQ ID NO: 286).

9. The multicistronic expression system of any one of claims 1-8, wherein (A) the signal peptide is present in the iCAR, optionally wherein the signal peptide of the iCAR comprises a native signal peptide native or a non-native signal peptide, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF, optionally wherein the signal peptide of the iCAR comprises a CD8 signal peptide, optionally wherein the CD8 signal peptide comprises the amino acid

sequence MALPVTALLLPLALLLHAARP (SEQ ID NO: 137), optionally wherein the CD8 signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGC TGCTAGACCA (SEQ ID NO: 416); and/or wherein (B) the hinge domain is present in the iCAR comprises, optionally wherein the hinge domain of the iCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the hinge domain of the iCAR comprises a LIR1 hinge, optionally wherein the LIR1 hinge comprises the amino acid sequence HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 417), optionally wherein the LIR1 hinge comprises is encoded by a polynucleotide sequence comprising the sequence CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAG CAGCCCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCT GACACCAACAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418); optionally wherein the hinge domain of the iCAR comprises a CD8 hinge, optionally wherein the CD8 hinge comprises the amino acid sequence TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271), optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGC AACCCCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGT TCACACTAGAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283); and/or wherein (C) the transmembrane domain of the iCAR is present, optionally wherein the transmembrane domain of the iCAR comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, LIR1, and BTLA, optionally wherein the transmembrane domain of the iCAR comprises a LIR1 transmembrane domain, optionally wherein the LIR1 transmembrane domain comprises the amino acid sequence VIGILVAVILLLLLLLLLLFLI (SEQ ID NO: 259), optionally wherein the LIR1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence

GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCT
GTTCCTGATC (SEQ ID NO: 260).

10. The multicistronic expression system of any one of claims 1-9, wherein the iCAR comprises the amino acid sequence:

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYD
MHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDNSKNTLYLQMNSL
RAEDTAVYYCTLRIKDWGQGTMVTVSSGGGGSGGGGSGGGGSDVVMTQSPLS
LPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPD
RFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQGTKLEIKngaaHPSDP
LELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVIL
LLLLLLLLFLILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAA
DAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPP
SPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSLTLRREATEPPPS
QEGPSPAVPSIYATLAIH (SEQ ID NO: 419), optionally wherein the iCAR is encoded by a polynucleotide sequence comprising the sequence
ATGGCTCTGCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGC
TGCTAGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCT
GGCGGATCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGAT
ACGATATGCACTGGGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGTC
CGTGATCTGGGGCAACGGCAACACACACTACCACAGCGCCCTGAAGTCCCG
GTTCACCATCTCCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAAC
AGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCACCCTGAGAATCAAG
GATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTGGAGGCGGAGGATCTG
GTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCATGACACAGAGCC
CTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCTGCAAGTC
TAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTCCAG
CAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGG
ATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCAC
CCTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCT
GCAAGGCATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATC
AAGAATGGCGCTGCACACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGAC
CTTCTGGCGGCCCTAGCAGCCCTACAACAGGACCTACAAGCACAAGCGGCCC
TGAGGACCAACCTCTGACACCAACAGGCAGCGATCCTCAGTCTGGACTGGGG

AGACATCTGGGCGTTGTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTT
GCTCCTGCTGCTTCTGTTCCTGATCCTGCGGCACAGAAGGCAGGGCAAGCAC
TGGACAAGCACCCAGAGAAAGGCCGACTTTCAGCATCCTGCTGGCGCCGTTG
GACCTGAGCCTACAGATAGAGGACTGCAGTGGCGGTCTAGCCCTGCCGCTGA
TGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCACACCCAGCCTGAGGAT
GGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACCCTCAGGCCGTG
ACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGCAAGCCCT
CCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCGAA
GAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGAT
GTGACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAG
CCTCCACCTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCAC
TCTGGCCATTCAC (SEQ ID NO: 420).

11. The multicistronic expression system of any one of claims 1-10, wherein the antigen-binding domain specific for FLT3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

the FLT3-VH comprises:
a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GGTFSSYAIS (SEQ ID NO: 360),
a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIIPIFGTANYAQKFQG (SEQ ID NO: 361), and
a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQAFDI (SEQ ID NO: 362), and
the FLT3-VL comprises:
a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASQSISSYLN (SEQ ID NO: 363),
a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and
a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and
wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein:

the FLT3-VH comprises the amino acid sequence EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPI FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQA FDIWGQGTTVTVSS (SEQ ID NO: 315); and

the FLT3-VL comprises the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIK (SEQ ID NO: 316), optionally wherein the FLT3-VH, and the FLT3-VL are separated by peptide linkers.

12. The multicistronic expression system of any one of claims 1-11, wherein the antigen-binding domain specific for CD33 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

the CD33-VH comprises:
a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402),
a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and
a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and
the CD33-VL comprises:
a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405),
a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and
a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and
wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein:

the CD33-VH comprises the amino acid sequence QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIY

PYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDY WGQGTLVTVSS (SEQ ID NO: 329); and

the CD33-VL comprises the amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAA SNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEI K (SEQ ID NO: 330), optionally wherein the CD33-VH, and the CD33-VL are separated by peptide linkers.

13. The multicistronic expression system of any one of claims 1-12, wherein the aCAR antigen binding domains comprises the structure (FLT3-VH) – L1 – (CD33-VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a third peptide linker, respectively; optionally wherein the L1, L2, and/or L3 are each independently selected from the group consisting of: SEQ ID Nos 230 – 248, optionally wherein the L1 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or GGGGSGGGGS (SEQ ID NO: 243), optionally wherein the L1 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGCGGCGGTGGCTCT (SEQ ID NO: 254) or the L1 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), optionally wherein the L2 peptide linker is the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247), optionally wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGG GC (SEQ ID NO: 249), optionally wherein the L2 peptide linker is the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243), optionally wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), optionally wherein the L3 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242)or GGGGSGGGGS (SEQ ID NO: 243), optionally wherein the L3 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGCGGATCC (SEQ ID NO: 255) or the L3 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258).

14. The multicistronic expression system of any one of claims 1-13, wherein the aCAR intracellular signaling domains that stimulate an immune response is selected from the group consisting of: CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278, FcεRI, DAP10, DAP12, CD66d, CD97, CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, LFA-1, CD7, LIGHT, NKG2C, B7-H3, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a SLAM protein, an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLAl, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and combinations thereof, optionally wherein the aCAR intracellular signaling domains that stimulate an immune response comprise a CD28 co-stimulatory domain and a CD3ζ signaling domain, optionally wherein the CD28 co-stimulatory domain comprises the amino acid sequence RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 287), optionally wherein the CD28 co-stimulatory domain is encoded by a polynucleotide sequence comprising the sequence AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCT AGACGGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAG ATTTCGCCGCCTACCGGTCC (SEQ ID NO: 288), optionally wherein the CD3ζ signaling domain comprises the amino acid sequence RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR (SEQ ID NO: 289), optionally wherein the CD3ζ signaling domain is encoded by a polynucleotide sequence comprising the sequence AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAG AACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTT TGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGG

AAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCG GAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGG GCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGAC GCCCTTCACATGCAGGCCCTGCCCCCTCGC (SEQ ID NO: 290), optionally wherein

(A) the hinge domain of the aCAR is present, optionally wherein the hinge domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the hinge domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 hinge comprises the amino acid sequence ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACD (SEQ ID NO: 272), optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCGTGTTTCTGCC CGCCAAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACA ATCGCCAGCCAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAGCTGCTG GCGGAGCCGTGCATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284), and/or wherein

(B) the transmembrane domain of the aCAR is present, optionally wherein the transmembrane domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the transmembrane domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 transmembrane comprises the amino acid sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206), optionally wherein the CD8 transmembrane comprises is encoded by a polynucleotide sequence comprising the sequence ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCT GGTCATCACCCTGTACTGCAACCACAGA (SEQ ID NO: 261); and/or wherein

(C) the aCAR signal peptide of the aCAR is present, optionally wherein the signal peptide of the aCAR is selected from the group consisting of: IgE, IL-12, IL-2, optimized

IL-2, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-CSFRa, optionally wherein the signal peptide of the aCAR comprises a GM-CSFRa signal peptide, optionally wherein the GM-CSFRa signal peptide comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIP (SEQ ID NO: 423), optionally wherein the GM-CSFRa signal peptide is encoded by a polynucleotide sequence comprising the sequence ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTTTCTGCTTATTCCT (SEQ ID NO: 424), optionally wherein the aCAR comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 414), optionally wherein the aCAR comprises the amino acid sequence MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISS

YLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATY
YCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCN
HRRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP
AYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO: 415).

15. An expression vector comprising the multicistronic expression system of any one of claims 1-14, optionally wherein the expression vector comprises the polynucleotide of SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 433, or SEQ ID NO: 432.

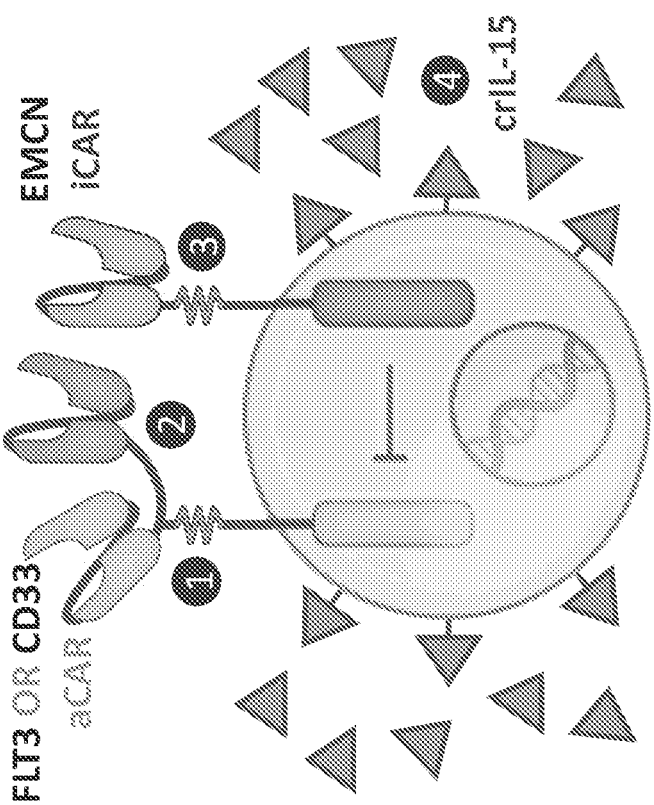
16. An isolated cell comprising the multicistronic expression system of any one of claims 1-14 or the expression vector of claim 15, optionally wherein the cell comprises an immune cell, optionally wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell, optionally wherein the cell is an NK cell.

17. A polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 433, SEQ ID NO: 432, and SEQ ID NO: 433.

18. A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells of claim 16 or the polynucleotide of any one of claims 17, optionally wherein the subject has cancer, optionally wherein the isolated cell is derived from the subject or is allogeneic with reference to the subject.

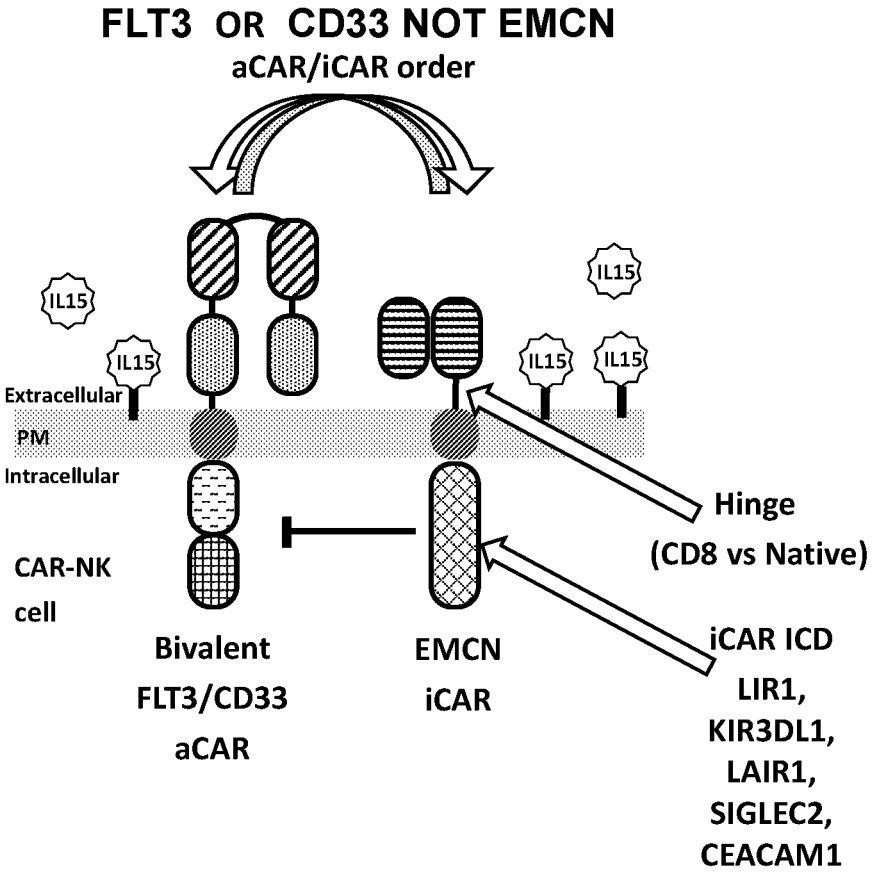
19. A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells of claim 16 or the polynucleotide claim 17, optionally wherein the isolated cell is derived from the subject or is allogeneic with reference to the subject.

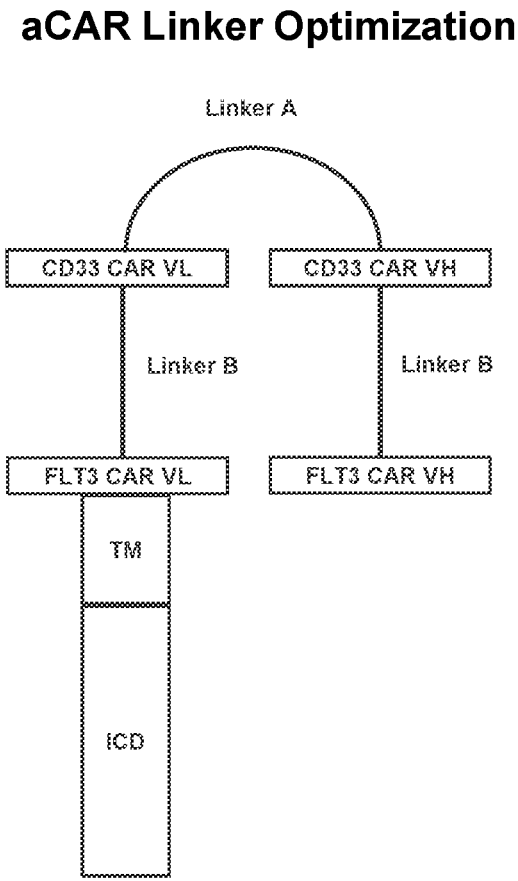
20. A method of making an engineered cell, comprising transducing an isolated cell with the multicistronic expression system of any one of claims 1-14, the expression vector of claim 15, or the polynucleotide of claim 17.



Gene Circuit	Logic Gating: FLT3 OR CD33 NOT EMCN
1	FLT3-targeting scFv; part of bivalent *activating* CAR (aCAR)
2	CD33-targeting scFv; part of bivalent *activating* CAR (aCAR)
3	EMCN *inhibitory* CAR (iCAR)
4	crIL-15 (novel IL-15 molecule)

FIG. 1





5 ICDs ✖ **3 Vectors** ✖ **3 Tags/Hinges** ✖ **2 Cargo Orders** = **90 constructs**

1. LIR1
2. LAIR1
3. KIR3DL1
4. SIGLEC2
5. CEACAM1

1. Retrovec
2. Sinvec SV40
3. Sinvec NFAT

1. aCAR/iCAR tags + CD8 hinge
2. aCAR/iCAR tags + native hinge from ICD
3. iCAR tag only + CD8 hinge

1. crIL-15_aCAR_iCAR
2. crIL-15_iCAR_aCAR

FIG. 2

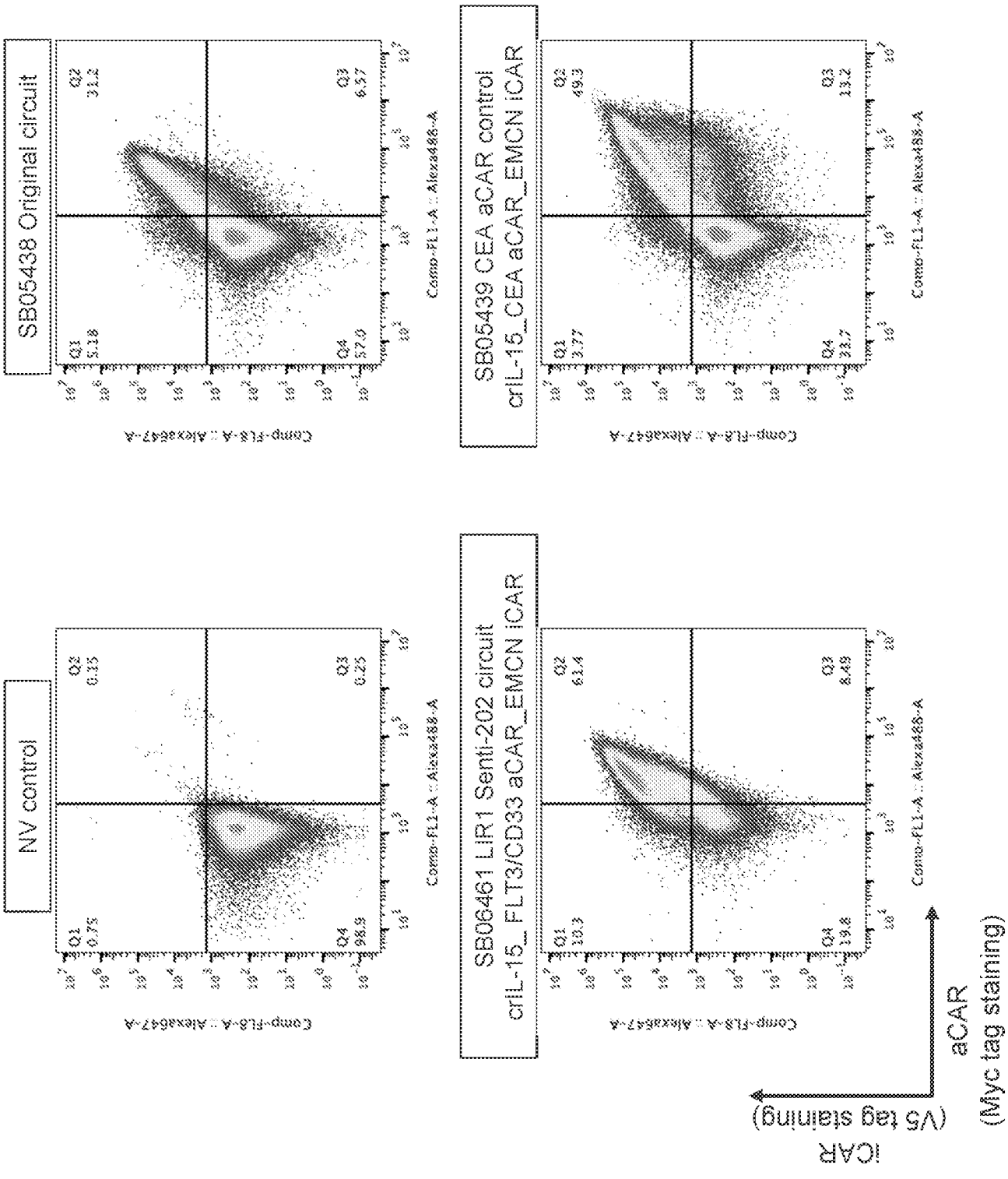

NV control
Q1 0.75
Q2 0.35
Q3 0.25
Q4 98.9
SB05438 Original circuit
Q1 5.18
Q2 31.2
Q3 6.57
Q4 57.0
SB06461 LIR1 Senti-202 circuit
criL-15_FLT3/CD33 aCAR_EMCN iCAR
Q1 10.3
Q2 62.4
Q3 8.49
Q4 19.8
SB05439 CEA aCAR control
criL-15_CEA aCAR_EMCN iCAR
Q1 3.77
Q2 49.3
Q3 13.2
Q4 33.7
Comp-FL8-A :: Alexa647-A
Comp-FL1-A :: Alexa488-A
iCAR
(V5 tag staining)
aCAR
(Myc tag staining)

FIG. 3

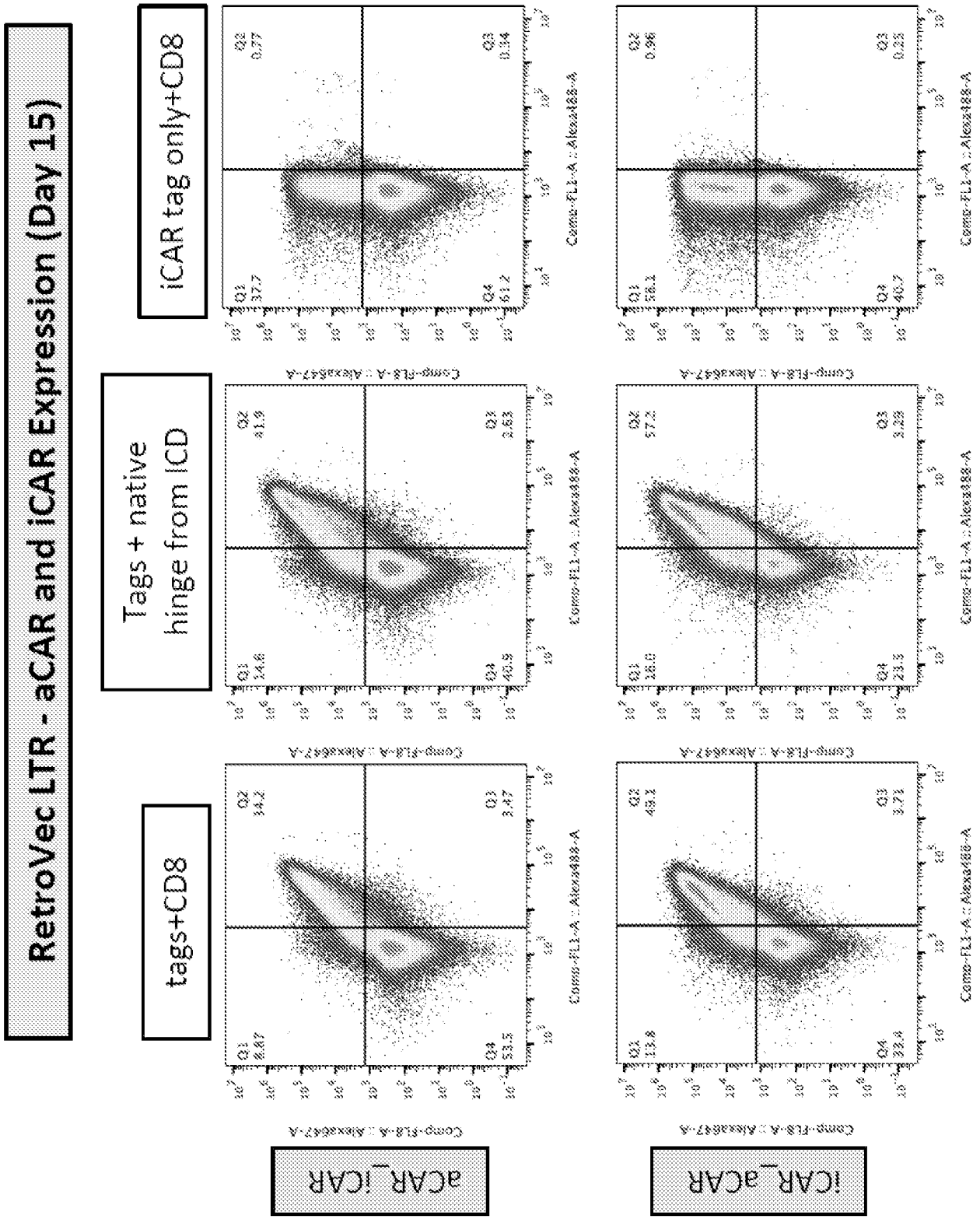

RetroVec LTR - aCAR and iCAR Expression (Day 15)
tags+CD8
Tags + native hinge from ICD
iCAR tag only+CD8
aCAR_iCAR
iCAR_aCAR

FIG. 4

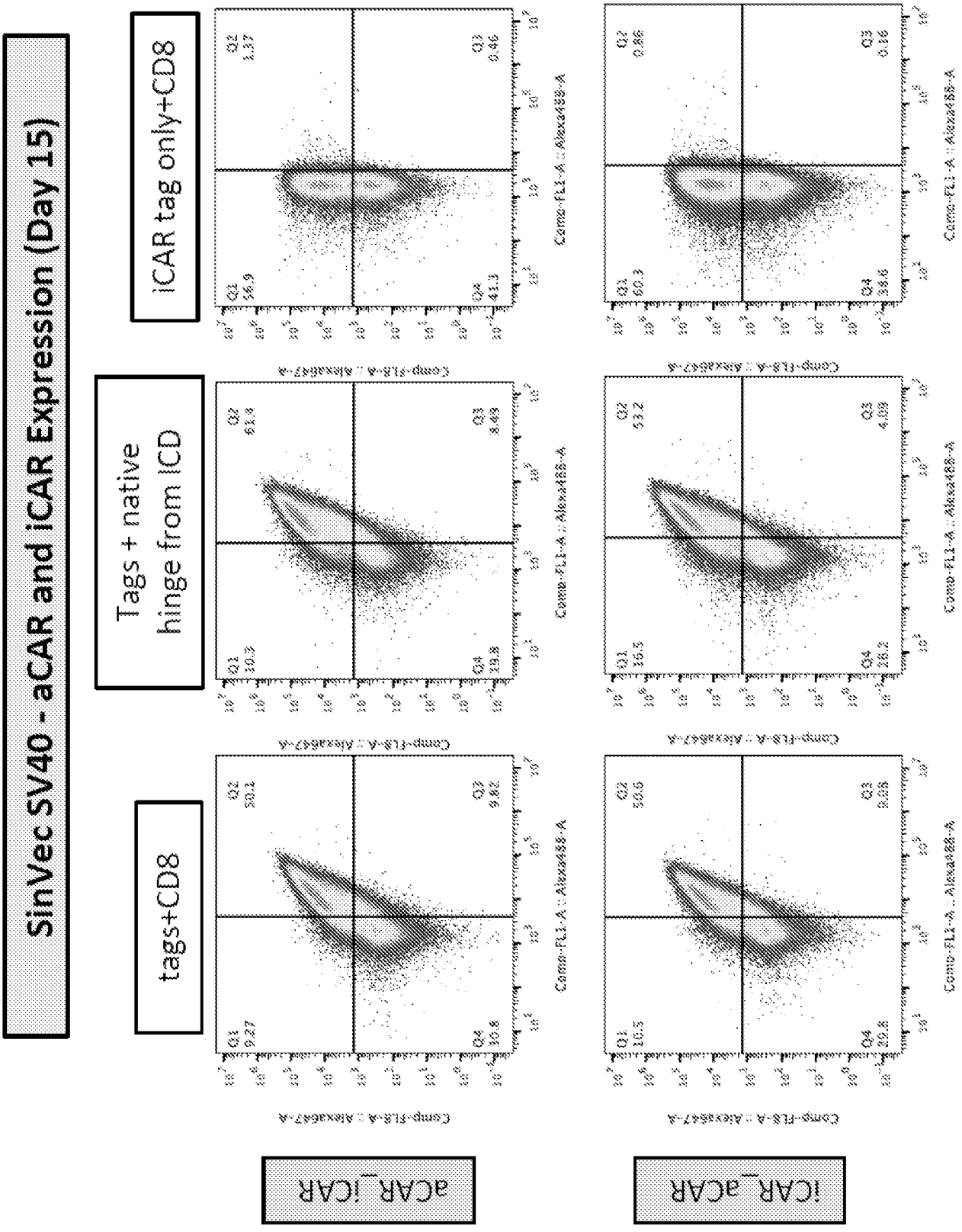

SinVec SV40 - aCAR and iCAR Expression (Day 15)
tags+CD8
Tags + native hinge from ICD
iCAR tag only+CD8
aCAR_iCAR
iCAR_aCAR

FIG. 5

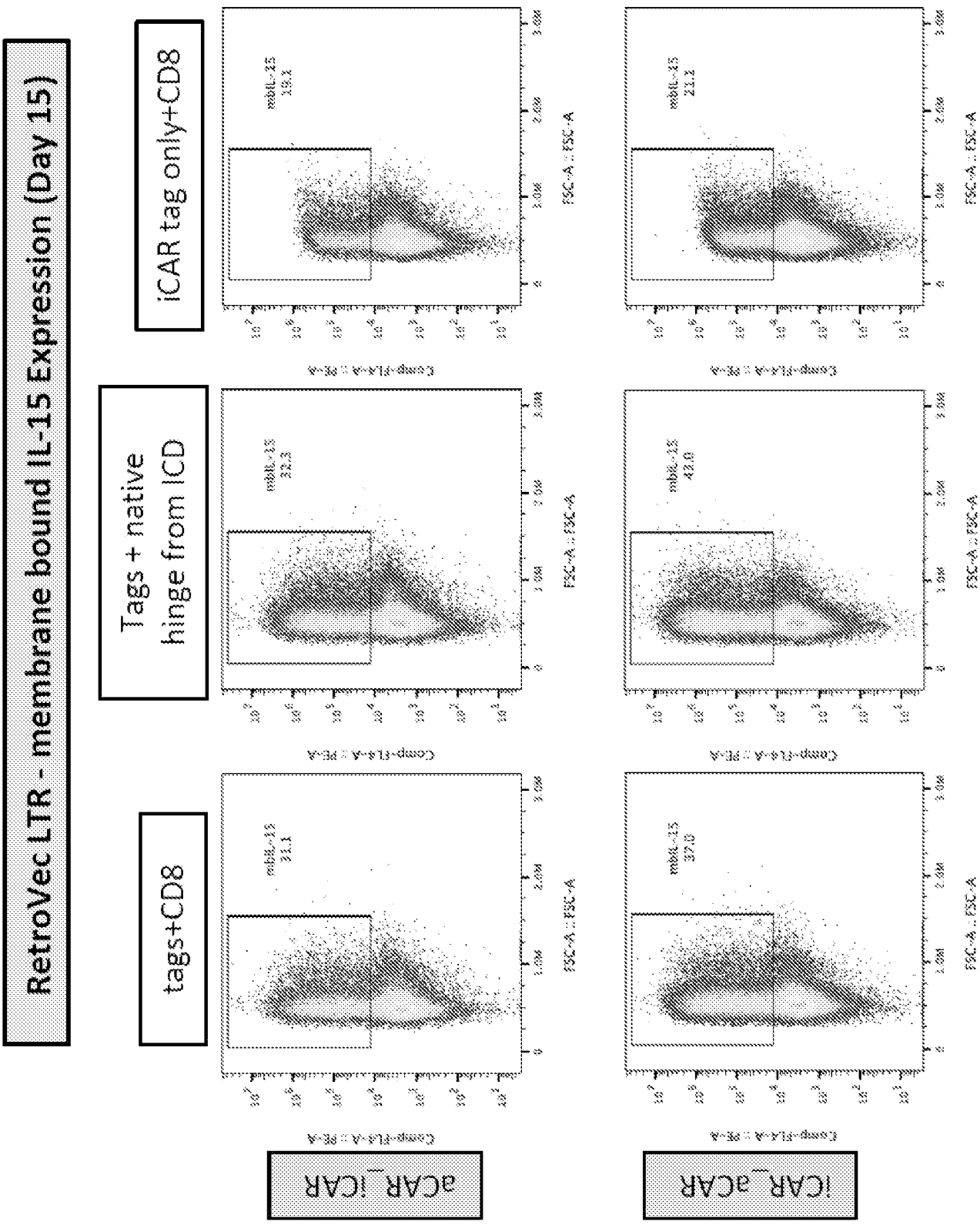

RetroVec LTR - membrane bound IL-15 Expression (Day 15)
tags+CD8
Tags + native hinge from ICD
iCAR tag only+CD8
aCAR_iCAR
iCAR_aCAR

FIG. 6

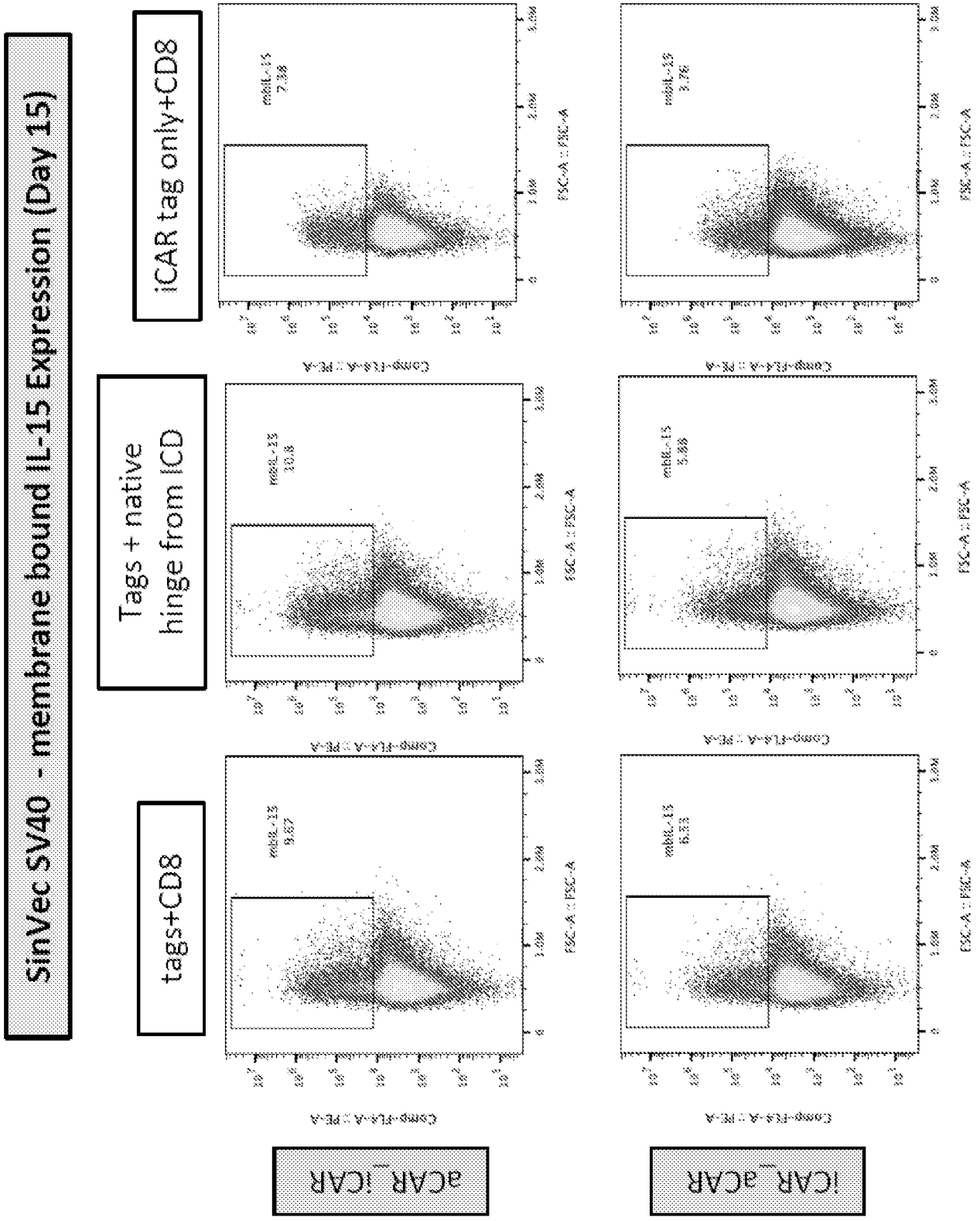

SinVec SV40 - membrane bound IL-15 Expression (Day 15)
tags+CD8
Tags + native hinge from ICD
iCAR tag only+CD8
aCAR_iCAR
iCAR_aCAR

FIG. 7

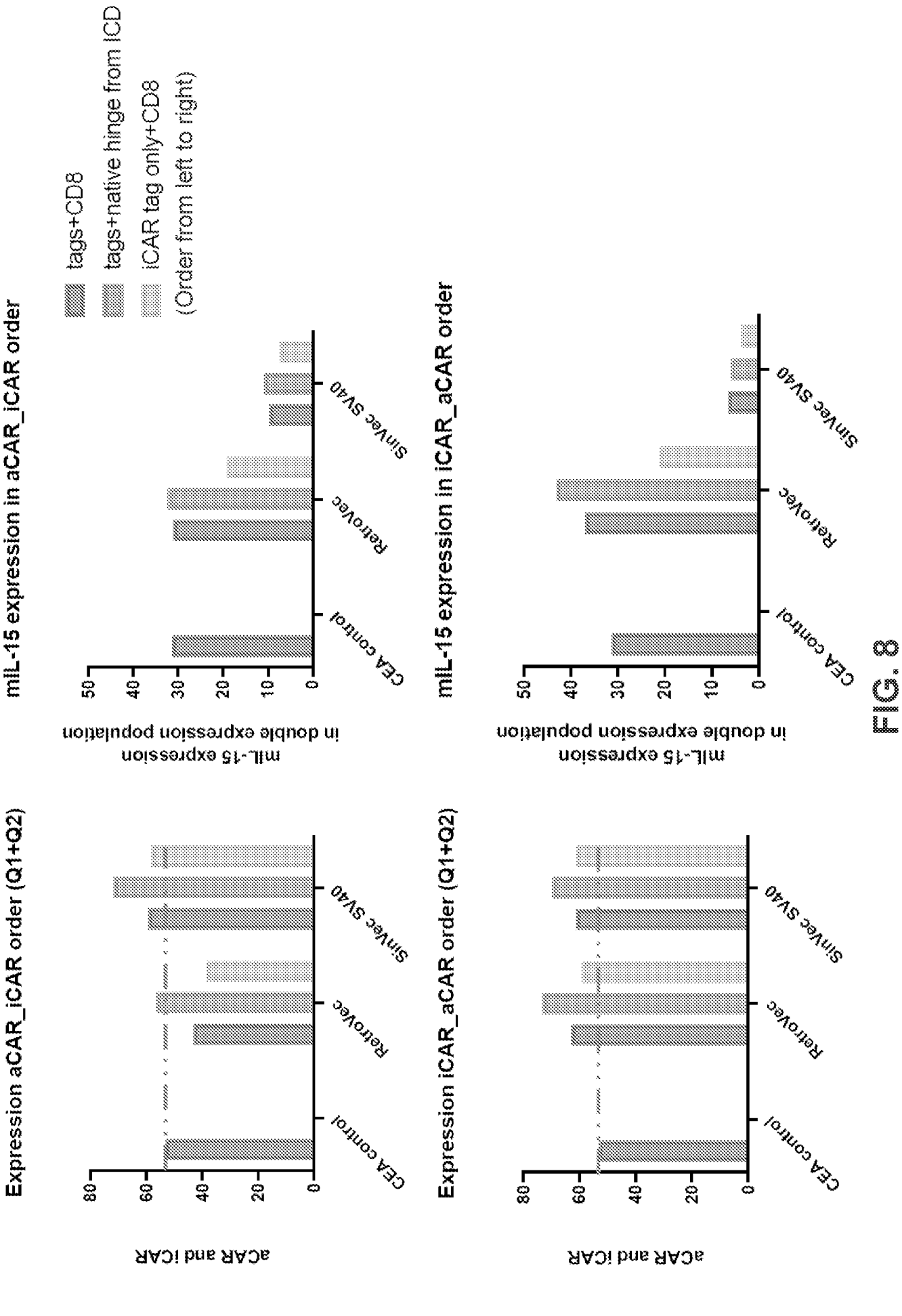

Expression aCAR_iCAR order (Q1+Q2)
aCAR and iCAR
0
20
40
60
80
CEA control
RetroVec
SinVec SV40
mIL-15 expression in aCAR_iCAR order
tags+CD8
tags+native hinge from ICD
iCAR tag only+CD8
(Order from left to right)
mIL-15 expression
in double expression population
0
10
20
30
40
50
CEA control
RetroVec
SinVec SV40
Expression iCAR_aCAR order (Q1+Q2)
aCAR and iCAR
0
20
40
60
80
CEA control
RetroVec
SinVec SV40
mIL-15 expression in iCAR_aCAR order
mIL-15 expression
in double expression population
0
10
20
30
40
50
CEA control
RetroVec
SinVec SV40

FIG. 8

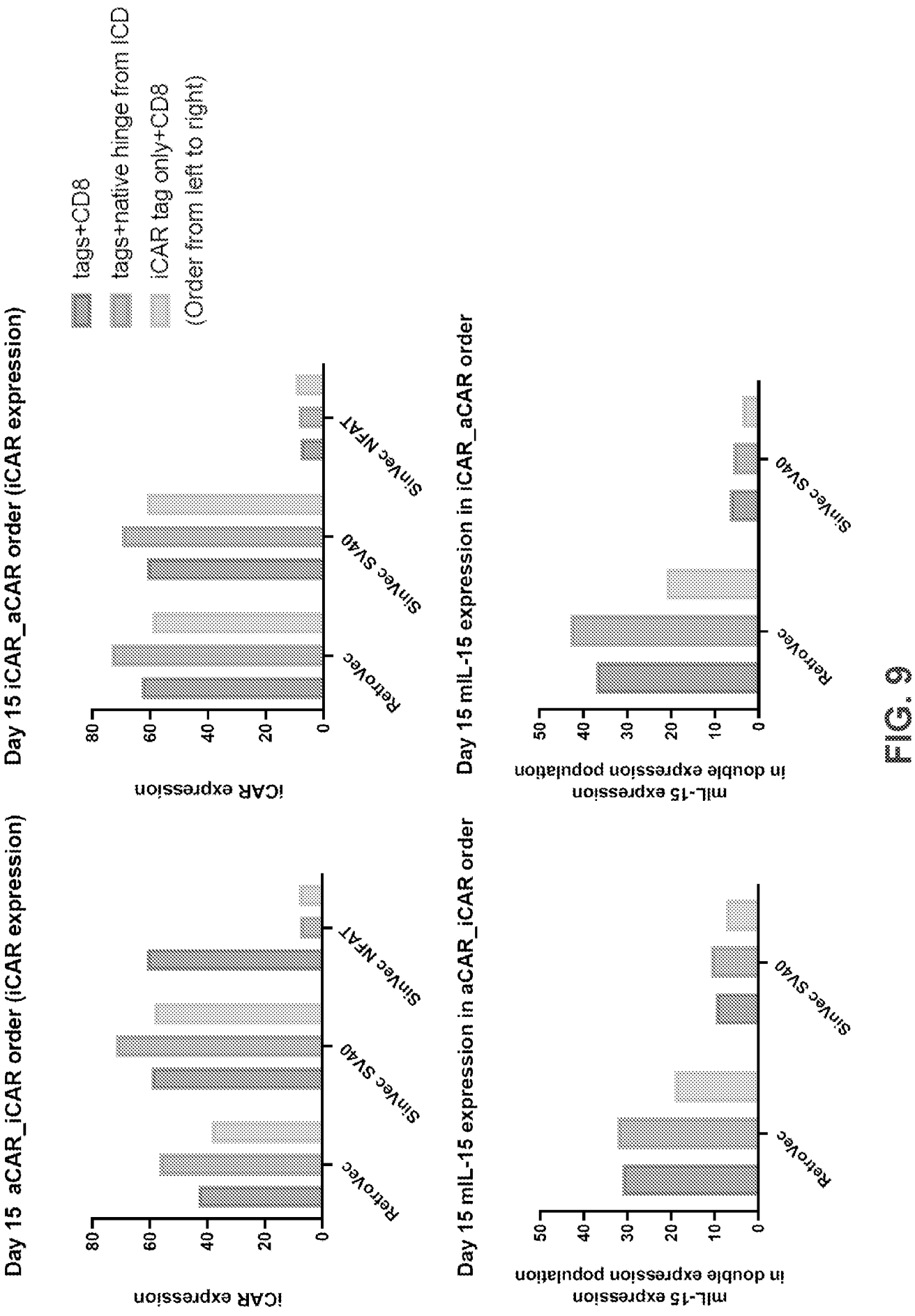
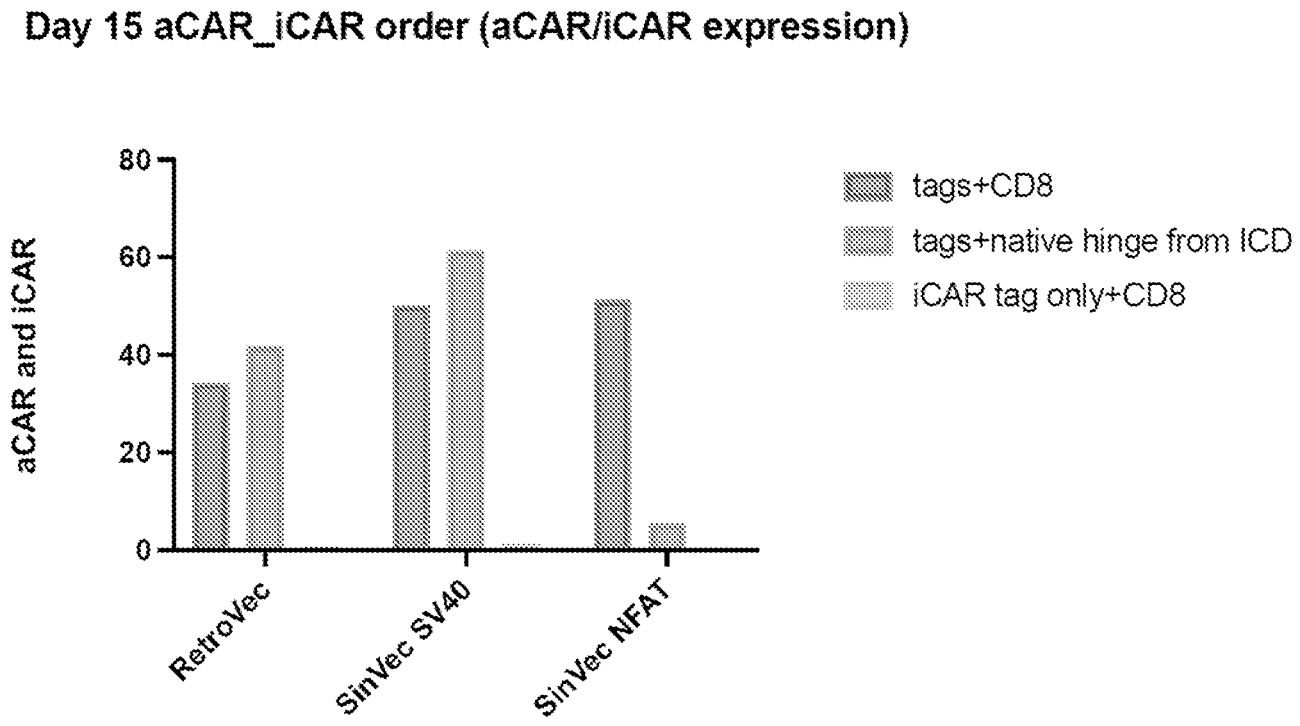

Day 15 aCAR_iCAR order (iCAR expression)
Day 15 iCAR_aCAR order (iCAR expression)
tags+CD8
tags+native hinge from ICD
iCAR tag only+CD8
(Order from left to right)
iCAR expression
0
20
40
60
80
RetroVec
SinVec SV40
SinVec NFAT
Day 15 miL-15 expression in aCAR_iCAR order
Day 15 miL-15 expression in iCAR_aCAR order
miL-15 expression
in double expression population
0
10
20
30
40
50
RetroVec
SinVec SV40

FIG. 9


Day 15 aCAR_iCAR order (aCAR/iCAR expression)
aCAR and iCAR
80
60
40
20
0
RetroVec
SinVec SV40
SinVec NFAT
tags+CD8
tags+native hinge from ICD
iCAR tag only+CD8

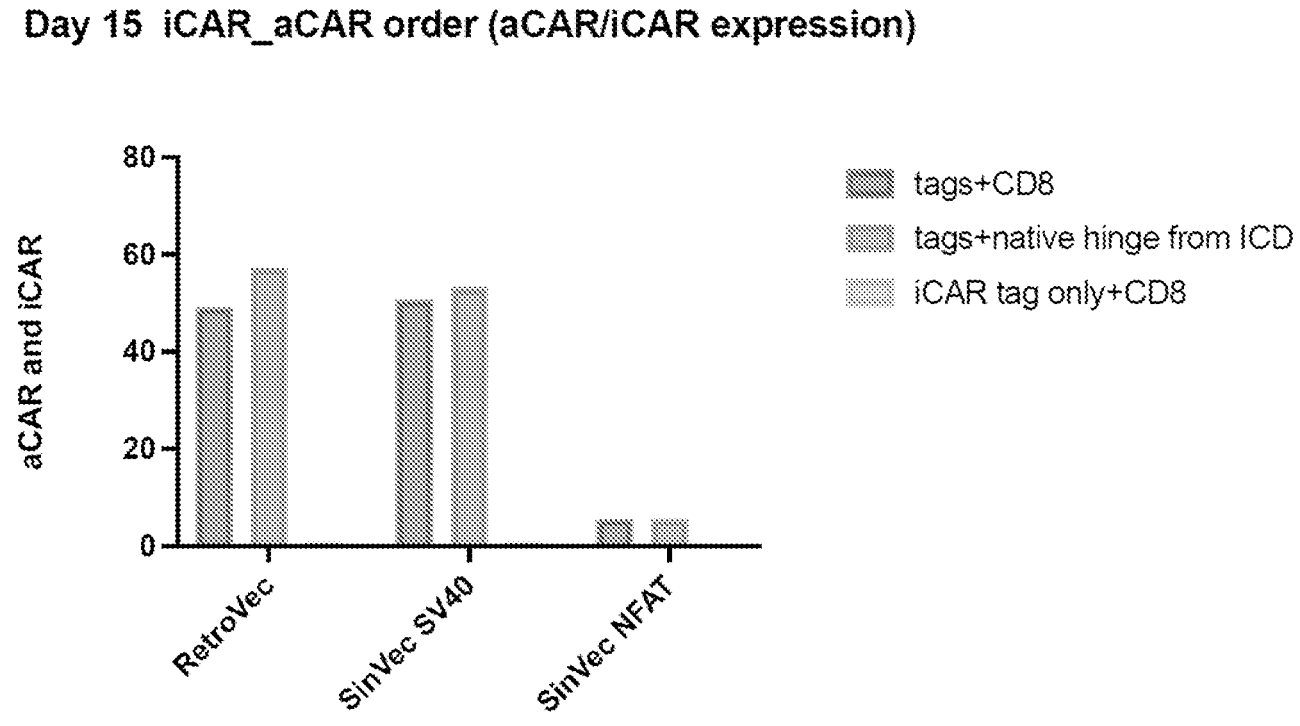

Day 15 iCAR_aCAR order (aCAR/iCAR expression)
aCAR and iCAR
80
60
40
20
0
RetroVec
SinVec SV40
SinVec NFAT
tags+CD8
tags+native hinge from ICD
iCAR tag only+CD8

FIG. 10

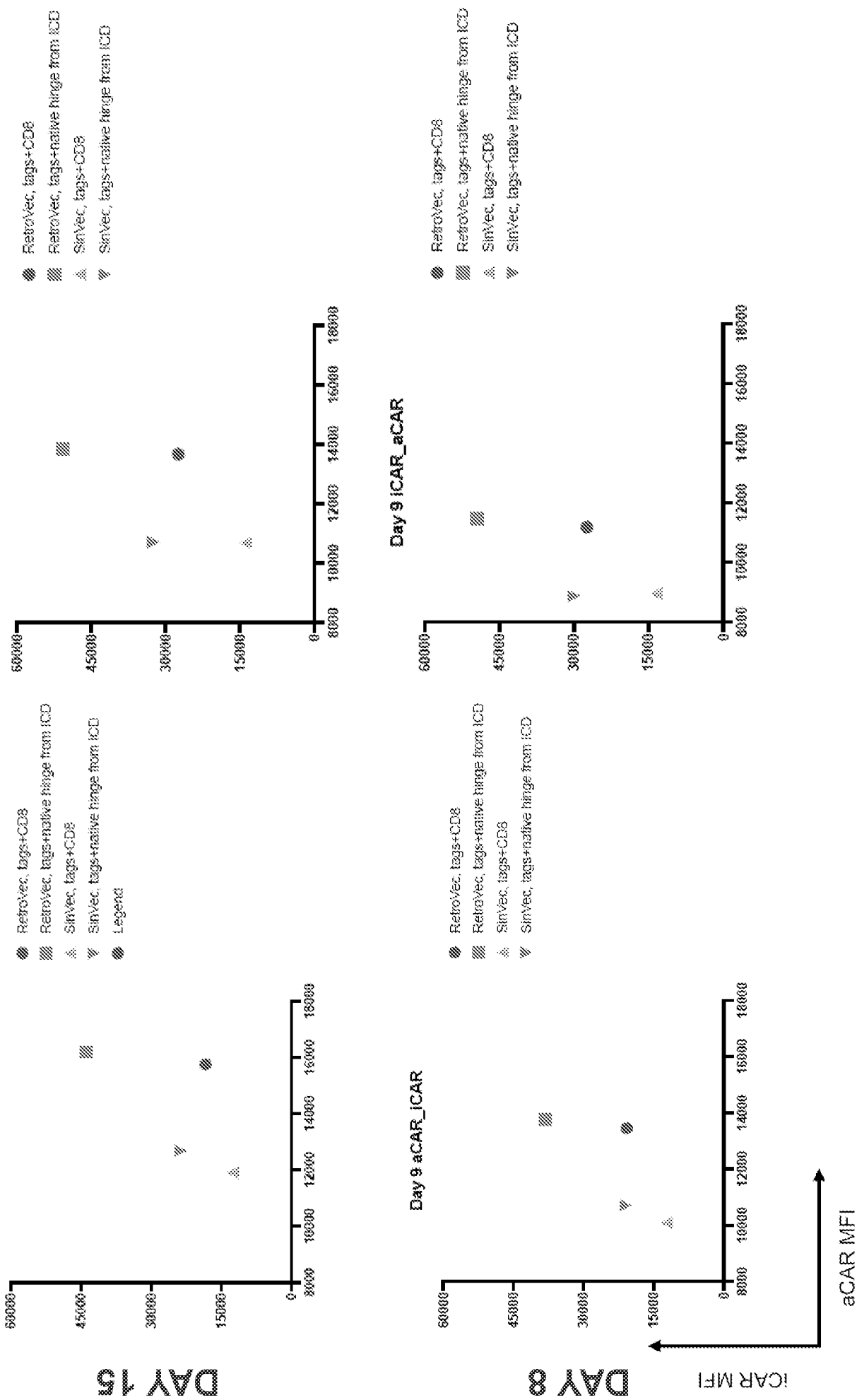

Day 15 aCAR_iCAR
Day 9 aCAR_iCAR
Day 15 iCAR_aCAR
Day 9 iCAR_aCAR
RetroVec, tags+CD8
RetroVec, tags+native hinge from ICD
SinVec, tags+CD8
SinVec, tags+native hinge from ICD
Legend
DAY 15
DAY 8
iCAR MFI
aCAR MFI
60000
45000
30000
15000
0
8000
10000
12000
14000
16000
18000

FIG. 11

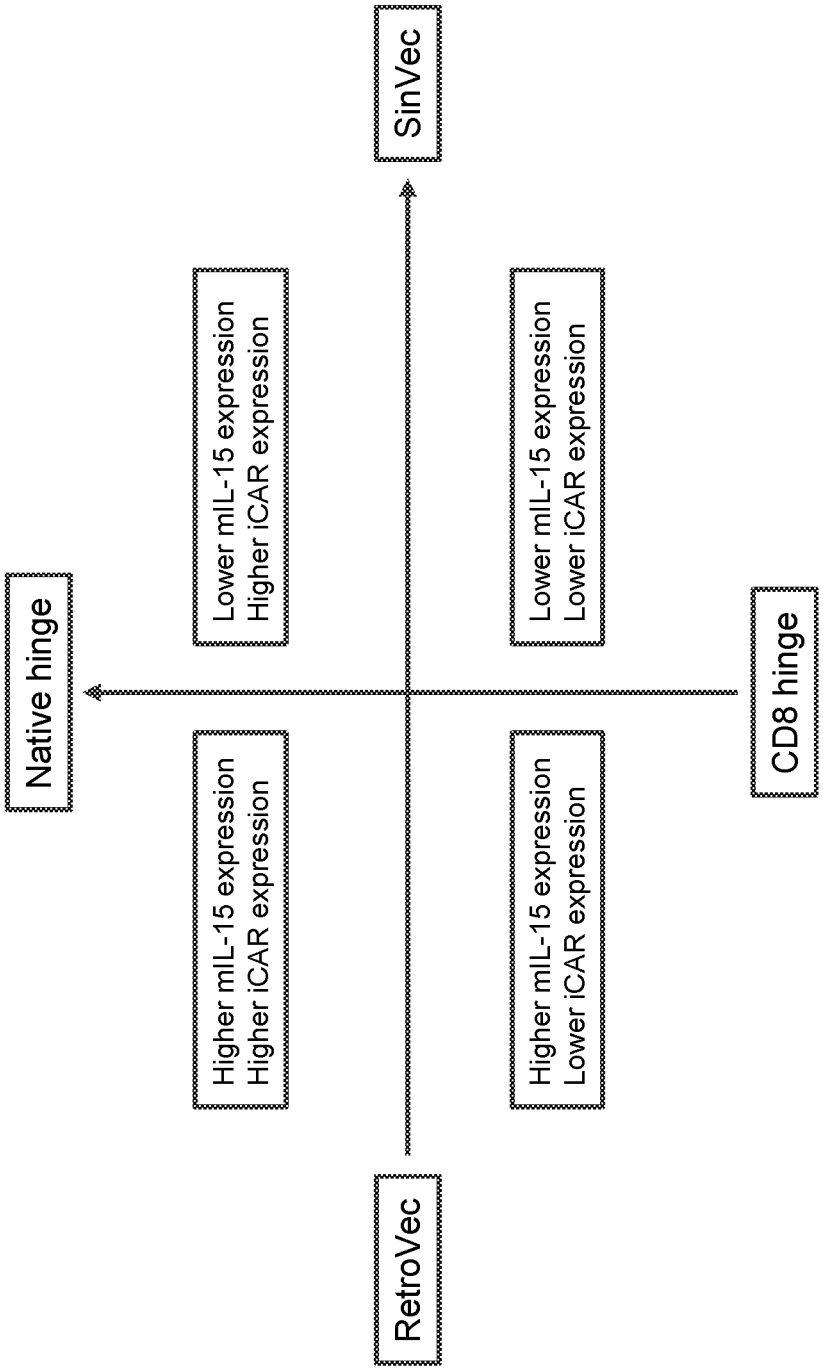
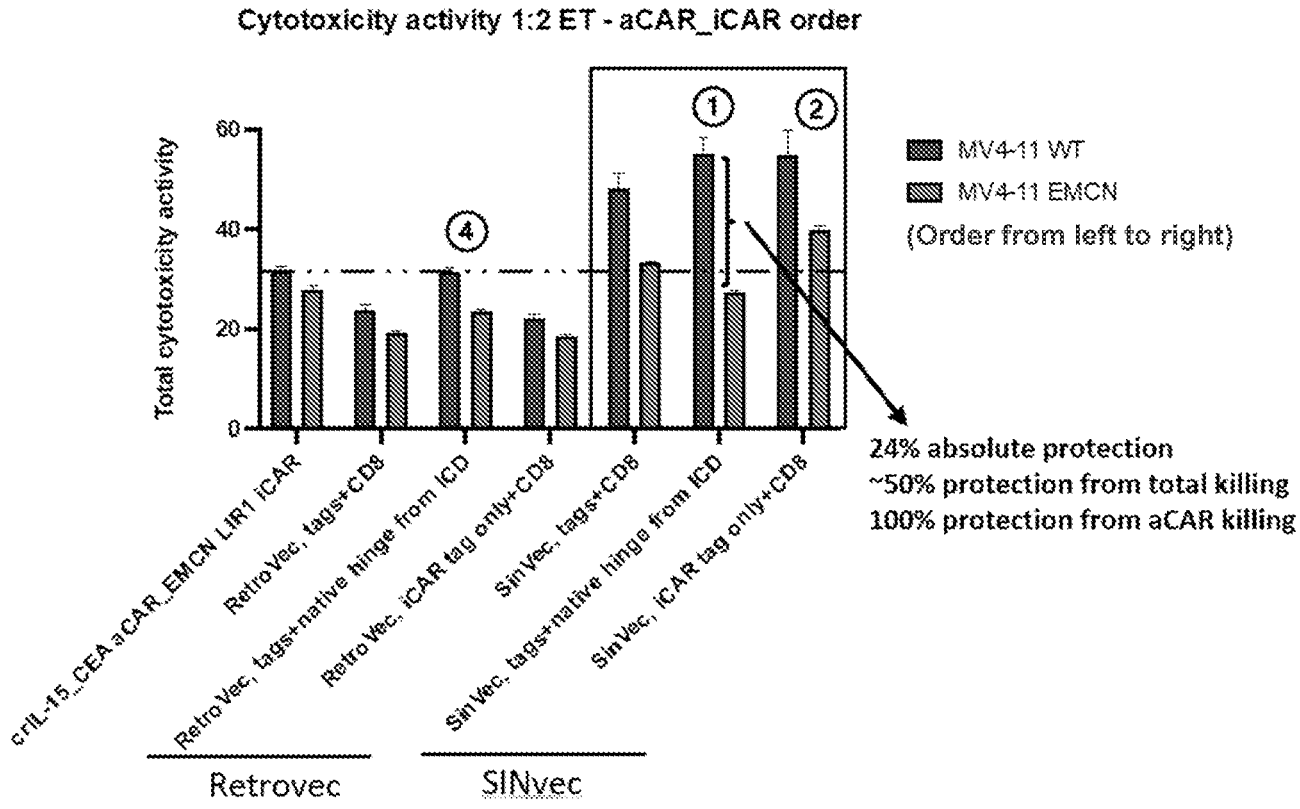

SinVec
RetroVec
Native hinge
CD8 hinge
Lower mIL-15 expression
Higher iCAR expression
Lower mIL-15 expression
Lower iCAR expression
Higher mIL-15 expression
Higher iCAR expression
Higher mIL-15 expression
Lower iCAR expression

FIG. 12


Cytotoxicity activity 1:2 ET - aCAR_iCAR order
Total cytotoxicity activity
60
40
20
0
1
2
4
MV4-11 WT
MV4-11 EMCN
(Order from left to right)
24% absolute protection
~50% protection from total killing
100% protection from aCAR killing
crIL-15_CEA aCAR_EMCN LIR1 iCAR
RetroVec, tags+CD8
RetroVec, tags+native hinge from ICD
RetroVec, iCAR tag only+CD8
SinVec, tags+CD8
SinVec, tags+native hinge from ICD
SinVec, iCAR tag only+CD8
Retrovec
SINvec

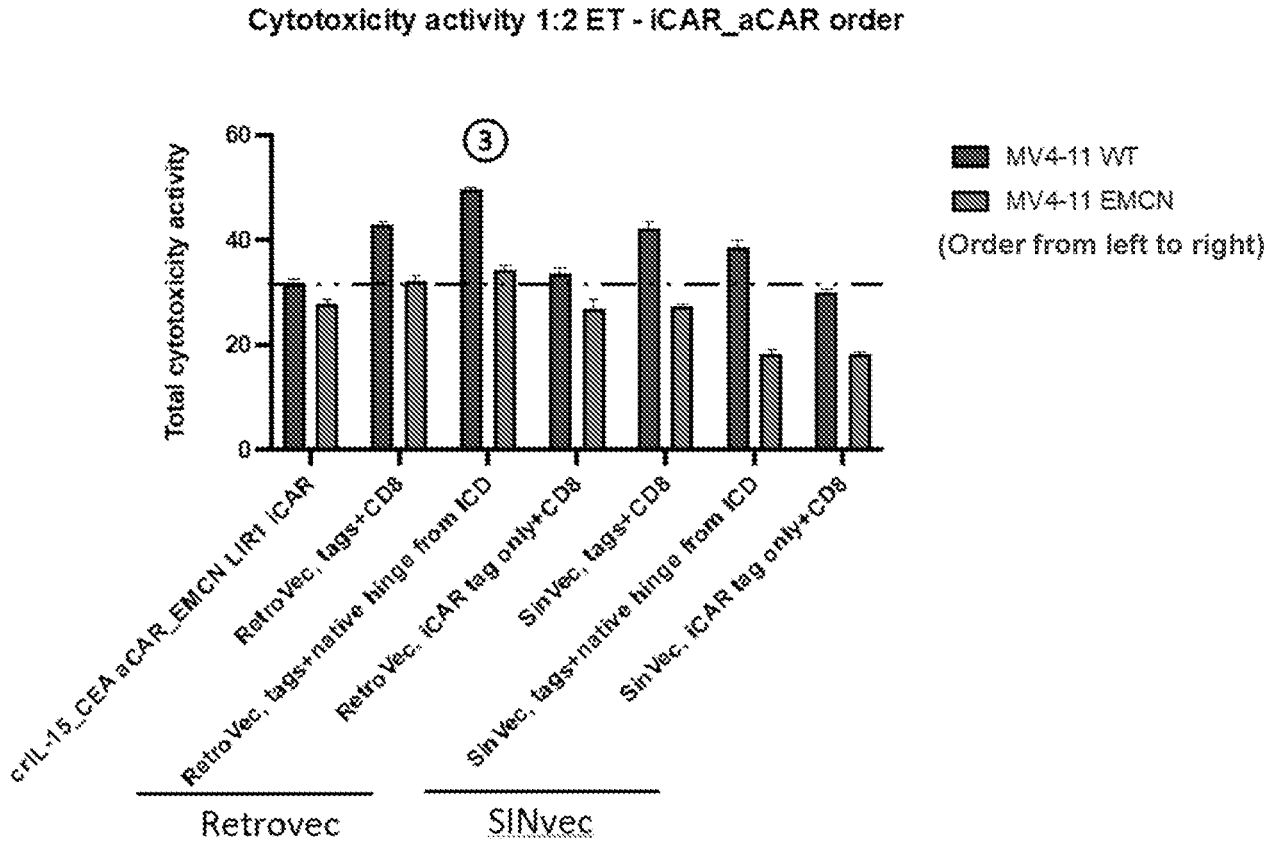

Cytotoxicity activity 1:2 ET - iCAR_aCAR order
Total cytotoxicity activity
60
40
20
0
3
MV4-11 WT
MV4-11 EMCN
(Order from left to right)
crIL-15_CEA aCAR_EMCN LIR1 iCAR
RetroVec, tags+CD8
RetroVec, tags+native hinge from ICD
RetroVec, iCAR tag only+CD8
SinVec, tags+CD8
SinVec, tags+native hinge from ICD
SinVec, iCAR tag only+CD8
Retrovec
SINvec

FIG. 13

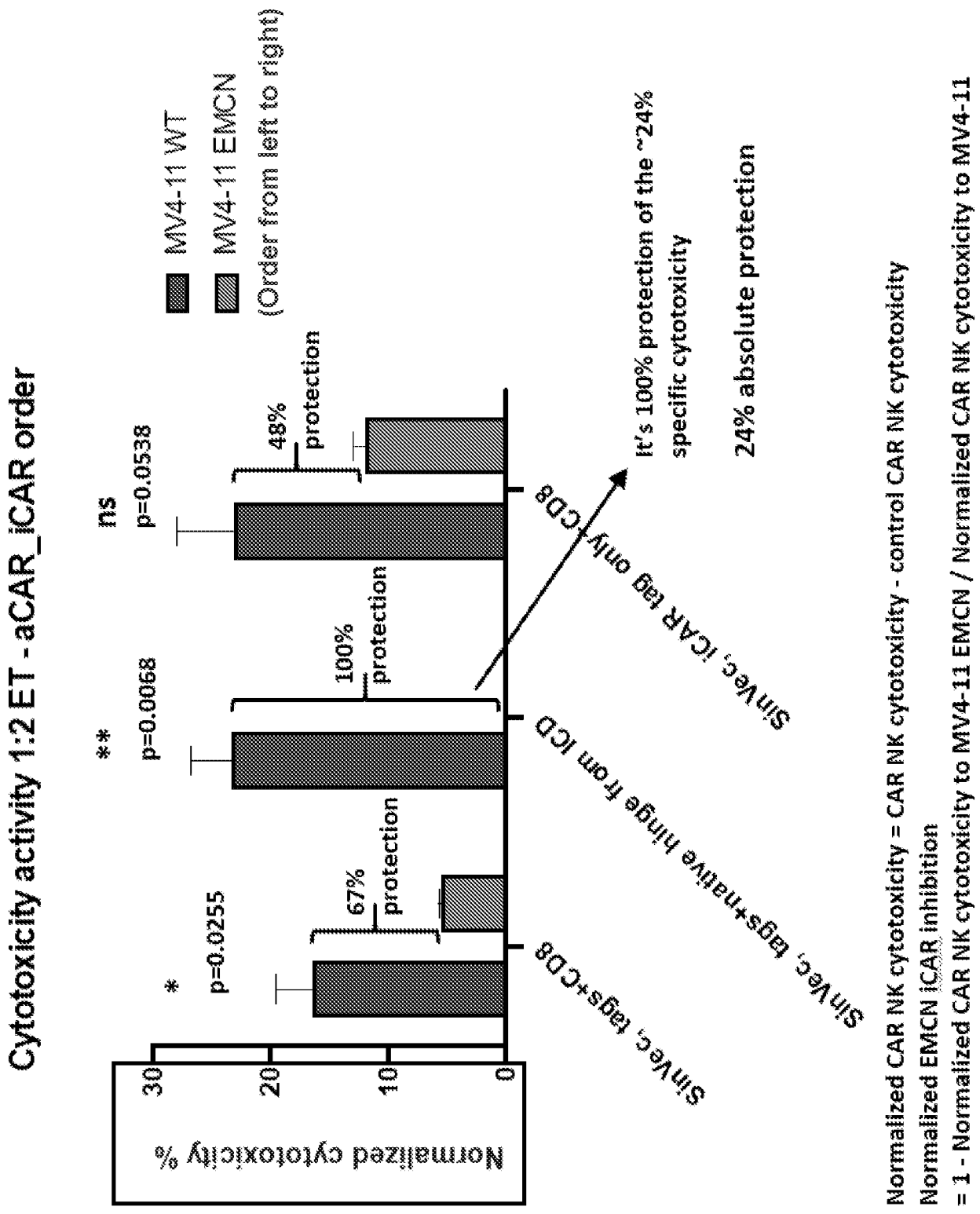

Cytotoxicity activity 1:2 ET - aCAR_iCAR order
MV4-11 WT
MV4-11 EMCN
(Order from left to right)
Normalized cytotoxicity %
0
10
20
30
*
p=0.0255
67% protection
**
p=0.0068
100% protection
ns
p=0.0538
48% protection
SinVec, tags+CD8
SinVec, tags+native hinge from ICD
SinVec, iCAR tag only CD8
It's 100% protection of the ~24% specific cytotoxicity
24% absolute protection
Normalized CAR NK cytotoxicity = CAR NK cytotoxicity - control CAR NK cytotoxicity
Normalized EMCN iCAR inhibition
= 1 - Normalized CAR NK cytotoxicity to MV4-11 EMCN / Normalized CAR NK cytotoxicity to MV4-11 WT

FIG. 14

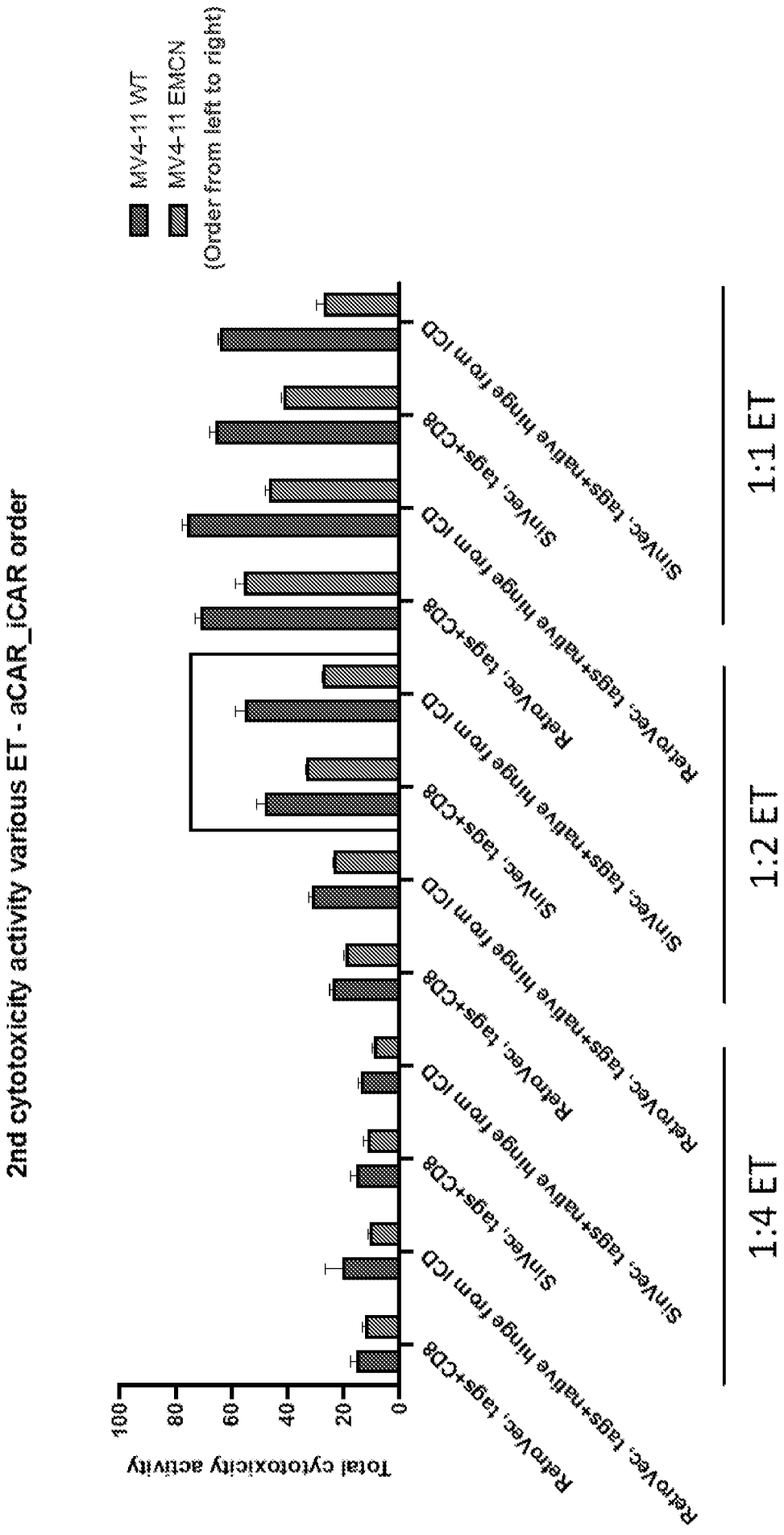
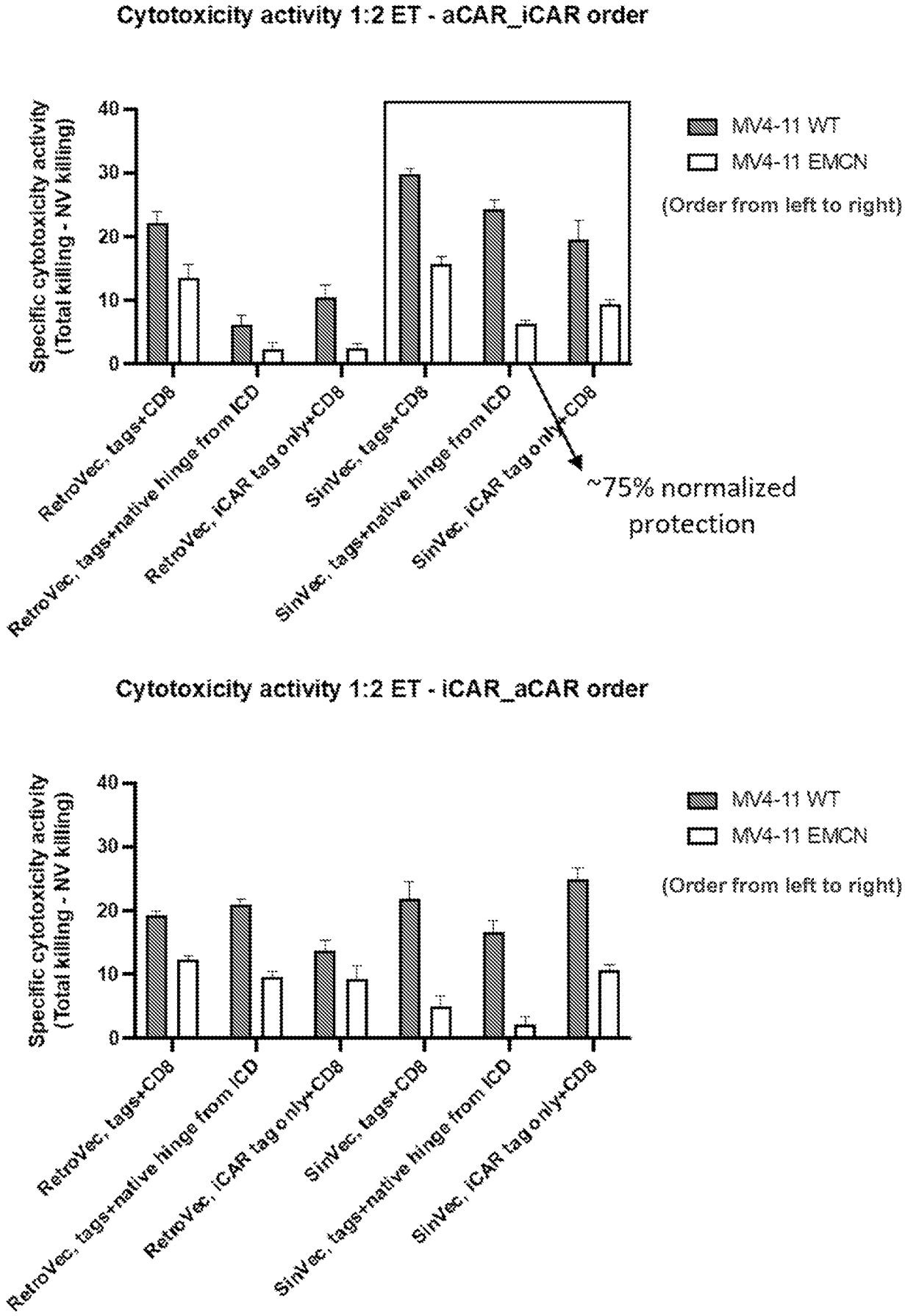

2nd cytotoxicity activity various ET - aCAR_iCAR order
MV4-11 WT
MV4-11 EMCN
(Order from left to right)
Total cytotoxicity activity
0
20
40
60
80
100
RetroVec, tags+CD8
RetroVec, tags+native hinge from ICD
SinVec, tags+CD8
SinVec, tags+native hinge from ICD
RetroVec, tags+CD8
RetroVec, tags+native hinge from ICD
SinVec, tags+CD8
SinVec, tags+native hinge from ICD
RetroVec, tags+CD8
RetroVec, tags+native hinge from ICD
SinVec, tags+CD8
SinVec, tags+native hinge from ICD
1:4 ET
1:2 ET
1:1 ET

FIG. 15



*Cytotoxicity activity is normalized by target cell only control and NV NK control

FIG. 16

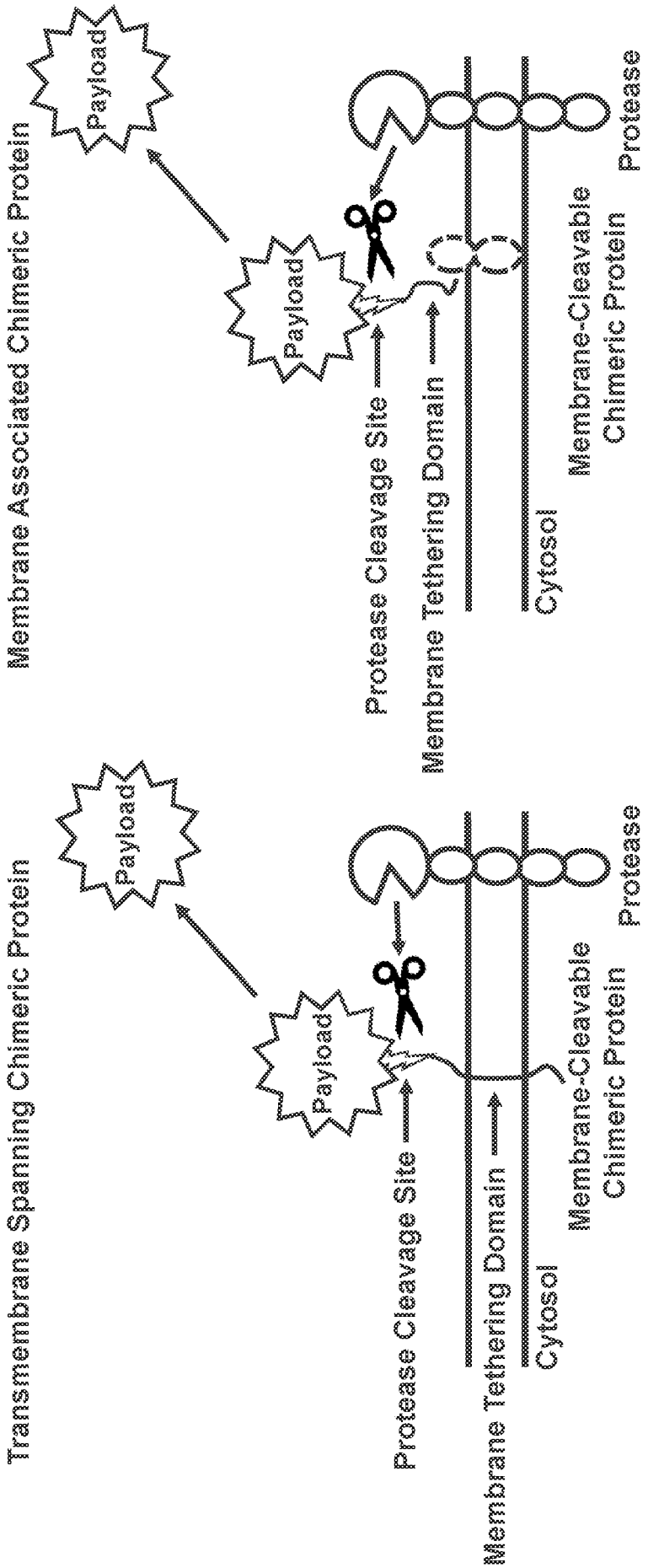

Membrane Associated Chimeric Protein
Payload
Payload
Protease Cleavage Site
Membrane Tethering Domain
Cytosol
Membrane-Cleavable Chimeric Protein
Protease
Transmembrane Spanning Chimeric Protein
Payload
Payload
Protease Cleavage Site
Membrane Tethering Domain
Cytosol
Membrane-Cleavable Chimeric Protein
Protease

FIG. 17

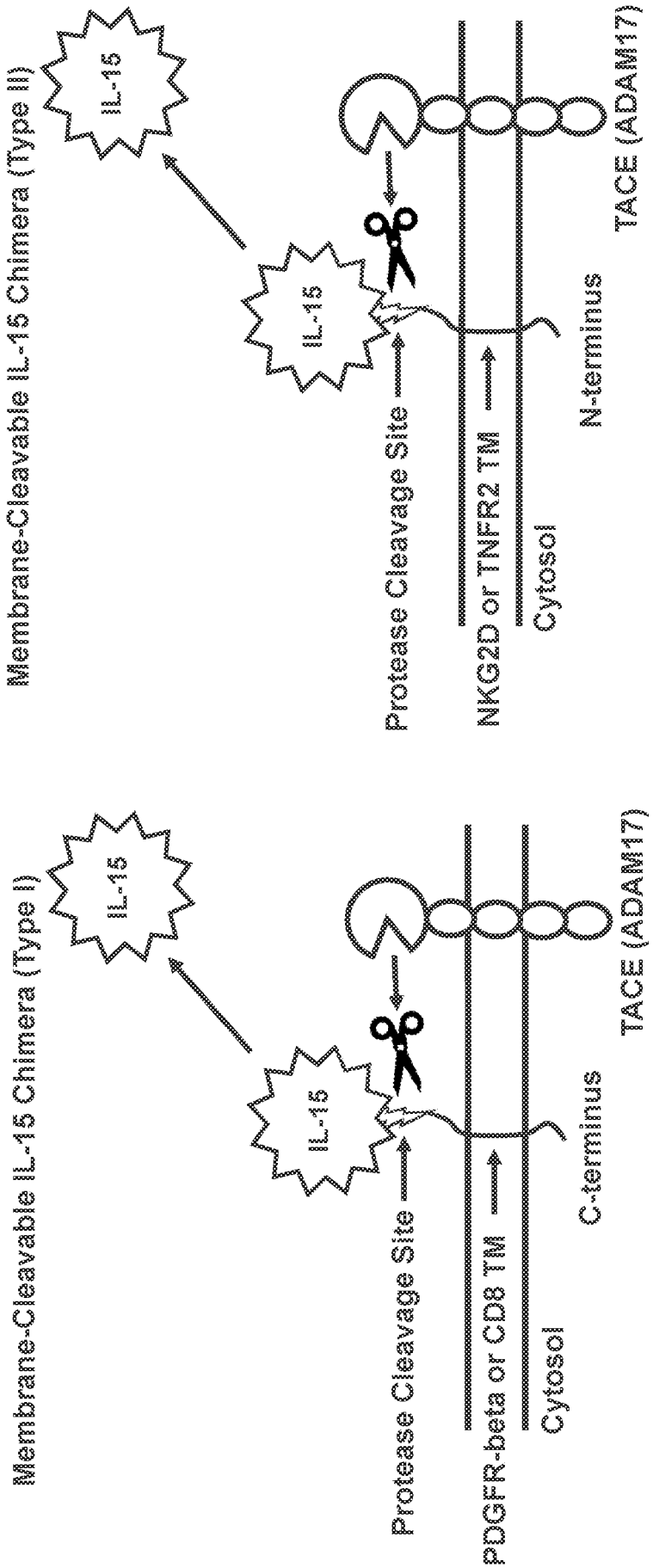

Membrane-Cleavable IL-15 Chimera (Type II)
IL-15
IL-15
Protease Cleavage Site
NKG2D or TNFR2 TM
Cytosol
N-terminus
TACE (ADAM17)
Membrane-Cleavable IL-15 Chimera (Type I)
IL-15
IL-15
Protease Cleavage Site
PDGFR-beta or CD8 TM
Cytosol
C-terminus
TACE (ADAM17)

FIG. 18

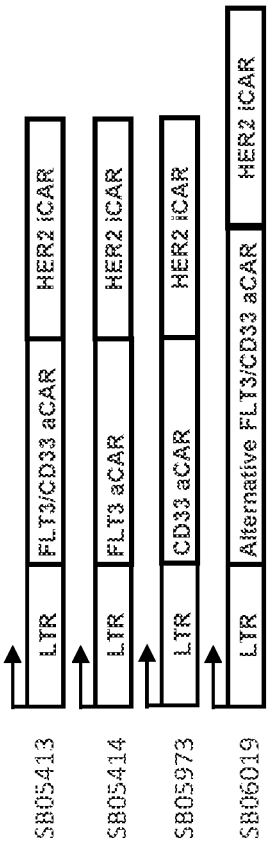

SB05413
LTR
FLT3/CD33 aCAR
HER2 iCAR
SB05414
LTR
FLT3 aCAR
HER2 iCAR
SB05973
LTR
CD33 aCAR
HER2 iCAR
SB06019
LTR
Alternative FLT3/CD33 aCAR
HER2 iCAR

FIG. 19

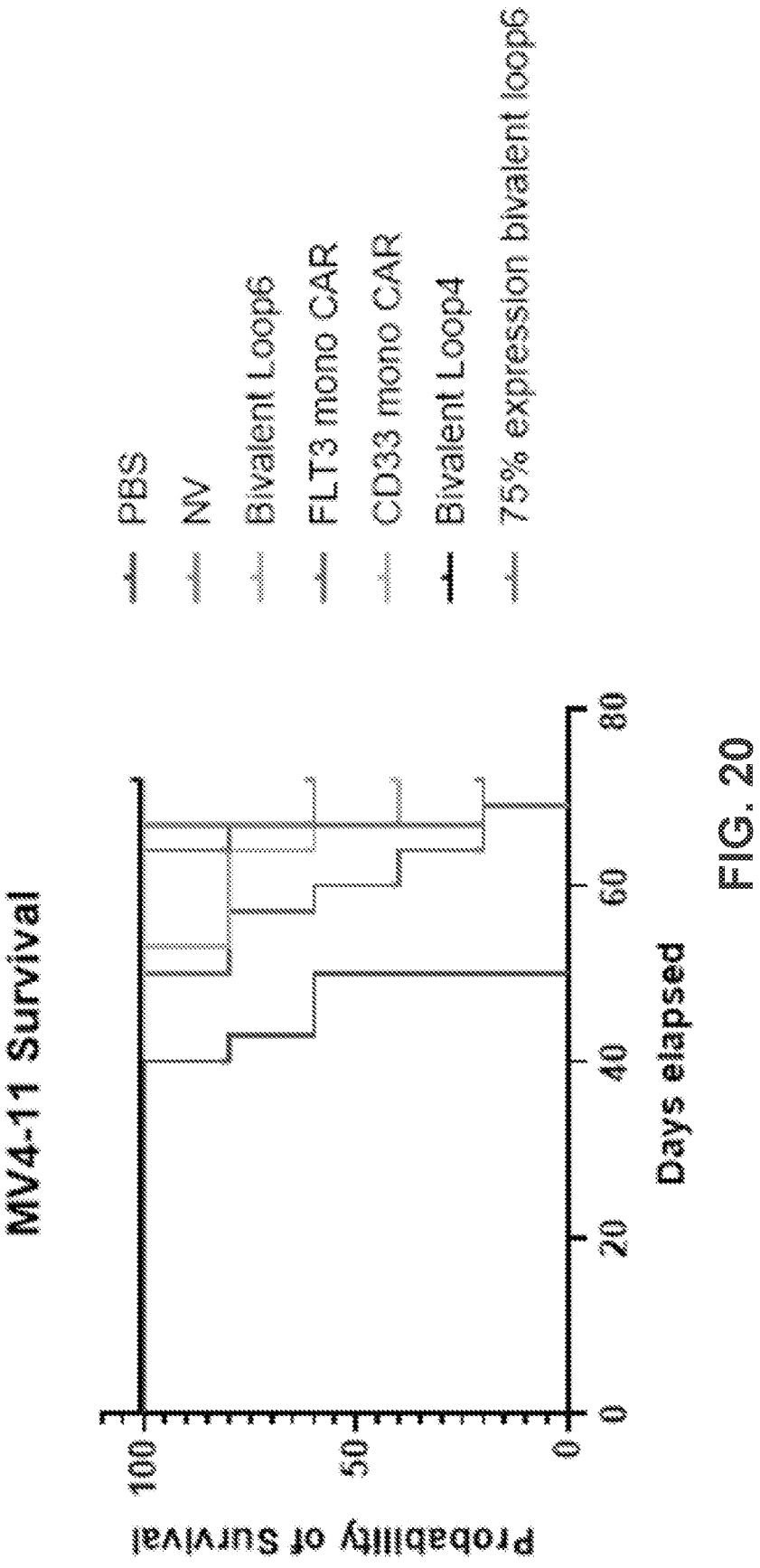

MV4-11 Survival
PBS
NV
Bivalent Loop6
FLT3 mono CAR
CD33 mono CAR
Bivalent Loop4
75% expression bivalent loop6
Probability of Survival
100
50
0
0
20
40
60
80
Days elapsed

FIG. 20

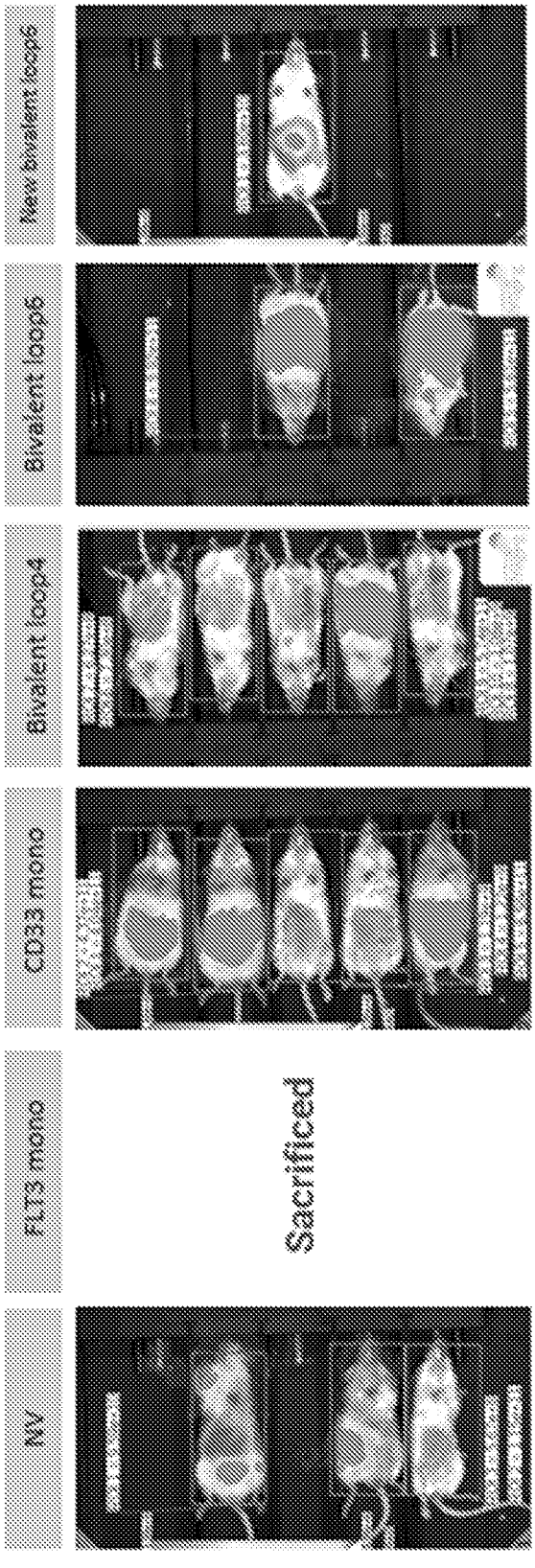

AML-01952 Imaging MV4-11 Day 71
PBS
MV
FLT3 mono
CD33 mono
Bivalent loop4
Bivalent loop6
New bivalent loops
Sacrificed
Sacrificed

FIG. 21

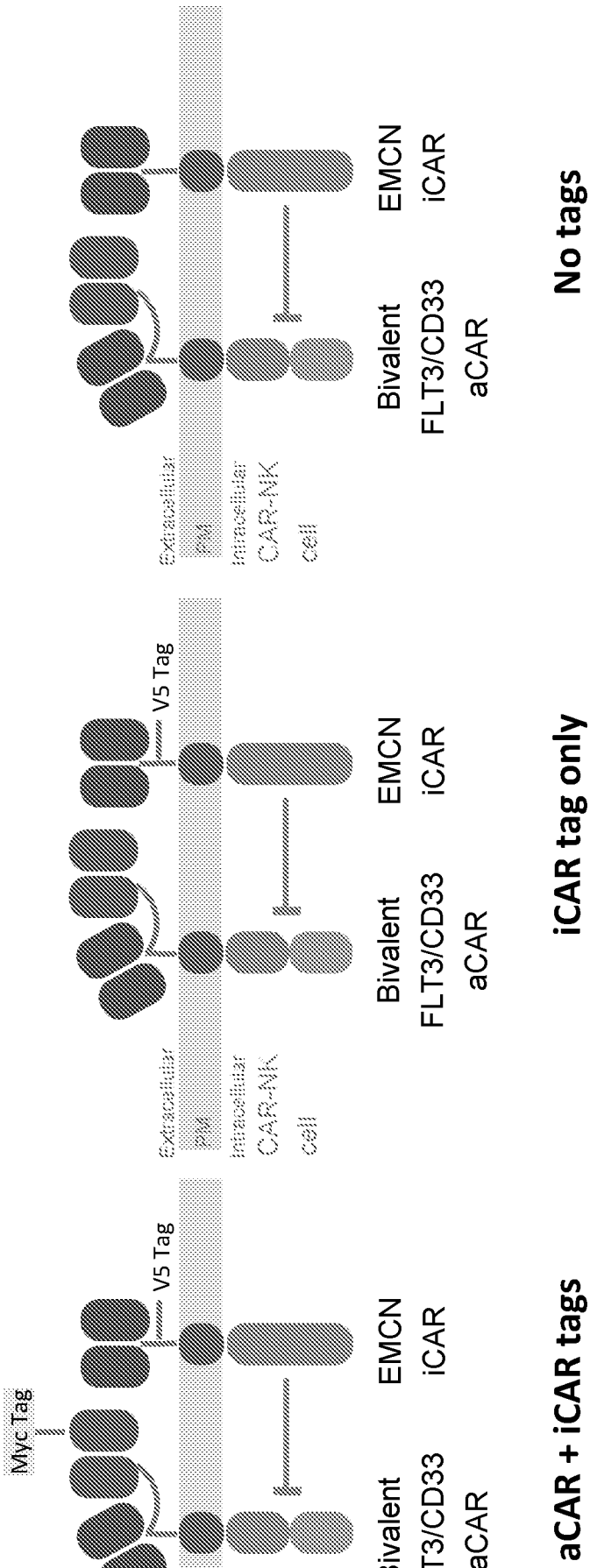
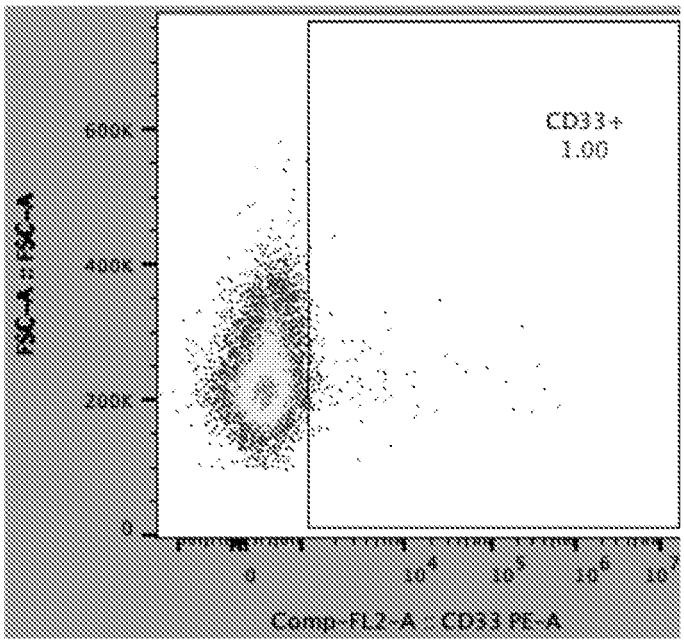
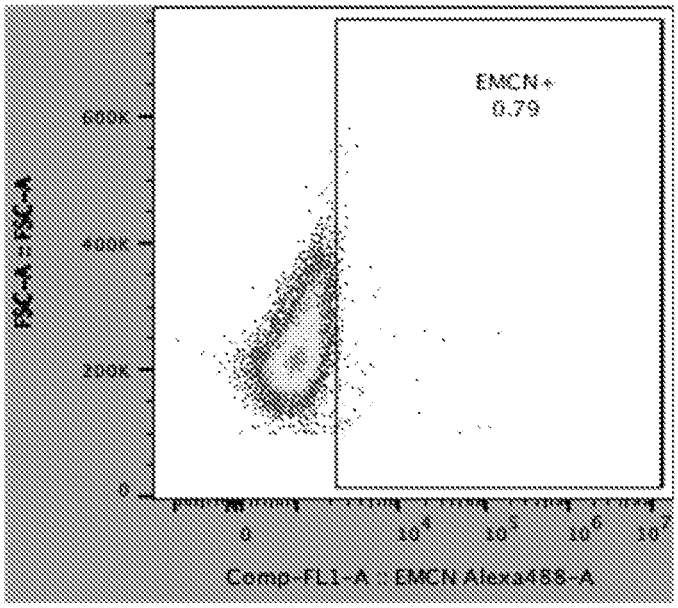

FLT3 OR CD33 NOT EMCN
Myc Tag
V5 Tag
Extracellular
PM
Intracellular
CAR-NK
cell
Bivalent
FLT3/CD33
aCAR
EMCN
iCAR
aCAR + iCAR tags
iCAR tag only
No tags

FIG. 22


CD33+
1.00
FSC-A : SSC-A
Comp-FL2-A :: CD33 PE-A


EMCN+
0.79
FSC-A : SSC-A
Comp-FL1-A :: EMCN Alexa488-A

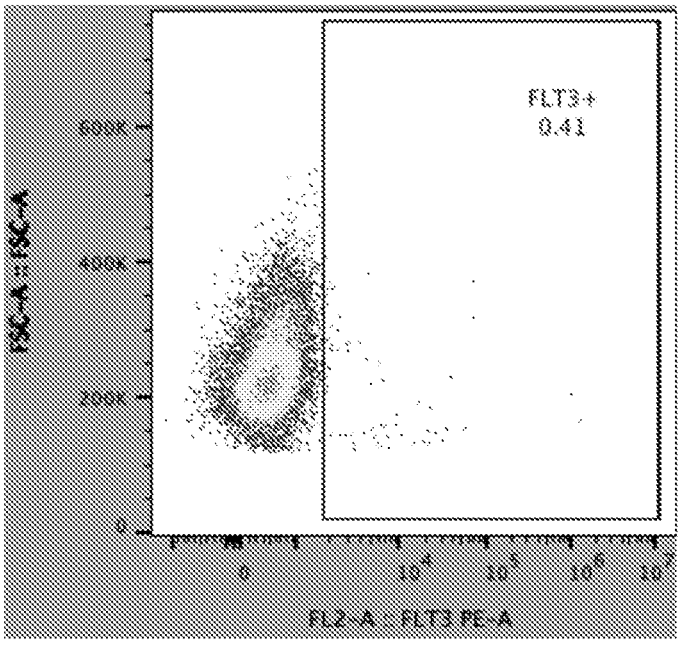

FLT3+
0.41
FSC-A : SSC-A
FL2-A :: FLT3 PE-A

FIG. 23

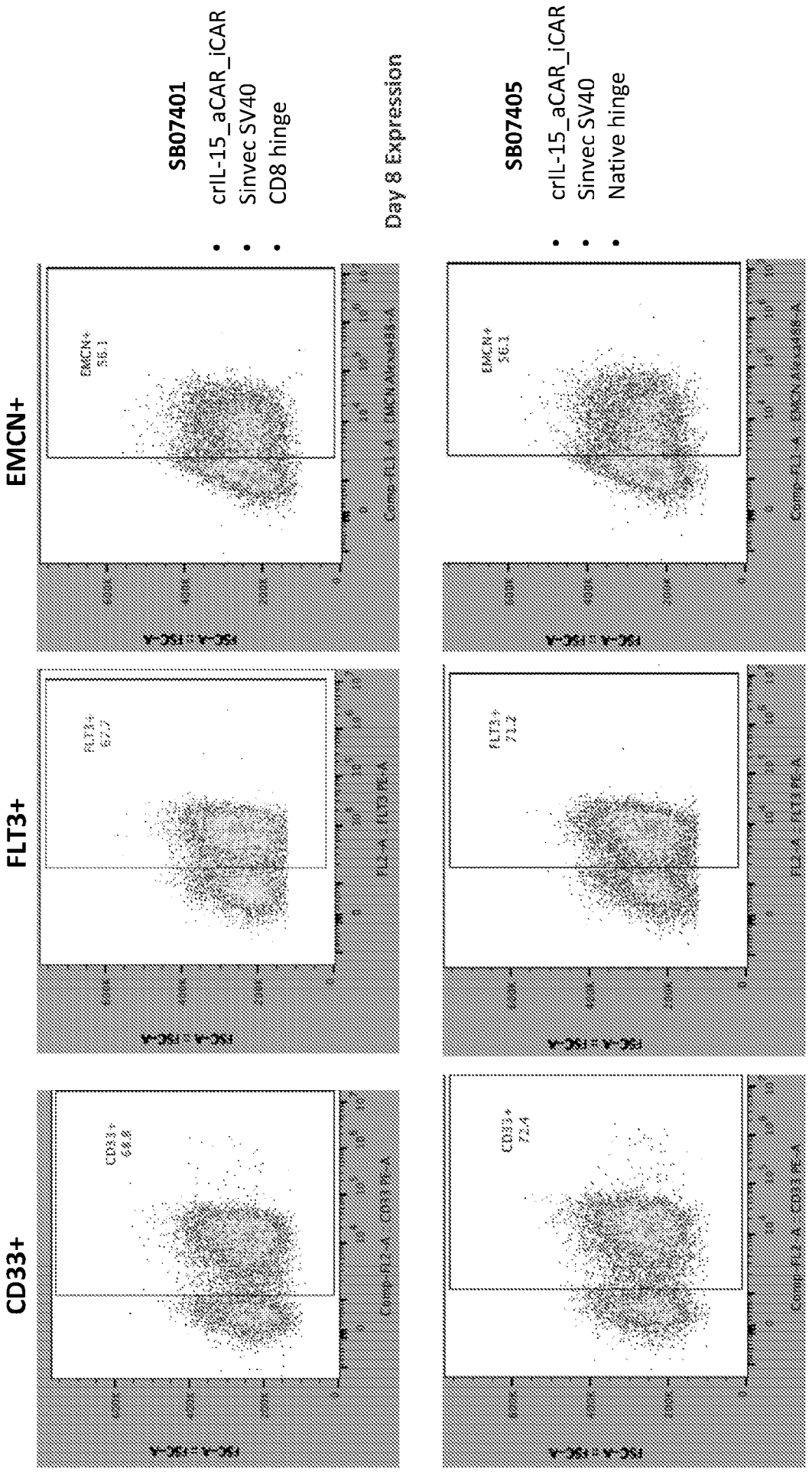

Day 8 Expression
SB07401
crIL-15_aCAR_iCAR
Sinvec SV40
CD8 hinge
SB07405
crIL-15_aCAR_iCAR
Sinvec SV40
Native hinge
EMCN+
FLT3+
CD33+

FIG. 24

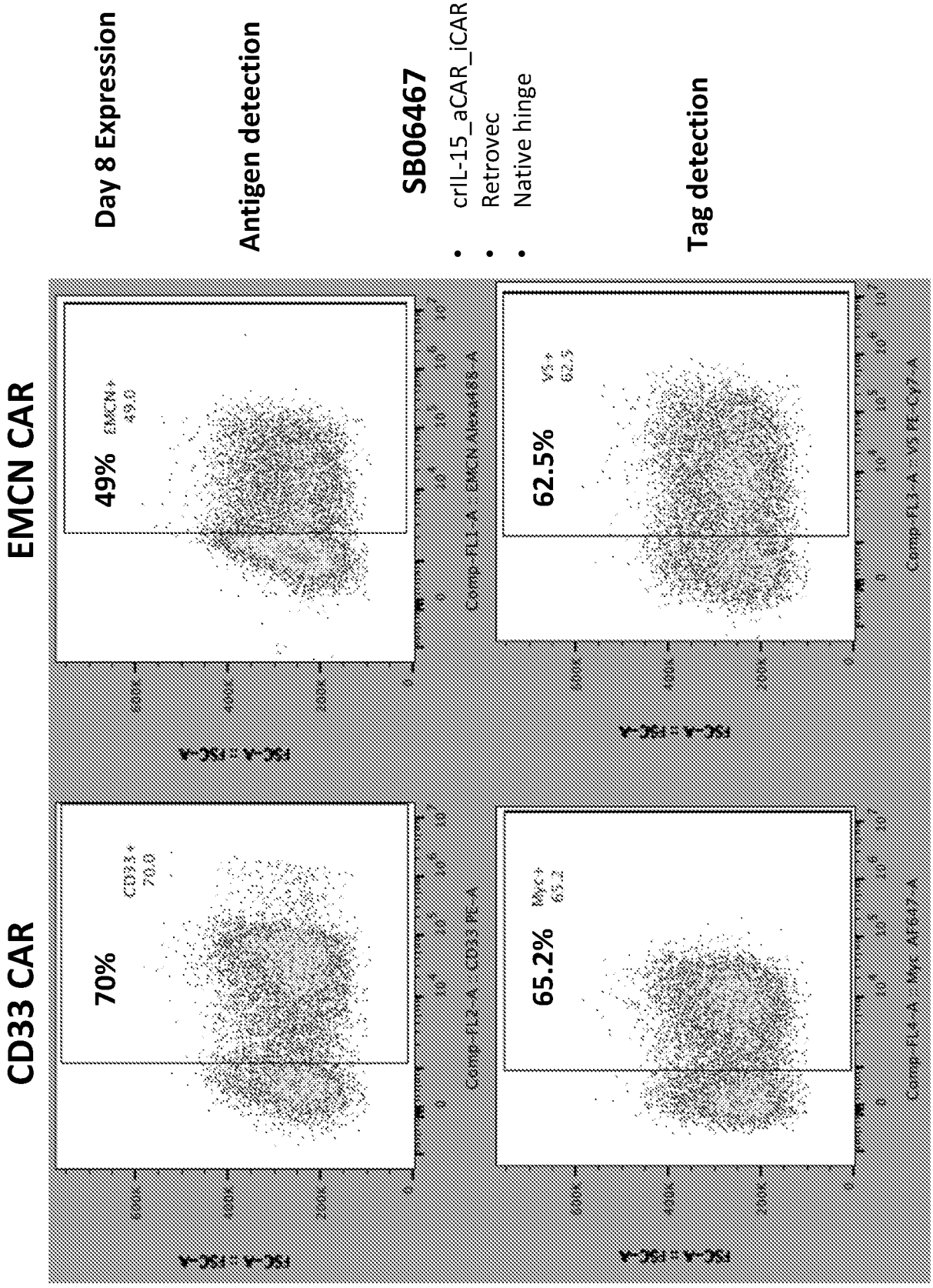

Day 8 Expression
Antigen detection
SB06467
crIL-15_aCAR_iCAR
Retrovec
Native hinge
Tag detection
EMCN CAR
CD33 CAR
49%
70%
62.5%
65.2%

FIG. 25

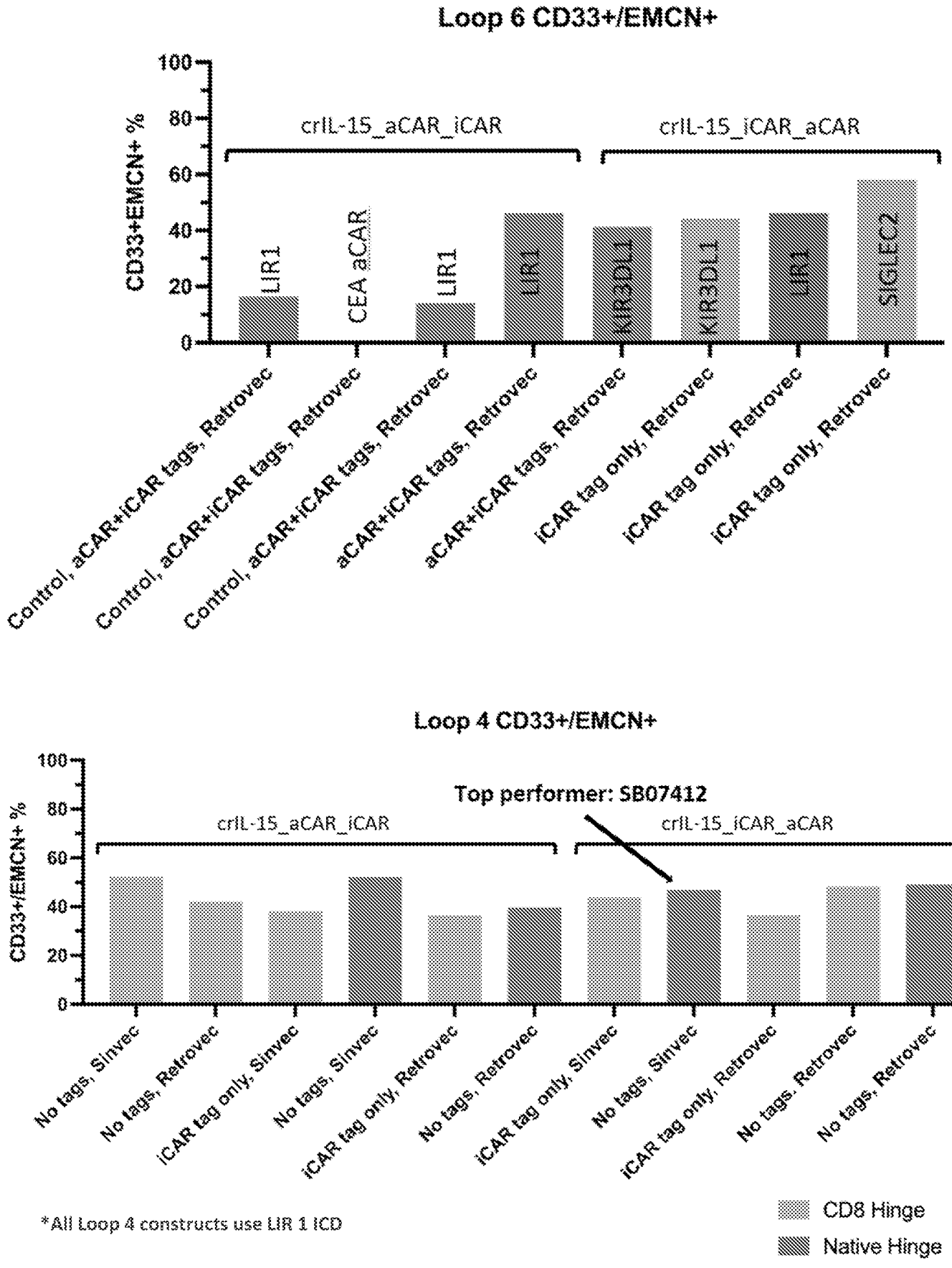

Loop 6 CD33+/EMCN+
CD33+EMCN+ %
100
80
60
40
20
0
crIL-15_aCAR_iCAR
crIL-15_iCAR_aCAR
LIR1
CEA aCAR
LIR1
LIR1
KIR3DL1
KIR3DL1
LIR1
SIGLEC2
Control, aCAR+iCAR tags, Retrovec
Control, aCAR+iCAR tags, Retrovec
Control, aCAR+iCAR tags, Retrovec
aCAR+iCAR tags, Retrovec
aCAR+iCAR tags, Retrovec
iCAR tag only, Retrovec
iCAR tag only, Retrovec
iCAR tag only, Retrovec
Loop 4 CD33+/EMCN+
Top performer: SB07412
CD33+/EMCN+ %
100
80
60
40
20
0
crIL-15_aCAR_iCAR
crIL-15_iCAR_aCAR
No tags, Sinvec
No tags, Retrovec
iCAR tag only, Sinvec
No tags, Sinvec
iCAR tag only, Retrovec
No tags, Retrovec
iCAR tag only, Sinvec
No tags, Sinvec
iCAR tag only, Retrovec
No tags, Retrovec
No tags, Retrovec
*All Loop 4 constructs use LIR 1 ICD
CD8 Hinge
Native Hinge

FIG. 26

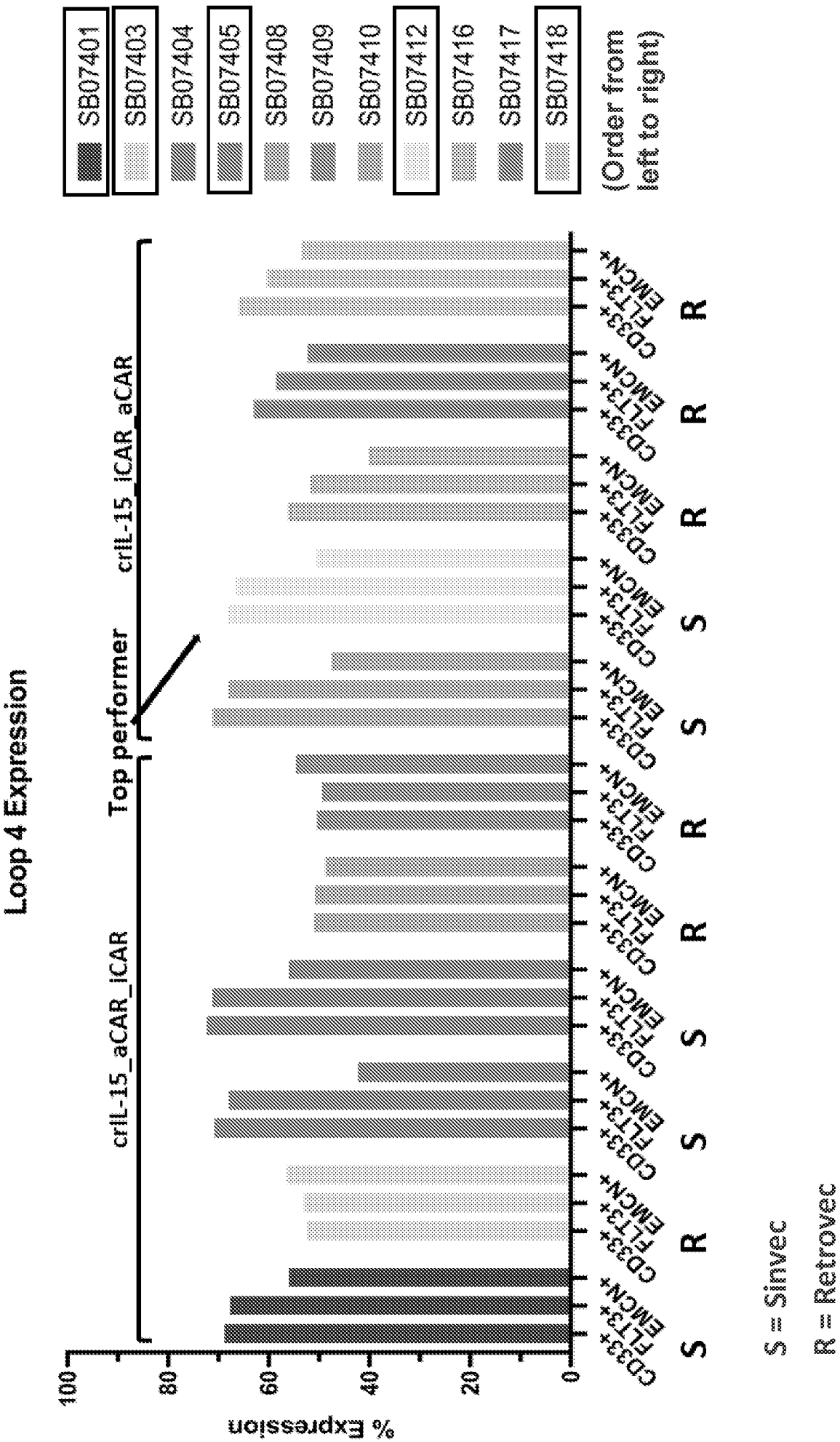

Loop 4 Expression
crIL-15_aCAR_iCAR
Top performer
crIL-15_iCAR_aCAR
% Expression
100
80
60
40
20
0
S R S S S R R S S R R R
CD33+ FLT3+ EMCN+
SB07401
SB07403
SB07404
SB07405
SB07408
SB07409
SB07410
SB07412
SB07416
SB07417
SB07418
(Order from left to right)
S = Sinvec
R = Retrovec

FIG. 27

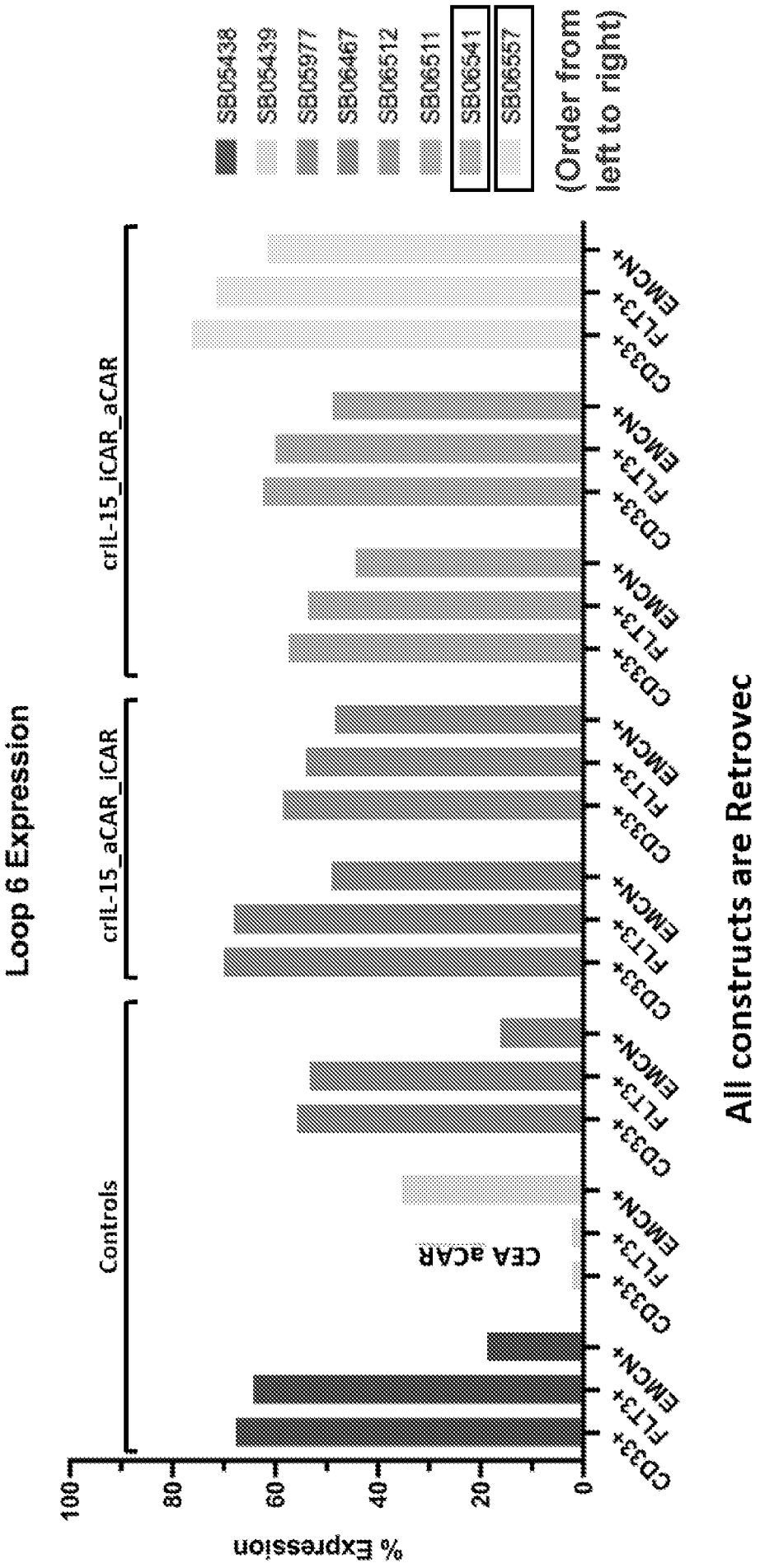

Loop 6 Expression
% Expression
100
80
60
40
20
0
Controls
CEA aCAR
criL-15_aCAR_iCAR
criL-15_iCAR_aCAR
CD33+ FLT3+ EMCN+
SB05438
SB05439
SB05977
SB06467
SB06512
SB06511
SB06541
SB06557
(Order from left to right)
All constructs are Retrovec

FIG. 28

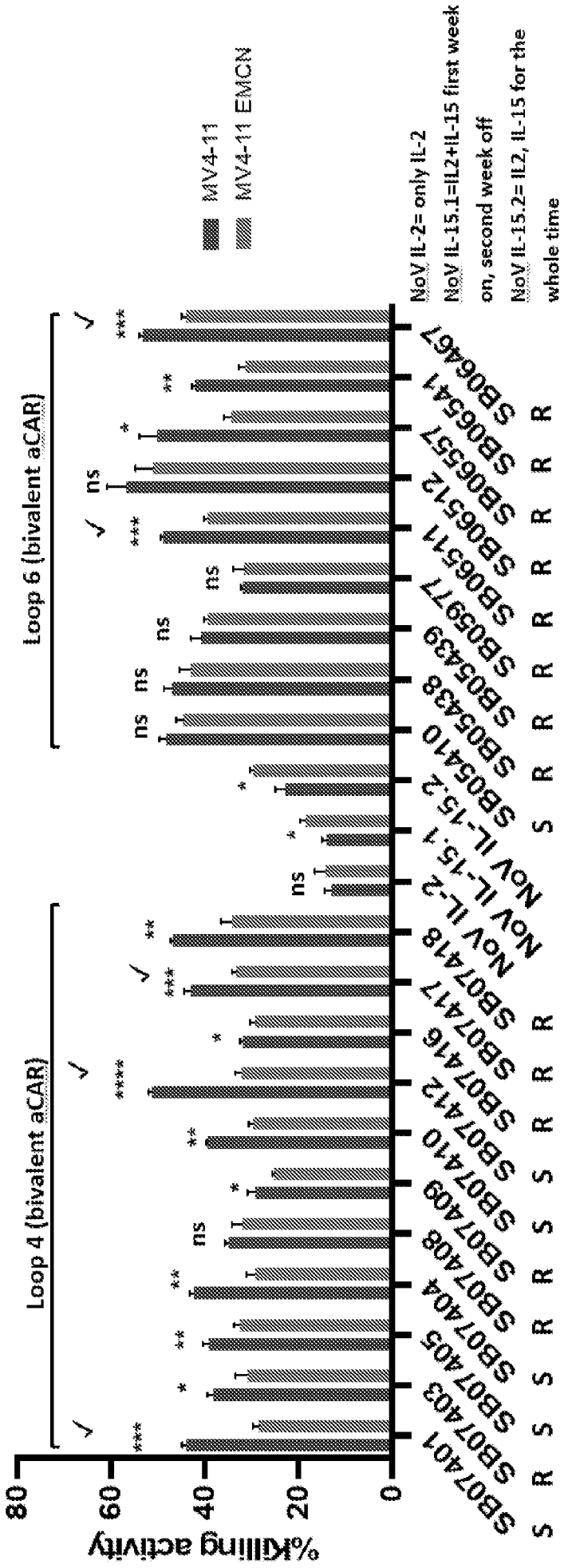

MV4-11, E:T=1.2, 20 h
%Killing activity
0
20
40
60
80
MV4-11
MV4-11 EMCN
Loop 4 (bivalent aCAR)
Loop 6 (bivalent aCAR)
NoV IL-2= only IL-2
NoV IL-15.1=IL2+IL-15 first week on, second week off
NoV IL-15.2= IL2, IL-15 for the whole time
S R S S S R R S S R R R R S R R R R S R R R R
SB07A01
SB07A03
SB07A05
SB07A04
SB07A08
SB07A09
SB07A10
SB07A12
SB07A16
SB07A17
SB07A18
NoV IL-2
NoV IL-15.1
NoV IL-15.2
SB05410
SB05438
SB05439
SB05977
SB06511
SB06512
SB06557
SB06541
SB06467
S = Sinvec
R = Retrovec

FIG. 29

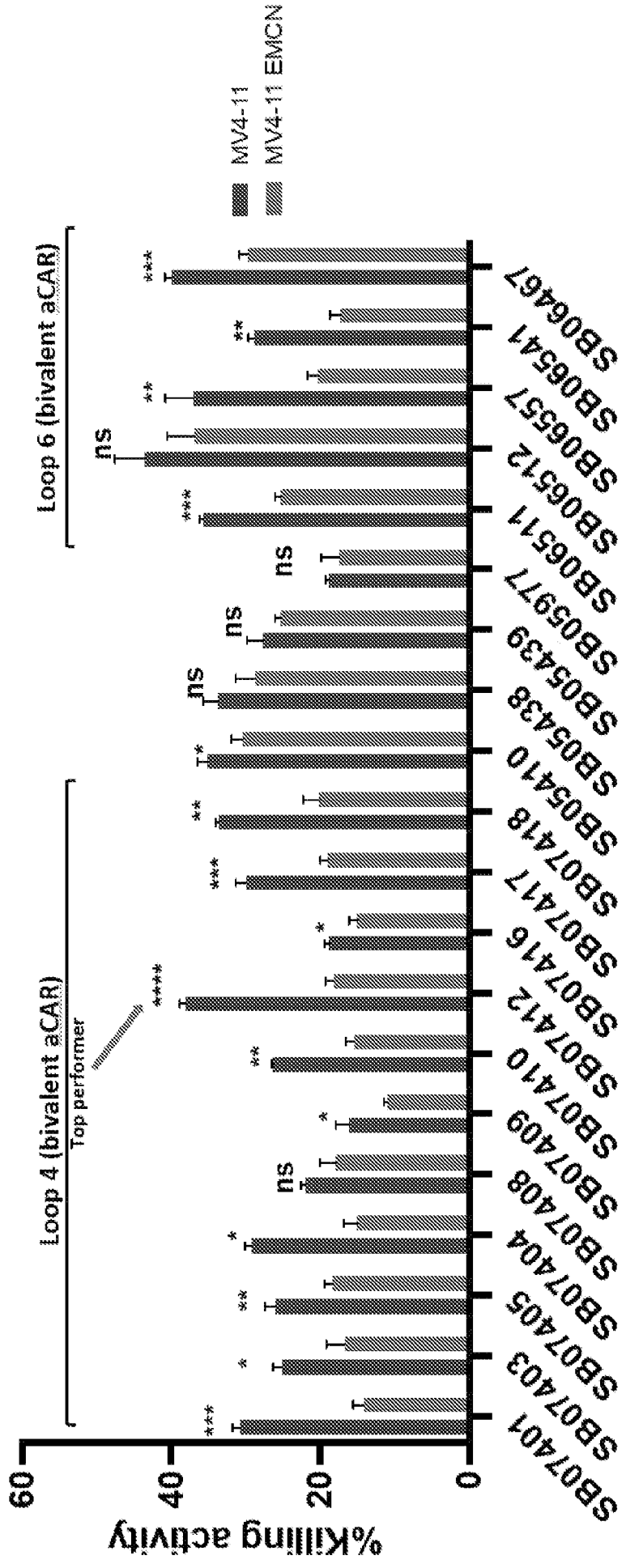

MV4-11 E.T 1.2 normalized to NV, 20 h
%Killing activity
0
20
40
60
MV4-11
MV4-11 EMCN
Loop 4 (bivalent aCAR)
Top performer
Loop 6 (bivalent aCAR)
SB074A01
SB074A03
SB074A05
SB074A04
SB074A08
SB074A09
SB074A10
SB074A12
SB074A16
SB074A17
SB074A18
SB054A10
SB054A38
SB054A39
SB059A77
SB065A11
SB065A12
SB065A57
SB065A41
SB064A67

FIG. 30

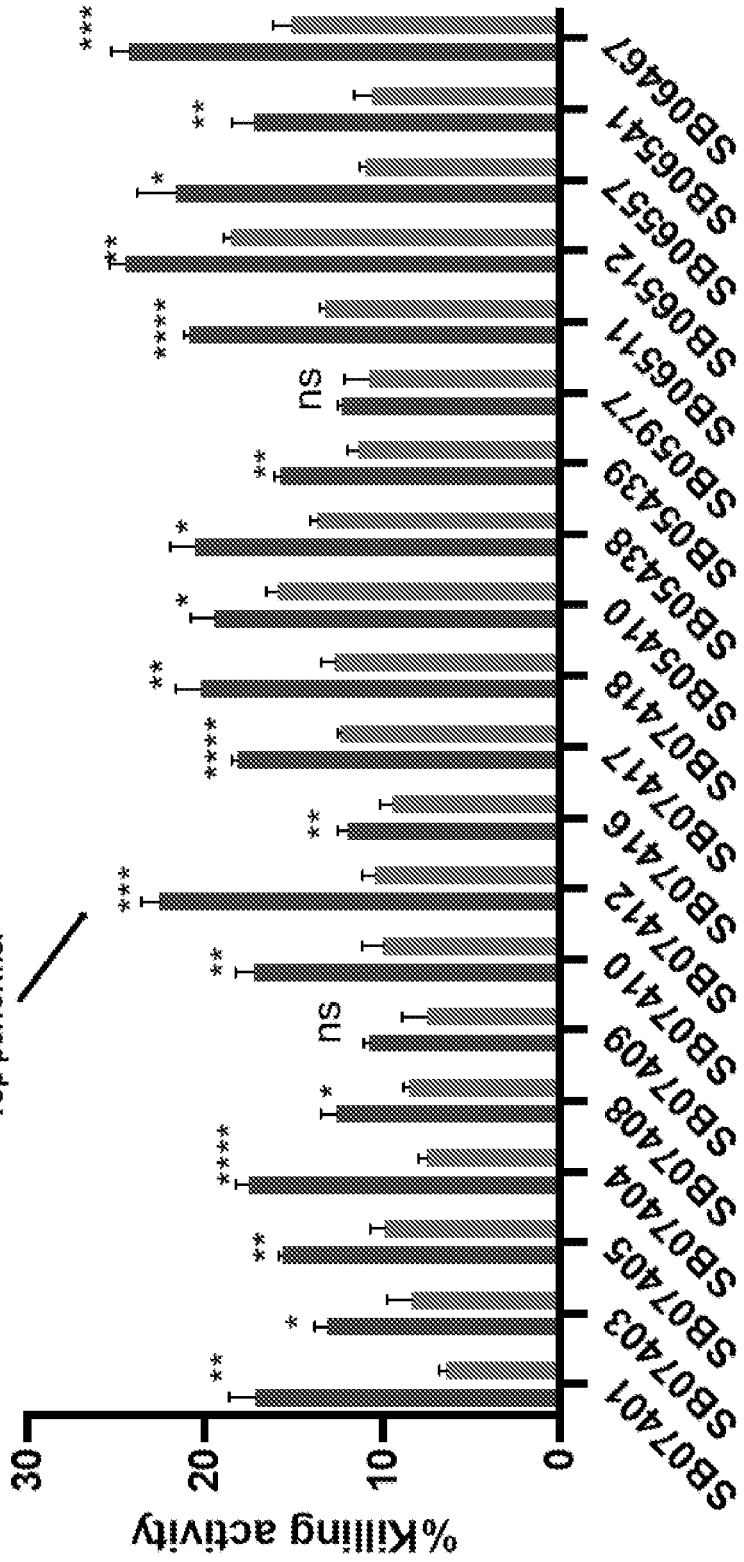

MV4-11 E.T 1.4 norm. to NV, 20h
%killing activity
30
20
10
0
MV4-11
MV4-11 EMCN
NoV IL-2= only IL-2
NoV IL-15.1=IL2+IL-15 first week on, second week off
NoV IL-15.2= IL2, IL-15 for the whole time
Top performer
ns
ns
SB07401
SB07403
SB07405
SB07404
SB07408
SB07409
SB07410
SB07412
SB07416
SB07417
SB07418
SB05410
SB05438
SB05439
SB05977
SB06511
SB06512
SB06557
SB06541
SB06467

FIG. 31

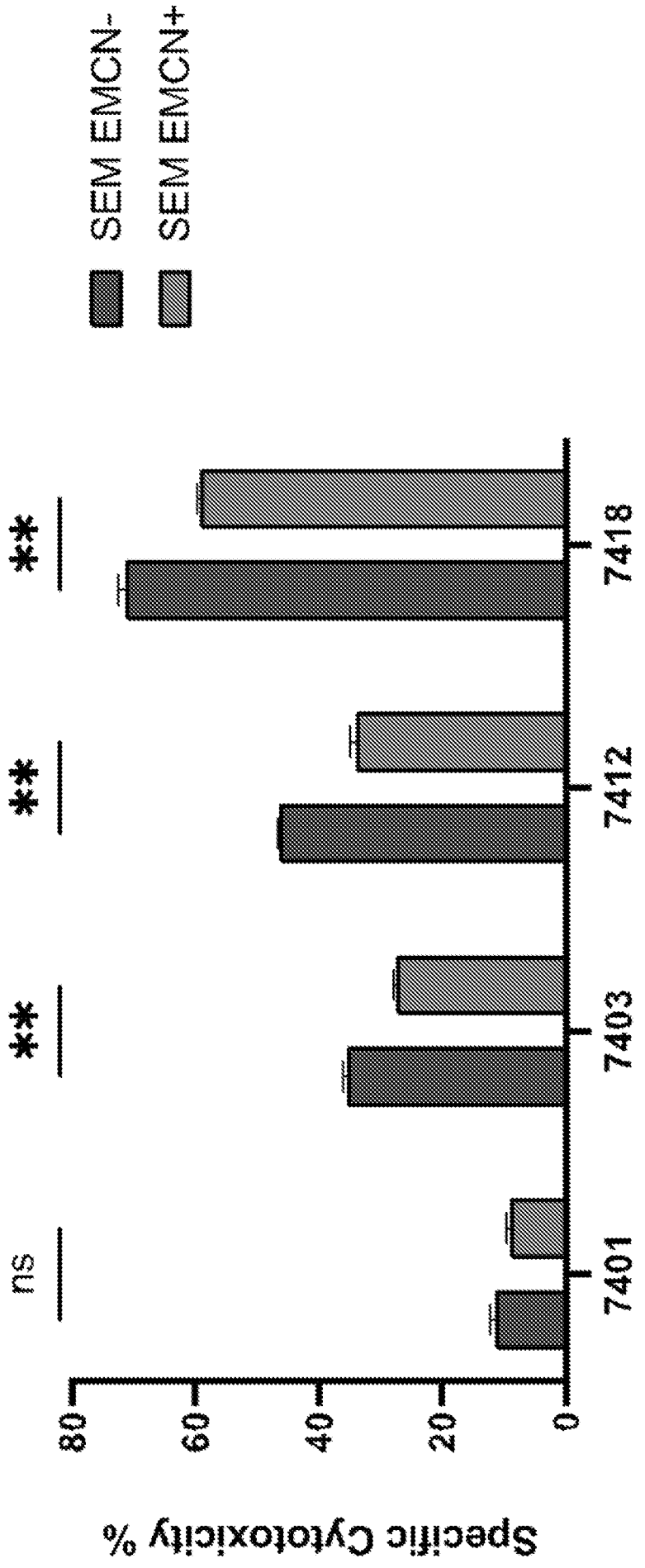

SEM EMCN-
SEM EMCN+
Specific Cytotoxicity %
80
60
40
20
0
ns
**
**
**
7401
7403
7412
7418

FIG. 32

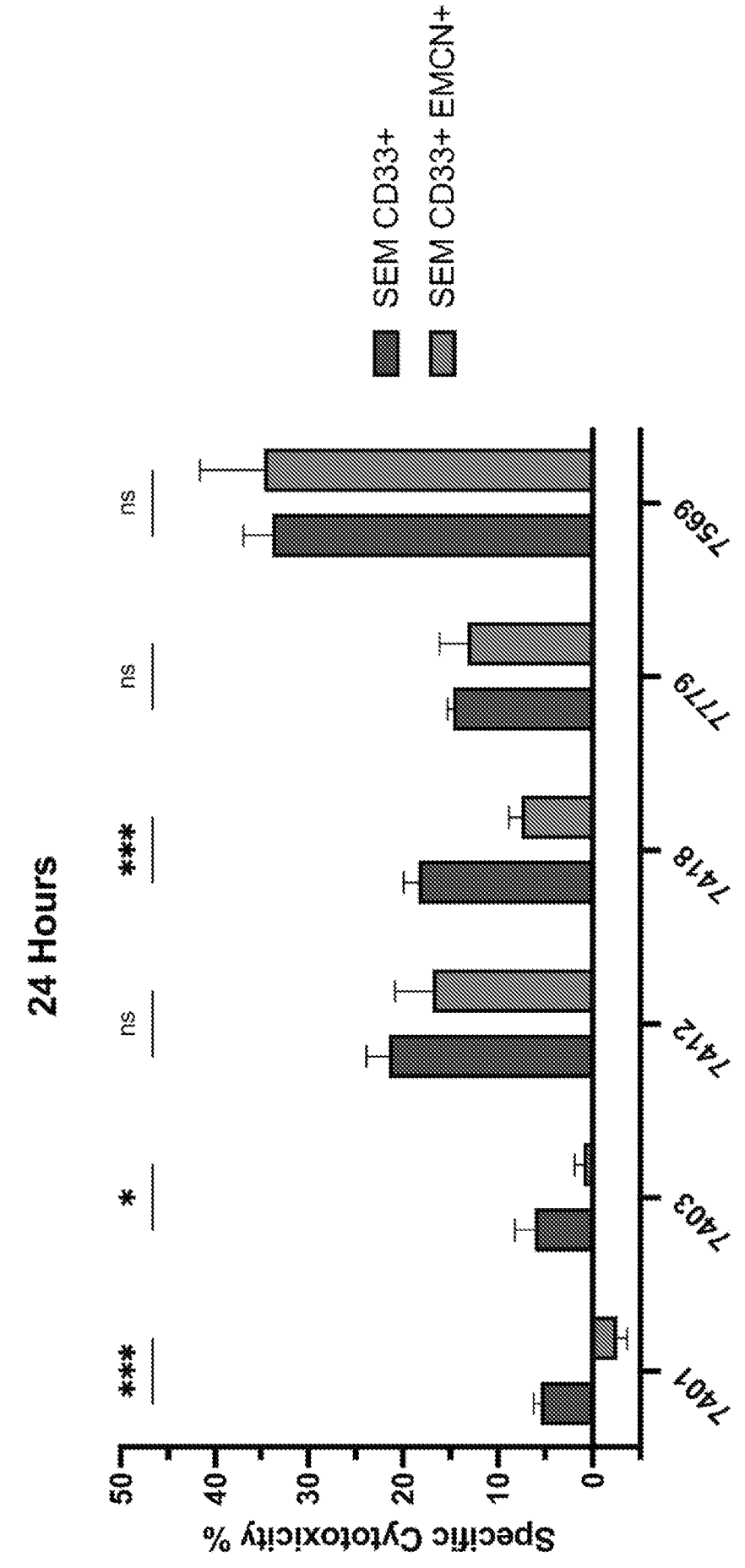

SEM CD33+
SEM CD33+ EMCN+
24 Hours
Specific Cytotoxicity %
50
40
30
20
10
0
****
*
ns
***
ns
ns
7401
7403
7412
7418
7779
7569

FIG. 33A

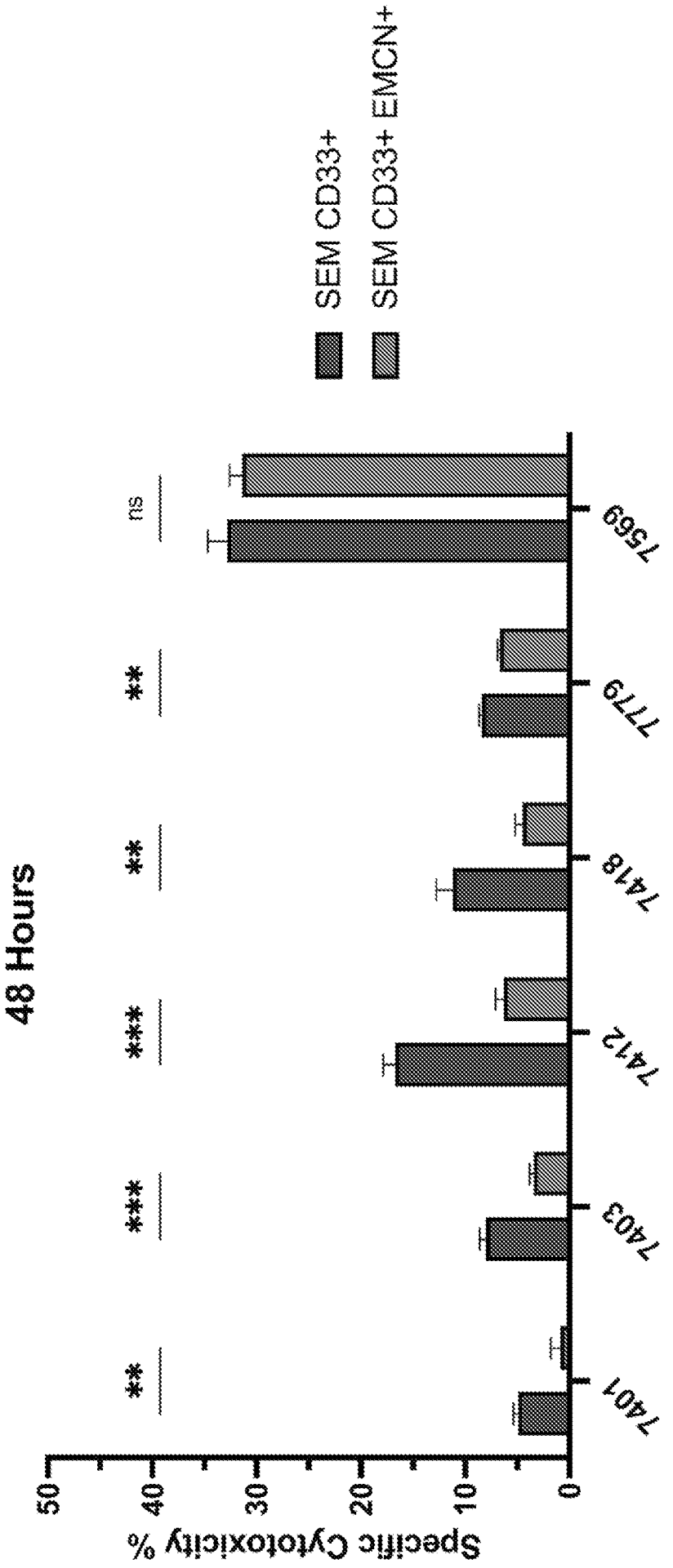

SEM CD33+
SEM CD33+ EMCN+
48 Hours
Specific Cytotoxicity %
0
10
20
30
40
50
7401
7403
7412
7418
7779
7569
**
***
***
**
**
ns

FIG. 33B

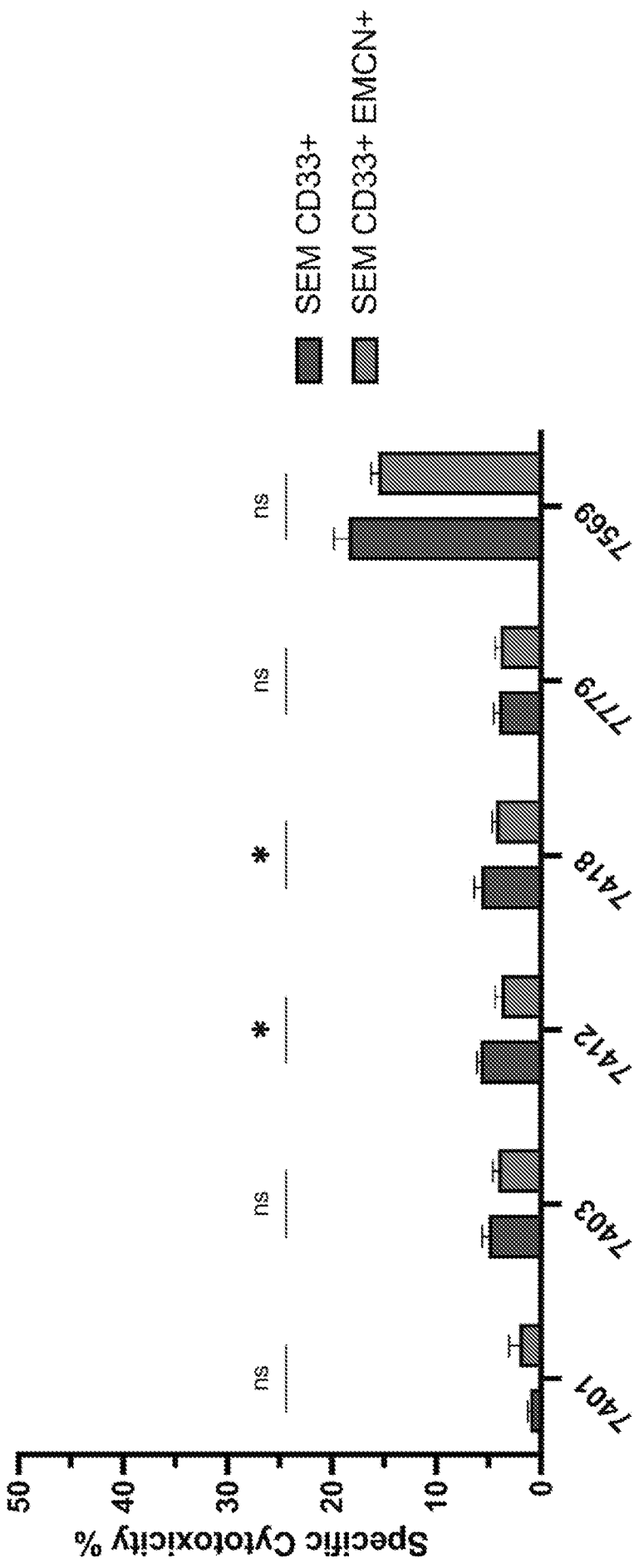

96 Hours
Specific Cytotoxicity %
0
10
20
30
40
50
SEM CD33+
SEM CD33+ EMCN+
ns
ns
*
*
ns
7401
7403
7412
7418
7779
7569

FIG. 33C

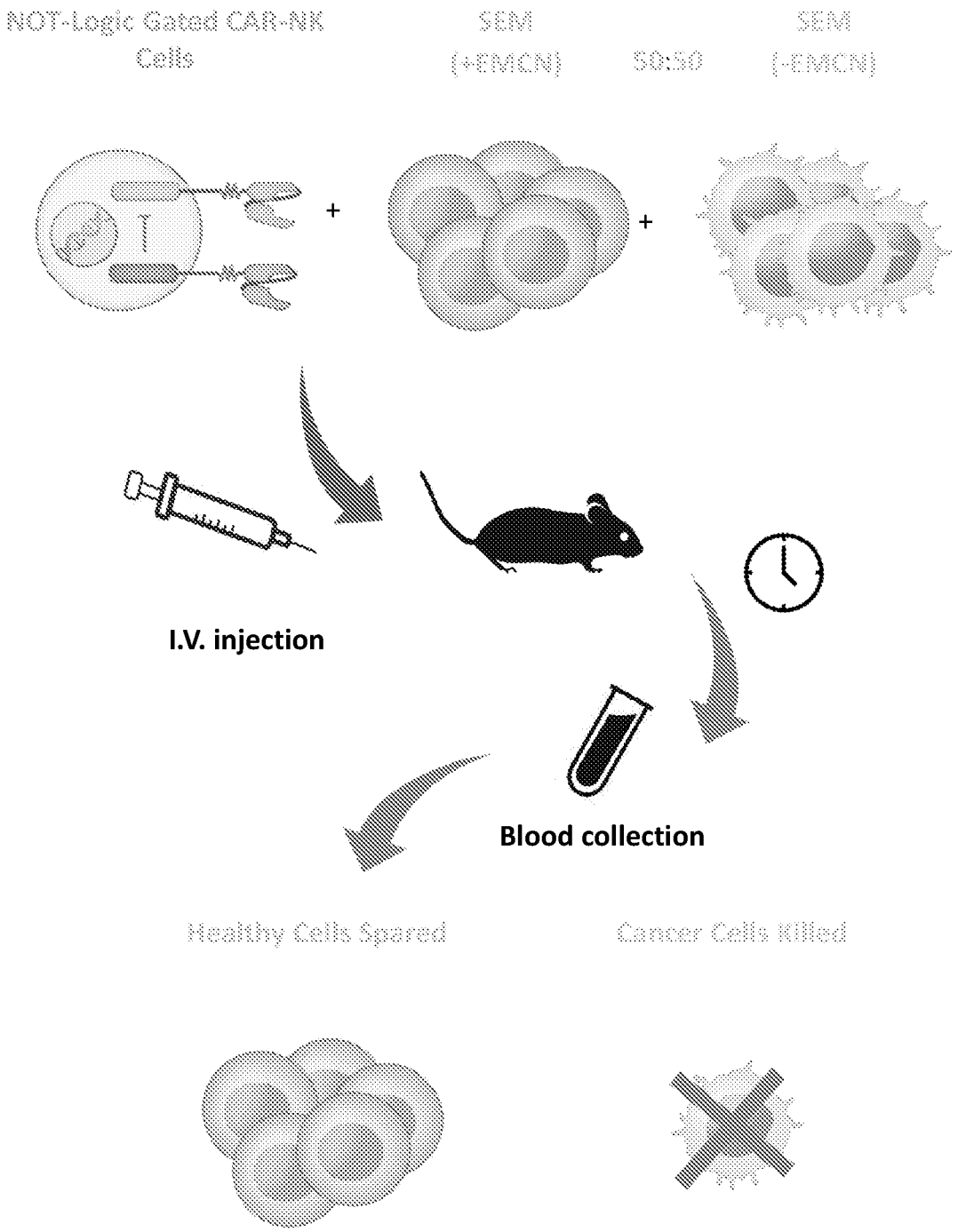

NOT-Logic Gated CAR-NK Cells
SEM (+EMCN)
50:50
SEM (-EMCN)
I.V. injection
Blood collection
Healthy Cells Spared
Cancer Cells Killed

FIG. 34A

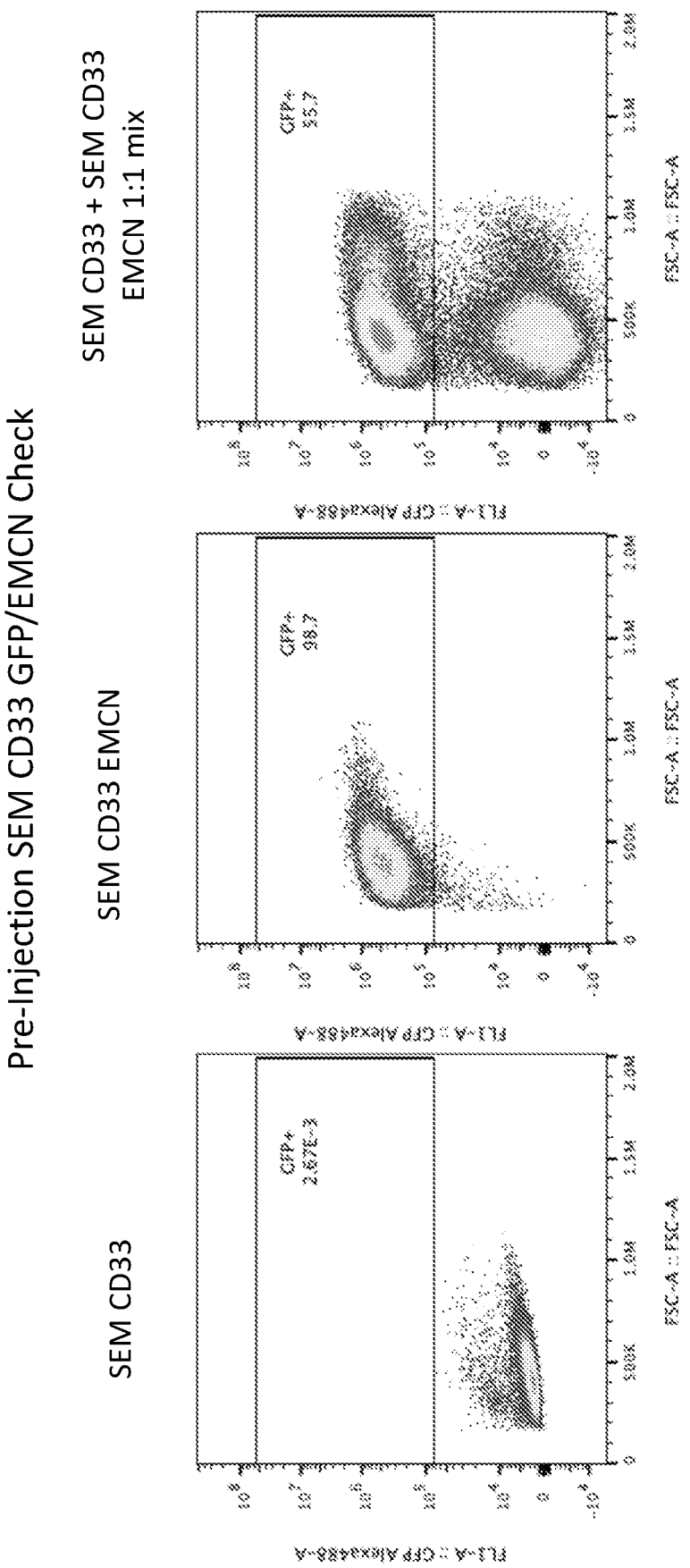

Pre-Injection SEM CD33 GFP/EMCN Check
SEM CD33
SEM CD33 EMCN
SEM CD33 + SEM CD33
EMCN 1:1 mix
GFP+
2.67E-3
GFP+
98.7
GFP+
55.7
FL1-A :: GFP Alexa488-A
FSC-A :: FSC-A

FIG. 34B

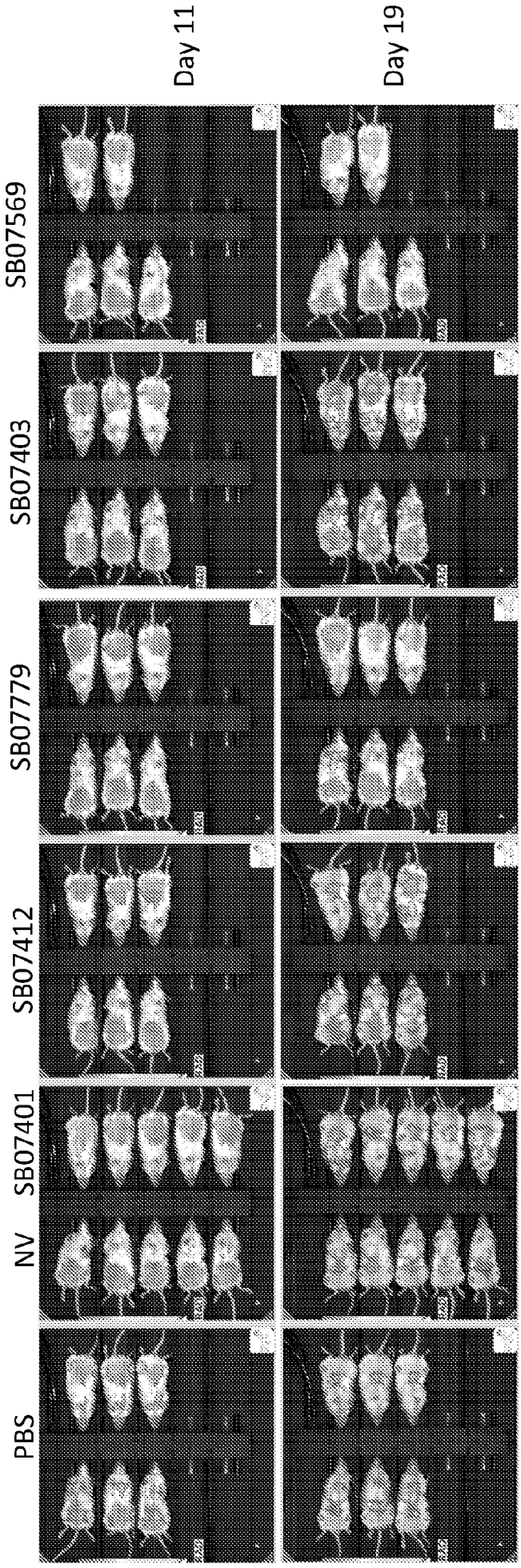

Day 11
Day 19
SB07569
SB07403
SB07779
SB07412
SB07401
NV
PBS

FIG. 35

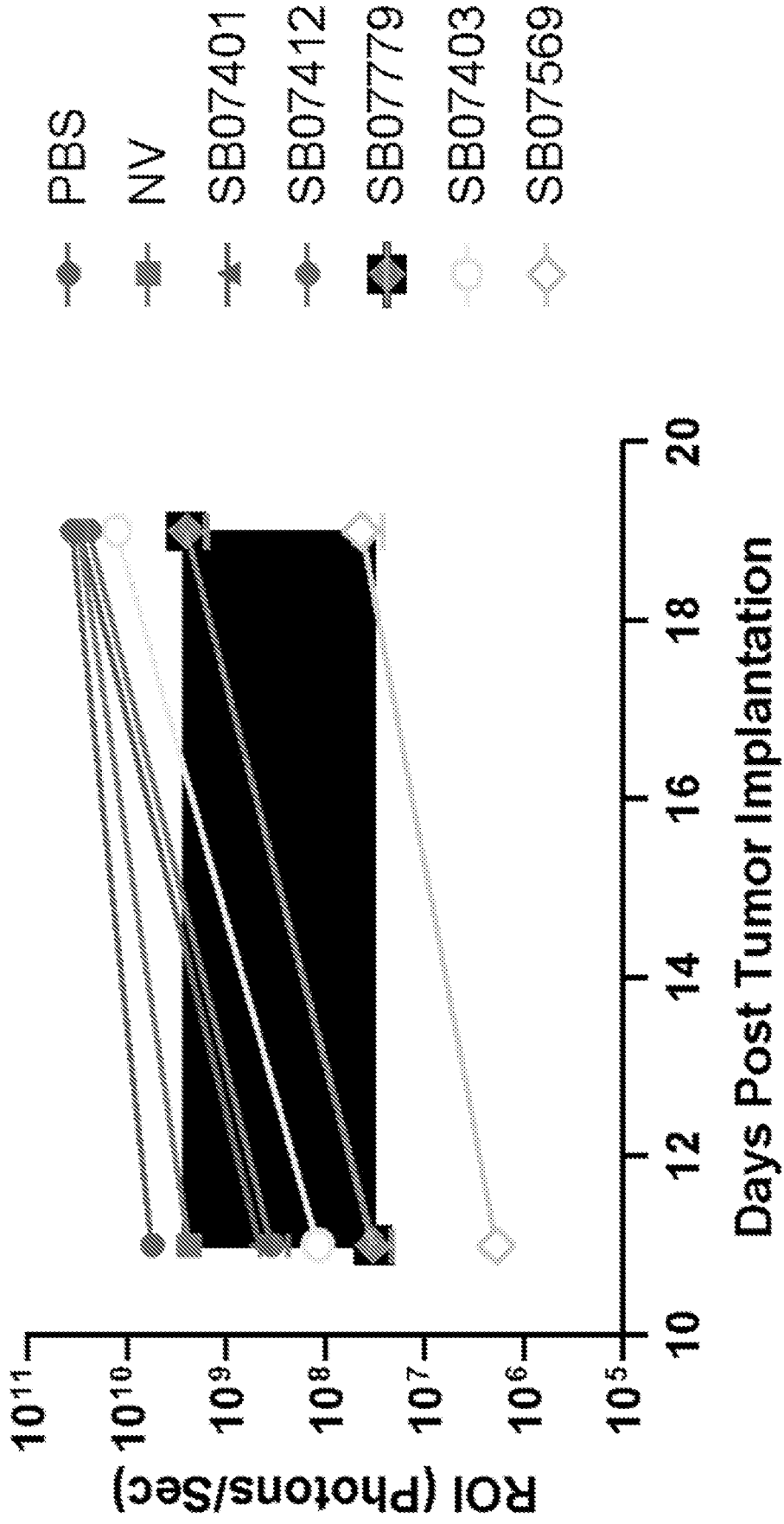

PBS
NV
SB07401
SB07412
SB07779
SB07403
SB07569
ROI (Photons/Sec)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10
12
14
16
18
20
Days Post Tumor Implantation

FIG. 36

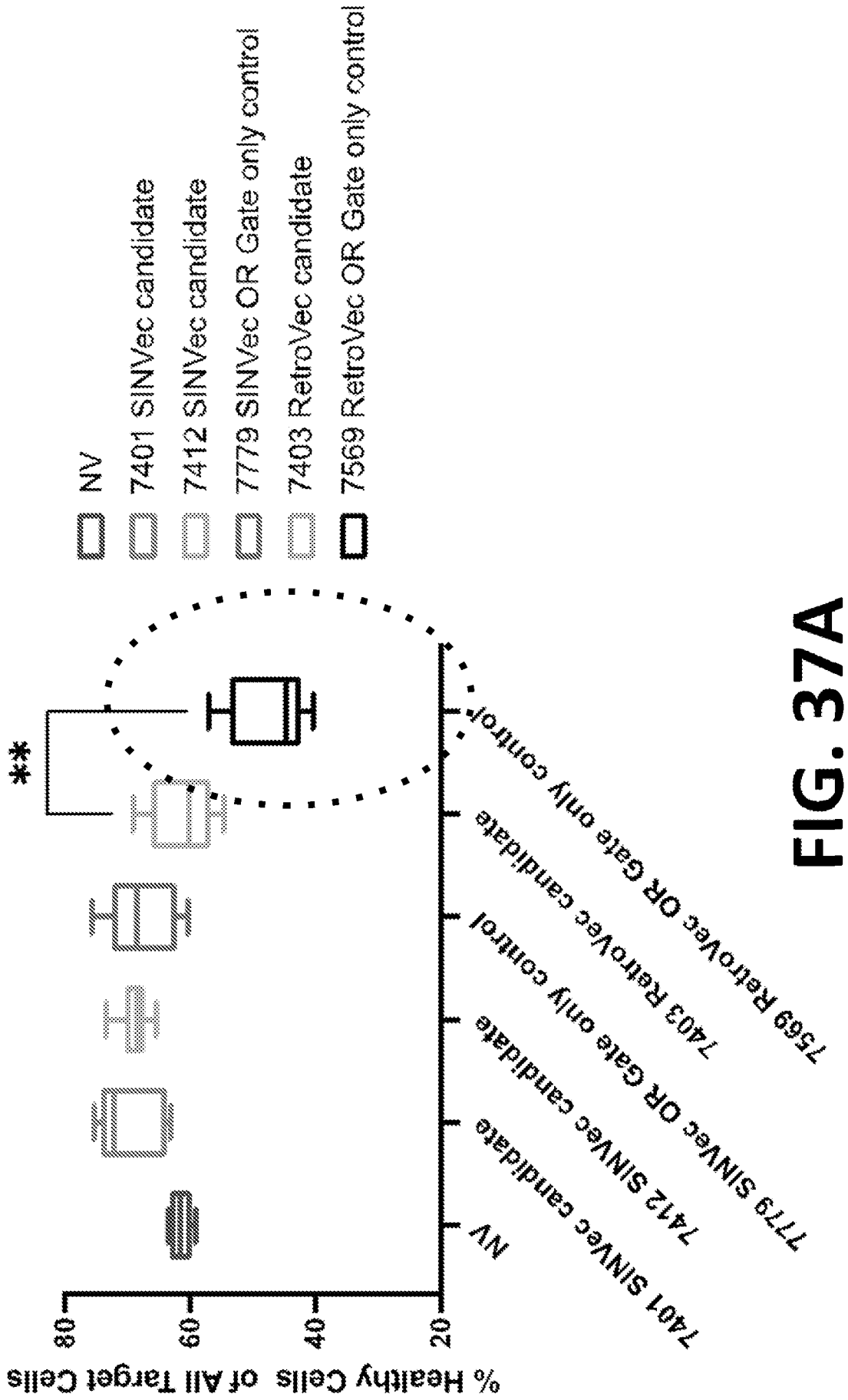

% Healthy Cells of All Target Cells
80
60
40
20
**
NV
7401 SINVec candidate
7412 SINVec candidate
7779 SINVec OR Gate only control
7403 RetroVec candidate
7569 RetroVec OR Gate only control
NV
7401 SINVec candidate
7412 SINVec candidate
7779 SINVec OR Gate only control
7403 RetroVec candidate
7569 RetroVec OR Gate only control

FIG. 37A

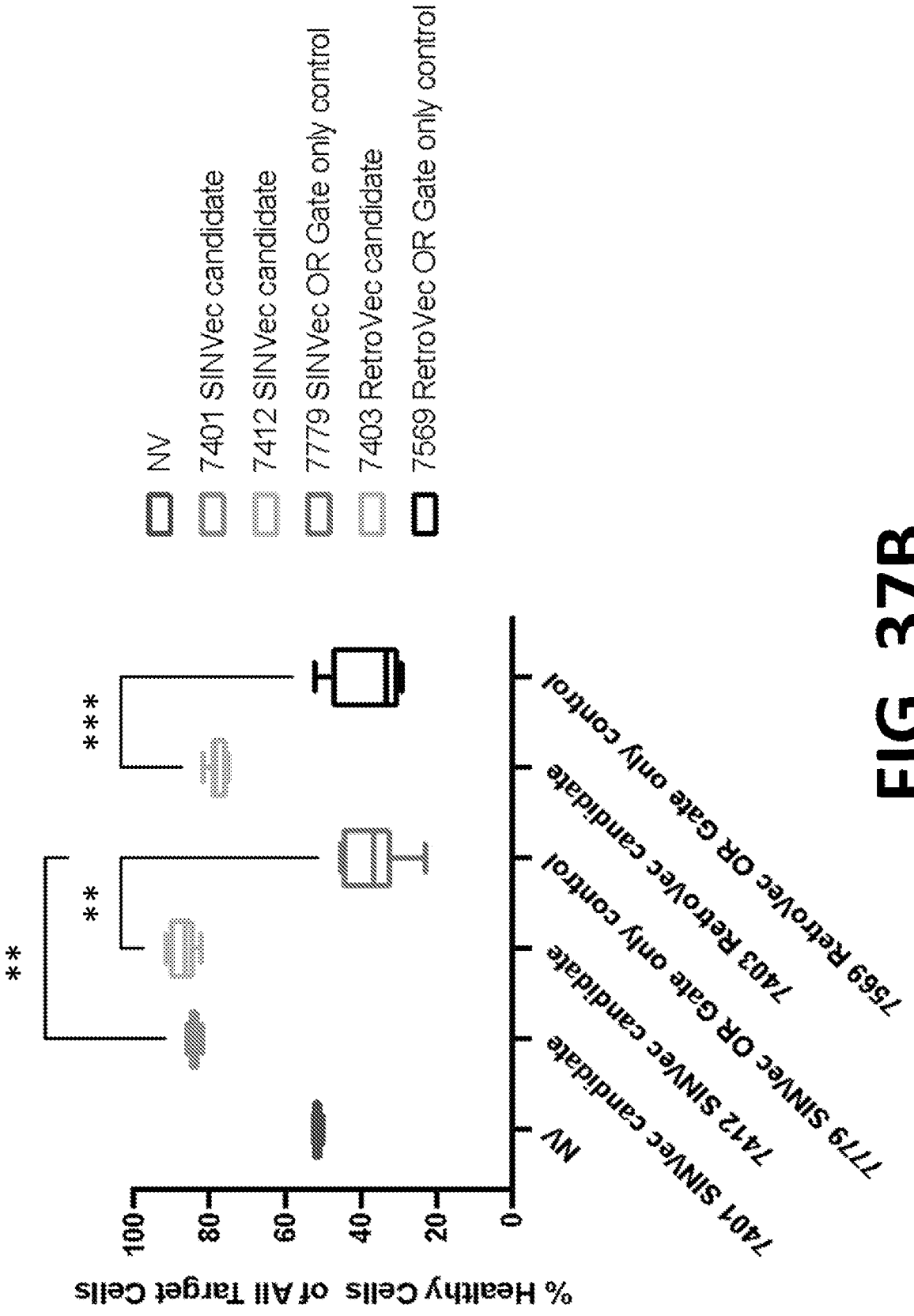

% Healthy Cells of All Target Cells
100
80
60
40
20
0
**
**
***
NV
7401 SINVec candidate
7412 SINVec candidate
7779 SINVec OR Gate only control
7403 RetroVec candidate
7569 RetroVec OR Gate only control
NV
7401 SINVec candidate
7412 SINVec candidate
7779 SINVec OR Gate only control
7403 RetroVec candidate
7569 RetroVec OR Gate only control

FIG. 37B

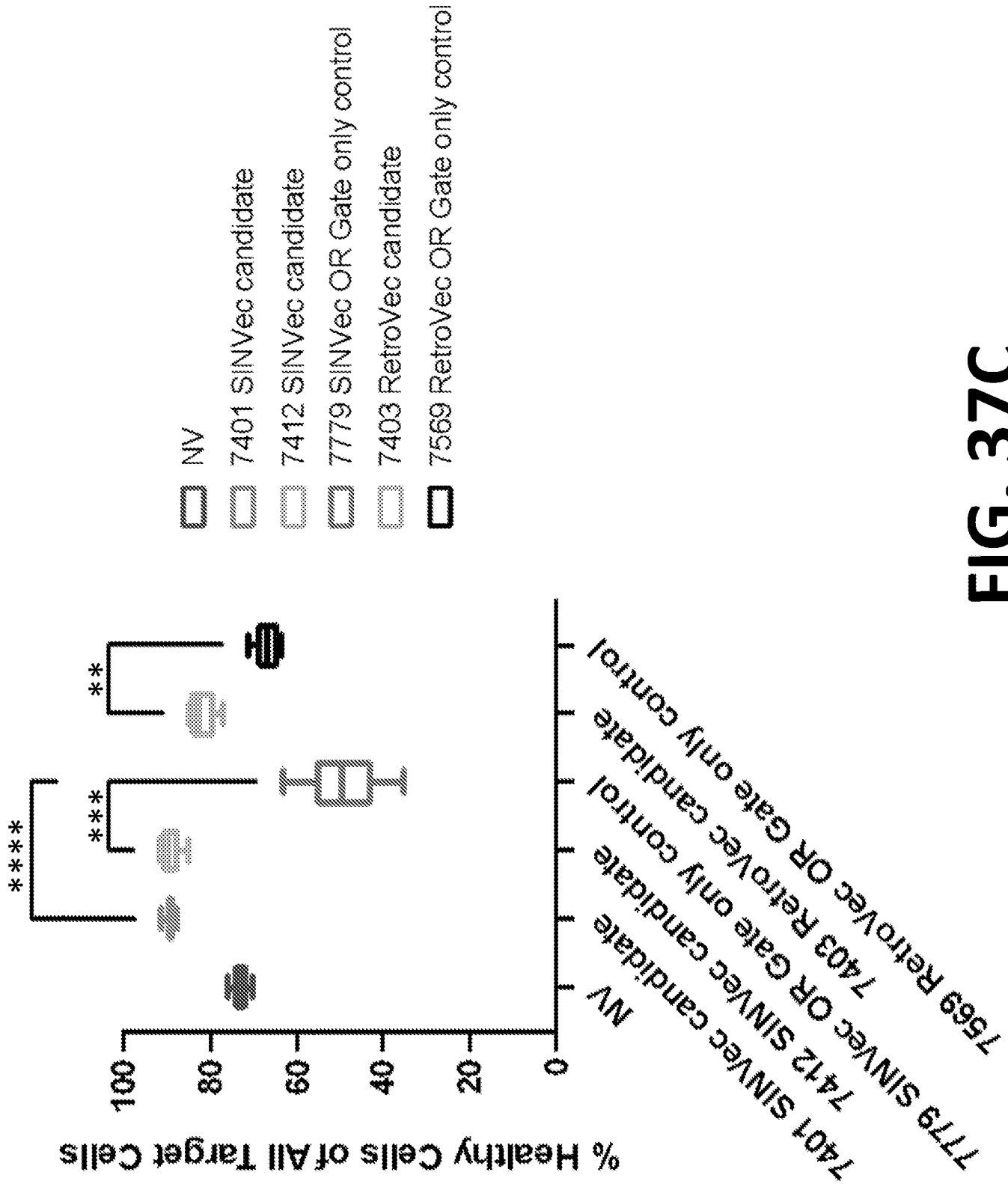

% Healthy Cells of All Target Cells
100
80
60
40
20
0
****
***
**
NV
7401 SINVec candidate
7412 SINVec candidate
7779 SINVec OR Gate only control
7403 RetroVec candidate
7569 RetroVec OR Gate only control
NV
7401 SINVec candidate
7412 SINVec candidate
7779 SINVec OR Gate only control
7403 RetroVec candidate
7569 RetroVec OR Gate only control

FIG. 37C

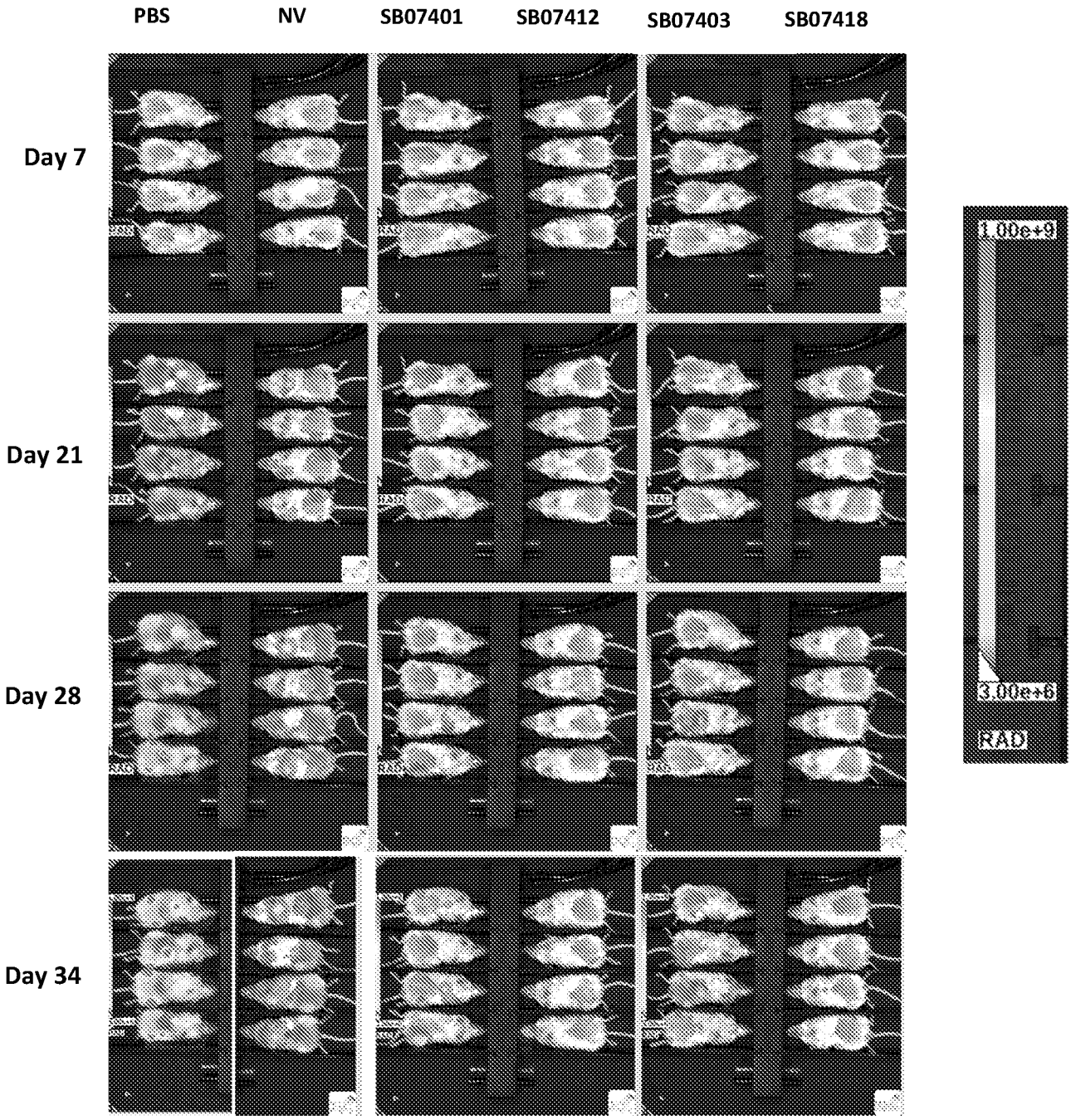

PBS
NV
SB07401
SB07412
SB07403
SB07418
Day 7
Day 21
Day 28
Day 34
1.00e+9
3.00e+6
RAD

FIG. 38A

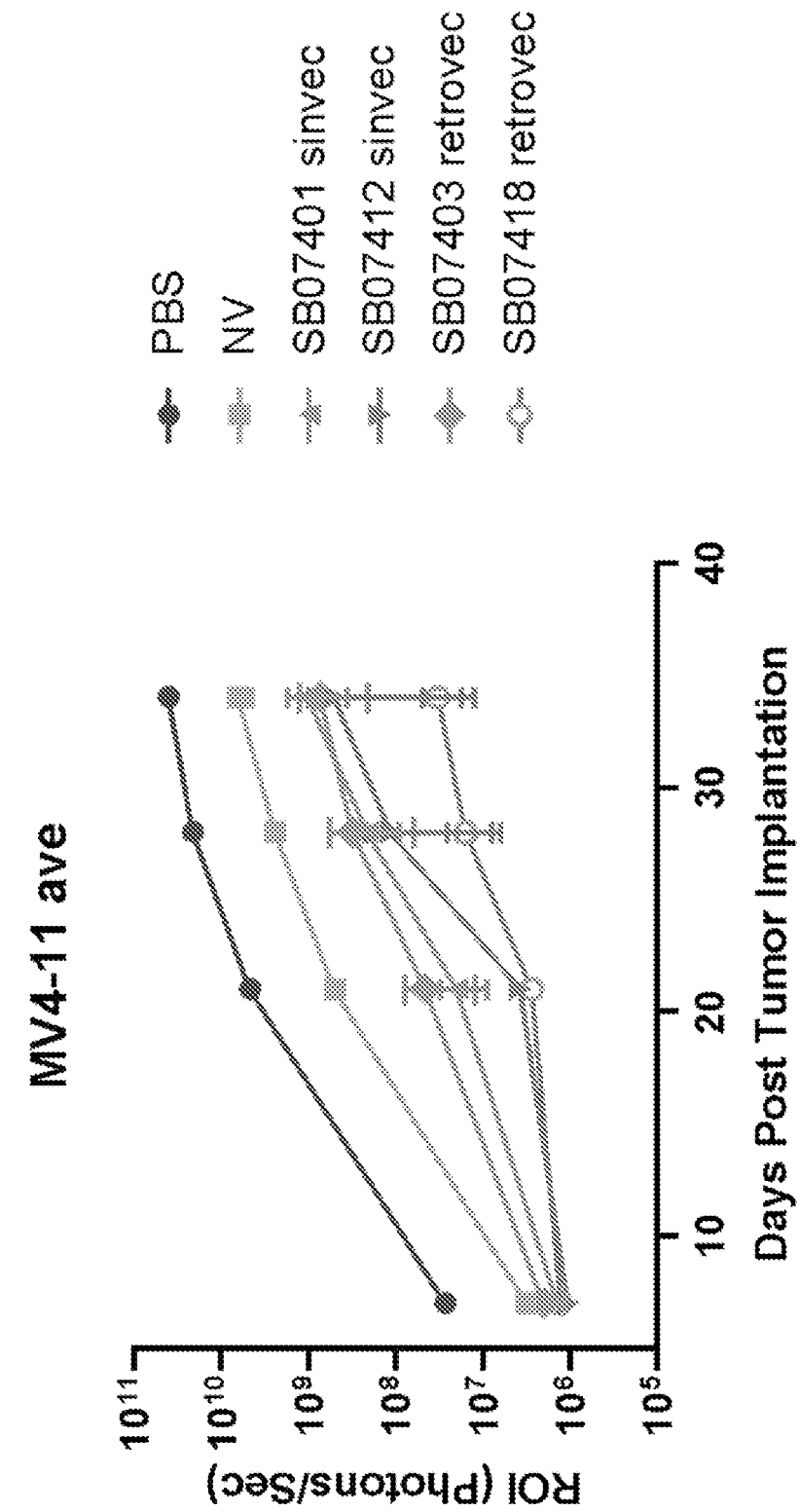

MV4-11 ave
PBS
NV
SB07401 sinvec
SB07412 sinvec
SB07403 retrovec
SB07418 retrovec
ROI (Photons/Sec)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10
20
30
40
Days Post Tumor Implantation

FIG. 38B

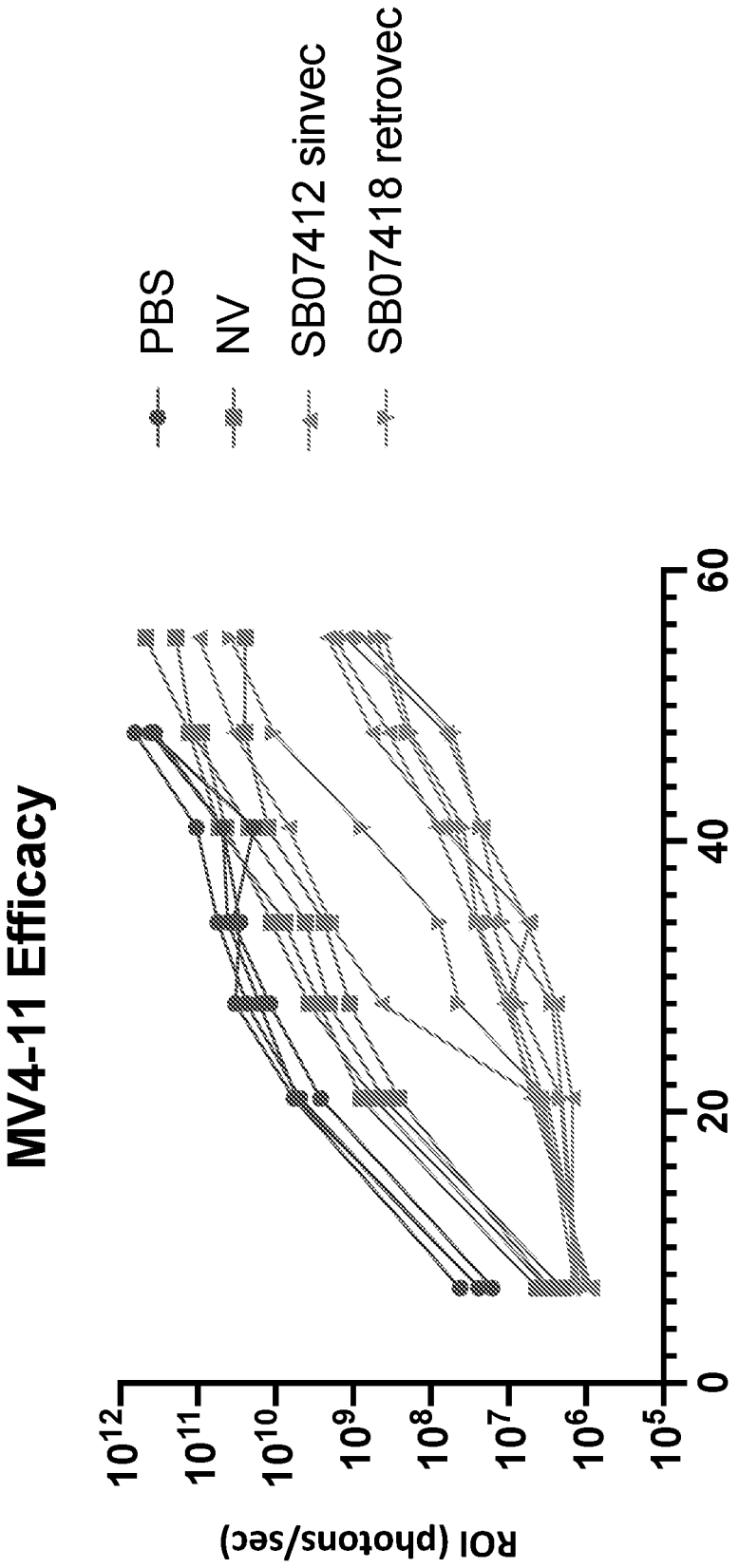

MV4-11 Efficacy
PBS
NV
SB07412 sinvec
SB07418 retrovec
ROI (photons/sec)
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
20
40
60
Days Post Tumor Implantation

FIG. 39A

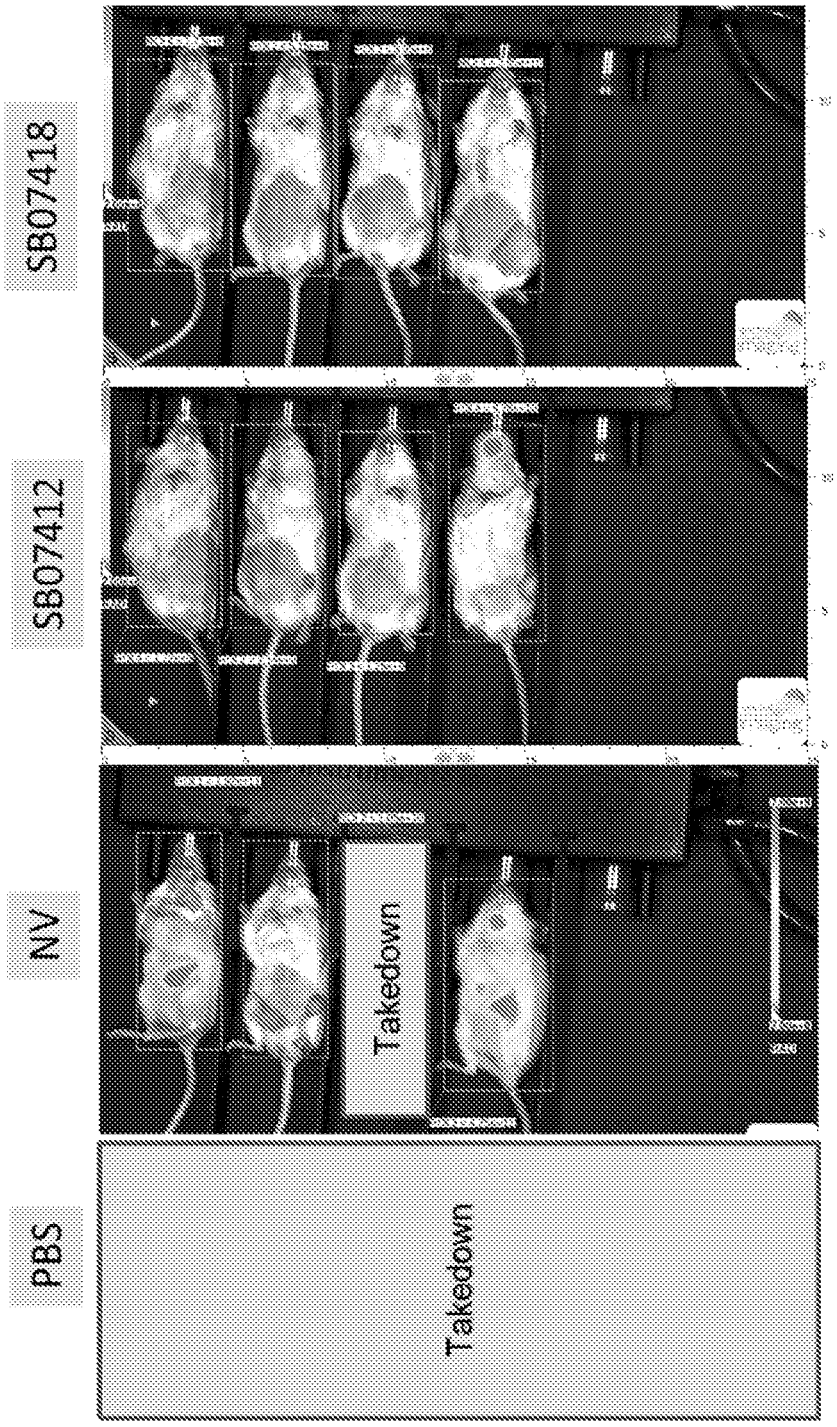
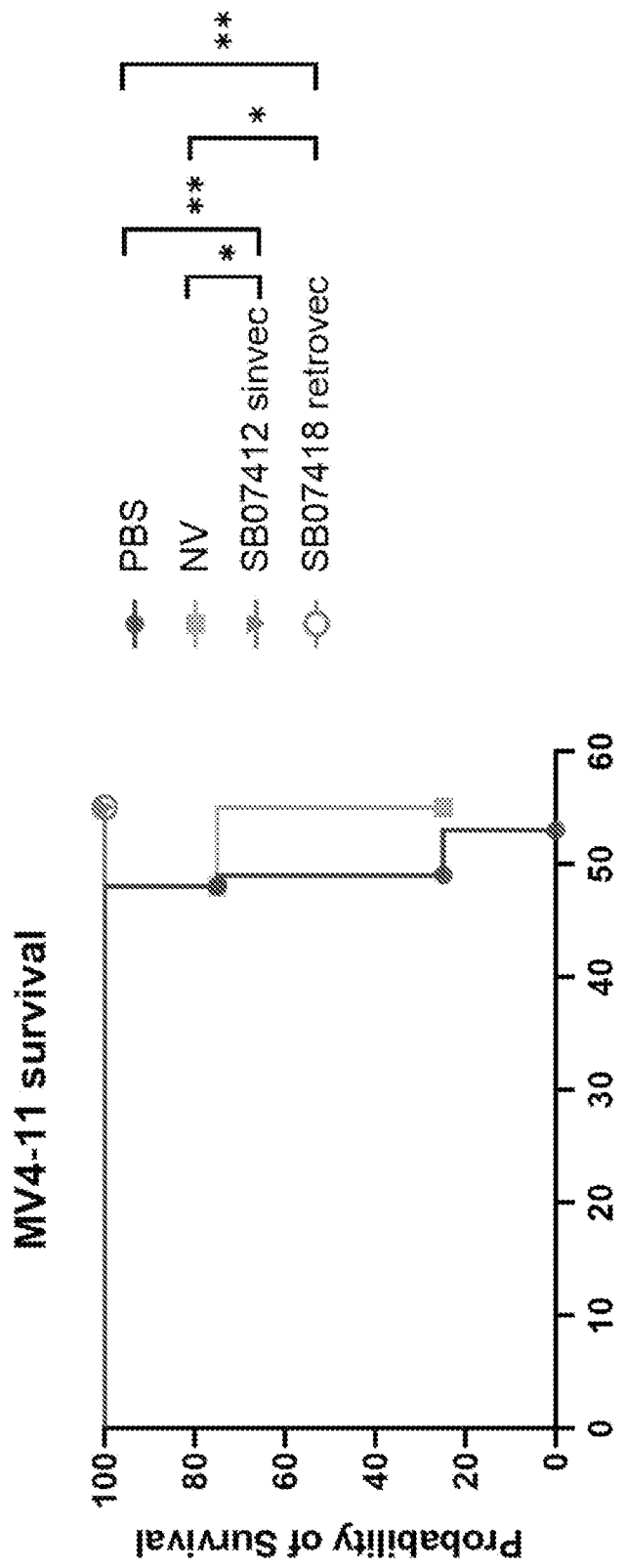
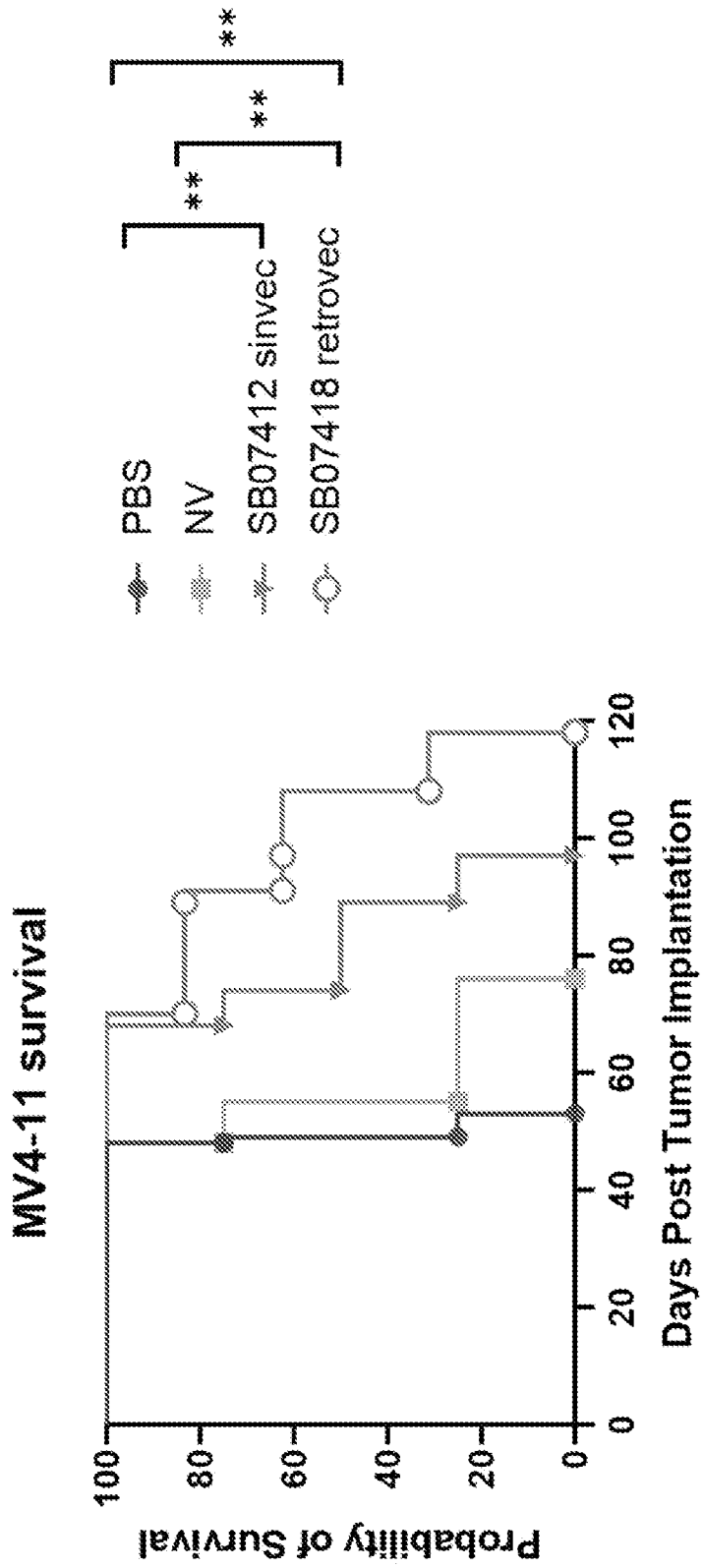


FIG. 39B



	PBS	NV	SB07412 sinvec	SB07418 retrovec
Median survival	49	55	Undefined	Undefined

FIG. 39C



	PBS	NV	SB07412 sinvec	SB07418 retrovec
Median survival	49	55	81.5	108

FIG. 39D

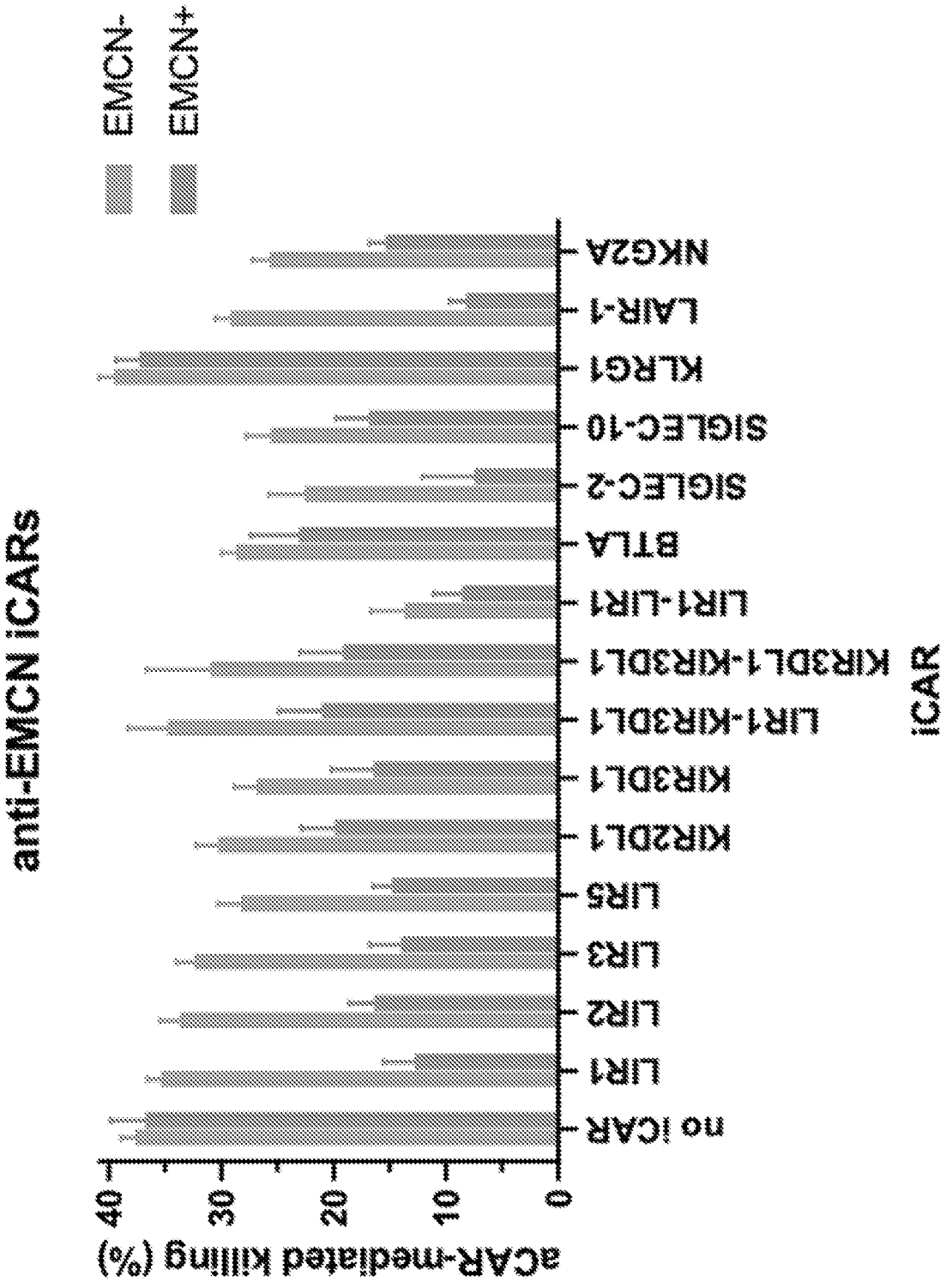

anti-EMCN iCARs
EMCN-
EMCN+
aCAR-mediated killing (%)
0
10
20
30
40
no iCAR
LIR1
LIR2
LIR3
LIR5
KIR2DL1
KIR3DL1
LIR1-KIR3DL1
KIR3DL1-KIR3DL1
LIR1-LIR1
BTLA
SIGLEC-2
SIGLEC-10
KLRG1
LAIR-1
NKG2A
iCAR

FIG. 40

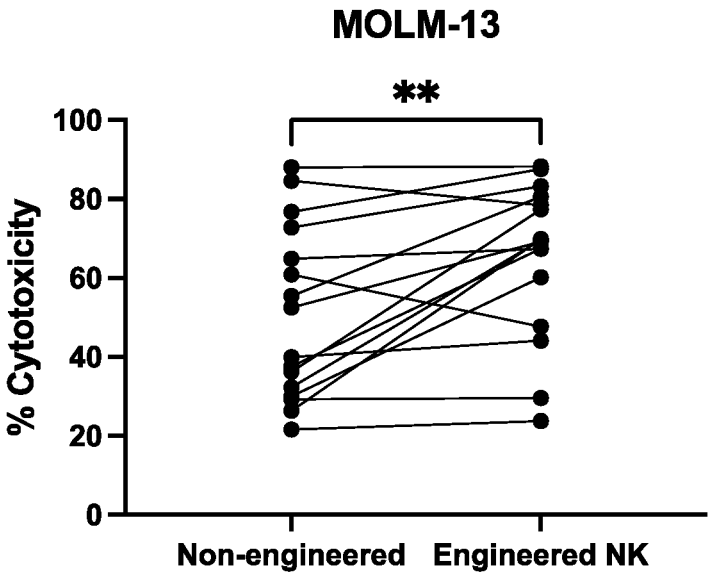


FIG 41C

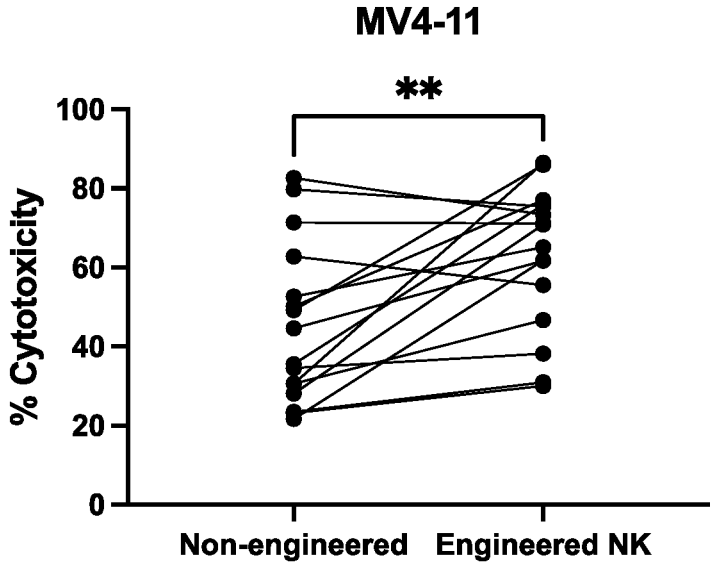


FIG 41B

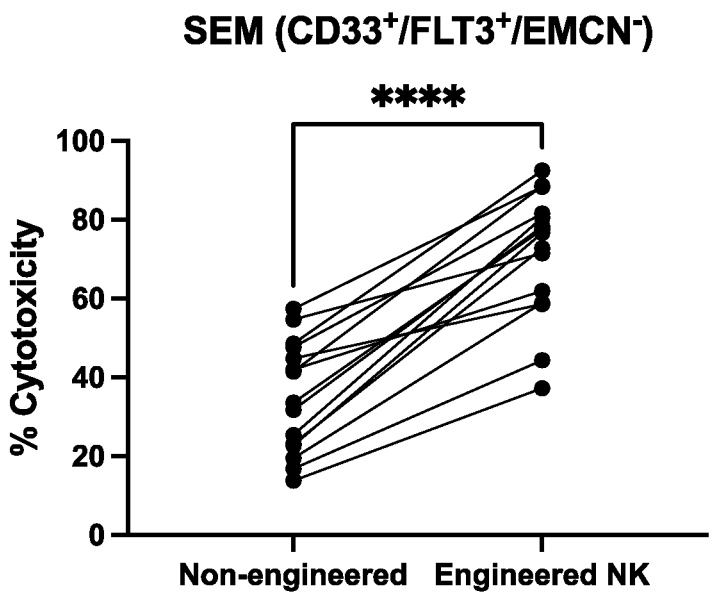


FIG 41A

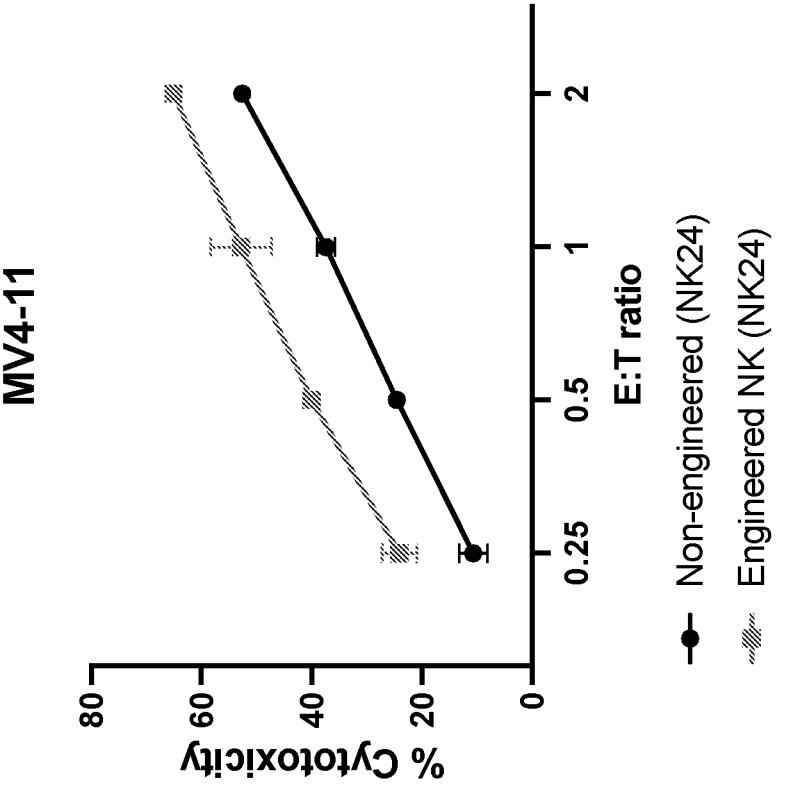


FIG. 41E

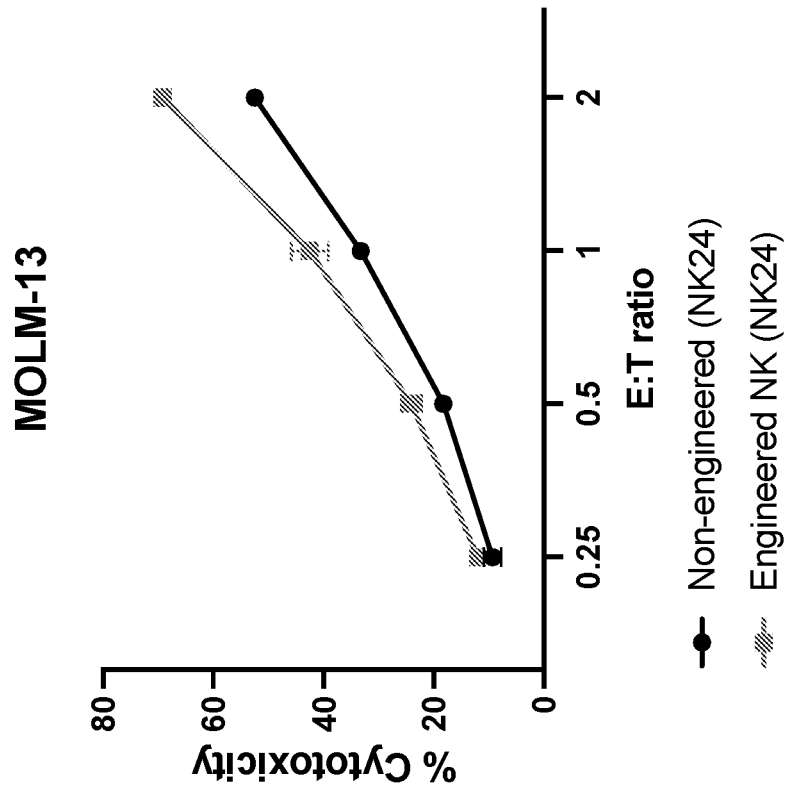


FIG. 41D

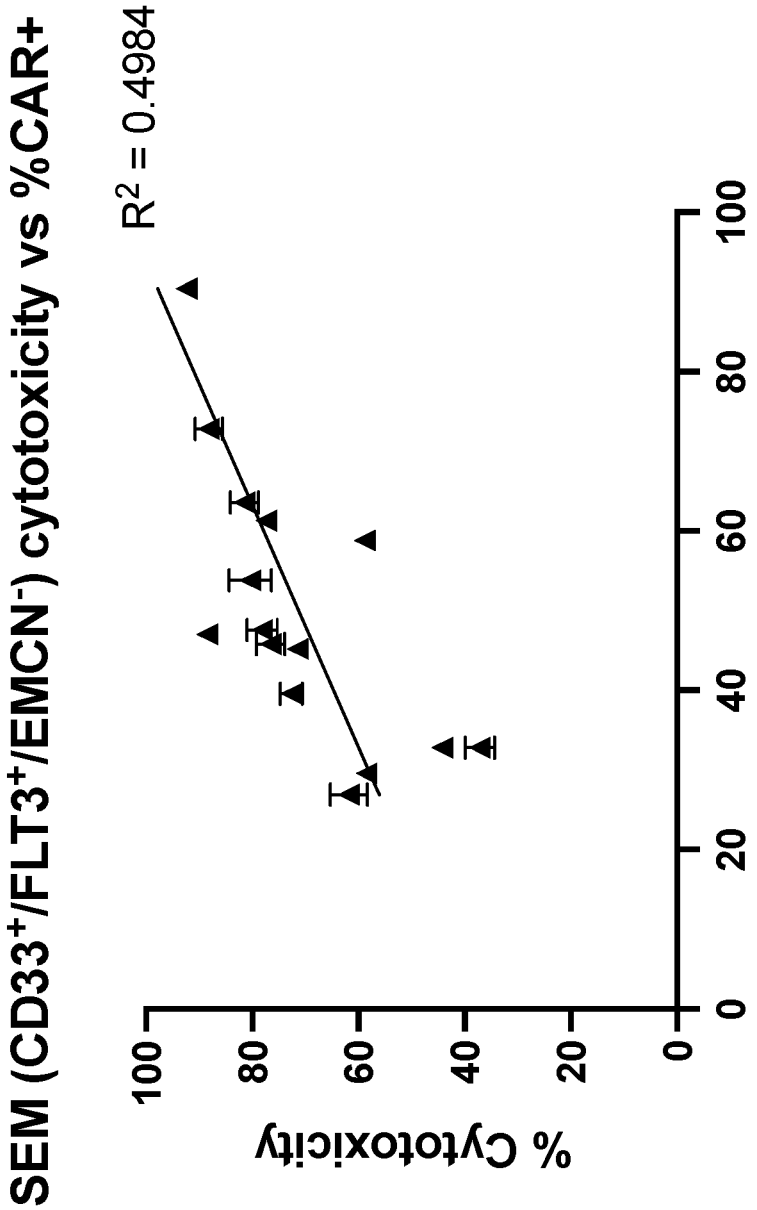

SEM (CD33+/FLT3+/EMCN-) cytotoxicity vs %CAR+
R2 = 0.4984
% Cytotoxicity
0
20
40
60
80
100
0
20
40
60
80
100
% CAR+

FIG. 41F

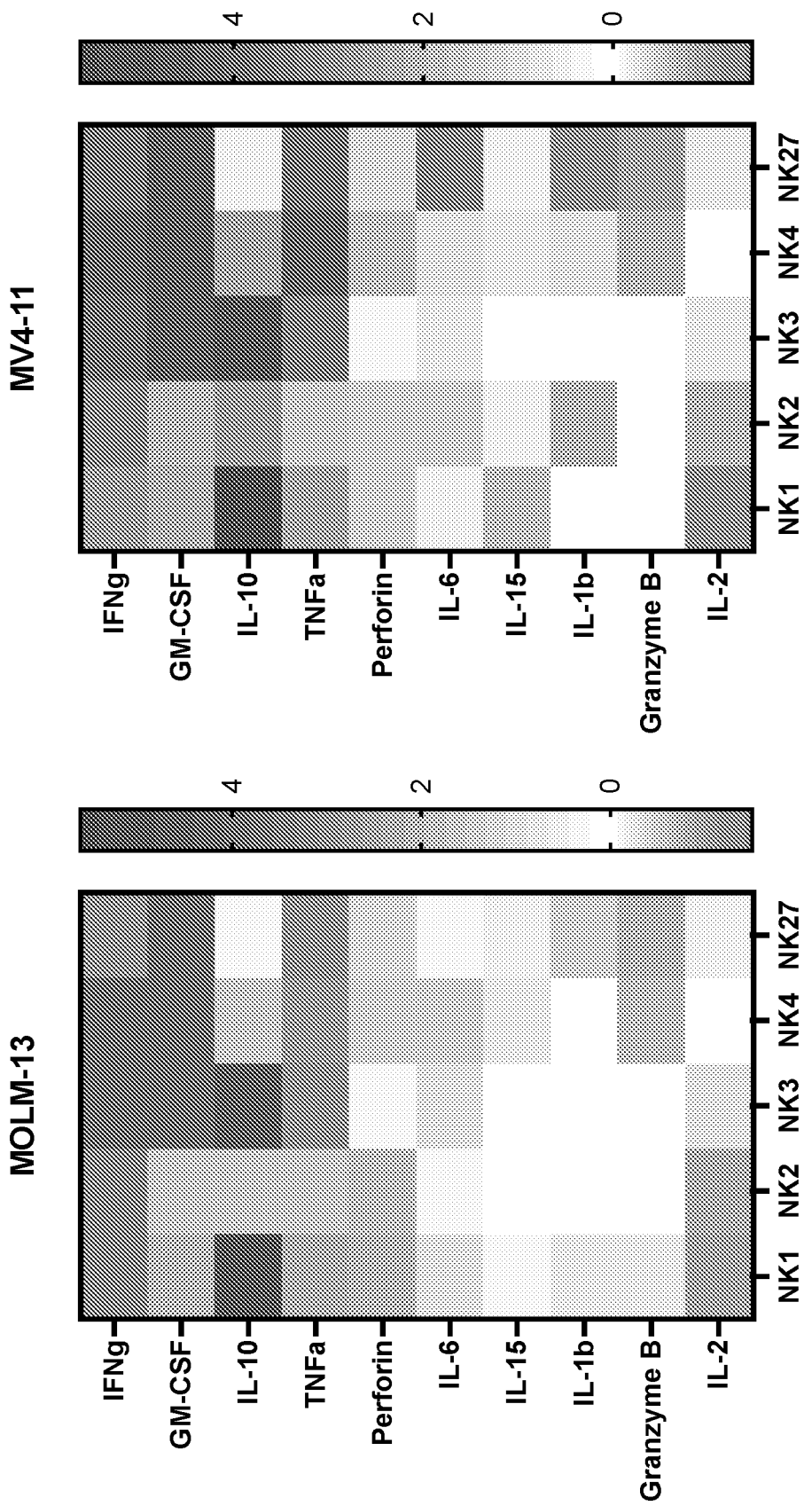

MV4-11
MOLM-13
IFNg
GM-CSF
IL-10
TNFa
Perforin
IL-6
IL-15
IL-1b
Granzyme B
IL-2
NK1
NK2
NK3
NK4
NK27
4
2
0

FIG. 42

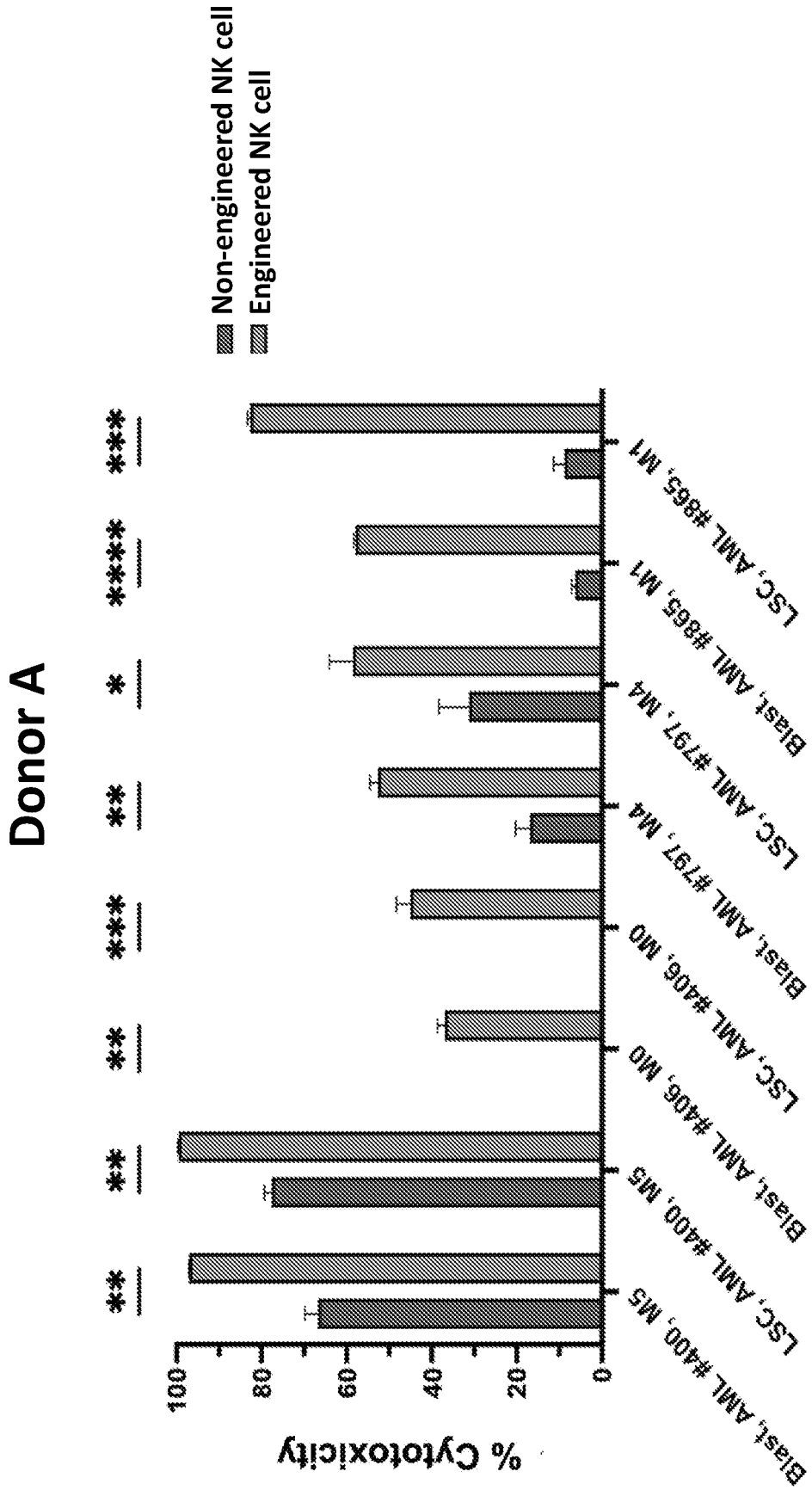

Donor A
Non-engineered NK cell
Engineered NK cell
% Cytotoxicity
0
20
40
60
80
100
Blast, AML #400, M5
LSC, AML #400, M5
Blast, AML #400, M5
LSC, AML #406, M0
Blast, AML #406, M0
LSC, AML #797, M4
Blast, AML #797, M4
LSC, AML #865, M1
Blast, AML #865, M1
LSC, AML #865, M1
**** ***** * ** **** ** ** ****

FIG. 43A

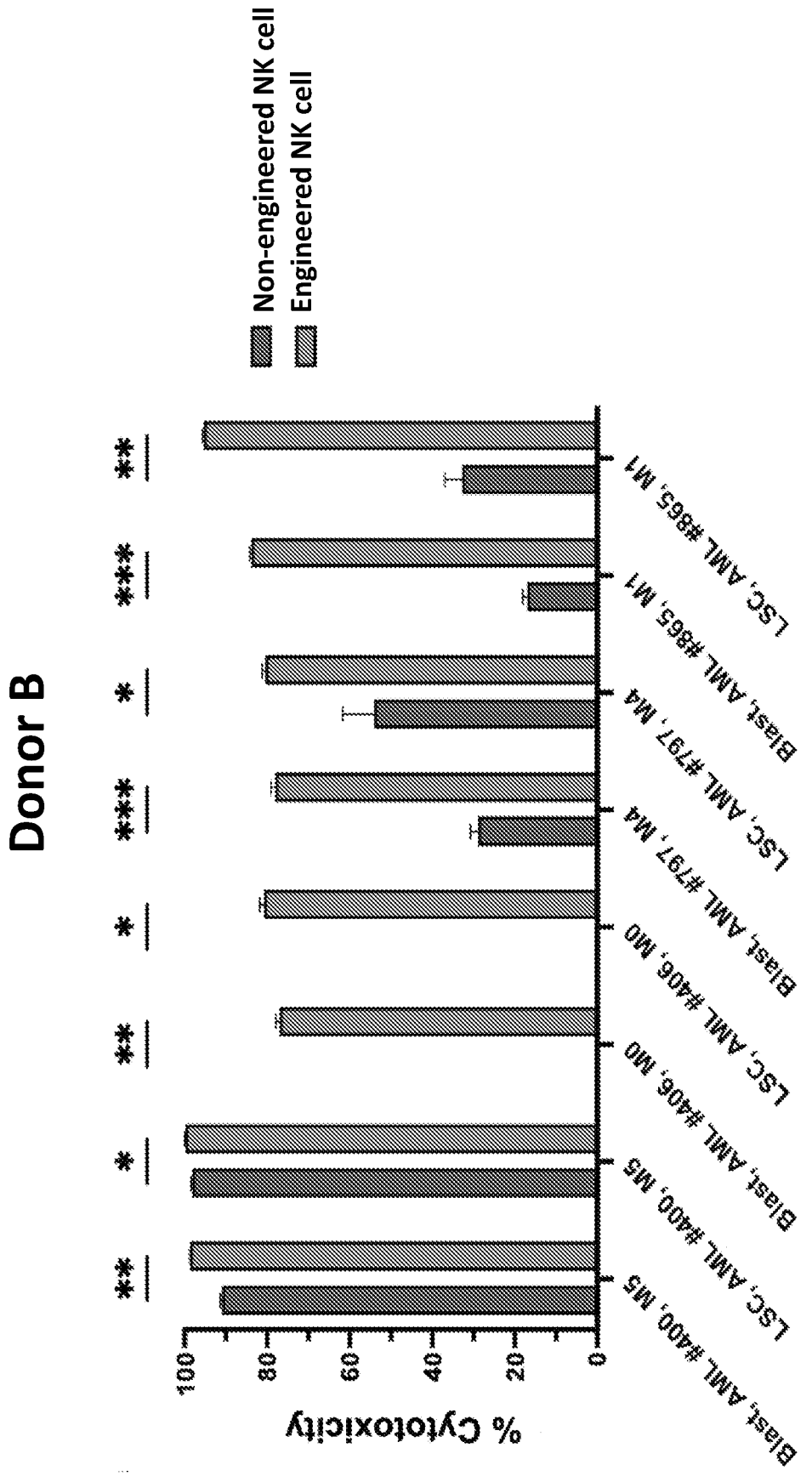

Donor B
Non-engineered NK cell
Engineered NK cell
% Cytotoxicity
0
20
40
60
80
100
Blast, AML #400, M5
LSC, AML #400, M5
Blast, AML #406, M0
LSC, AML #406, M0
Blast, AML #797, M4
LSC, AML #797, M4
Blast, AML #865, M1
LSC, AML #865, M1
**
*
**
*
***
*
***
**

FIG. 43B

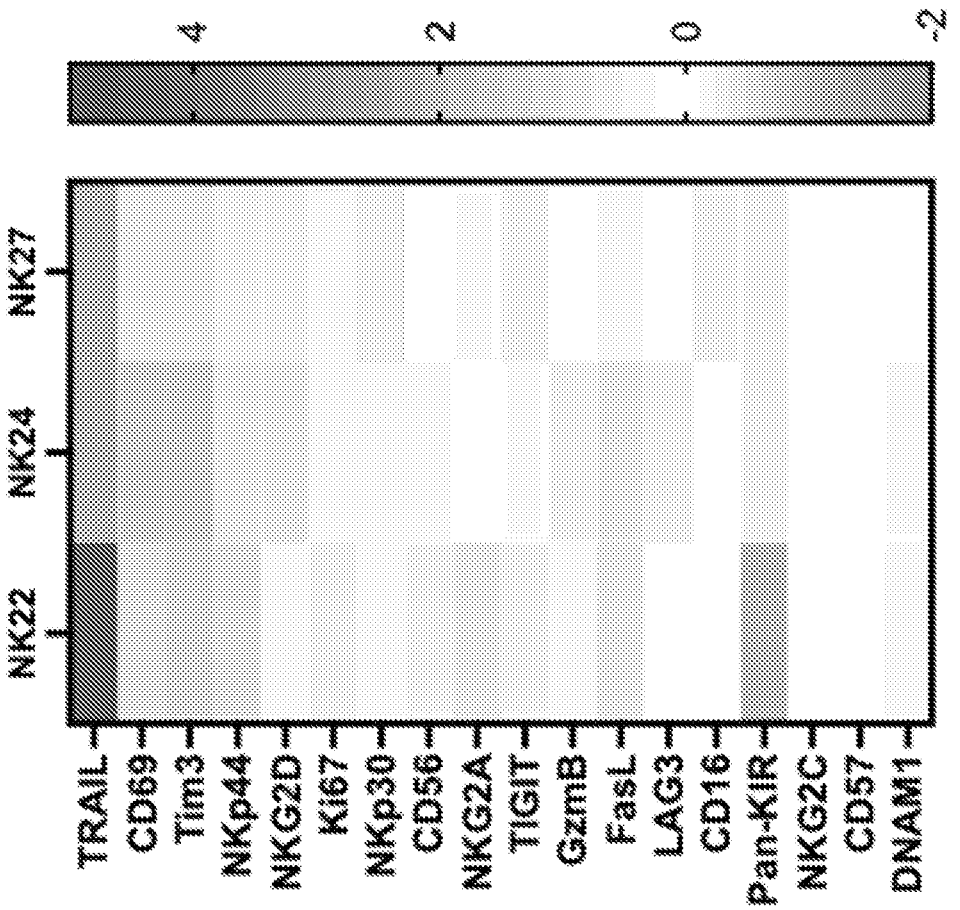

4
2
0
-2
NK22
NK24
NK27
TRAIL
CD69
Tim3
NKp44
NKG2D
Ki67
NKp30
CD56
NKG2A
TIGIT
GzmB
FasL
LAG3
CD16
Pan-KIR
NKG2C
CD57
DNAM1

FIG. 44

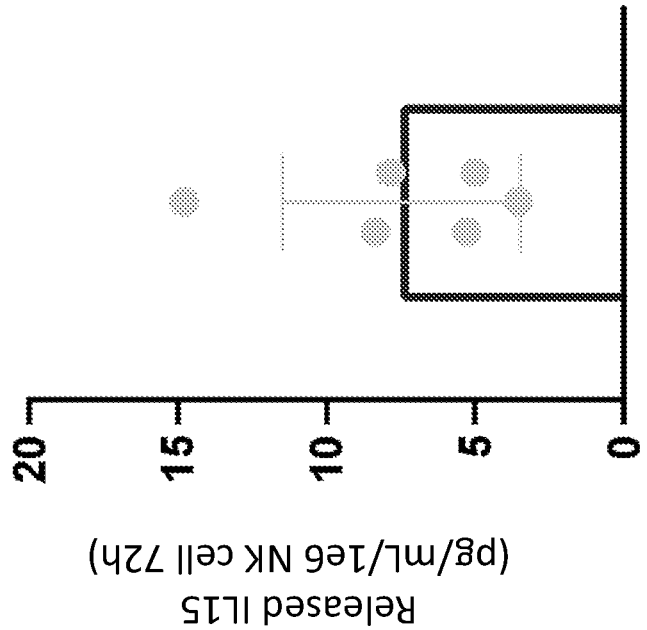


FIG. 45B

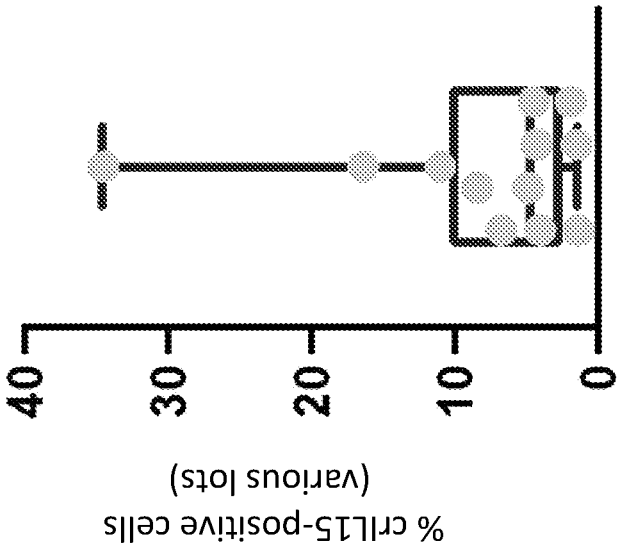


FIG. 45A

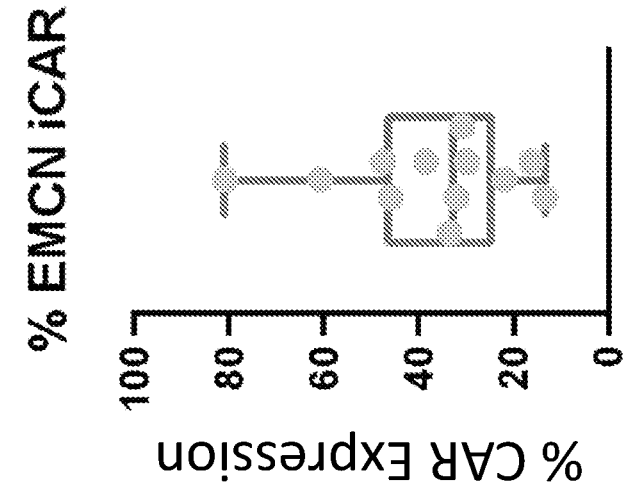


FIG. 45D

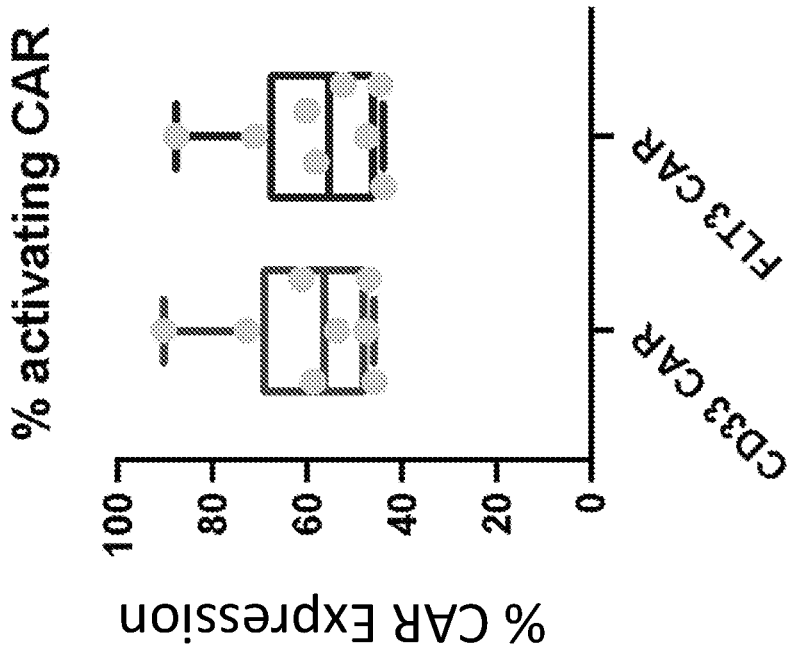


FIG. 45C

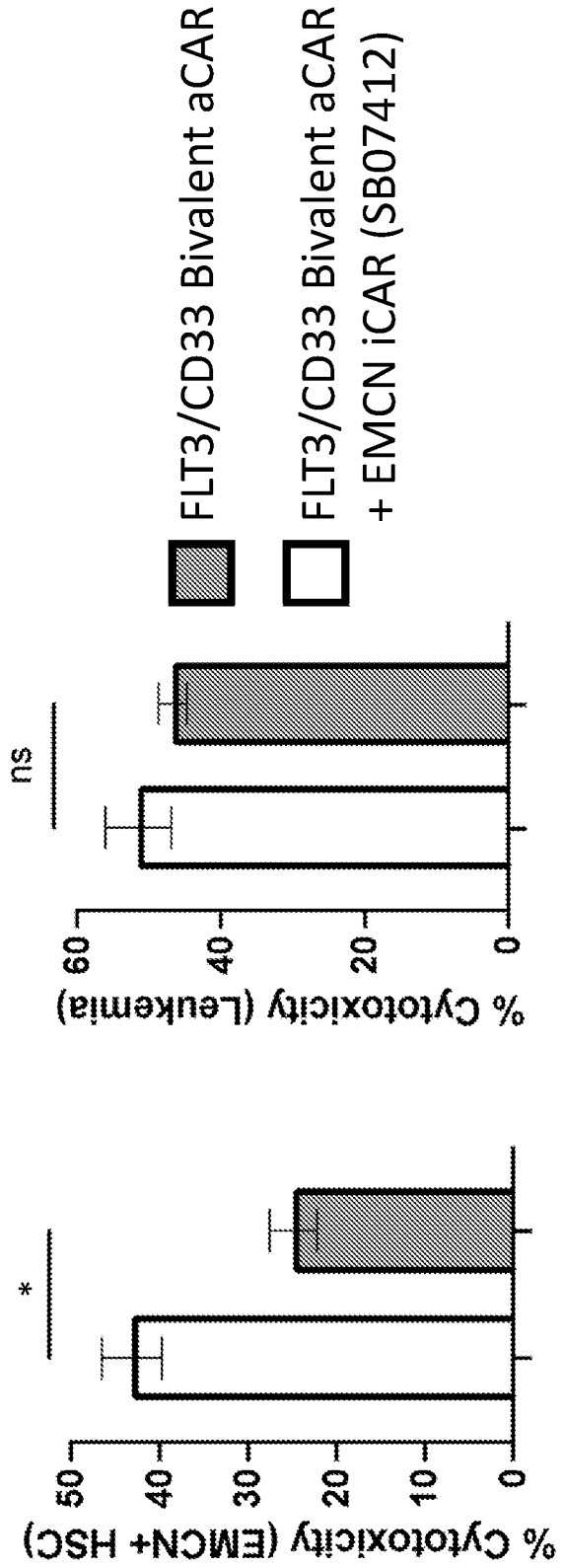


FIG. 46A

FIG. 46B

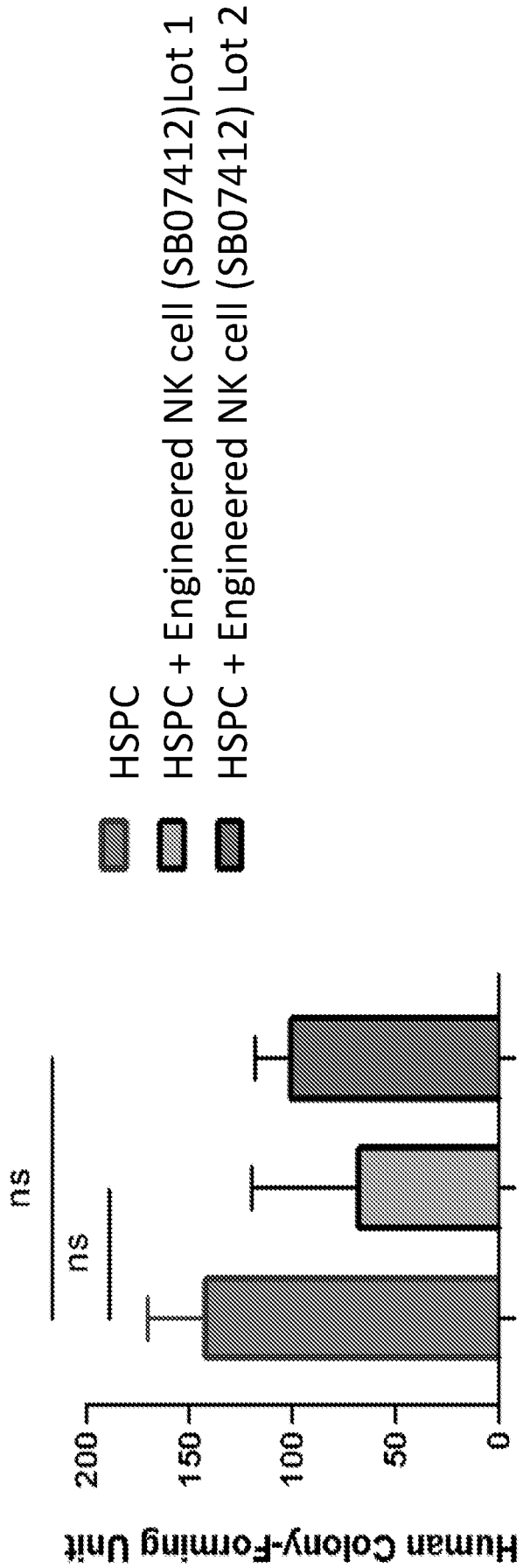

HSPC
HSPC + Engineered NK cell (SB07412)Lot 1
HSPC + Engineered NK cell (SB07412) Lot 2
ns
ns
200
150
100
50
0
Human Colony-Forming Unit

FIG. 46C



FIG. 46D

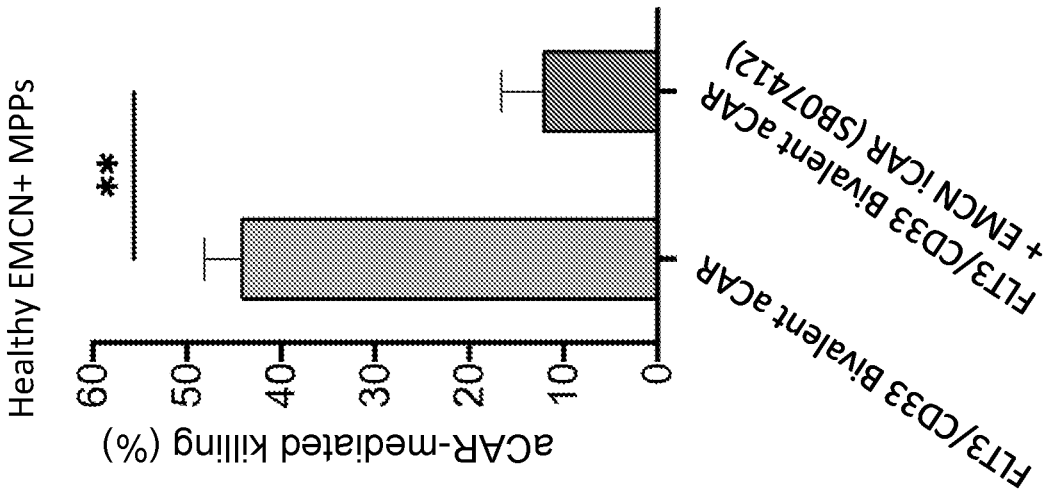


FIG. 46E

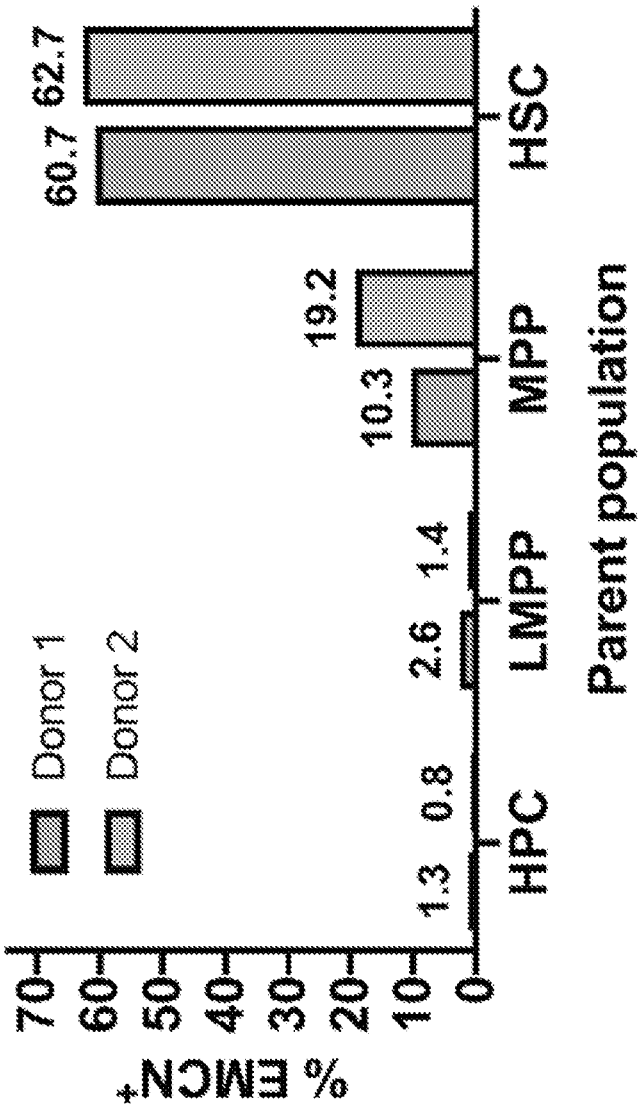

% EMCN+
70
60
50
40
30
20
10
0
Donor 1
Donor 2
1.3
0.8
2.6
1.4
10.3
19.2
60.7
62.7
HPC
LMPP
MPP
HSC
Parent population

FIG. 47

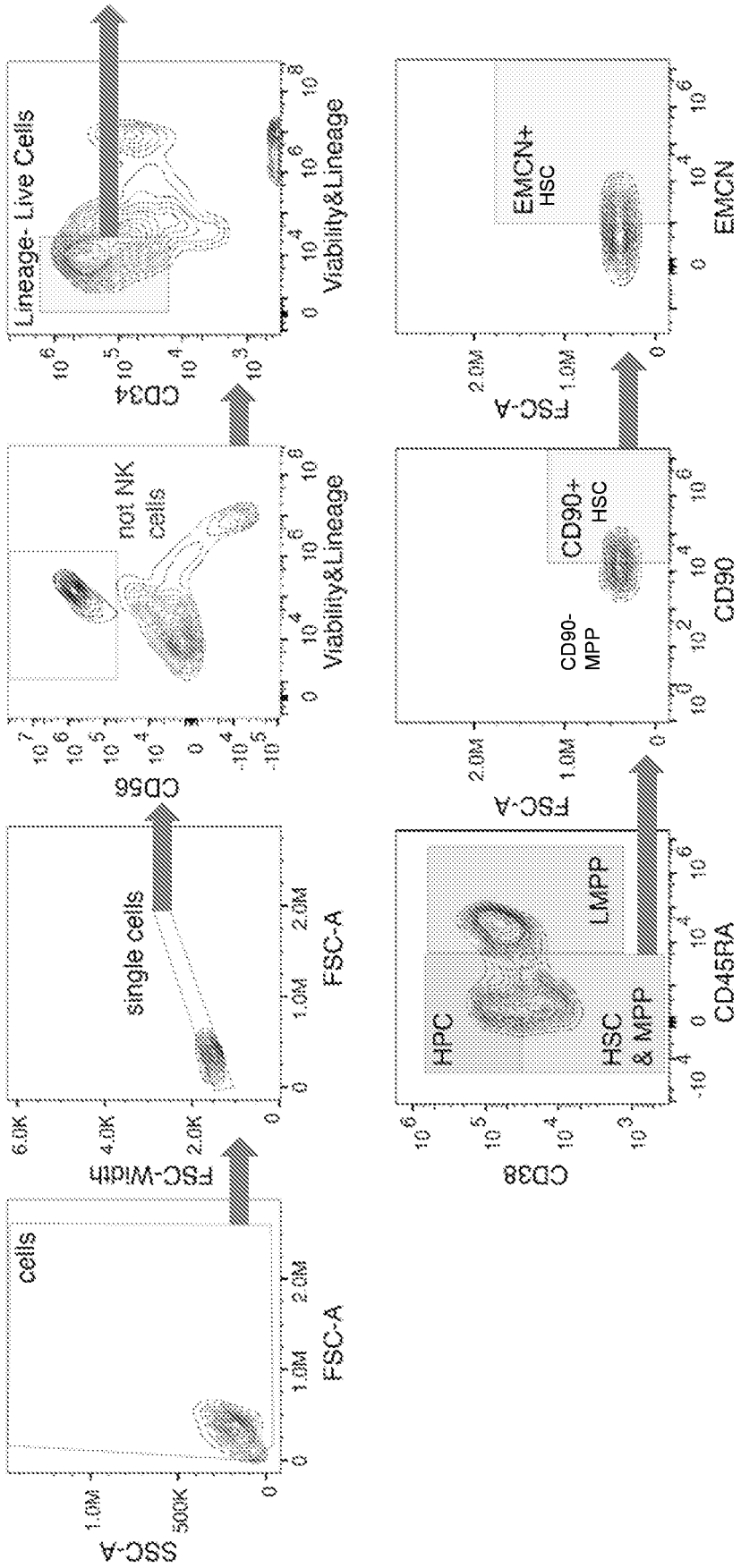

SSC-A
FSC-A
cells
FSC-Width
FSC-A
single cells
CD56
Viability&Lineage
not NK cells
CD34
Viability&Lineage
Lineage- Live Cells
CD38
CD45RA
HPC
HSC & MPP
LMPP
FSC-A
CD90
CD90+ HSC
CD90- MPP
FSC-A
EMCN
EMCN+ HSC

FIG. 48