

MODIFIED CD47 PROTEINS AND THEIR USES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of United States Provisional Application No. 63/534,134, filed August 23, 2023, and United States Provisional Application No. 63/534,136, filed August 23, 2023; the contents of which are incorporated herein by reference in their entirety.

FIELD OF INVENTION

[0002] The present disclosure generally relates to engineered CD47 proteins and uses thereof. Also disclosed are polynucleotides encoding the engineered CD47 proteins, vectors comprising the polynucleotides, cells comprising the engineered proteins and/or the vectors, and compositions comprising the engineered CD47 proteins.

[0003] International application PCT/US2021/065157 and US applications 63/282,961, 63/270,956, and 63/222,954 are incorporated by reference in their entireties. International application PCT/US2023/013364 and US application 63/311,143 are incorporated by reference in their entireties.

SUMMARY

[0004] CD47 is a transmembrane protein that, in humans, is encoded by the CD47 gene (Fig. 1). It is a member of the immunoglobulin (Ig) superfamily. CD47 has a molecular weight of about ~50 kDa. It is glycosylated and ubiquitously expressed by virtually all cells in the human body (Fig. 2). It has a single IgV-like domain at its N-terminus, a highly hydrophobic stretch with five membrane-spanning segments, and an alternatively spliced cytoplasmic tail at its C-terminus (Fig. 4). In addition, it has two extracellular regions and two intracellular regions between neighboring membrane-spanning segments. The signal peptide, when it exists on a CD47 isoform, is located at the N-terminus of the IgV-like domain. The human CD47 gene has six naturally-occurring transcripts, five of which each encode a protein isoform of CD47 (Ensembl, Gene: CD47). As such, there are five protein isoforms of human CD47, each with differential expression across various cell and tissue types.

[0005] CD47 is involved in a range of cellular processes, including apoptosis, proliferation, adhesion, and migration. CD47 interacts with multiple extracellular ligands, such as TSP-1, integrins, other CD47 proteins, and SIRPα. The CD47/SIRPα interaction regulates a multitude of intercellular interactions in many body systems, such as the immune system where it regulates lymphocyte homeostasis, dendritic cell (DC) maturation and activation, proper localization of certain DC subsets in secondary lymphoid organs, and cellular transmigration. CD47 on cells, including on donor cells in the context of transplantation or cell therapy applications, can function as a “marker of self” and regulate phagocytosis by binding to SIRPα on the surface of circulating immune cells to deliver an inhibitory “don’t kill me” signal. CD47-SIRPα binding results in phosphorylation of immunoreceptor tyrosine-based inhibition motifs (ITIMs) on SIRPα, which triggers recruitment of the SHP1 and SHP2 Src homology phosphatases. These phosphatases, in turn, inhibit accumulation of myosin II at the phagocytic synapse, preventing phagocytosis (Fujioka et al., 1996). Phagocytosis of target cells by macrophages is ultimately regulated by a balance of activating signals (e.g., FcγR, CRT, LRP-1) and inhibitory signals (e.g., SIRPα-CD47). Elevated expression of CD47 can help the cell evade immune surveillance, subsequent destruction, and innate immune cell killing. Thus, CD47 can be used as a tolerogenic factor to induce immune tolerance when there is pathological or undesirable activation of an otherwise normal immune response. This can occur, for example, when a patient develops an immune reaction to donor antigens after receiving an allogeneic

transplantation or an allogeneic cell therapy, or when the body responds inappropriately to self-antigens implicated in autoimmune diseases. However, there is a need in the art to improve on such uses of CD47.

[0006] In one aspect, the present disclosure provides engineered CD47 proteins comprising one or more amino acid substitutions and/or insertions relative to a wild-type human CD47 protein. In some embodiments, an engineered CD47 protein of the present disclosure exhibits decreased binding with TSP-1 relative to a wild-type human CD47 protein. In some embodiments, an engineered CD47 protein of the present disclosure exhibits decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein.

[0007] In another aspect, an engineered CD47 protein of the present disclosure exhibits improved binding with SIRPα relative to a wild-type CD47 protein.

[0008] The present disclosure provides, in another aspect, engineered CD47 proteins that have fewer amino acids than the wild-type full-length human CD47 protein. Such engineered proteins afford more efficient cell engineering approaches, including delivery via integrating gene therapy vectors.

[0009] In one aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein the engineered CD47 protein exhibits: decreased binding with TSP-1 relative to a wild-type CD47 protein; and/or decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein.

[0010] In another aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein the genetically engineered cells: exhibit decreased CD47/TSP-1-mediated undesirable effects relative to a control cell; and/or exhibit decreased CD47/TSP-1-mediated exhaustion relative to a control cell; and/or exhibit decreased CD47-mediated undesirable effects relative to a control cell; and/or exhibit increased CD47-mediated desirable effects relative to a control cell; and/or exhibit increased longevity relative to a control cell; and/or exhibit increased persistence relative to a control cell; and/or exhibit increased expansion relative to a control cell, and/or exhibit increased response to its intended therapeutic target, and/or exhibit increased hypoimmunity relative to a control cell; and/or exhibit increased immune evasion relative to a control cell; and/or exhibit increased ability to evade a host immune response relative to a control cell; and/or exhibit increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; and/or express the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would experience one or more unwanted effects.

[0011] In another aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein the engineered CD47 protein exhibits improved binding with SIRPα relative to a wild-type CD47 protein.

[0012] In another aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein contacting a cell expressing SIRPα with the genetically engineered cell increases CD47/SIRPα-mediated signaling in the cell expressing SIRPα relative to contacting the cell expressing SIRPα with a control cell.

[0013] In another aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein contacting a cell expressing SIRPα with the genetically engineered cell increases CD47/SIRPα-mediated signaling in the cell expressing the engineered CD47 relative to contacting the cell expressing SIRPα with a control cell.

[0014] In another aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein contacting a cell expressing SIRPα with the genetically engineered cell increases

CD47/SIRPα-mediated signaling in both the cell expressing SIRPα and the cell expressing CD47 relative to contacting the cell expressing SIRPα with a control cell.

[0015] In another aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein the genetically engineered cells: exhibit decreased CD47-mediated undesirable effects relative to a control cell; and/or exhibit increased CD47-mediated desirable effects relative to a control cell; and/or exhibit increased longevity relative to a control cell; and/or exhibit increased persistence relative to a control cell; and/or exhibit increased expansion relative to a control cell; and/or exhibit increased response to its intended therapeutic target, and/or exhibit increased hyp immunity relative to a control cell; and/or exhibit improved immune evasion relative to a control cell; and/or exhibit increased ability to evade a host immune response relative to a control cell; and/or exhibit increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; and/or express the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would be killed by one or more immune cells.

[0016] In one aspect, the present disclosure provides genetically engineered cells comprising an engineered CD47 protein, wherein the engineered CD47 protein exhibits: decreased binding with TSP-1 relative to a wild-type CD47 protein; decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein; and improved binding with SIRPα relative to a wild-type CD47 protein; and wherein the genetically engineered cells: exhibit decreased CD47/TSP-1-mediated undesirable effects relative to a control cell; exhibit decreased CD47-mediated undesirable effects relative to a control cell; exhibit decreased CD47/TSP-1-mediated exhaustion relative to a control cell; exhibit decreased CD47-mediated undesirable effects relative to a control cell; exhibit increased CD47-mediated desirable effects relative to a control cell; exhibit increased longevity relative to a control cell; exhibit increased persistence relative to a control cell; exhibit increased expansion relative to a control cell; exhibit increased response to its intended therapeutic target; exhibit increased hyp immunity relative to a control cell; exhibit increased immune evasion relative to a control cell; exhibit increased ability to evade a host immune response relative to a control cell; exhibit increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; express the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would experience one or more unwanted effects and would be killed by one or more immune cells; and wherein contacting a cell expressing SIRPα with the genetically engineered cell increases CD47/SIRPα-mediated signaling in both the cell expressing SIRPα and the cell expressing CD47 relative to contacting the cell expressing SIRPα with a control cell.

[0017] In another aspect, the present disclosure provides compositions comprising genetically engineered cells of the present disclosure.

[0018] In another aspect, the present disclosure provides pharmaceutical compositions comprising (i) a genetically engineered cell of the present disclosure, and (ii) a pharmaceutically acceptable excipient.

[0019] In another aspect, the present disclosure provides methods of treating a disease in a subject, the method comprising administering to the subject a genetically engineered cell of the present disclosure, a composition of the present disclosure, or a pharmaceutical composition of the present disclosure.

[0020] In another aspect, the present disclosure provides populations of cells comprising a genetically engineered cell of the present disclosure for use in treating a disease in a subject.

[0021] In another aspect, the present disclosure provides uses of a genetically modified cell of the present disclosure, a population of cells of the present disclosure, a composition of the present disclosure, or a pharmaceutical composition of the present disclosure for use in treating a disease in a subject.

[0022] In another aspect, the present disclosure provides uses of a genetically modified cell of the present disclosure, a population of cells of the present disclosure, a composition of the present disclosure, or a pharmaceutical composition of the present disclosure in the manufacture of a medicament for the treatment of a disease.

[0023] In another aspect, the present disclosure provides methods of making a genetically engineered cell comprising an engineered CD47 protein and a nucleic acid construct encoding the engineered CD47 protein, the method comprising: delivering to a cell a nucleic acid construct comprising a first transgene encoding the engineered CD47 protein, thereby making a genetically engineered cell.

[0024] In another aspect, the present disclosure provides a nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered protein, wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains. In some embodiments, the one or more nucleic acid sequences comprise a nucleic acid sequence at least 80% identical to SEQ ID NO: 17, SEQ ID NO: 16, or SEQ ID NO: 15.

[0025] In another aspect, the present disclosure provides an engineered CD47 protein or a nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered CD47 protein comprising (a) one or more extracellular domains; and (b) one or more membrane tethers. In some embodiments, the one or more extracellular domains comprise: an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6; and/or an amino acid substitution at position S83 of SEQ ID NO: 2, an amino acid substitution at position S65 of SEQ ID NO: 3, an amino acid substitution at position S83 of SEQ ID NO: 1, or an amino acid substitution at position S38 of SEQ ID NO: 6; and/or an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 1 or SEQ ID NO: 6; and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.

[0026] In another aspect, the present disclosure provides an engineered CD47 protein or a nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered CD47 protein, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 4 or SEQ ID NO: 5.

[0027] In another aspect, the present disclosure provides an engineered protein encoded by a nucleic acid construct as described herein.

[0028] In another aspect, the present disclosure provides an engineered protein, wherein the engineered protein is or comprises an amino acid sequence at least 80% identical to SEQ ID NO: 5, and wherein the engineered protein does not comprise one or more full-length CD47 intracellular domains.

[0029] In another aspect, the present disclosure provides an engineered protein, wherein the engineered protein is or comprises an amino acid sequence at least 80% identical to SEQ ID NO: 4, and wherein the engineered protein does not comprise one or more full-length CD47 intracellular domains.

[0030] In another aspect, the present disclosure provides an engineered CD47 protein comprising: (a) one or more extracellular domains; and (b) one or more membrane tethers; wherein the one or more extracellular domains comprise: an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6; and/or an amino acid substitution at position S83 of SEQ ID NO: 2, an amino acid substitution at position S65 of SEQ ID NO: 3, an amino acid substitution at position S83 of SEQ ID NO: 1, or an amino acid substitution at position S38 of SEQ ID NO: 6; and/or an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 3

SEQ ID NO: 1 or SEQ ID NO: 6; and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.

[0031] In another aspect, the present disclosure provides a genetically engineered cell comprising an engineered CD47 protein as described herein.

[0032] In another aspect, the present disclosure provides a composition comprising a genetically engineered cell as disclosed herein.

[0033] In another aspect, the present disclosure provides a method comprising administering to a subject a genetically engineered cell, a composition, or a pharmaceutical composition described herein.

[0034] In another aspect, the present disclosure provides a method of treating a disease in a subject, the method comprising administering to the subject a genetically engineered cell, a composition, or a pharmaceutical composition described herein.

[0035] In another aspect, the present disclosure provides a population of cells comprising a genetically engineered cell as described herein for use in treating a disease in a subject.

[0036] In another aspect, the present disclosure provides a composition or pharmaceutical composition for use in treating a disease in a subject.

[0037] In another aspect, the present disclosure provides a use of a genetically engineered cell, a population of cells, a composition, or a pharmaceutical composition as described herein for use in treating a disease in a subject.

[0038] In another aspect, the present disclosure provides a use of a genetically engineered cell, a population of cells, a composition, or a pharmaceutical composition as described herein in the manufacture of a medicament for the treatment of a disease.

[0039] In another aspect, the present disclosure provides a method of making a genetically engineered cell comprising a nucleic acid construct, the method comprising: delivering to a cell a nucleic acid construct as described herein, thereby making a genetically engineered cell.

BRIEF DESCRIPTION OF THE DRAWING

[0040] **Figure 1** provides a map of the human CD47 gene and illustrates the regions in the human CD47 gene that are protein coding. This map comes from the Ensembl genome database.

[0041] **Figure 2A** and **Figure 2B** provide the isoform expression of CD47 ENSG00000196776.14 CD47 molecule (Source: HGNC Symbol;Acc:HGNC: 1682) and illustrate expression of each isoform in various human tissues and human cell types. This data comes from the Genotype-Tissue Expression (GTEx) project database.

[0042] **Figure 3** provides the predicted CD47 transmembrane domains and the human CD47 protein topology. In particular, Fig. 3 provides the predicted locations of various domains in the human CD47 protein. This prediction comes from the Universal Protein Resource (UniProt).

[0043] **Figure 4** provides the predicted human CD47 tertiary structure from the AlphaFold Protein Structure Database.

[0044] **Figure 5A, Figure 5B, Figure 5C, and Figure 5D** provide a sequence alignment of Isoform 201, Isoform 202, Isoform 203, Isoform 205, and Isoform 206 of the human CD47 protein.

[0045] **Figure 6** provides an exemplary graph showing viral titers, as assessed via the Ella automated immunoassay system, of LVV comprising exemplary CD47 truncated variants.

[0046] **Figure 7** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry). Various CD47 variants, including several comprising GPI anchors, were tested.

[0047] **Figure 8** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry). Various CD47 variants, including several comprising truncated intracellular domains and alternative transmembrane domains, were tested.

[0048] **Figure 9** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry). Various CD47 variants, including those comprising truncated intracellular domains and alternative hinge domains, were tested.

[0049] **Figure 10** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry). Various CD47 variants, including those comprising truncated intracellular domains and alternative hinge domains, were tested.

[0050] **Figure 11** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry). Various CD47 variants, including those comprising truncated intracellular domains and alternative hinge domains, were tested.

[0051] **Figure 12A, Figure 12B, Figure 12C, Figure 12D, and Figure 12E** provide exemplary graphs showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry).

[0052] **Figure 13** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry).

[0053] **Figure 14** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry).

[0054] **Figure 15** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by SIRPa-FC flow cytometry).

[0055] **Figure 16** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by SIRPa-FC flow cytometry).

[0056] **Figure 17** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by SIRPa-FC flow cytometry).

[0057] **Figure 18A** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry).

[0058] **Figure 18B** provides an exemplary graph showing the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by SIRPa-FC flow cytometry).

[0059] **Figure 19** provides schematics of various exemplary CD47 variants tested, including several variants comprising truncated intracellular domains and alternative transmembrane domains.

[0060] **Figure 20** provides graphs showing Gibco iPSC expression of CD47 (left) and isotype control (right) after treatment with control or with truncated CD47 LVV, as assessed by flow cytometry.

[0061] **Figure 21A and Figure 21B** provide exemplary graphs showing Gibco iPSC surface expression of HLA-I (top row), HLA-II (middle row), and CD47 (bottom row) after treatment with control or with truncated CD47 LVV (as assessed by anti-HLA-I, anti-HLA-II, or anti-CD47 flow cytometry, respectively, relative to isotype control). Surface expression of CD47 was also assessed with Quantibrite.

[0062] **Figure 22A** and **Figure 22B** provide exemplary graphs showing killing of Gibco iPSC target cells expressing CD47 LVV by natural killer (NK) effector cells (top row), in the absence of effector cells (middle row), or after treatment with TritonX (bottom row), as assessed by XCELLIGENCE assay.

DETAILED DESCRIPTION

I. Definitions

[0063] All publications, patents, and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

[0064] In the present disclosure, unless otherwise specified, the scientific and technical terms used herein have the meanings generally understood by a person skilled in the art. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present disclosure, the preferred methods and materials are described herein. Accordingly, the terms defined herein are more fully described by reference to the Specification as a whole.

[0065] As used herein, the singular terms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise.

[0066] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or"). Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted.

[0067] The term "about," as used herein when referring to a measurable value such as a sequence length and the like, is meant to encompass variations of 5%, 1 %, 0.5%, or even 0.1 % of the specified amount.

[0068] Unless the context requires otherwise, the terms "comprise," "comprises," and "comprising," or similar terms are intended to mean a non-exclusive inclusion, such that a recited list of elements or features does not include those stated or listed elements solely, but may include other elements or features that are not listed or stated.

[0069] Unless otherwise indicated, nucleic acids are written left to right in the 5' to 3' orientation; and amino acid sequences are written left to right in amino to carboxy orientation, respectively.

[0070] It is to be understood that this disclosure is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context in which they are used by those skilled in the art.

[0071] As used herein, "affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). The affinity of a molecule for its partner can generally be represented by the equilibrium dissociation constant (K_D) (or its inverse equilibrium association constant, K_A). Affinity can be measured by common methods known in the art, including those described herein. See, for example, Pope M.E., Soste M.V., Eyford B.A., Anderson N.L., Pearson T.W., (2009) *J. Immunol. Methods*. 341(1-2):86-96 and methods described therein.

[0072] As used herein, the terms "percent identity" and "% identity," as applied to nucleic acid or polynucleotide sequences, refer to the percentage of residue matches between at least two nucleic acid or polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

[0073] Percent identity between nucleic acid or polynucleotide sequences may be determined using a suite of commonly used and freely available sequence comparison algorithms provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S. F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, Md., and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>.

[0074] Nucleic acid or polynucleotide sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al. (1991) Nucleic Acid Res 19:5081; Ohtsuka et al. (1985) J Biol Chem 260:2605-2608; Cassol et al. (1992); Rossolini et al. (1994) Mol Cell Probes 8:91-98). The term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. The term nucleic acid is used interchangeably with polynucleotide, and (in appropriate contexts) gene, cDNA, and mRNA encoded by a gene.

[0075] As used herein, "percent (%) amino acid sequence identity" with respect to a peptide, polypeptide or protein sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in another peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent amino acid sequence identity in the current disclosure is measured using BLAST software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0076] An amino acid substitution refers to the replacement of one amino acid in a polypeptide with another amino acid. Exemplary substitutions are shown in **Table 1**. Amino acid substitutions may be introduced into a protein of interest and the products screened for a desired activity, for example, retained/improved biological activity.

Table 1. Exemplary Amino Acid Substitutions

Original Residue	Exemplary Substitutions
Ala (A)	Val; Leu; Ile
Arg (R)	Lys; Gln; Asn
Asn (N)	Gln; His; Asp, Lys; Arg
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn; Glu
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln; Lys; Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg; Gln; Asn
Met (M)	Leu; Phe; Ile
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Val; Ser

Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe; Thr; Ser
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine

[0077] Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

[0078] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. The term, "corresponding to" with reference to nucleotide or amino acid positions of a sequence, such as set forth in the Sequence Listing, refers to nucleotides or amino acid positions identified upon alignment with a target sequence based on structural sequence alignment or using a standard alignment algorithm, such as the GAP algorithm. For example, corresponding residues of a similar sequence (e.g., a fragment or species variant) can be determined by alignment to a reference sequence by structural alignment methods. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid residues as guides.

[0079] As used herein, an isoform of the human CD47 protein refers to a protein that is translated from the human CD47 gene and is processed by alternative splicing.

[0080] As used herein, a wild-type human CD47 protein refers to a human CD47 protein that is naturally occurring in vivo, such as a wild-type human CD47 protein encoded in/by the human genome. A wild-type human CD47 protein could be any of the isoforms of the naturally occurring human CD47 protein. The amino acid sequences of the five currently known naturally occurring isoforms of CD47 (isoforms 201, 202, 203, 205, 206) are set forth in SEQ ID NOs: 1, 2, 4-5. Isoform CD47-202 (SEQ ID NO:2) is the full-length wild-type human CD47 protein as it is translated containing its signal sequence. The sequence of the mature CD47-202 isoform lacking its signal sequence is set forth in SEQ ID NO:3. A wild-type human CD47 protein may or may not have a signal peptide when it is expressed. For instance, CD47-206 lacks a signal peptide when it is translated. A wild-type human CD47 protein may or may not be glycosylated. A wild-type human CD47 protein could be a proteoglycan.

[0081] As used herein, an engineered CD47 protein refers to a CD47 protein that is not naturally occurring in any species. In other words, an engineered CD47 protein is not a wild-type CD47 protein in any species. In some embodiments, the engineered CD47 protein is an engineered human CD47 protein, meaning it is engineered by using the human wild-type CD47 protein as a starting material and making one or more of the modifications described herein. In some embodiments, the engineered CD47 protein is an engineered humanized CD47 protein, meaning it is engineered by using a non-human (e.g., murine) CD47 protein as a starting material and by humanizing the non-human CD47 sequence in addition to making one or more of the other modifications described herein. In some embodiments, the engineered CD47 protein is an engineered partially-humanized CD47 protein, meaning it is engineered by using a non-human (e.g., murine) CD47 protein as a starting material and by humanizing a portion of the non-human CD47 sequence in addition to making one or more of the other modifications described herein.

[0082] As used herein, an engineered CD47 protein refers to a protein that is not a CD47 protein encoded in/by a native genome, e.g., not a wild-type CD47 protein. Non-limiting examples of engineered CD47 proteins include an engineered CD47 protein having (i) a human CD47 extracellular domain or a portion thereof, at least one human

CD47 transmembrane domain or a portion thereof, and a portion of a human CD47 intracellular domain comprising a deletion of at least one amino acid, wherein the deletion is not a C-terminal deletion of 18 amino acids, (ii) a portion of a human CD47 extracellular domain, at least one human CD47 transmembrane domain or a portion thereof, and a portion of a human CD47 intracellular domain comprising a C-terminal deletion of 18 amino acids, (iii) a human CD47 extracellular domain or a portion thereof, at least one and fewer than five human CD47 transmembrane domain(s) or portion(s) thereof, and a portion of a human CD47 intracellular domain comprising a C-terminal deletion of 18 amino acids, (iv) a human CD47 extracellular domain or a portion thereof, at least one human CD47 transmembrane domain or a portion thereof, and a signal peptide, wherein the engineered CD47 protein does not comprise an intracellular domain, (v) a human CD47 extracellular domain, and at least one human CD47 transmembrane domain or a portion thereof, wherein the engineered CD47 protein does not comprise an intracellular domain, (vi) a human CD47 extracellular domain or a portion thereof, and at least one and fewer than five human CD47 transmembrane domain(s) or portion(s) thereof, wherein the engineered CD47 protein does not comprise an intracellular domain, (vii) a human CD47 extracellular domain or a portion thereof, and at least one human CD47 transmembrane domain or a portion thereof, no intracellular domain, or a human CD47 intracellular domain comprising a deletion of at least one amino acid, wherein the engineered CD47 protein has an amino acid sequence that has at most 99% identity to SEQ ID NO:1 and SEQ ID NO:6.

[0083] As used herein, the term "exogenous" in the context of a polynucleotide or polypeptide being expressed is intended to mean that the referenced molecule or the referenced polypeptide is introduced into the cell of interest. The polypeptide can be introduced, for example, by introduction of an encoding nucleic acid into the genetic material of the cells such as by integration into a chromosome or as non-chromosomal genetic material such as a plasmid or expression vector. Therefore, the term as it is used in reference to expression of an encoding nucleic acid refers to introduction of the encoding nucleic acid in an expressible form into the cell. An exogenous polynucleotide can be inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some embodiments, exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector. In some embodiments, the exogenous polynucleotide is inserted into target locus of at least one allele of the cell.

[0084] An "exogenous" molecule is a molecule, construct, factor and the like that is not normally present in a cell, but can be introduced into a cell by one or more genetic, biochemical or other methods. "Normal presence in the cell" is determined with respect to the particular developmental stage and environmental conditions of the cell. Thus, for example, a molecule that is present only during embryonic development of neurons is an exogenous molecule with respect to an adult neuron cell. An exogenous molecule can comprise, for example, a functioning version of a malfunctioning endogenous molecule or a malfunctioning version of a normally-functioning endogenous molecule.

[0085] An exogenous molecule or factor can be, among other things, a small molecule, such as is generated by a combinatorial chemistry process, or a macromolecule such as a protein, nucleic acid, carbohydrate, lipid, glycoprotein, lipoprotein, polysaccharide, any modified derivative of the above molecules, or any complex comprising one or more of the above molecules. Nucleic acids include DNA and RNA; can be single- or double-stranded; can be linear, branched or circular; and can be of any length. Nucleic acids include those capable of forming duplexes, as well as triplex-forming nucleic acids. See, for example, U.S. Pat. Nos. 5,176,996 and 5,422,251. Proteins include, but are not limited to, DNA-binding proteins, transcription factors, chromatin remodeling factors, methylated DNA binding proteins,

polymerases, methylases, demethylases, acetylases, deacetylases, kinases, phosphatases, integrases, recombinases, ligases, topoisomerases, gyrases, and helicases.

[0086] An exogenous molecule or construct can be the same type of molecule as an endogenous molecule, *e.g.*, an exogenous protein or nucleic acid. In such instances, the exogenous molecule is introduced into the cell at greater concentrations than that of the endogenous molecule in the cell. In some instances, an exogenous nucleic acid can comprise an infecting viral genome, a plasmid or episome introduced into a cell, or a chromosome that is not normally present in the cell. Methods for the introduction of exogenous molecules into cells are known to those of skill in the art and include, but are not limited to, lipid-mediated transfer (*i.e.*, liposomes, including neutral and cationic lipids), electroporation, direct injection, cell fusion, particle bombardment, calcium phosphate co-precipitation, DEAE-dextran-mediated transfer and viral vector-mediated transfer.

[0087] The term “genetic modification” and its grammatical equivalents as used herein can refer to one or more alterations of a nucleic acid, *e.g.*, the nucleic acid within an organism’s genome. For example, genetic modification can refer to alterations, additions, and/or deletion of genes or portions of genes or other nucleic acid sequences. A genetically modified cell can also refer to a cell with an added, deleted, and/or altered gene or portion of a gene. A genetically modified cell can also refer to a cell with an added nucleic acid sequence that is not a gene or gene portion. Genetic modifications include, for example, both transient knock-in or knock-down mechanisms, and mechanisms that result in permanent knock-in, knock-down, or knock-out of target genes or portions of genes or nucleic acid sequences. Genetic modifications include, for example, both transient knock-in and mechanisms that result in permanent knock-in of nucleic acids sequences. Genetic modifications also include, for example, reduced or increased transcription, reduced or increased mRNA stability, reduced or increased translation, and reduced or increased protein stability.

[0088] As used herein, a portion of a peptide has fewer amino acids than the reference peptide, and has at least one amino acid from that peptide.

[0089] As used herein, a composition refers to any mixture of two or more products, substances, or compounds, including cells.

II. CD47 proteins

Overview of CD47 protein

[0090] This disclosure relates to engineered CD47 proteins and uses thereof.

[0091] CD47, also known as integrin-associated protein (IAP) or MER6, is a transmembrane protein that, in humans, is encoded by the human CD47 gene (SEQ ID NO:19) (Fig. 1). CD47 is a member of the immunoglobulin (Ig) superfamily and is involved in a range of cellular processes, including apoptosis, proliferation, adhesion, and migration.

[0092] Human CD47 is about ~50 kDa. It is glycosylated and ubiquitously expressed by virtually all cells in the human body (Fig. 2). Historical literature suggests that isoform 202 (*i.e.*, CD47-202, SEQ ID NO:2) is mainly expressed in the brain, but recent GTEx expression data do not support this conclusion (Fig. 2). As shown in Example 1 herein, isoform CD47-202 (SEQ ID NO:2, SEQ ID NO:14) and isoform CD47-201 (SEQ ID NO:1, SEQ ID NO:13) are expressed at relatively equal levels in Gibco and Rues2 human stem cell lines. Isoforms CD47-206 (SEQ ID NO:6, SEQ ID NO:18), 205 (SEQ ID NO:5, SEQ ID NO:17), 204 (SEQ ID NO:16) also appear to be highly expressed in these stem cell lines. No evidence of isoform CD47-203 (SEQ ID NO:4, SEQ ID NO:15) in stem cell lines was detected.

[0093] Human CD47 has a single IgV-like domain at its N-terminus, a highly hydrophobic stretch with five membrane-spanning segments, and an alternatively spliced cytoplasmic tail at its C-terminus (Fig. 4). In addition, it has

two extracellular regions and two intracellular regions between neighboring membrane-spanning segments. The signal peptide, when it exists on a CD47 isoform, is located at the N-terminus of the IgV-like domain.

[0094] As used herein, a human CD47 extracellular domain refers to the IgV-like domain at the N-terminus of the human CD47 protein. Structurally, the human CD47 extracellular domain is the N-terminal portion of the human CD47 protein that is located outside a cell when the human CD47 protein is anchored in the cell membrane. In some embodiments, the human CD47 extracellular domain has an amino acid sequence corresponding to amino acids 19-141 of SEQ ID NO:2, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 19-141 of SEQ ID NO:2. In some embodiments, the human CD47 extracellular domain has an amino acid sequence corresponding to amino acids 19-141 of SEQ ID NO:2, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 19-141 of SEQ ID NO:2.

[0095] As used herein, a human CD47 intracellular domain refers to the cytoplasmic tail at the C-terminus of the human CD47 protein. Structurally, the human CD47 intracellular domain is the C-terminal portion of the human CD47 protein that is located inside a cell when the human CD47 protein is anchored in the cell membrane. The human CD47 intracellular domain is alternatively spliced *in vivo*. In some embodiments, the human CD47 intracellular domain has an amino acid sequence corresponding to amino acids 290-323 of SEQ ID NO:2, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 290-323 of SEQ ID NO:2. In some embodiments, the human CD47 intracellular domain has an amino acid sequence corresponding to amino acids 290-323 of SEQ ID NO:2, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 290-323 of SEQ ID NO:2.

[0096] As used herein, a human CD47 transmembrane domain refers to one of the membrane-spanning segments of the human CD47 protein. In some embodiments, the human CD47 transmembrane domain has an amino acid sequence corresponding to amino acids 142-162, 177-197, 208-228, 236-257, or 269-289 of SEQ ID NO:2, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 142-162, 177-197, 208-228, 236-257, or 269-289 of SEQ ID NO:2. In some embodiments, the human CD47 transmembrane domain has an amino acid sequence corresponding to amino acids 142-162, 177-197, 208-228, 236-257, or 269-289 of SEQ ID NO:2, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 142-162, 177-197, 208-228, 236-257, or 269-289 of SEQ ID NO:2.

[0097] As used herein, a signal peptide refers to the short peptide present at the N-terminus of the CD47 protein when the protein is initially translated. Signal peptides are usually cleaved off from a protein by a signal peptidase during or immediately after insertion into a cell membrane. Signal peptides function to prompt a cell to translocate the protein, usually to the plasma membrane. In some embodiments, the signal peptide for a human CD47 protein has an amino acid sequence corresponding to amino acids 1-18 of SEQ ID NO:2, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 1-18 of SEQ ID NO:2. In some embodiments, the signal peptide for a human CD47 protein has an amino acid sequence corresponding to amino acids 1-18 of SEQ ID NO:2, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 1-18 of SEQ ID NO:2.

[0098] The human CD47 gene has six transcripts, five of which encode a protein isoform of CD47 (Ensembl, Gene: CD47). The six transcripts are named CD47-201, CD47-202, CD47-203, CD47-204, CD47-205, and CD47-206 (Ensembl, Gene: CD47, ENSG00000196776). The coding DNA sequence (CDS) of the six transcripts are as set forth in

SEQ ID NO: 13-18, respectively. The amino acid sequences of the five protein isoforms are as set forth in SEQ ID NO:1, 2, 4, 5, 6, respectively (Table 2).

[0099] Transcript CD47-202 (SEQ ID NO:14) encodes isoform CD47-202 (SEQ ID NO:2), which has 323 amino acids. CD47-202 is the longest transcript of the human CD47 gene. It is designated as the representative transcript in the Ensembl database. In identifying the representative transcript, Ensembl aims to identify the transcript that, on balance, has the highest coverage of conserved exons, highest expression, longest coding sequence and is represented in other key resources, such as NCBI and UniProt. All splice junctions of the CD47-202 transcript are supported by at least one non-suspect mRNA.

[0100] Transcript CD47-201 (SEQ ID NO:13) encodes isoform CD47-201 (SEQ ID NO:1), which has 305 amino acids. Isoform CD47-201 has a C-terminal truncation of 18 amino acids from isoform CD47-202. All splice junctions of the CD47-201 transcript are supported by at least one non-suspect mRNA.

[0101] Transcript CD47-203 (SEQ ID NO:15) encodes isoform CD47-203 (SEQ ID NO:4), which has 86 amino acids. The only support for the transcript model is from a single expressed sequence tag (EST).

[0102] Transcript CD47-204 (SEQ ID NO:16) does not encode protein. All splice junctions of this transcript are supported by at least one non-suspect mRNA

[0103] Transcript CD47-205 (SEQ ID NO:17) encodes isoform CD47-205 (SEQ ID NO:5), which has 109 amino acids. Isoform 205 comprises 3 transmembrane domains and a truncated intracellular domain from isoform CD47-202 (SEQ ID NO:2). The best supporting mRNA for the transcript model is flagged as suspect or the support is from multiple ESTs.

[0104] Transcript CD47-206 (SEQ ID NO:18) encodes isoform CD47-206 (SEQ ID NO:6), which has 183 amino acids. Isoform 206 comprises a truncated extracellular domain and 5 transmembrane domains from isoform CD47-202 (SEQ ID NO:2).

[0105] The amino acid sequences of the five isoforms are listed in **Table 2**. The amino acids corresponding to the various domains in the human CD47 protein are also identified in Table 2 and depicted in Fig. 5. "Intracellular connection" refers to the intracellular region connecting neighboring transmembrane domains, which is positioned inside of a cell (i.e., not outside the cell and not within the cell membrane) but are not positioned at the N-terminus or the C-terminus of the engineered CD47 protein. "Extracellular connection" refers to the extracellular region connecting neighboring transmembrane domains, which is positioned outside of a cell (i.e., not inside the cell and not within the cell membrane). As used herein, the CD47 "intracellular domain" does not include the intracellular connections. Also as used herein, the CD47 "extracellular domain" does not include the extracellular connections.

Table 2. Amino Acid SEQ ID NOs for CD47 Domains

	CD47-201 SEQ ID NO:1	CD47-202 SEQ ID NO:2	CD47-203 SEQ ID NO:4	CD47-205 SEQ ID NO:5	CD47-206 SEQ ID NO:6
Signal peptide	1-18	1-18	N/A	N/A	N/A
Extracellular domain	19-141	19-141			1-96
Transmembrane domain 1	142-162	142-162			97-117
Intracellular connection 1	163-176	163-176			118-131

Transmembrane domain 2	177-197	177-197			132-152
Extracellular connection 1	198-207	198-207		1-4	153-162
Transmembrane domain 3	208-228	208-228		5-25	163-183
Intracellular connection 2	229-235	229-235		26-32	
Transmembrane domain 4	236-256	236-256		33-53	
Extracellular connection 2	257-268	257-268		54-65	
Transmembrane domain 5	269-289	269-289		66-86	
Intracellular domain	290-305	290-323		87-109	

Engineered CD47 proteins

[0106] The present disclosure provides engineered CD47 proteins comprising one or more amino acid substitutions and/or insertions relative to a wild-type human CD47 protein. In some embodiments, the one or more amino acid substitutions and/or insertions relative to a wild-type human CD47 protein are described in WO2016179399A1 (incorporated by reference herein). In some embodiments, the one or more amino acid substitutions and/or insertions relative to a wild-type human CD47 protein are described Kaur, Sukhbir, et al. "Heparan sulfate modification of the transmembrane receptor CD47 is necessary for inhibition of T cell receptor signaling by thrombospondin-1." *Journal of Biological Chemistry* 286.17 (2011): 14991-15002 (incorporated by reference herein). In some embodiments, the one or more amino acid substitutions and/or insertions relative to a wild-type human CD47 protein are described in Ho, Chia Chi M., et al. "Velcro" engineering of high affinity CD47 ectodomain as signal regulatory protein α (SIRPα) antagonists that enhance antibody-dependent cellular phagocytosis." *Journal of Biological Chemistry* 290.20 (2015): 12650-12663 (incorporated by reference herein).

[0107] In some embodiments, an engineered CD47 protein of the present disclosure exhibits decreased binding with TSP-1 relative to a wild-type human CD47 protein. In some embodiments, an engineered CD47 protein of the present disclosure exhibits decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein. In some embodiments, decreased binding to TSP-1 is due to a lack of a heparan sulfate proteoglycan modification on the engineered CD47 protein.

[0108] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid substitution at position S82 of SEQ ID NO: 2. In some embodiments, the term "an engineered CD47 protein comprising an S64A mutation" (or grammatical variants of the term) is used to refer to an engineered CD47 protein of the present disclosure that comprises: an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of

SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6.

[0109] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid substitution at position S83 of SEQ ID NO: 2, an amino acid substitution at position S65 of SEQ ID NO: 3, an amino acid substitution at position S83 of SEQ ID NO: 1, or an amino acid substitution at position S38 of SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid substitution at position S83 of SEQ ID NO: 2.

[0110] In some embodiments, an engineered CD47 protein of the present disclosure comprises: amino acid substitutions at positions S82 and S83 of SEQ ID NO: 2, amino acid substitutions at positions S64 and S65 of SEQ ID NO: 3, amino acid substitutions at positions S82 and S83 of SEQ ID NO: 1, or amino acid substitutions at positions S37 and S38 of SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises amino acid substitutions at positions S82 and S83 of SEQ ID NO: 2.

[0111] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, or an amino acid substitution of S37A relative to SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid substitution of S82A relative to SEQ ID NO: 2.

[0112] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2, an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3, an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, or an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6.

[0113] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid substitution of S83A relative to SEQ ID NO: 2, an amino acid substitution of S65A relative to SEQ ID NO: 3, an amino acid substitution of S83A relative to SEQ ID NO: 1, or an amino acid substitution of S38A relative to SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid substitution of S83A relative to SEQ ID NO: 2.

[0114] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2, an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3, an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, or an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2.

[0115] In some embodiments, an engineered CD47 protein of the present disclosure comprises: a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, and an amino acid substitution of S37A relative to SEQ ID NO: 6; and a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83A relative to SEQ ID NO: 2, an amino acid substitution of S65A relative to SEQ ID NO: 3, an amino acid substitution of S83A relative to SEQ ID NO: 1, and an amino acid substitution of

S38A relative to SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises amino acid substitutions of S82A and S83A relative to SEQ ID NO: 2.

[0116] In some embodiments, an engineered CD47 protein of the present disclosure comprises: a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2, an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3, an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, and an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6; and a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2, an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3, an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, and an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[0117] In some embodiments, an engineered CD47 protein of the present disclosure comprises: a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2; and a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2.

[0118] In some embodiments, an engineered CD47 protein of the present disclosure comprises an elongated N-terminus, which increases the buried surface area between the engineered CD47 protein and SIRPα, resulting in increasing affinity. In some embodiments, an engineered CD47 protein of the present disclosure exhibits improved binding with SIRPα relative to a wild-type CD47 protein.

[0119] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid sequence having at least 80% identity to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6; and an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises: an amino acid sequence having at least 80% identity to SEQ ID NO: 2; and an insertion of one or more amino acids relative to SEQ ID NO: 2.

[0120] In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of three amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of three amino acids relative to SEQ ID NO: 2.

[0121] In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2.

[0122] In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 or SEQ ID NO: 1. In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2.

[0123] In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1. In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2.

[0124] In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2.

[0125] In some embodiments, an engineered CD47 protein of the present disclosure comprises: an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2, or an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 1 and an amino acid substitution of Q19P relative to SEQ ID NO: 1. In some embodiments, an engineered CD47 protein of the present disclosure comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2.

[0126] In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2.

[0127] In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence comprising SEQ ID NO: 346. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence comprising SEQ ID NO: 347. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence comprising, from N-terminus to C-terminus: an amino acid sequence comprising SEQ ID NO: 20; an amino acid sequence comprising SEQ ID NO: 348; and an amino acid sequence comprising SEQ ID NO: 3. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence comprising, from N-terminus to C-terminus: an amino acid sequence comprising SEQ ID NO: 20; an amino acid sequence comprising SEQ ID NO: 348; and an amino acid substitution of Q1P relative to SEQ ID NO: SEQ ID NO: 3. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence comprising, from N-terminus to C-terminus: an amino acid sequence comprising SEQ ID NO: 348; and an amino acid sequence comprising SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0128] In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence comprising: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, or an amino acid substitution of S37A relative to SEQ ID NO: 6; an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1; and an amino acid substitution of Q19P relative to SEQ ID NO: 2 or SEQ ID NO: 1. In some embodiments, an engineered CD47 protein of the present disclosure comprises an amino acid sequence comprising: an amino acid substitution of S82A relative to SEQ ID NO: 2; an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2; and an amino acid substitution of Q19P relative to SEQ ID NO: 2.

[0129] The present disclosure also provides engineered CD47 proteins that have fewer amino acids than the wild-type full-length human CD47 protein. Such engineered proteins afford more efficient cell engineering approaches, including delivery via integrating gene therapy vectors.

[0130] The wild-type full-length human CD47 protein, as used herein, refers to the isoform CD47-202 as disclosed in the Ensembl database as of the filing date of this patent application. The wild-type full-length human CD47 protein has an amino acid sequence of SEQ ID NO:2, wherein amino acids 1-18 are the signal peptide, amino acids 19-141 are the extracellular domain, amino acids 142-162, 177-197, 208-228, 236-257, 269-289 are the five transmembrane domains (Fig. 3), and amino acids 290-323 are the intracellular domain. Amino acids 163-176 and 229-

235 are the two intracellular connections between the transmembrane domains, and amino acids 198-207 and 257-268 are the two extracellular connections between the transmembrane domains (Fig. 3).

[0131] In some embodiments, the engineered CD47 protein is a C-terminally truncated version of isoform 202 (SEQ ID NO:2). For example, in some embodiments, the C-terminal truncation is consecutive and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, or 161 amino acid(s) long. Preferably, the C-terminal truncation is 158, 123, 92, 64, 31, or 95 amino acids long, resulting in an engineered CD47 protein having an amino acid sequence as set for in SEQ ID NO:7-12, respectively. In some embodiments, the engineered CD47 protein having a C-terminal truncation of SEQ ID NO:2 further has an N-terminal truncation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140 consecutive amino acid(s).

[0132] In some embodiments, the engineered CD47 protein is a C-terminally truncated version of isoform 201 (SEQ ID NO:1). For example, in some embodiments, the C-terminal truncation is consecutive and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, or 143 amino acid(s) long. In some embodiments, the engineered CD47 protein having a C-terminal truncation of SEQ ID NO:1 further has an N-terminal truncation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, or 95 consecutive amino acid(s).

[0133] In some embodiments, the engineered CD47 protein is a C-terminally truncated version of isoform 206 (SEQ ID NO:6). For example, in some embodiments, the C-terminal truncation is consecutive and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, or 66 amino acid(s) long. In some embodiments, the engineered CD47 protein having a C-terminal truncation of SEQ ID NO:6 further has an N-terminal truncation of 1, 2, 3, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, or 140 consecutive amino acid(s).

[0134] In some embodiments, the engineered CD47 protein comprises a minimal intracellular domain. As used herein, a minimal intracellular domain refers to an intracellular domain that has the minimum number of amino acids required to preserve SIRPα binding of the engineered CD47 protein.

[0135] In some embodiments, the engineered CD47 protein comprises a minimal extracellular domain. As used herein, a minimal extracellular domain refers to an extracellular domain that has the minimum number of amino acids required for the engineered CD47 protein to bind to SIRPα.

[0136] In an aspect, the present disclosure provides an engineered CD47 protein that comprises a human CD47 extracellular domain or a portion thereof and at least one human CD47 transmembrane domain, wherein when an intracellular domain exists, it is a human CD47 intracellular domain with a deletion of at least one amino acid. In some embodiments, when there are more than one transmembrane domains in the engineered CD47 protein, each of the transmembrane domains are interconnected with intracellular and/or extracellular connection(s).

[0137] In an aspect, the present disclosure provides an engineered CD47 protein that consists essentially of a human CD47 extracellular domain or a portion thereof and at least one human CD47 transmembrane domain. In some embodiments, when there are more than one transmembrane domain in the engineered CD47 protein, each of the transmembrane domains are interconnected with intracellular and/or extracellular connection(s). As used herein, the term "consisting essentially of" includes the specified elements and any additional elements that do not abrogate SIRPα binding of the engineered CD47 protein.

[0138] In an aspect, the present disclosure provides an engineered CD47 protein that consists of a human CD47 extracellular domain or a portion thereof and at least one human CD47 transmembrane domain. In some embodiments, when there are more than one transmembrane domain in the engineered CD47 protein, each of the transmembrane domains are interconnected with intracellular and/or extracellular connection(s).

[0139] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain or a portion thereof, at least one human CD47 transmembrane domain or a portion thereof, and a portion of a human CD47 intracellular domain comprising a deletion of at least one amino acid, wherein the deletion is not a C-terminal deletion of 18 amino acids.

[0140] In some embodiments, the engineered CD47 protein comprises a portion of a human CD47 extracellular domain, at least one human CD47 transmembrane domain or a portion thereof, and a portion of a human CD47 intracellular domain comprising a C-terminal deletion of 18 amino acids.

[0141] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain or a portion thereof, at least one and fewer than five human CD47 transmembrane domain(s) or portion(s) thereof, and a portion of a human CD47 intracellular domain comprising a C-terminal deletion of 18 amino acids.

[0142] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain or a portion thereof, at least one human CD47 transmembrane domain or a portion thereof, and a signal peptide, wherein the engineered CD47 protein does not comprise an intracellular domain.

[0143] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain, and at least one human CD47 transmembrane domain or a portion thereof, wherein the engineered CD47 protein does not comprise an intracellular domain.

[0144] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain or a portion thereof, and at least one and fewer than five human CD47 transmembrane domain(s) or portion(s) thereof, wherein the engineered CD47 protein does not comprise an intracellular domain.

[0145] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain, five human CD47 transmembrane domains and a human CD47 intracellular domain with a C-terminal deletion of 18 amino acids, wherein the amino acid sequence of the engineered CD47 protein is not SEQ ID NO:1.

[0146] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain and five human CD47 transmembrane domain, wherein the amino acid sequence of the engineered CD47 protein is not SEQ ID NO:6.

[0147] In some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain or a portion thereof, at least one human CD47 transmembrane domain or a portion thereof, and no intracellular domain or a human CD47 intracellular domain comprising a deletion of at least one amino acid, wherein the amino acid sequence of the engineered CD47 protein has at most 99% identity with SEQ ID NO:1 and SEQ ID NO:6. In other words, in some embodiments, the engineered CD47 protein comprises a human CD47 extracellular domain or a portion thereof, at least one human CD47 transmembrane domain or a portion thereof, and no intracellular domain or a human CD47 intracellular domain comprising a deletion of at least one amino acid, wherein the amino acid sequence of the engineered CD47 protein has 99% identity or less to SEQ ID NO:1 and has 99% identity or less to SEQ ID NO:6.

[0148] In some embodiments, the human CD47 extracellular domain in the engineered CD47 protein is a wild-type human CD47 extracellular domain. In some embodiments, the wild-type domain has an amino acid sequence corresponding to amino acids 19-141 of SEQ ID NO:1, or to amino acids 1-96 of SEQ ID NO:6.

[0149] In some embodiments, the human CD47 extracellular domain in the engineered CD47 protein has an amino acid sequence with at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to amino acids 19-141 of SEQ ID NO:1, or to amino acids 1-96 of SEQ ID NO:6. In some embodiments, the human CD47 extracellular domain in the engineered CD47 protein has an amino acid sequence with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to amino acids 19-141 of SEQ ID NO:1, or to amino acids 1-96 of SEQ ID NO:6.

[0150] In some embodiments, the human CD47 extracellular domain in the engineered CD47 protein is structurally equivalent to a wild-type human CD47 extracellular domain.

[0151] As used herein, "structurally equivalent" refers to two amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity. Preferably, the sequence variation does not change the engineered CD47 protein's biological activity. In some embodiments, the sequence variation does not prevent the engineered human protein from binding to SIRPα. In some embodiments, the sequence variation does not prevent the engineered human protein from being a tolerogenic factor. In some embodiments, at least a portion of the sequence variation may occur through conservative amino acid substitution(s).

[0152] In some embodiments, an engineered protein of the present disclosure comprises one or more membrane tethers. In some embodiments, one or more membrane tethers are or comprise a transmembrane domain. In some embodiments, a transmembrane domain comprises a GPCR transmembrane domain selected from the group consisting of: 5-hydroxytryptamine (serotonin) receptor 1A (HTR1A), 5-hydroxytryptamine (serotonin) receptor 1B (HTR1B), 5-hydroxytryptamine (serotonin) receptor 1D (HTR1D), 5-hydroxytryptamine (serotonin) receptor 1E (HTR1E), 5-hydroxytryptamine (serotonin) receptor 1F (HTR1F), 5-hydroxytryptamine (serotonin) receptor 2A (HTR2A), 5-hydroxytryptamine (serotonin) receptor 2B (HTR2B), 5-hydroxytryptamine (serotonin) receptor 2C (HTR2C), 5-hydroxytryptamine (serotonin) receptor 4 (HTR4), 5-hydroxytryptamine (serotonin) receptor 5A (HTR5A), 5-hydroxytryptamine (serotonin) receptor 5B (HTR5BP), 5-hydroxytryptamine (serotonin) receptor 6 (HTR6), 5-hydroxytryptamine (serotonin) receptor 7, adenylate cyclase-coupled (HTR7), cholinergic receptor, muscarinic 1

(CHRM1), cholinergic receptor, muscarinic 2 (CHRM2), cholinergic receptor, muscarinic 3 (CHRM3), cholinergic receptor, muscarinic 4 (CHRM4), cholinergic receptor, muscarinic 5 (CHRM5), adenosine A1 receptor (ADORA1), adenosine A2a receptor (ADORA2A), adenosine A2b receptor (ADORA2B), adenosine A3 receptor (ADORA3), adhesion G protein-coupled receptor A1 (ADGRA1), adhesion G protein-coupled receptor A2 (ADGRA2), adhesion G protein-coupled receptor A3 (ADGRA3), adhesion G protein-coupled receptor B1 (ADGRB1), adhesion G protein-coupled receptor B2 (ADGRB2), adhesion G protein-coupled receptor B3 (ADGRB3), cadherin EGF LAG seven-pass G-type receptor 1 (CELSR1), cadherin EGF LAG seven-pass G-type receptor 2 (CELSR2), cadherin EGF LAG seven-pass G-type receptor 3 (CELSR3), adhesion G protein-coupled receptor D1 (ADGRD1), adhesion G protein-coupled receptor D2 (ADGRD2), adhesion G protein-coupled receptor E1 (ADGRE1), adhesion G protein-coupled receptor E2 (ADGRE2), adhesion G protein-coupled receptor E3 (ADGRE3), adhesion G protein-coupled receptor E4 (ADGRE4P), adhesion G protein-coupled receptor E5 (ADGRE5), adhesion G protein-coupled receptor F1 (ADGRF1), adhesion G protein-coupled receptor F2 (ADGRF2), adhesion G protein-coupled receptor F3 (ADGRF3), adhesion G protein-coupled receptor F4 (ADGRF4), adhesion G protein-coupled receptor F5 (ADGRF5), adhesion G protein-coupled receptor G1 (ADGRG1), adhesion G protein-coupled receptor G2 (ADGRG2), adhesion G protein-coupled receptor G3 (ADGRG3), adhesion G protein-coupled receptor G4 (ADGRG4), adhesion G protein-coupled receptor G5 (ADGRG5), adhesion G protein-coupled receptor G6 (ADGRG6), adhesion G protein-coupled receptor G7 (ADGRG7), adhesion G protein-coupled receptor L1 (ADGRL1), adhesion G protein-coupled receptor L2 (ADGRL2), adhesion G protein-coupled receptor L3 (ADGRL3), adhesion G protein-coupled receptor L4 (ADGRL4), adhesion G protein-coupled receptor V1 (ADGRV1), adrenoceptor alpha 1A (ADRA1A), adrenoceptor alpha 1B (ADRA1B), adrenoceptor alpha 1D (ADRA1D), adrenoceptor alpha 2A (ADRA2A), adrenoceptor alpha 2B (ADRA2B), adrenoceptor alpha 2C (ADRA2C), adrenoceptor beta 1 (ADRB1), adrenoceptor beta 2 (ADRB2), adrenoceptor beta 3 (ADRB3), angiotensin II receptor type 1 (AGTR1), angiotensin II receptor type 2 (AGTR2), apelin receptor (APLNR), G protein-coupled bile acid receptor 1 (GPBAR1), neuromedin B receptor (NMBR), gastrin releasing peptide receptor (GRPR), bombesin like receptor 3 (BRS3), bradykinin receptor B1 (BDKRB1), bradykinin receptor B2 (BDKRB2), calcitonin receptor (CALCR), calcitonin receptor like receptor (CALCRL), calcium sensing receptor (CASR), G protein-coupled receptor, class C (GPRC6A), cannabinoid receptor 1 (brain) (CNR1), cannabinoid receptor 2 (CNR2), chemerin chemokine-like receptor 1 (CMKLR1), chemokine (C—C motif) receptor 1 (CCR1), chemokine (C—C motif) receptor 2 (CCR2), chemokine (C—C motif) receptor 3 (CCR3), chemokine (C—C motif) receptor 4 (CCR4), chemokine (C—C motif) receptor 5 (gene/pseudogene) (CCR5), chemokine (C—C motif) receptor 6 (CCR6), chemokine (C—C motif) receptor 7 (CCR7), chemokine (C—C motif) receptor 8 (CCR8), chemokine (C—C motif) receptor 9 (CCR9), chemokine (C—C motif) receptor 10 (CCR10), chemokine (C—X—C motif) receptor 1 (CXCR1), chemokine (C—X—C motif) receptor 2 (CXCR2), chemokine (C—X—C motif) receptor 3 (CXCR3), chemokine (C—X—C motif) receptor 4 (CXCR4), chemokine (C—X—C motif) receptor 5 (CXCR5), chemokine (C—X—C motif) receptor 6 (CXCR6), chemokine (C—X3-C motif) receptor 1 (CX3CR1), chemokine (C motif) receptor 1 (XCR1), atypical chemokine receptor 1 (Duffy blood group) (ACKR1), atypical chemokine receptor 2 (ACKR2), atypical chemokine receptor 3 (ACKR3), atypical chemokine receptor 4 (ACKR4), chemokine (C—C motif) receptor-like 2 (CCRL2), cholecystokinin A receptor (CCKAR), cholecystokinin B receptor (CCKBR), G protein-coupled receptor 1 (GPR1), bombesin like receptor 3 (BRS3), G protein-coupled receptor 3 (GPR3), G protein-coupled receptor 4 (GPR4), G protein-coupled receptor 6 (GPR6), G protein-coupled receptor 12 (GPR12), G protein-coupled receptor 15 (GPR15), G protein-coupled receptor 17 (GPR17), G protein-coupled receptor 18 (GPR18), G protein-coupled receptor 19 (GPR19), G protein-coupled receptor 20 (GPR20), G protein-coupled receptor 21 (GPR21), G protein-coupled receptor 22 (GPR22), G protein-coupled receptor 25 (GPR25), G protein-coupled receptor 26 (GPR26), G protein-coupled receptor 27 (GPR27), G protein-coupled receptor 31 (GPR31), G protein-coupled receptor 32 (GPR32), G

protein-coupled receptor 33 (gene/pseudogene) (GPR33), G protein-coupled receptor 34 (GPR34), G protein-coupled receptor 35 (GPR35), G protein-coupled receptor 37 (endothelin receptor type B-like) (GPR37), G protein-coupled receptor 37 like 1 (GPR37L1), G protein-coupled receptor 39 (GPR39), G protein-coupled receptor 42 (gene/pseudogene) (GPR42), G protein-coupled receptor 45 (GPR45), G protein-coupled receptor 50 (GPR50), G protein-coupled receptor 52 (GPR52), G protein-coupled receptor 55 (GPR55), G protein-coupled receptor 61 (GPR61), G protein-coupled receptor 62 (GPR62), G protein-coupled receptor 63 (GPR63), G protein-coupled receptor 65 (GPR65), G protein-coupled receptor 68 (GPR68), G protein-coupled receptor 75 (GPR75), G protein-coupled receptor 78 (GPR78), G protein-coupled receptor 79 (GPR79), G protein-coupled receptor 82 (GPR82), G protein-coupled receptor 83 (GPR83), G protein-coupled receptor 84 (GPR84), G protein-coupled receptor 85 (GPR85), G protein-coupled receptor 87 (GPR87), G protein-coupled receptor 88 (GPR88), G protein-coupled receptor 101 (GPR101), G protein-coupled receptor 119 (GPR119), G protein-coupled receptor 132 (GPR132), G protein-coupled receptor 135 (GPR135), G protein-coupled receptor 139 (GPR139), G protein-coupled receptor 141 (GPR141), G protein-coupled receptor 142 (GPR142), G protein-coupled receptor 146 (GPR146), G protein-coupled receptor 148 (GPR148), G protein-coupled receptor 149 (GPR149), G protein-coupled receptor 150 (GPR150), G protein-coupled receptor 151 (GPR151), G protein-coupled receptor 152 (GPR152), G protein-coupled receptor 153 (GPR153), G protein-coupled receptor 160 (GPR160), G protein-coupled receptor 161 (GPR161), G protein-coupled receptor 162 (GPR162), G protein-coupled receptor 171 (GPR171), G protein-coupled receptor 173 (GPR173), G protein-coupled receptor 174 (GPR174), G protein-coupled receptor 176 (GPR176), G protein-coupled receptor 182 (GPR182), G protein-coupled receptor 183 (GPR183), leucine-rich repeat containing G protein-coupled receptor 4 (LGR4), leucine-rich repeat containing G protein-coupled receptor 5 (LGR5), leucine-rich repeat containing G protein-coupled receptor 6 (LGR6), MAS1 proto-oncogene (MAS1), MAS1 proto-oncogene like (MAS1L), MAS related GPR family member D (MRGPRD), MAS related GPR family member E (MRGPRE), MAS related GPR family member F (MRGPRF), MAS related GPR family member G (MRGPRG), MAS related GPR family member X1 (MRGPRX1), MAS related GPR family member X2 (MRGPRX2), MAS related GPR family member X3 (MRGPRX3), MAS related GPR family member X4 (MRGPRX4), opsin 3 (OPN3), opsin 4 (OPN4), opsin 5 (OPN5), purinergic receptor P2Y (P2RY8), purinergic receptor P2Y (P2RY10), trace amine associated receptor 2 (TAAR2), trace amine associated receptor 3 (gene/pseudogene) (TAAR3), trace amine associated receptor 4 (TAAR4P), trace amine associated receptor 5 (TAAR5), trace amine associated receptor 6 (TAAR6), trace amine associated receptor 8 (TAAR8), trace amine associated receptor 9 (gene/pseudogene) (TAAR9), G protein-coupled receptor 156 (GPR156), G protein-coupled receptor 158 (GPR158), G protein-coupled receptor 179 (GPR179), G protein-coupled receptor, class C (GPRC5A), G protein-coupled receptor, class C (GPRC5B), G protein-coupled receptor, class C (GPRC5C), G protein-coupled receptor, class C (GPRC5D), frizzled class receptor 1 (FZD1), frizzled class receptor 2 (FZD2), frizzled class receptor 3 (FZD3), frizzled class receptor 4 (FZD4), frizzled class receptor 5 (FZD5), frizzled class receptor 6 (FZD6), frizzled class receptor 7 (FZD7), frizzled class receptor 8 (FZD8), frizzled class receptor 9 (FZD9), frizzled class receptor 10 (FZD10), smoothened, frizzled class receptor (SMO), complement component 3a receptor 1 (C3AR1), complement component 5a receptor 1 (C5AR1), complement component 5a receptor 2 (C5AR2), corticotropin releasing hormone receptor 1 (CRHR1), corticotropin releasing hormone receptor 2 (CRHR2), dopamine receptor D1 (DRD1), dopamine receptor D2 (DRD2), dopamine receptor D3 (DRD3), dopamine receptor D4 (DRD4), dopamine receptor D5 (DRD5), endothelin receptor type A (EDNRA), endothelin receptor type B (EDNRB), formyl peptide receptor 1 (FPR1), formyl peptide receptor 2 (FPR2), formyl peptide receptor 3 (FPR3), free fatty acid receptor 1 (FFAR1), free fatty acid receptor 2 (FFAR2), free fatty acid receptor 3 (FFAR3), free fatty acid receptor 4 (FFAR4), G protein-coupled receptor 42 (gene/pseudogene) (GPR42), gamma-aminobutyric acid (GABA) B receptor, 1 (GABBR1), gamma-aminobutyric acid (GABA) B receptor, 2 (GABBR2), galanin receptor 1 (GALR1), galanin

receptor 2 (GALR2), galanin receptor 3 (GALR3), growth hormone secretagogue receptor (GHSR), growth hormone releasing hormone receptor (GHRHR), gastric inhibitory polypeptide receptor (GIPR), glucagon like peptide 1 receptor (GLP1R), glucagon-like peptide 2 receptor (GLP2R), glucagon receptor (GCGR), secretin receptor (SCTR), follicle stimulating hormone receptor (FSHR), luteinizing hormone/choriogonadotropin receptor (LHCGR), thyroid stimulating hormone receptor (TSHR), gonadotropin releasing hormone receptor (GNRHR), gonadotropin releasing hormone receptor 2 (pseudogene) (GNRHR2), G protein-coupled receptor 18 (GPR18), G protein-coupled receptor 55 (GPR55), G protein-coupled receptor 119 (GPR119), G protein-coupled estrogen receptor 1 (GPER1), histamine receptor H1 (HRH1), histamine receptor H2 (HRH2), histamine receptor H3 (HRH3), histamine receptor H4 (HRH4), hydroxycarboxylic acid receptor 1 (HCAR1), hydroxycarboxylic acid receptor 2 (HCAR2), hydroxycarboxylic acid receptor 3 (HCAR3), KISS1 receptor (KISS1R), leukotriene B4 receptor (LTB4R), leukotriene B4 receptor 2 (LTB4R2), cysteinyl leukotriene receptor 1 (CYSLTR1), cysteinyl leukotriene receptor 2 (CYSLTR2), oxoecicosanoid (OXE) receptor 1 (OXER1), formyl peptide receptor 2 (FPR2), lysophosphatidic acid receptor 1 (LPAR1), lysophosphatidic acid receptor 2 (LPAR2), lysophosphatidic acid receptor 3 (LPAR3), lysophosphatidic acid receptor 4 (LPAR4), lysophosphatidic acid receptor 5 (LPAR5), lysophosphatidic acid receptor 6 (LPAR6), sphingosine-1-phosphate receptor 1 (S1PR1), sphingosine-1-phosphate receptor 2 (S1PR2), sphingosine-1-phosphate receptor 3 (S1PR3), sphingosine-1-phosphate receptor 4 (S1PR4), sphingosine-1-phosphate receptor 5 (S1PR5), melanin concentrating hormone receptor 1 (MCHR1), melanin concentrating hormone receptor 2 (MCHR2), melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor) (MC1R), melanocortin 2 receptor (adrenocorticotrophic hormone) (MC2R), melanocortin 3 receptor (MC3R), melanocortin 4 receptor (MC4R), melanocortin 5 receptor (MC5R), melatonin receptor 1A (MTNR1A), melatonin receptor 1B (MTNR1B), glutamate receptor, metabotropic 1 (GRM1), glutamate receptor, metabotropic 2 (GRM2), glutamate receptor, metabotropic 3 (GRM3), glutamate receptor, metabotropic 4 (GRM4), glutamate receptor, metabotropic 5 (GRM5), glutamate receptor, metabotropic 6 (GRM6), glutamate receptor, metabotropic 7 (GRM7), glutamate receptor, metabotropic 8 (GRM8), motilin receptor (MLNR), neuromedin U receptor 1 (NMUR1), neuromedin U receptor 2 (NMUR2), neuropeptide FF receptor 1 (NPFFR1), neuropeptide FF receptor 2 (NPFFR2), neuropeptide S receptor 1 (NPSR1), neuropeptides B/W receptor 1 (NPBWR1), neuropeptides B/W receptor 2 (NPBWR2), neuropeptide Y receptor Y1 (NPY1R), neuropeptide Y receptor Y2 (NPY2R), neuropeptide Y receptor Y4 (NPY4R), neuropeptide Y receptor Y5 (NPY5R), neuropeptide Y receptor Y6 (pseudogene) (NPY6R), neurotensin receptor 1 (high affinity) (NTSR1), neurotensin receptor 2 (NTSR2), opioid receptor, delta 1 (OPRD1), opioid receptor, kappa 1 (OPRK1), opioid receptor, mu 1 (OPRM1), opiate receptor-like 1 (OPRL1), hypocretin (orexin) receptor 1 (HCRTR1), hypocretin (orexin) receptor 2 (HCRTR2), G protein-coupled receptor 107 (GPR107), G protein-coupled receptor 137 (GPR137), olfactory receptor family 51 subfamily E member 1 (OR51E1), transmembrane protein, adipocyte associated 1 (TPRA1), G protein-coupled receptor 143 (GPR143), G protein-coupled receptor 157 (GPR157), oxoglutarate (alpha-ketoglutarate) receptor 1 (OXGR1), purinergic receptor P2Y (P2RY1), purinergic receptor P2Y (P2RY2), pyrimidinergic receptor P2Y (P2RY4), pyrimidinergic receptor P2Y (P2RY6), purinergic receptor P2Y (P2RY11), purinergic receptor P2Y (P2RY12), purinergic receptor P2Y (P2RY13), purinergic receptor P2Y (P2RY14), parathyroid hormone 1 receptor (PTH1R), parathyroid hormone 2 receptor (PTH2R), platelet-activating factor receptor (PTAFR), prokineticin receptor 1 (PROKR1), prokineticin receptor 2 (PROKR2), prolactin releasing hormone receptor (PRLHR), prostaglandin D2 receptor (DP) (PTGDR), prostaglandin D2 receptor 2 (PTGDR2), prostaglandin E receptor 1 (PTGER1), prostaglandin E receptor 2 (PTGER2), prostaglandin E receptor 3 (PTGER3), prostaglandin E receptor 4 (PTGER4), prostaglandin F receptor (PTGFR), prostaglandin 12 (prostacyclin) receptor (IP) (PTGIR), thromboxane A2 receptor (TBXA2R), coagulation factor II thrombin receptor (F2R), F2R like trypsin receptor 1 (F2RL1), coagulation factor II thrombin receptor like 2 (F2RL2), F2R

like thrombin/trypsin receptor 3 (F2RL3), pyroglutamylated RFamide peptide receptor (QRFPR), relaxin/insulin-like family peptide receptor 1 (RXFP1), relaxin/insulin-like family peptide receptor 2 (RXFP2), relaxin/insulin-like family peptide receptor 3 (RXFP3), relaxin/insulin-like family peptide receptor 4 (RXFP4), somatostatin receptor 1 (SSTR1), somatostatin receptor 2 (SSTR2), somatostatin receptor 3 (SSTR3), somatostatin receptor 4 (SSTR4), somatostatin receptor 5 (SSTR5), succinate receptor 1 (SUCNR1), tachykinin receptor 1 (TACR1), tachykinin receptor 2 (TACR2), tachykinin receptor 3 (TACR3), taste 1 receptor member 1 (TAS1R1), taste 1 receptor member 2 (TAS1R2), taste 1 receptor member 3 (TAS1R3), taste 2 receptor member 1 (TAS2R1), taste 2 receptor member 3 (TAS2R3), taste 2 receptor member 4 (TAS2R4), taste 2 receptor member 5 (TAS2R5), taste 2 receptor member 7 (TAS2R7), taste 2 receptor member 8 (TAS2R8), taste 2 receptor member 9 (TAS2R9), taste 2 receptor member 10 (TAS2R10), taste 2 receptor member 13 (TAS2R13), taste 2 receptor member 14 (TAS2R14), taste 2 receptor member 16 (TAS2R16), taste 2 receptor member 19 (TAS2R19), taste 2 receptor member 20 (TAS2R20), taste 2 receptor member 30 (TAS2R30), taste 2 receptor member 31 (TAS2R31), taste 2 receptor member 38 (TAS2R38), taste 2 receptor member 39 (TAS2R39), taste 2 receptor member 40 (TAS2R40), taste 2 receptor member 41 (TAS2R41), taste 2 receptor member 42 (TAS2R42), taste 2 receptor member 43 (TAS2R43), taste 2 receptor member 45 (TAS2R45), taste 2 receptor member 46 (TAS2R46), taste 2 receptor member 50 (TAS2R50), taste 2 receptor member 60 (TAS2R60), thyrotropin-releasing hormone receptor (TRHR), trace amine associated receptor 1 (TAAR1), urotensin 2 receptor (UTS2R), arginine vasopressin receptor 1A (AVPR1A), arginine vasopressin receptor 1B (AVPR1B), arginine vasopressin receptor 2 (AVPR2), oxytocin receptor (OXTR), adenylate cyclase activating polypeptide 1 (pituitary) receptor type I (ADCYAP1R1), vasoactive intestinal peptide receptor 1 (VIPR1), vasoactive intestinal peptide receptor 2 (VIPR2), and any variant thereof. In some embodiments, a transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNCR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.

[0153] Plasma membrane proteins can be attached to the peripheral membrane or can be integral membrane proteins. See, for example, a review in Komath SS, Fujita M, Hart GW, et al. Glycosylphosphatidylinositol Anchors. In: Varki A, Cummings RD, Esko JD, et al., editors. *Essentials of Glycobiology*. 4th edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2022. Chapter 12. GPI anchorage refers to the attachment of glycosylphosphatidylinositol, or GPI, to the C-terminus of a protein during posttranslational modification. In some embodiments, a heterologous membrane attachment sequence is a GPI anchor attachment sequence. Proteins that are attached to GPI anchors via their C-terminus are typically found in the outer lipid bilayer. GPI anchors are alternatives to the single transmembrane domain of type-I integral membrane proteins.

[0154] A heterologous GPI anchor attachment sequence can be derived from any known GPI-anchored protein (reviewed in Ferguson MAJ, Kinoshita T, Hart GW. Glycosylphosphatidylinositol Anchors. In: Varki A, Cummings RD, Esko JD, et al., editors. *Essentials of Glycobiology*. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 11). In some embodiments, a heterologous GPI anchor attachment sequence is a GPI anchor attachment sequence from CD14, CD16, CD48, DAF/CD55, CD59, CD80, CD87, or TRAIL-R3. In some embodiments, a heterologous GPI anchor attachment sequence is derived from DAF/CD55. In some embodiments, a heterologous GPI anchor attachment sequence is derived from CD59. In some embodiments, a heterologous GPI anchor attachment sequence is derived from TRAIL-R3. In illustrative embodiments, a heterologous GPI anchor attachment sequence is derived from DAF/CD55, CD59, or TRAIL-R3. In some embodiments, one or both of the activation elements include a heterologous signal sequence to help direct expression of the activation element to the cell membrane. Any signal sequence that is active in the packaging cell line can be used. In some

embodiments, a signal sequence is a DAF/CD55 signal sequence. In some embodiments, a signal sequence is a CD59 signal sequence. In some embodiments, a signal sequence is a TRAIL-R3 signal sequence.

[0155] In some embodiments, the engineered CD47 protein comprises one or more wild-type human CD47 transmembrane domains. In some embodiments, the wild-type domain has an amino acid sequence corresponding to amino acids 142-162, 177-197, 208-228, 236-257, or 269-289 of SEQ ID NO:2.

[0156] In some embodiments, the engineered CD47 protein comprises one or more transmembrane domains that are structurally equivalent to a wild-type human CD47 transmembrane domain. In some embodiments, the engineered CD47 protein comprises one or more transmembrane domains having an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to amino acids 142-162, 177-197, 208-228, 236-257, or 269-289 of SEQ ID NO:2.

[0157] In some embodiments, the human CD47 intracellular domain in the engineered CD47 protein is a wild-type human CD47 intracellular domain. In some embodiments, the wild-type intracellular domain has an amino acid sequence corresponding to amino acids 290-323 of SEQ ID NO:2.

[0158] In some embodiments, the human CD47 intracellular domain in the engineered CD47 protein is structurally equivalent to a wild-type human CD47 intracellular domain. In some embodiments, the human CD47 intracellular domain in the engineered CD47 protein has an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to amino acids 290-323 of SEQ ID NO:2.

[0159] In some embodiments, the engineered CD47 protein comprises an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO:7.

[0160] In some embodiments, the engineered CD47 protein comprises an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO:8.

[0161] In some embodiments, the engineered CD47 protein comprises an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO:9.

[0162] In some embodiments, the engineered CD47 protein comprises an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO:12.

[0163] In some embodiments, the engineered CD47 protein comprises an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO:10.

[0164] In some embodiments, the engineered CD47 protein comprises an amino acid sequence with at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence set forth in SEQ ID NO:11.

[0165] In some embodiments, the engineered CD47 protein is a transmembrane protein. A transmembrane protein is an integral membrane protein that spans the entirety of the cell membrane and has both intracellular and extracellular portions. As used herein, "intracellular portion" can include the CD47 intracellular domain and the intracellular connections, when present in the molecule. As used herein, "extracellular portion" can include the CD47 extracellular domain and the extracellular connections, when present in the molecule.

[0166] As used herein, a wild-type human CD47 extracellular domain refers to the extracellular domain of any one of the wild-type human CD47 protein isoforms. A wild-type human CD47 transmembrane domain refers to a transmembrane domain of any one of the wild-type human CD47 protein isoforms. A wild-type human CD47 intracellular domain refers to the intracellular domain of any one of the wild-type human CD47 protein isoforms.

[0167] In some embodiments, the engineered CD47 protein is an engineered human CD47 protein, an engineered humanized CD47 protein, or an engineered partially-humanized CD47 protein.

[0168] As used herein, "humanized" or "humanization" means that the amino acid sequence of the engineered CD47 protein is modified to reduce its immunogenicity in humans. As used herein, "partially-humanized" or "partial humanization" means that a portion of the amino acid sequence of the engineered CD47 protein is modified to reduce the engineered CD47 protein's immunogenicity in humans. For example, in some embodiments, the extracellular domain of the engineered CD47 protein is modified to reduce the engineered CD47 protein's immunogenicity in humans. Humanization is usually achieved by modifying a protein sequence from a non-human source to increase its similarity to its counterpart protein produced naturally in humans. Two major approaches have been used to humanize proteins: rational design and empirical methods. The rational design methods are characterized by protein structural modeling, generating a few variants of the protein and assessing their binding or any other property of interest. In contrast to the rational design methods, empirical methods do not require the structure information of the protein. They depend on the generation of large combinatorial libraries and selection of the desired variants by enrichment technologies such as phage, ribosome or yeast display, or by high throughput screening techniques. These methods rest on selection rather than making assumptions on the impact of mutations on the protein structure. For example, in some embodiments, humanization of the engineered CD47 protein comprises grafting the SIRP α binding region in the engineered CD47 protein onto a human CD47 protein. In other embodiments, humanization of the engineered CD47 protein comprises introducing one or more point mutations in the engineered CD47 protein so that one or more residue(s) in the engineered CD47 protein is substituted with the corresponding residue in a human CD47 protein.

[0169] In some embodiments, an engineered protein of the present disclosure comprises a SIRP α interaction motif comprising a SIRP α antibody. In some embodiments, a SIRP α antibody is selected from the antibodies listed in Table 3 of Int'l App. No. PCT/US2023/013364 incorporated herein by reference in its entirety.

Glycosylation

[0170] The human CD47 protein is glycosylated. Protein glycosylation involves the covalent attachment of glycans (also called carbohydrates, saccharides, or sugars) to a protein. Based on the amino acid side-chain atoms to which glycans are linked, most protein glycosylations fall within two categories: N-linked glycosylation and O-linked glycosylation. In N-linked glycosylation, glycans are attached to the side-chain nitrogen atoms of asparagine residues in a conserved consensus sequence Asn-Xaa-Ser/Thr (Xaa $\neq$ Pro), whereas in O-linked glycosylation, glycans are attached to the side-chain oxygen atoms of hydroxyl amino acids, primarily serine and threonine residues.

[0171] The IgV domain of wild type human CD47 protein is N-glycosylated and modified with O-linked glycosaminoglycans. The human CD47 protein can be expressed as a proteoglycan with a molecular weight of >250kDa, having both heparan and chondroitin sulfate glycosaminoglycan (GAG) chains at Ser⁶⁴ and Ser⁷⁹ (Kaur et al., J. Biological Chemistry, 2011). Heparan sulfate (HSGAG) and chondroitin sulfate (CSGAG) are synthesized in the Golgi apparatus, where protein cores made in the rough endoplasmic reticulum are post-translationally modified with O-linked glycosylation by glycosyltransferases forming proteoglycans. In addition, N-linked glycosylation has been identified at four of the five potential modification sites (N¹⁶, N³², N⁵⁵, and N⁹³) in the human CD47 protein (Hatherley et al., Cell, 2008). The numbering of amino acid in this paragraph is based on SEQ ID NO 3 (i.e., mature, full length, wild type CD47).

[0172] In some embodiments, the engineered CD47 protein comprises fewer glycosylation modification sites than a wild-type human CD47 protein. A glycosylation modification site refers to a sequence of consecutive amino acids in a protein that can serve as the attachment site for a glycan. Glycosylation modification sites are also called sequons.

In some embodiments, one or more amino acid(s) within the glycosaminoglycan modification site of a wild-type human CD47 protein is deleted or substituted in the engineered CD47 protein. In some embodiments, the engineered CD47 protein has 0, 1, 2, 3, 4, 5, or 6 glycosylation site(s).

[0173] In some embodiments, the engineered CD47 protein comprises fewer glycosylation modifications than a wild-type human CD47 protein. The glycosylation modifications include, but are not limited to, N-glycosylation, O glycosylation, phosphoserine glycosylation, and C-glycosylation. In some embodiments, the engineered CD47 protein has 0, 1, 2, 3, 4, or 5 glycosylation modification(s).

[0174] In some embodiments, the engineered CD47 protein comprises fewer glycosaminoglycan modification sites than a wild-type human CD47 protein. A glycosaminoglycan modification site refers to a sequence of consecutive amino acids in a protein that can serve as the attachment site for a glycosaminoglycan. In some embodiments, one or more amino acid(s) within the glycosaminoglycan modification site of a wild-type human CD47 protein is deleted or substituted in the engineered CD47 protein. In some embodiments, the engineered CD47 protein has 0 or 1 glycosaminoglycan modification site.

[0175] In some embodiments, the engineered CD47 protein comprises fewer glycosaminoglycan chains than a wild-type human CD47 protein. Glycosaminoglycan chains include, but are not limited to, heparan sulfate (HSGAG), chondroitin sulfate (CSGAG), keratan sulfate, and hyaluronic acid. In some embodiments, the engineered CD47 protein has 0 or 1 glycosaminoglycan side chain.

[0176] In some embodiments, the engineered CD47 protein comprises fewer than two heparan and/or chondroitin sulfate glycosaminoglycan modification sites. In some embodiments, one or more amino acid(s) within the glycosaminoglycan modification sites of a wild-type human CD47 protein is deleted or substituted in the engineered CD47 protein. In some embodiments, the engineered CD47 protein comprises one heparan and/or chondroitin sulfate glycosaminoglycan modification site. In some embodiments, the engineered CD47 protein comprises no heparan and/or chondroitin sulfate glycosaminoglycan modification sites.

[0177] In some embodiments, the engineered CD47 protein comprises fewer than two heparan and/or chondroitin sulfate glycosaminoglycan chains. In some embodiments, the engineered CD47 protein has 0 or 1 heparan and/or chondroitin sulfate glycosaminoglycan chains.

[0178] In some embodiments, the engineered CD47 protein comprises fewer N-linked glycosylation sites than a wild-type human CD47 protein. An N-linked glycosylation site is a sequence of consecutive amino acids in a protein that can serve as the attachment site for a saccharide, particularly an N-glycan. In some embodiments, the engineered CD47 protein has 0, 1, 2, 3, or 4 N-linked glycosylation site(s).

[0179] In some embodiments, the engineered CD47 protein comprises fewer N-linked glycosylation modifications than a wild-type human CD47 protein. In some embodiments, the engineered CD47 protein has 0, 1, 2, or 3 N-linked glycosylation modification(s).

Biological Activity of Engineered CD47 protein

[0180] In some embodiments the engineered CD47 protein is functionally equivalent to a wild-type human CD47 protein. As used herein, "functionally equivalent" refers to having at least one type of biological activity of a wild-type human CD47 protein. The types of biological activity of a wild-type human CD47 protein include, but are not limited to, the ability to interact with TSP-1, integrins, another CD47 protein, or SIRPα. Preferably, the engineered CD47 protein can bind to SIRPα.

Interaction with TSP-1

[0181] A major secreted ligand for CD47 is thrombospondin-1 (TSP-1). TSP-1 is a homotrimeric glycoprotein, and its C-terminal domain mediates binding to CD47 (Isenberg et al., 2009). At least two additional members of the thrombospondin family bind to CD47, albeit with lower affinity. TSP-1 is the prototypic member of the thrombospondin family of extracellular matrix glycoproteins, which are implicated in regulating cell motility, proliferation, and differentiation. The extracellular IgV domain of CD47 is a receptor for the C-terminal cell-binding domain (CBD) of TSP-1. Mutagenesis studies established that heparan sulfate modification of CD47 is required for high affinity interaction of TSP-1 with CD47 (Kaur et al., 2011).

[0182] In some embodiments, the engineered CD47 protein lacks one or more thrombospondin-1 binding site(s) compared to a wild-type human CD47 protein. In some embodiments, one or more amino acid(s) within the TSP-1 site of a wild-type human CD47 protein is deleted or substituted in the engineered CD47 protein. In some embodiments, the engineered CD47 protein lacking one or more TSP-1 binding sites is not glycosylated by one or more heparan sulfate.

[0183] In some embodiments, the engineered CD47 protein binds to TSP-1 with lower affinity compared to a wild-type human CD47 protein. In some embodiments, the engineered CD47 protein binds to TSP-1 with a K_D higher than about 0.1 μ M, 0.2 μ M, 0.3 μ M, 0.4 μ M, 0.5 μ M, 0.6 μ M, 0.7 μ M, 0.8 μ M, 0.9 μ M, 1 μ M, 2 μ M, 3 μ M, 4 μ M, 5 μ M, 6 μ M, 7 μ M, 8 μ M, 9 μ M, 10 μ M, 20 μ M, 30 μ M, 40 μ M, 50 μ M, 60 μ M, 70 μ M, 80 μ M, 90 μ M, 100 μ M. In some embodiments, the engineered CD47 protein cannot bind to TSP-1.

Interaction with integrins

[0184] Early studies of CD47 were based on the use of monoclonal antibodies (mAbs) raised against the CD47 protein purified from placenta. These studies showed a role of CD47 in enhancing the IgG-mediated phagocytosis response in the presence of RGD-containing ligands, such as fibronectin, fibrinogen, vitronectin, or collagen type IV. The same mAbs were also found to block neutrophil transendothelial migration stimulated by interleukin 8 (IL-8) or the bacterial peptide N-formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe) and to inhibit neutrophil migration across tumor-necrosis-factor- α (TNF α) stimulated endothelial cells. These studies revealed that CD47 on both the neutrophils and the endothelial cells was important. Generation of other anti-CD47mAbs, raised against epithelial membrane preparations, showed that CD47 is present at the basolateral membrane of epithelial cell monolayers, that mAbs blocking CD47 on either neutrophils or the epithelial cells delay neutrophil trans-epithelial migration, and that efficient neutrophil chemotaxis correlates with an increased neutrophil cell surface expression of CD47. The basement membrane protein entactin, which contains an RGD sequence, was also found to stimulate neutrophil adhesion and chemotaxis in a CD47-dependent manner in vitro. Generation of CD47-deficient mice further proved the importance of this protein in regulating neutrophil inflammatory responses, by showing an increased sensitivity to bacterial infection due to a delayed neutrophil accumulation in bacterial peritonitis. CD47-deficient neutrophils also show a strongly impaired RGD-stimulated neutrophil adhesion, phagocytosis, and respiratory burst. For $\alpha_v\beta_3$ integrin-mediated cellular responses to the extracellular matrix protein vitronectin, CD47 was found to be required for $\alpha_v\beta_3$ -mediated binding to vitronectin-coated beads, but not $\alpha_v\beta_3$ -mediated adhesion to vitronectin-coated surfaces. In addition to its original association with $\alpha_v\beta_3$ integrins, CD47 has also been shown to interact with and regulate the integrins $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ on platelets, the $\alpha_2\beta_1$ integrin on smooth muscle cells, the $\alpha_4\beta_1$ integrin on sickle red blood cells and B lymphocytes, the $\alpha_6\beta_1$ integrin in microglia, and the α_5 integrin in chondrocytes.

[0185] In some embodiments, the engineered CD47 protein lacks one or more integrin binding site(s) compared to a wild-type human CD47 protein. In some embodiments, one or more amino acid(s) within the integrin binding site of a wild-type human CD47 protein is deleted or substituted in the engineered CD47 protein.

[0186] In some embodiments, the engineered CD47 protein binds to integrin with lower affinity compared to a wild-type human CD47 protein. In some embodiments, the engineered CD47 protein binds to integrin with a K_D higher than about 0.1 μ M, 0.2 μ M, 0.3 μ M, 0.4 μ M, 0.5 μ M, 0.6 μ M, 0.7 μ M, 0.8 μ M, 0.9 μ M, 1 μ M, 2 μ M, 3 μ M, 4 μ M, 5 μ M, 6 μ M, 7 μ M, 8 μ M, 9 μ M, 10 μ M, 20 μ M, 30 μ M, 40 μ M, 50 μ M, 60 μ M, 70 μ M, 80 μ M, 90 μ M, 100 μ M. In some embodiments, the engineered CD47 protein cannot bind to integrin.

[0187] In some embodiments, the integrin is selected from the group consisting of $\alpha_v\beta_3$ integrin, $\alpha IIb\beta_3$ integrin, $\alpha_2\beta_1$ integrin, $\alpha_4\beta_1$ integrin, $\alpha_6\beta_1$ integrin, and α_5 integrin.

Interaction with SIRP proteins

[0188] The signal regulator protein (SIRP) family contains three members, and of these SIRP α and SIRP γ are known CD47 receptors. SIRP proteins belong to the Ig family of cell surface glycoproteins, and the first member identified was SIRP α (also known as SHPS-1, CD172a, BIT, MFR, or P84). SIRP α is highly expressed in myeloid cells and neurons, but also in endothelial cells and fibroblasts, and has three extracellular Ig-like domains, one distal IgV-like domain, and two membrane proximal IgC-like domains. In addition, an alternatively spliced form having only one IgV domain has also been reported. In its intracellular tail, SIRP α has two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which when phosphorylated, can bind the Src homology 2 (SH2) domain-containing protein-tyrosine phosphatases SHP-1 and SHP-2. Additional cytoplasmic binding partners for SIRP α are the adaptor molecules Src kinase-associated protein of 55 kDa homolog/SKAP2 (SKAP55hom/R), Fyn-binding protein/SLP-76-associated phosphoprotein of 130 kDa (FYB/SLAP-130), and the tyrosine kinase PYK2. SIRP α is also a substrate for the kinase activity of the insulin, EGF, and bPDGF receptors. The overexpression of SIRP α in fibroblasts decreases proliferation and other downstream events in response to insulin, EGF, and bPDGF. Since SIRP α is also constitutively associated with the M-CSF receptor c-fms, SIRP α overexpression partially reverses the v-fms phenotype.

[0189] CD47 is a ligand for SIRP α . The glycosylation of CD47 or SIRP α does not seem to be necessary for their interaction, but the level of N-glycosylation of SIRP α has an impact on the interaction, such that over glycosylation reduces the binding of CD47. The long-range disulfide bond between Cys³³ in the CD47 IgV domain and Cys²⁶³ in the CD47 transmembrane domain is also important to establish an orientation of the CD47 IgV domain that enhances its binding to SIRP α . The numbering of amino acids is based on SEQ ID NO:3 in this paragraph (i.e., the mature, wild-type, full-length CD47 protein).

[0190] The structure of CD47 and the CD47/SIRP α complex have been revealed (Hatherley et al., 2008). Two β -strands, corresponding to amino acids 92-100 and 103-113 of SEQ ID NO:3, were identified to be at the core of the CD47/SIRP α interaction. Amino acids 97 (Glu), 99 (Thr), 100 (Glu), 103 (Arg), 104 (Glu), and 106 (Glu) of SEQ ID NO:3 were identified as contact residues in the CD47/SIRP α interaction and play a role in CD47's binding affinity for SIRP α (Hatherley et al., 2008). In preferred embodiments, the two β -strands and the six contact residues within them are retained in the engineered CD47 proteins disclosed herein to maintain SIRP α binding capability.

[0191] In some embodiments, the engineered CD47 protein comprises at least one SIRP α interaction motif in its extracellular domain. In some embodiments, the amino acids corresponding to amino acids 97, 99, 100, 103, 104, 106 of SEQ ID NO:3 are retained in the engineered CD47 protein. In some embodiments, the two β -strands are retained in the engineered CD47 protein.

[0192] In some embodiments, the engineered CD47 protein comprises a disulfide bond between a cysteine within the human CD47 extracellular domain or portion thereof and a cysteine within or between the human CD47 transmembrane domain(s). In some embodiments, the two cysteines are Cys³³ in the extracellular domain and the Cys²⁶³ in the transmembrane domain, wherein the numbering is based on SEQ ID NO:3.

[0193] In some embodiments, the engineered CD47 protein can bind to SIRPα. In some embodiments, the engineered CD47 protein can bind to SIRPα with a binding affinity that is similar to a wild-type human CD47 protein. In some embodiments, the engineered CD47 protein binds to SIRPα with a K_D lower than about 0.01 μM, 0.02 μM, 0.03 μM, 0.04 μM, 0.05 μM, 0.06 μM, 0.07 μM, 0.08 μM, 0.09 μM, 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1 μM, 2 μM, 3 μM, 4 μM, 5 μM. In some embodiments, the engineered CD47 protein can bind to SIRPα with a higher binding affinity than a wild-type human CD47 protein.

[0194] Direct binding studies demonstrate that TSP-1 inhibits SIRPα binding to cells expressing CD47 (Isenberg et al., 2009). This data is consistent with competitive binding of TSP-1 and SIRPα to a single site on CD47, steric inhibition of binding to distinct but proximal sites, or allosteric inhibition. Glycosylation at Ser⁶⁴ with a heparan sulfate chain is necessary for TSP-1 binding but not SIRPα binding (Soto-Pantoja et al., 2015). Therefore, in some embodiments, the engineered CD47 protein that lacks glycosylation at Ser⁶⁴ has enhanced SIRPα binding capacity because TSP-1 binding is hindered.

[0195] The CD47/SIRPα interaction regulates a multitude of intercellular interactions in many body systems, such as the immune system where it regulates lymphocyte homeostasis, dendritic cell (DC) maturation and activation, proper localization of certain DC subsets in secondary lymphoid organs, and cellular transmigration. The CD47/SIRPα interaction also regulates cells of the nervous system. An interaction between these two proteins also plays an important role in bone remodeling. Cellular responses regulated by the CD47/SIRPα interaction are often dependent on a bidirectional signaling through both receptors.

[0196] CD47 on host cells can function as a "marker of self" and regulate phagocytosis by binding to SIRPα on the surface of circulating immune cells to deliver an inhibitory "don't kill me" signal. As disclosed above, SIRPα encodes an Ig-superfamily receptor expressed on the surface of macrophages and dendritic cells, whose cytoplasmic region contains immunoreceptor tyrosine-based inhibition motifs (ITIMs) that can trigger a cascade to inhibit phagocytosis. CD47-SIRPα binding results in phosphorylation of ITIMs on SIRPα, which triggers recruitment of the SHP1 and SHP2 Src homology phosphatases. These phosphatases, in turn, inhibit accumulation of myosin II at the phagocytic synapse, preventing phagocytosis (Fujioka et al., 1996). Phagocytosis of target cells by macrophages is ultimately regulated by a balance of activating signals (FcγR, CRT, LRP-1) and inhibitory signals (SIRPα-CD47). Elevated expression of CD47 can help the cell evade immune surveillance and subsequent destruction. Elevated expression of CD47 can help the cell evade innate immune cell killing.

[0197] In some embodiments, the engineered CD47 protein is a tolerogenic factor. As used herein, tolerogenic factor is an agent that induces immune tolerance when there is pathological or undesirable activation of the normal immune response. This can occur, for example, when a patient develops an immune reaction to donor antigens after receiving an allogeneic transplantation or an allogeneic cell therapy, or when the body responds inappropriately to self-antigens implicated in autoimmune diseases. In some embodiments, "tolerogenic factor" includes hypoinmunity factors, complement inhibitors, and other factors that modulate or affect the ability of a cell to be recognized by the immune system of a host or recipient subject upon administration, transplantation, or engraftment. In some embodiments, the tolerogenic factor is genetically modified to achieve additional functions.

[0198] In some embodiments, the engineered CD47 protein can inhibit phagocytosis, release of cytotoxic agents, and/or other mechanisms of cell-mediated killing.

Interactions between CD47 molecules

[0199] It has also been shown that CD47 mediates cell adhesion interactions in the absence of any known CD47 ligands. This cell-cell adhesion, which requires CD47 but not any of its known ligands, suggests that homotypic binding can also occur between the IgV domains of CD47 on opposing cells (Rebres et al., 2005). This interaction may require an unidentified trypsin-sensitive protein (X) to mediate cell-cell adhesion, but the potential should be considered that this cell-cell interaction and homotypic binding of proteolytically shed CD47 IgV domain (Maile et al., 2010) or CD47 in exosomes (Kaur et al., 2014) to cell surface CD47 could elicit CD47 signal transduction. However, direct evidence for CD47-CD47 binding and signaling resulting from homotypic CD47 binding is lacking. In some embodiments, the engineered CD47 protein cannot interact with another CD47 molecule.

III. Polynucleotides

[0200] In another aspect, the present disclosure provides a polynucleotide encoding the engineered CD47 protein disclosed herein.

[0201] A polynucleotide encoding the engineered CD47 protein disclosed herein can be obtained by methods known in the art. For example, the polynucleotide can be obtained from cloned DNA (e.g., from a DNA library), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA or fragments thereof, purified from the desired cell. When the polynucleotides are produced by recombinant means, any method known to those skilled in the art for identification of nucleic acids that encode desired genes can be used. Any method available in the art can be used to obtain a full length (i.e. encompassing the entire coding region) cDNA or genomic DNA encoding a desired human CD47 protein, such as from a cell or tissue source. Modified or variant polynucleotides, including truncated forms of CD47 such as provided herein, can be engineered from a wildtype polynucleotide using standard recombinant DNA methods. Polynucleotides can be cloned or isolated using any available methods known in the art for cloning and isolating nucleic acid molecules. Such methods include PCR amplification of nucleic acids and screening of libraries, including nucleic acid hybridization screening, antibody-based screening, and activity-based screening.

[0202] Methods for amplification of polynucleotides can be used to isolate polynucleotides encoding a desired protein, including for example, polymerase chain reaction (PCR) methods. PCR can be carried out using any known methods or procedures in the art. Exemplary methods include use of a Perkin-Elmer Cetus thermal cyclor and Taq polymerase (Gene Amp). A nucleic acid containing gene of interest can be used as a source material from which a desired polypeptide-encoding nucleic acid molecule can be amplified. For example, DNA and mRNA preparations, cell extracts, tissue extracts from an appropriate source (e.g. testis, prostate, breast), fluid samples (e.g. blood, serum, saliva), samples from healthy and/or diseased subjects can be used in amplification methods. The source can be from any eukaryotic species including, but not limited to, vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, and other primate sources. Nucleic acid libraries also can be used as a source material. Primers can be designed to amplify a desired polynucleotide. For example, primers can be designed based on expressed sequences from which a desired polynucleotide is generated. Primers can be designed based on back-translation of a polypeptide amino acid sequence. If desired, degenerate primers can be used for amplification. Oligonucleotide primers that hybridize to sequences at the 3' and 5' termini of the desired sequence can be used as primers to amplify by PCR from a nucleic acid

sample. Primers can be used to amplify the entire full-length polynucleotide, or a truncated sequence thereof. Nucleic acid molecules generated by amplification can be sequenced and confirmed to encode a desired polypeptide.

IV. Vectors

[0203] In another aspect, the present disclosure provides a vector comprising a polynucleotide that encodes the engineered CD47 protein disclosed herein.

[0204] Any methods known to those skilled in the art for the insertion of DNA fragments into a vector can be used to construct expression vectors comprising a polynucleotide disclosed herein. These methods can include in vitro recombinant DNA and synthetic techniques and in vivo (genetic) recombination. The polynucleotide disclosed herein can be operably linked to control sequences in the expression vector(s) to ensure the expression of the engineered CD47 protein. Such control sequences may include, but are not limited to, leader or signal sequences, promoters (e.g., naturally associated or heterologous promoters), ribosomal binding sites, enhancer or activator elements, translational start and termination sequences, and transcription start and termination sequences, and are chosen to be compatible with the host cell chosen to express the engineered CD47 protein. Constitutive or inducible promoters as known in the art are also contemplated. The promoters may be either naturally occurring promoters, hybrid promoters that combine elements of more than one promoter, or synthetic promoters. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome such as in a gene locus. In some embodiment, the expression vector includes a selectable marker gene to allow the selection of transformed host cells. Some embodiments, include an expression vector comprising a nucleotide sequence encoding a variant polypeptide operably linked to at least one regulatory control sequence. Regulatory control sequence for use herein include promoters, enhancers, and other expression control elements. In some embodiments, an expression vector is designed for the choice of the host cell to be transformed, the particular variant polypeptide desired to be expressed, the vector's copy number, the ability to control that copy number, and/or the expression of any other protein encoded by the vector, such as antibiotic markers.

[0205] Examples of suitable mammalian promoters include, for example, promoters from the following genes: elongation factor 1 alpha (EF1 α) promoter, CAG promoter, ubiquitin/S27a promoter of the hamster (WO 97/15664), Simian vacuolating virus 40 (SV40) early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, the long terminal repeat region of Rous Sarcoma Virus (RSV), mouse mammary tumor virus promoter (MMTV), Moloney murine leukemia virus Long Terminal repeat region, and the early promoter of human Cytomegalovirus (CMV). Examples of other heterologous mammalian promoters are the actin, immunoglobulin or heat shock promoter(s). In additional embodiments, promoters for use in mammalian host cells can be obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 Jul. 1989), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40). In further embodiments, heterologous mammalian promoters are used. Examples include the actin promoter, an immunoglobulin promoter, and heat-shock promoters. The early and late promoters of SV40 are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature 273: 113-120 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII restriction enzyme fragment (Greenaway et al., Gene 18: 355-360 (1982)). The foregoing references are incorporated by reference in their entirety.

[0206] In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide.

[0207] The vector can include, but is not limited to, viral vectors and plasmid DNA. Viral vectors can include, but are not limited to, adenoviral vectors, lentiviral vectors, retroviral vectors, and adeno-associated viral vectors. Commonly, expression vectors contain selection markers such as ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance, or neomycin resistance to permit detection of those cells transformed with the desired DNA sequences. Suitable vectors, promoter, and enhancer elements are known in the art; many are commercially available for generating subject recombinant constructs. In some embodiments, the vector is a polycistronic vector. In some embodiments, the vector is a bicistronic vector or a tricistronic vector. Bicistronic or multicistronic expression vectors may include (1) multiple promoters fused to each of the open reading frames; (2) insertion of splicing signals between genes; (3) fusion of genes whose expressions are driven by a single promoter; and (4) insertion of proteolytic cleavage sites between genes (self-cleavage peptide) or insertion of internal ribosomal entry sites (IRESs) between genes.

[0208] A polycistronic vector is used to co-express multiple genes in the same cell. Two strategies are most commonly used to construct a multicistronic vector. First, an Internal Ribosome Entry Site (IRES) element is typically used for bi-cistronic vectors. The IRES element, acting as another ribosome recruitment site, allows initiation of translation from an internal region of the mRNA. Thus, two proteins are translated from one mRNA. IRES elements are quite large (usually 500-600 bp) (Pelletier et al., 1988; Jang et al., 1988). The engineered CD47 proteins disclosed herein have a smaller size compared to the wild-type full-length human CD47, and thus could be used with IRES element in a multicistronic vectors having limited packaging capacity.

[0209] The second strategy relies on "self-cleaving" 2A peptides. These peptides, first discovered in picornaviruses, are short (about 20 amino acids) and produce equimolar levels of multiple genes from the same mRNA. The term "self-cleaving" is not entirely accurate, as these peptides are thought to function by making the ribosome skip the synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (Kim et al., 2011). The "cleavage" occurs between the glycine and proline residues found on the C-terminus. Thus, the upstream cistron will have a few additional residues added to the end, while the downstream cistron will start with the proline.

[0210] In some embodiments, the polycistronic vectors used in the context of this disclosure are the polycistronic vectors described in US applications 63/270,956 and 63/222,954.

[0211] In some embodiments, the vector herein is a nucleic acid molecule capable of transferring or transporting another nucleic acid molecule, including into the cell or into the genome of a cell. The transferred nucleic acid is generally linked to, *e.g.*, inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell or may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (*e.g.*, DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, *e.g.*, replication defective retroviruses and lentiviruses. Non-viral vectors may require a delivery vehicle to facilitate entry of the nucleic acid molecule into a cell.

[0212] A viral vector can comprise a nucleic acid molecule that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also host cell components in addition to nucleic acid(s). A viral vector can comprise, *e.g.*, a virus or viral particle capable of

transferring a nucleic acid into a cell, or to the transferred nucleic acid (*e.g.*, as naked DNA). Viral vectors and transfer plasmids can comprise structural and/or functional genetic elements that are primarily derived from a virus. A retroviral vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus.

[0213] In some vectors described herein, at least part of one or more protein coding regions that contribute to or are essential for replication may be absent compared to the corresponding wild-type virus. This makes the viral vector replication-defective. In some embodiments, the vector is capable of transducing a target non-dividing host cell and/or integrating its genome into a host genome.

[0214] In some embodiments, the retroviral nucleic acid comprises one or more of (*e.g.*, all of): a 5' promoter (*e.g.*, to control expression of the entire packaged RNA), a 5' LTR (*e.g.*, that includes R (polyadenylation tail signal) and/or U5 which includes a primer activation signal), a primer binding site, a psi packaging signal, a RRE element for nuclear export, a promoter directly upstream of the transgene to control transgene expression, a transgene (or other exogenous agent element), a polypurine tract, and a 3' LTR (*e.g.*, that includes a mutated U3, a R, and U5). In some embodiments, the retroviral nucleic acid further comprises one or more of a cPPT, a WPRE, and/or an insulator element (*e.g.*, as described in Browning et al., "Insulators to Improve the Safety of Retroviral Vectors for HIV Gene Therapy," *Biomedicines*, 4(1):4 (2016)).

[0215] A retrovirus typically replicates by reverse transcription of its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. The structure of a wild-type retrovirus genome often comprises a 5' long terminal repeat (LTR) and a 3' LTR, between or within which are located a packaging signal to enable the genome to be packaged, a primer binding site, integration sites to enable integration into a host cell genome and gag, pol and env genes encoding the packaging components which promote the assembly of viral particles. More complex retroviruses have additional features, such as rev and RRE sequences in HIV, which enable the efficient export of RNA transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell. In the provirus, the viral genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are involved in proviral integration and transcription. LTRs also serve as enhancer-promoter sequences and can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a psi sequence located at the 5' end of the viral genome.

[0216] The LTRs themselves are typically similar (*e.g.*, identical) sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

[0217] For the viral genome, the site of transcription initiation is typically at the boundary between U3 and R in one LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the other LTR. U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses comprise any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: *tot*, *rev*, *tax* and *rex*.

[0218] With regard to the structural genes gag, pol and env themselves, gag encodes the internal structural protein of the virus. Gag protein is proteolytically processed into the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The pol gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The env gene encodes the surface (SU) glycoprotein and

the transmembrane (TM) protein of the virion, which form a complex that interacts specifically with cellular receptor proteins. This interaction promotes infection, *e.g.*, by fusion of the viral membrane with the cell membrane.

[0219] In a replication-defective retroviral vector genome gag, pol and env may be absent or not functional. The R regions at both ends of the RNA are typically repeated sequences. U5 and U3 represent unique sequences at the 5' and 3' ends of the RNA genome respectively. Retroviruses may also contain additional genes which code for proteins other than gag, pol and env. Examples of additional genes include (in HIV), one or more of vif, vpr, vpx, vpu, tat, rev and nef. EIAV has (amongst others) the additional gene S2.

[0220] Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV) and human immunodeficiency virus (HIV).

[0221] In some embodiments the retrovirus is a Gammaretrovirus. In some embodiments the retrovirus is an Epsilonretrovirus. In some embodiments the retrovirus is an Alpharetrovirus. In some embodiments the retrovirus is a Betaretrovirus. In some embodiments the retrovirus is a Deltaretrovirus. In some embodiments the retrovirus is a Spumaretrovirus. In some embodiments the retrovirus is an endogenous retrovirus. In some embodiments the retrovirus is a lentivirus.

[0222] In some embodiments, a retroviral or lentivirus vector further comprises one or more insulator elements, *e.g.*, an insulator element described in Browning et al., "Insulators to Improve the Safety of Retroviral Vectors for HIV Gene Therapy," *Biomedicines*, 4(1):4 (2016). In various embodiments, the vectors comprise a promoter operably linked to a polynucleotide encoding an exogenous agent. The vectors may have one or more LTRs, wherein either LTR comprises one or more modifications, such as one or more nucleotide substitutions, additions, or deletions. The vectors may further comprise one or more accessory elements to increase transduction efficiency (*e.g.*, a cPPT/FLAP), viral packaging (*e.g.*, a Psi (Y) packaging signal, RRE), and/or other elements that increase exogenous gene expression (*e.g.*, poly (A) sequences), and may optionally comprise a WPRE or HPRE. In some embodiments, a lentiviral nucleic acid comprises one or more of, *e.g.*, all of, *e.g.*, from 5' to 3', a promoter (*e.g.*, CMV), an R sequence (*e.g.*, comprising TAR), a U5 sequence (*e.g.*, for integration), a PBS sequence (*e.g.*, for reverse transcription), a DIS sequence (*e.g.*, for genome dimerization), a psi packaging signal, a partial gag sequence, an RRE sequence (*e.g.*, for nuclear export), a cPPT sequence (*e.g.*, for nuclear import), a promoter to drive expression of the exogenous agent, a gene encoding the exogenous agent, a WPRE sequence (*e.g.*, for efficient transgene expression), a PPT sequence (*e.g.*, for reverse transcription), an R sequence (*e.g.*, for polyadenylation and termination), and a U5 signal (*e.g.*, for integration).

[0223] Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In some embodiments, HIV based vector backbones (i.e., HIV cis-acting sequence elements) are used. A lentivirus vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus.

[0224] In embodiments, a lentivirus vector (*e.g.*, lentiviral expression vector) may comprise a lentiviral transfer plasmid (*e.g.*, as naked DNA) or an infectious lentiviral particle. With respect to elements such as cloning sites, promoters, regulatory elements, heterologous nucleic acids, etc., it is to be understood that the sequences of these elements can be present in RNA form in lentiviral particles and can be present in DNA form in DNA plasmids.

[0225] In embodiments, a lentivirus vector is a vector with sufficient retroviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle capable of infecting a target cell. Infection of the target cell can comprise reverse transcription and integration into the target cell genome. The recombinant lentivirus vector (RLV) typically carries non-viral coding sequences which are to be delivered by the vector to the target cell. In embodiments, an RLV is incapable of independent replication to produce infectious retroviral particles within the target cell. Usually the RLV lacks a functional gag-pol and/or env gene and/or other genes involved in replication. The vector may be configured as a split-intron vector, *e.g.*, as described in PCT patent application WO 99/15683, which is herein incorporated by reference in its entirety.

[0226] In some embodiments, the lentivirus vector comprises a minimal viral genome, *e.g.*, the viral vector has been manipulated so as to remove the non-essential elements and to retain the essential elements in order to provide the required functionality to infect, transduce and deliver a nucleotide sequence of interest to a target host cell, *e.g.*, as described in WO 98/17815, which is herein incorporated by reference in its entirety.

[0227] A minimal lentiviral genome may comprise, *e.g.*, (5')R-U5-one or more first nucleotide sequences-U3-R(3'). However, the plasmid vector used to produce the lentiviral genome within a source cell can also include transcriptional regulatory control sequences operably linked to the lentiviral genome to direct transcription of the genome in a source cell. These regulatory sequences may comprise the natural sequences associated with the transcribed retroviral sequence, *e.g.*, the 5' U3 region, or they may comprise a heterologous promoter such as another viral promoter, for example the CMV promoter. Some lentiviral genomes comprise additional sequences to promote efficient virus production. For example, in the case of HIV, rev and RRE sequences may be included.

V. Cells

[0228] In another aspect, the present disclosure provides a cell comprising a polynucleotide encoding an engineered CD47 protein as disclosed herein, and/or a vector comprising a polynucleotide that encodes an engineered CD47 protein as disclosed herein.

[0229] In another aspect, the present disclosure provides a cell comprising an engineered CD47 protein as disclosed herein. In some embodiments, the engineered CD47 protein is introduced into a cell in the form of a nucleic acid molecule encoding the engineered CD47 protein. The process of introducing the nucleic acid molecule into the cell can be achieved by any suitable technique. Suitable techniques include calcium phosphate or lipid-mediated transfection, electroporation, and transduction or infection using a viral vector. In some embodiments, the nucleic acid molecule comprises DNA. In some embodiments, the nucleic acid molecule comprises a modified DNA. In some embodiments, the nucleic acid molecule comprises mRNA. In some embodiments, the nucleic acid molecule comprises a modified mRNA.

[0230] In some embodiments, the engineered CD47 protein is delivered using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide.

[0231] In some embodiments, the engineered CD47 protein is delivered using one or more gene editing systems. In some embodiments, the gene editing system is CRISPR/Cas. In some embodiments, the gene editing system is one or more of the CRISPR/Cas systems described herein. In some embodiments, the engineered CD47 protein is delivered using Transcription Activator-Like Effector Nucleases (TALEN) methodologies. In some embodiments, the gene editing system is one or more of the TALEN methodologies described herein.

- [0232] In some embodiments, the engineered CD47 protein is delivered using zinc finger nuclease (ZFN). In some embodiments, the gene editing system is one or more of the ZFN methodologies described herein.
- [0233] In some embodiments, the engineered CD47 protein is delivered using a meganuclease. In some embodiments, the gene editing system is one or more of the meganuclease methodologies described herein.
- [0234] In some embodiments, the cell is a stem cell.
- [0235] In some embodiments, the cell is a pluripotent stem cell. Pluripotent stem cells are cells that have the capacity to self-renew by dividing and to develop into the three primary germ cell layers of the early embryo and therefore into all cells of the adult body, but not extra-embryonic tissues such as the placenta. Embryonic stem cells and induced pluripotent stem cells are pluripotent stem cells.
- [0236] In some embodiments, the cell is an embryonic stem cell (ESC). Embryonic stem cells are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo.
- [0237] In some embodiments, the cell is an induced pluripotent stem cell (iPSC). iPSCs are derived from adult somatic cells that have been genetically reprogrammed back into an embryonic-like pluripotent state that enables the development of an unlimited source of any type of cell needed for therapeutic purposes.
- [0238] "Pluripotent stem cells" as used herein have the potential to differentiate into any of the three germ layers: endoderm (*e.g.*, the stomach lining, gastrointestinal tract, lungs, etc.), mesoderm (*e.g.*, muscle, bone, blood, urogenital tissue, etc.) or ectoderm (*e.g.*, epidermal tissues and nervous system tissues). The term "pluripotent stem cells," as used herein, also encompasses induced pluripotent stem cells (iPSCs or iPS cells), or a type of pluripotent stem cell derived from a non-pluripotent cell. In some embodiments, a pluripotent stem cell is produced or generated from a cell that is not a pluripotent cell. In other words, pluripotent stem cells can be direct or indirect progeny of a non-pluripotent cell. Examples of parent cells include somatic cells that have been reprogrammed to induce a pluripotent, undifferentiated phenotype by various means. Such "iPS" or "iPSC" cells can be created by inducing the expression of certain regulatory genes or by the exogenous application of certain proteins. Methods for the induction of iPS cells are known in the art and are further described below. (See, *e.g.*, Zhou *et al.*, *Stem Cells* 27 (11): 2667-74 (2009); Huangfu *et al.*, *Nature Biotechnol.* 26 (7): 795 (2008); Woltjen *et al.*, *Nature* 458 (7239): 766-770 (2009); and Zhou *et al.*, *Cell Stem Cell* 8:381-384 (2009); each of which is incorporated by reference herein in their entirety.) As used herein, "hiPSCs" are human induced pluripotent stem cells. In some embodiments, "pluripotent stem cells," as used herein, also encompasses mesenchymal stem cells (MSCs), and/or embryonic stem cells (ESCs).
- [0239] In some embodiments, the cell is a pancreatic islet cell. In some embodiments, the cell is a primary pancreatic islet cell.
- [0240] In some embodiments, the cell is differentiated from a pluripotent stem cell. In some embodiments, the pluripotent stem cell is an iPSC or an ESC.
- [0241] In some embodiments, the cell is a T cell. In some embodiments, the cell is a primary T cell. In some embodiments, the cell is a T cell comprising a chimeric antigen receptor (CAR), such as comprising a polynucleotide encoding a CAR and/or comprising the CAR, or otherwise expressing the CAR from the polynucleotide. In some embodiments, the cell is a CAR-T cell. In some embodiments, the T cell is differentiated from a pluripotent stem cell. In some embodiments, the pluripotent stem cell is an iPSC or an ESC.
- [0242] A T cell is a type of lymphocyte. T cells are one of the white blood cells of the immune system and play a central role in the adaptive immune response. CAR-T cells are T cells that have been genetically engineered to produce an artificial T cell receptor. Chimeric antigen receptors (CARs, also known as chimeric immunoreceptors, chimeric T cell receptors or artificial T cell receptors) are receptor proteins that have been engineered to give T cells the

ability to target a specific protein. The receptors are chimeric because they combine both antigen-binding and T cell activating functions into a single receptor. CAR-T cells can be both CD4+ and CD8+, with a 1-to-1 ratio of both cell types providing synergistic antitumor effects. CAR-T cells can be derived from T cells in a patient's own blood (autologous) or derived from the T cells of another healthy donor (allogeneic). T cells can be obtained from a number of sources including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, e.g., FICOLL™ separation, antibody-conjugated bead-based methods such as MACS™ separation (Miltenyi).

[0243] In some embodiments, the cell is selected from, but not limited to, cardiac cells, cardiac progenitor cells, neural cells, glial progenitor cells, endothelial cells, T cells, B cells, pancreatic islet cells, retinal pigmented epithelium cells, hepatocytes, thyroid cells, skin cells, blood cells, plasma cells, platelets, renal cells, epithelial cells, CAR-T cells, NK cells, and CAR-NK cells.

[0244] In some embodiments, the cell is a primary cell. Primary cells are isolated directly from human or animal tissue using enzymatic or mechanical methods. Once isolated, they are placed in an artificial environment in plastic or glass containers supported with specialized medium containing essential nutrients and growth factors to support proliferation. Primary cells could be of two types: adherent or suspension. Adherent cells require attachment for growth and are said to be anchorage-dependent cells. Adherent cells are usually derived from tissues of organs. Suspension cells do not require attachment for growth and are said to be anchorage-independent cells. Most suspension cells are isolated from the blood system, but some tissue-derived cells can also be used in suspension, such as hepatocytes or intestinal cells. Although primary cells usually have a limited lifespan, they offer a number of advantages compared to cell lines. Primary cell culture enables researchers to study donors and not just cells. Several factors such as age, medical history, race, and sex can be considered when building an experimental model. With a growing trend towards personalized medicine, such donor variability and tissue complexity can be achieved with use of primary cells, but are difficult to replicate with cell lines that are more systematic and uniform in nature and do not capture the true diversity of a living tissue.

[0245] In some embodiments, the cell is a differentiated cell. Differentiated cells are cells that have undergone differentiation. They are mature cells that perform a specialized function. Some examples of differentiated cells are epithelial cells, skin fibroblasts, endothelial cells lining the blood vessels, smooth muscle cells, liver cells, nerve cells, human cardiac muscle cells, etc. Generally, these cells have a unique morphology, metabolic activity, membrane potential, and responsiveness to signals facilitating their function in a body tissue or organ.

Hypoimmunogenic cells

[0246] In some embodiments, the cells described herein are hypoimmunogenic cells. As used herein to characterize a cell, the term "hypoimmunogenic" generally means that such cell is less prone to innate or adaptive immune rejection by a subject into which such cells are transplanted, e.g., the cell is less prone to allorejection by a subject into which such cells are transplanted. For example, in some embodiments, relative to a cell of the same cell type that does not comprise the modifications, such a hypoimmunogenic cell may be about 2.5%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, 99%, 100%, or any amount in between, less prone to innate or adaptive immune rejection by a subject into which such cells are transplanted. In some embodiments, genome editing technologies are used to modulate the expression of MHC I and MHC II genes, and thus, contribute to generation of a

hypoimmunogenic cell. In some embodiments, a hypoimmunogenic cell evades immune rejection in an MHC-mismatched allogeneic recipient. In some embodiments, differentiated cells produced from the hypoimmunogenic stem cells outlined herein evade immune rejection when administered (*e.g.*, transplanted or grafted) to an MHC-mismatched allogeneic recipient. In some embodiments, a hypoimmunogenic cell is protected from T cell-mediated adaptive immune rejection and/or innate immune cell rejection. Detailed descriptions of hypoimmunogenic cells, methods of producing such cells, and methods of using such cells are found in WO2016183041 filed May 9, 2015; WO2018132783 filed January 14, 2018; WO2018176390 filed March 20, 2018; WO2020018615 filed July 17, 2019; WO2020018620 filed July 17, 2019; PCT/US2020/44635 filed July 31, 2020; US62/881,840 filed August 1, 2019; US62/891,180 filed August 23, 2019; US63/016,190, filed April 27, 2020; and US63/052,360 filed July 15, 2020, WO2023158836, filed February 2023, the disclosures including the examples, sequence listings, and figures of which are incorporated herein by reference in their entirety.

[0247] Hypoimmunogenicity of a cell can be determined by evaluating the immunogenicity of the cell such as the cell's ability to elicit adaptive and innate immune responses or to avoid eliciting such adaptive and innate immune responses. Such immune response can be measured using assays recognized by those skilled in the art. In some embodiments, an immune response assay measures the effect of a hypoimmunogenic cell on T cell proliferation, T cell activation, T cell killing, donor specific antibody generation, NK cell proliferation, NK cell activation, and macrophage activity. In some cases, hypoimmunogenic cells and derivatives thereof undergo decreased killing by T cells and/or NK cells upon administration to a subject. In some instances, the cells and derivatives thereof show decreased macrophage engulfment compared to an unmodified or wild-type cell. In some embodiments, a hypoimmunogenic cell elicits a reduced or diminished immune response in a recipient subject compared to a corresponding unmodified wild-type cell. In some embodiments, a hypoimmunogenic cell is nonimmunogenic or fails to elicit an immune response in a recipient subject.

[0248] In some embodiments, the expression of major histocompatibility (MHC) class I and MHC class II proteins are disrupted in the cell. MHC class I and class II proteins play a role in the adaptive branch of the immune system. Both classes of proteins share the task of presenting peptides on the cell surface for recognition by T cells. Immunogenic peptide-MHC class I (pMHCI) complexes are presented on nucleated cells and are recognized by cytotoxic CD8+ T cells. The presentation of peptide-MHC class II (pMHCII) by antigen-presenting cells (*e.g.*, dendritic cells (DCs), macrophages, or B cells), on the other hand, can activate CD4+ T cells, leading to the coordination and regulation of effector cells. In all cases, it is a clonotypic T cell receptor that interacts with a given pMHC complex, potentially leading to sustained cell-cell contact formation and T cell activation.

[0249] In some embodiments the cells described herein express reduced levels of MHC class I proteins relative to a wild-type or control cell. In some embodiments, the cells express reduced levels of MHC class II proteins relative to a wild-type or control cell. In some embodiments, the cells express reduced levels of both MHC class I and class II proteins relative to a wild-type or control cell.

[0250] In some embodiments, the cells do not express any MHC class I proteins. In some embodiments, the cells do not express any MHC class II proteins. In some embodiments, the cells do not express any MHC class I and do not express any MHC class II proteins.

[0251] In some embodiments, the MHC proteins discussed herein are HLA proteins.

[0252] "HLA" or "human leukocyte antigen" complex is a gene complex encoding the MHC proteins in humans. The cell-surface proteins that make up the HLA complex are responsible for the regulation of the immune response to antigens. In humans, there are two MHCs, class I and class II, or "HLA-I" and "HLA-II." HLA-I includes three

proteins, HLA-A, HLA-B, and HLA-C, which present peptides from the inside of the cell, and antigens presented by the HLA-I complex attract killer T-cells (also known as CD8+ T-cells or cytotoxic T cells). The HLA-I proteins are associated with β -2 microglobulin (B2M). HLA-II includes five proteins, HLA-DP, HLA-DM, HLA-DOB, HLA-DQ, and HLA-DR, which present antigens from outside the cell to T lymphocytes. The HLA-II proteins are associated with Class II transactivator (CIITA). This stimulates CD4+ cells (also known as T-helper cells). It should be understood that the use of either "MHC" or "HLA" is not meant to be limiting, though the term "HLA" typically indicates that the genes are from humans. Thus, as it relates to mammalian cells, these terms may be used interchangeably herein.

[0253] In some embodiments, the expression of MHC class II proteins is reduced by knocking out or by reducing expression of CIITA. In some embodiments, the expression of MHC II genes is modulated (*e.g.*, reduced or eliminated) by targeting and modulating (*e.g.*, reducing or eliminating) Class II transactivator (CIITA) expression. In some embodiments, the modulation occurs using a CRISPR/Cas system. CIITA is a member of the LR or nucleotide binding domain (NBD) leucine-rich repeat (LRR) family of proteins and regulates the transcription of MHC II by associating with the MHC enhanceosome. In some embodiments, the polynucleotide sequence being targeted for modulation is a variant of CIITA, a homolog of CIITA, or an ortholog of CIITA.

[0254] In some embodiments, reduced or eliminated expression of CIITA reduces or eliminates expression of one or more of the following MHC class II molecules: HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR.

[0255] In some embodiments, the cells described herein comprise gene modifications at the gene locus encoding the CIITA protein. In other words, the cells comprise a genetic modification at the CIITA locus. In some instances, the nucleotide sequence encoding the CIITA protein is set forth in RefSeq. No. NM_000246.4 and NCBI Genbank No. U18259. In some instances, the CIITA gene locus is described in NCBI Gene ID No. 4261. In certain cases, the amino acid sequence of CIITA is depicted as NCBI GenBank No. AAA88861.1. Additional descriptions of the CIITA protein and gene locus can be found in Uniprot No. P33076, HGNC Ref. No. 7067, and OMIM Ref. No. 600005.

[0256] In some embodiments, the hypoinmunogenic cells outlined herein comprise a genetic modification targeting the CIITA gene. In some embodiments, the genetic modification targeting the CIITA gene is generated by a rare-cutting endonuclease comprising a Cas protein or a polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid sequence for specifically targeting the CIITA gene. In some embodiments, the at least one guide ribonucleic acid sequence for specifically targeting the CIITA gene is selected from the group consisting of SEQ ID NOS:5184-36352 of Table 12 of WO2016183041, which is herein incorporated by reference. In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject. In some embodiments, an exogenous nucleic acid encoding a polypeptide as disclosed herein (*e.g.*, a chimeric antigen receptor, CD47, or another tolerogenic factor disclosed herein) is inserted at the CIITA gene.

[0257] Assays to test whether the CIITA gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the CIITA gene can be confirmed by PCR and the reduction of HLA-II expression can be confirmed by FACS analysis. In another embodiment, CIITA protein expression is detected using a Western blot of cells lysates probed with antibodies to the CIITA protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell by viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some embodiments, the

exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0258] In some embodiments, the expression of MHC class I proteins is reduced by knocking out or by reducing expression of B2M. In some embodiments, the expression of MHC-I genes is modulated (*e.g.*, reduced or eliminated) by targeting and modulating (*e.g.*, reducing or eliminating) expression of the accessory chain B2M. In some embodiments, the modulation occurs using a CRISPR/Cas system. By modulating (*e.g.*, reducing or deleting) expression of B2M, surface trafficking of MHC-I molecules is blocked and the cell rendered hypoimmunogenic. In some embodiments, the cell has a reduced ability to induce an innate and/or an adaptive immune response in a recipient subject.

[0259] In some embodiments, the polynucleotide sequence being targeted for modulation is a variant of B2M, a homolog of B2M, or an ortholog of B2M.

[0260] In some embodiments, decreased or eliminated expression of B2M reduces or eliminates expression of one or more of the following MHC I molecules: HLA-A, HLA-B, and HLA-C.

[0261] In some embodiments, the cells described herein comprise gene modifications at the gene locus encoding the B2M protein. In other words, the cells comprise a genetic modification at the B2M locus. In some instances, the nucleotide sequence encoding the B2M protein is set forth in RefSeq. No. NM_004048.4 and Genbank No. AB021288.1. In some instances, the B2M gene locus is described in NCBI Gene ID No. 567. In certain cases, the amino acid sequence of B2M is set forth in NCBI GenBank No. BAA35182.1. Additional descriptions of the B2M protein and gene locus can be found in Uniprot No. P61769, HGNC Ref. No. 914, and OMIM Ref. No. 109700.

[0262] In some embodiments, the hypoimmunogenic cells outlined herein comprise a genetic modification targeting the B2M gene. In some embodiments, the genetic modification targeting the B2M gene is generated by a rare-cutting endonuclease comprising a Cas protein or a polynucleotide encoding a Cas protein, and at least one guide ribonucleic acid sequence for specifically targeting the B2M gene. In some embodiments, the at least one guide ribonucleic acid sequence for specifically targeting the B2M gene is selected from the group consisting of SEQ ID NOS:81240-85644 of Table 15 of WO2016183041, which is herein incorporated by reference. In some embodiments, an exogenous nucleic acid encoding a polypeptide as disclosed herein (*e.g.*, a chimeric antigen receptor, CD47, or another tolerogenic factor disclosed herein) is inserted at the B2M gene. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some embodiments, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0263] Assays to test whether the B2M gene has been inactivated are known and described herein. In some embodiments, the resulting genetic modification of the B2M gene can be confirmed by PCR and the reduction of HLA-I expression can be confirmed by FACS analysis. In another embodiment, B2M protein expression is detected using a Western blot of cells lysates probed with antibodies to the B2M protein. In another embodiment, reverse transcriptase polymerase chain reactions (RT-PCR) are used to confirm the presence of the inactivating genetic modification.

[0264] In some embodiments of the T cells, iPSCs, or ESCs described herein, T cell receptor alpha chain (TRAC) and/or T cell receptor beta chain (TRBC) genes are knocked out, or their expression is reduced in the cells.

[0265] The T-cell receptor (TCR) is a protein complex found on the surface of T cells that is responsible for recognizing fragments of antigen as peptides bound to MHC molecules. The TCR is a disulfide-linked membrane-anchored heterodimeric protein normally consisting of the highly variable alpha (α) and beta (β) chains (encoded by TRAC and TRBC genes, respectively) expressed as part of a complex with the invariant CD3 chain molecules. T cells expressing this receptor are referred to as $\alpha\beta$ (or $\alpha\beta$) T cells, though a minority of T cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, referred to as $\gamma\delta$ T cells. Each chain is composed of two extracellular domains: a variable region and a constant region, both of these immunoglobulin superfamily (IgSF) domains forming antiparallel β -sheets. The constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the variable region binds to the peptide/MHC complex. It has been reported that knockout of endogenous TRAC and/or TRBC genes could increase expression and function of T cells expressing transgenic T cell receptor. In some embodiments the cells described herein express reduced levels of MHC class I proteins and /or MHC class II proteins relative to a wild-type or control cell.

[0266] In some embodiments, the cells comprise increased expression of wild-type and/or engineered CD47 protein relative to a wild-type cell or a control cell of the same cell type. In some embodiments, the wild-type cell or the control cell is a starting material. As used herein, a starting material refers to a raw material upon which one or more of the modifications described herein are made in order to produce the engineered CD47 protein as described herein, the polynucleotide encoding the engineered CD47 protein as described herein, the vector as described herein, the cell comprising the engineered CD47 protein as described herein, or the composition comprising the engineered CD47 protein or the cell as described herein.

[0267] As used herein, a "wild-type cell" or a "wt cell" means any cell found in nature. In some embodiments, wild-type cells include primary cells and T cells found in nature. As used herein, a "control cell" is a cell whose CD47 gene is unaltered, but in which other modifications may be made. In some embodiments, the control cell is an engineered cell that may contain nucleic acid changes resulting in reduced expression of MHC I protein and/or MHC II protein and/or T-cell receptors. In some embodiments, the control cell is an engineered cell that has B2M knocked out, or comprises reduced expression of B2M. In some embodiments, the control cell is an engineered cell that has CIITA knocked out, or comprises reduced expression of CIITA. In some embodiments, the control cell is an engineered cell that has TRAC and/or TRBC knocked out, or comprises reduced expression of TRAC and/or TRBC.

[0268] In some embodiments, the control cell is an iPSC, an ESC, or a progeny that contains nucleic acid changes resulting in pluripotency. In some embodiments, the control cell is an iPSC, an ESC, or a progeny that has B2M knocked out, or comprises reduced expression of B2M. In some embodiments, the control cell is an iPSC, an ESC, or a progeny that has CIITA knocked out, or comprises reduced expression of CIITA. In some embodiments, the control cell is an iPSC, an ESC, or a progeny that has TRAC and/or TRBC knocked out, or comprises reduced expression of TRAC and/or TRBC.

[0269] In some embodiments, the control cell is a primary T cell or a progeny that contains nucleic acid changes resulting in reduced expression of MHC I protein and/or MHC II protein and/or T-cell receptors. In some embodiments, the control cell is a primary T cell or a progeny that has B2M knocked out, or comprises reduced expression of B2M. In some embodiments, the control cell is a primary T cell or a progeny that has CIITA knocked out, or comprises reduced expression of CIITA. In some embodiments, the control cell is a primary T cell or a progeny that has TRAC and/or TRBC knocked out, or comprises reduced expression of TRAC and/or TRBC.

[0270] In some embodiments, the starting material is a primary cell collected from a donor. In some embodiments, the starting material is a primary blood cell collected from a donor, *e.g.*, via a leukopak. For example, in

some embodiments, the starting material are unmodified T cells obtained from a donor. In some embodiments, the starting material is an iPSC cell line. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes other than human CD47 gene.

[0271] In some embodiments, the engineered and hypoimmunogenic cells described are derived from an iPSC or a progeny thereof. As used herein, the term "derived from an iPSC or a progeny thereof" encompasses the initial iPSC that is generated and any subsequent progeny thereof. As used herein, the term "progeny" encompasses, *e.g.*, a first-generation progeny, *i.e.*, the progeny is directly derived from, obtained from, obtainable from or derivable from the initial iPSC by, *e.g.*, traditional propagation methods. The term "progeny" also encompasses further generations such as second, third, fourth, fifth, sixth, seventh, or more generations, *i.e.*, generations of cells which are derived from, obtained from, obtainable from or derivable from the former generation by, *e.g.*, traditional propagation methods. The term "progeny" also encompasses modified cells that result from the modification or alteration of the initial iPSC or a progeny thereof.

[0272] In some embodiments, knocking down (*e.g.*, decreasing, eliminating, or inhibiting) gene expression can be achieved by RNA silencing or RNA interference (RNAi). Useful RNAi methods include those that utilize synthetic RNAi molecules, short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), short hairpin RNAs (shRNAs), microRNAs (miRNAs), and other transient knock down methods recognized by those skilled in the art. Reagents for RNAi including sequence specific shRNAs, siRNA, miRNAs and the like are commercially available.

[0273] The term "engineered cell" as used herein refers to a cell that has been altered in at least some way by human intervention, including, for example, by genetic alterations or modifications such that the engineered cell differs from a wild-type cell.

[0274] The terms "decrease," "reduced," "reduction," and "decreased" are all used herein generally to mean a lowering by a statistically significant amount. However, for avoidance of doubt, "decrease," "reduced," "reduction," "decreased" means a lowering by at least 10% as compared to a reference level, for example a lowering by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90%, or up to and including a 100% lowering (*i.e.* absent level as compared to a reference sample), or any lowering between 10-100% as compared to a reference level. In some embodiments, the cells are engineered to have reduced expression of one or more targets relative to an unaltered or unmodified wild-type cell.

[0275] In some embodiments, the provided modified cells are modified such that they are able to evade immune recognition and responses when administered to a patient (*e.g.*, recipient subject). The cells can evade killing by immune cells *in vitro* and *in vivo*. In some embodiments, the cells evade killing by macrophages and NK cells. In some embodiments, the cells are ignored by immune cells or a subject's immune system. In other words, the cells administered in accordance with the methods described herein are not detectable by immune cells of the immune system. In some embodiments, the cells are cloaked and therefore avoid immune rejection.

[0276] Methods of determining whether a modified cell provided herein evades immune recognition include, but are not limited to, IFN- γ Elispot assays, microglia killing assays, cell engraftment animal models, cytokine release assays, ELISAs, killing assays using bioluminescence imaging or chromium release assay or Xcelligence analysis, mixed-lymphocyte reactions, immunofluorescence analysis, etc.

[0277] In some embodiments, the immunogenicity of the cells is evaluated in a complement-dependent cytotoxicity (CDC) assay. CDC can be assayed *in vitro* by incubating cells with IgG or IgM antibodies targeting an HLA-independent antigen expressed on the cell surface in the presence of serum containing complement and analyzing cell

killing. In some embodiments, CDC can be assayed by incubating cells with ABO blood type incompatible serum, wherein the cells comprise A antigens or B antigens, and the serum comprises antibodies against the A antigens and/or B antigens of the cells.

[0278] In some embodiments, once the modified cells have been modified or generated as described herein, they may be assayed for their hypoimmunogenicity. Any of a variety of assays can be used to assess if the cells are hypoimmunogenic or can evade the immune system. Exemplary assays include any as is described in WO2016183041 and WO2018132783.

[0279] In some embodiments, the modified cells described herein survive in a host without stimulating the host immune response for one week or more (e.g., one week, two weeks, one month, two months, three months, 6 months, one year, two years, three years, four years, five years or more, e.g., for the life of the cell and/or its progeny). The cells maintain expression of the transgenes and/or are deleted or reduced in expression of target genes for as long as they survive in the host. In some aspects, if the transgenes are no longer expressed and/or if target genes are expressed the modified cells may be removed by the host's immune system. In some embodiments, the persistence or survival of the modified cells may be monitored after their administration to a recipient by further expressing a transgene encoding a protein that allows the cells to be detected in vivo (e.g., a fluorescent protein, such as GFP, a truncated receptor or other surrogate marker or other detectable marker).

[0280] The hypoimmunogenic cells are administered in a manner that permits them to engraft to the intended tissue site and reconstitute or regenerate the functionally deficient area. In some embodiments, the hypoimmunogenic cells are assayed for engraftment (e.g., successful engraftment). In some embodiments, the engraftment of the hypoimmunogenic cells is evaluated after a pre-selected amount of time. In some embodiments, the engrafted cells are monitored for cell survival. For example, the cell survival may be monitored via bioluminescence imaging (BLI), wherein the cells are transduced with a luciferase expression construct for monitoring cell survival. In some embodiments, the engrafted cells are visualized by immunostaining and imaging methods known in the art. In some embodiments, the engrafted cells express known biomarkers that may be detected to determine successful engraftment. For example, flow cytometry may be used to determine the surface expression of particular biomarkers. In some embodiments, the hypoimmunogenic cells are engrafted to the intended tissue site as expected (e.g., successful engraftment of the hypoimmunogenic cells). In some embodiments, the hypoimmunogenic cells are engrafted to the intended tissue site as needed, such as at a site of cellular deficiency. In some embodiments, the hypoimmunogenic cells are engrafted to the intended tissue site in the same manner as a cell of the same type not comprising the modifications.

[0281] In some embodiments, administering the populations of modified cells (e.g., modified beta islet cells comprising modifications including reduced CD142 expression) or combinations (e.g., administering a population of modified cells in combination with an anti-coagulant agent) improves survival and engraftment by allowing cells to avoid or reduce IBMIR that occurs as a result of exposure of the cells to blood during transplant. In some embodiments, the reduction in IBMIR reduces the amount of cell loss (e.g., loss of transplanted islets) that occurs during transplant.

[0282] In some embodiments, the hypoimmunogenic cells are assayed for function. In some embodiments, the hypoimmunogenic cells are assayed for function prior to their engraftment to the intended tissue site. In some embodiments, the hypoimmunogenic cells are assayed for function following engraftment to the intended tissue site. In some embodiments, the function of the hypoimmunogenic cells is evaluated after a pre-selected amount. In some embodiments, the function of the engrafted cells is evaluated by the ability of the cells to produce a detectable phenotype. For example, engrafted beta islet cells function may be evaluated based on the restoration of lost glucose control due to diabetes. In some embodiments, the function of the hypoimmunogenic cells is as expected (e.g.,

successful function of the hypoinmunogenic cells while avoiding antibody-mediated rejection). In some embodiments, the function of the hypoinmunogenic cells is as needed, such as sufficient function at a site of cellular deficiency while avoiding antibody-mediated rejection. In some embodiments, the modified cells function in the same manner as a non-modified cell of the same type.

[0283] In some embodiments, genetically engineered cells of the present disclosure comprise an engineered CD47 protein of the present disclosure, wherein the engineered CD47 protein exhibits: decreased binding with TSP-1 relative to a wild-type CD47 protein; and/or decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein. In some embodiments, genetically engineered cells of the present disclosure comprise an engineered CD47 protein of the present disclosure and: exhibit decreased CD47/TSP-1-mediated undesirable effects relative to a control cell; and/or exhibit decreased CD47/TSP-1-mediated exhaustion relative to a control cell; and/or exhibit decreased CD47-mediated undesirable effects relative to a control cell; and/or exhibit increased CD47-mediated desirable effects relative to a control cell; and/or exhibit increased longevity relative to a control cell; and/or exhibit increased persistence relative to a control cell; and/or exhibit increased expansion relative to a control cell, and/or exhibit increased response to its intended therapeutic target, and/or exhibit increased hypoinmunity relative to a control cell; and/or exhibit increased immune evasion relative to a control cell; and/or exhibit increased ability to evade a host immune response relative to a control cell; and/or exhibit increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; and/or express the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would experience one or more unwanted effects. In some embodiments, a control cell comprises a wild-type cell, an unmodified cell, a cell that has not been engineered to express CD47 or a cell that has been engineered to express wild-type CD47. In some embodiments, a first expression level comprises 1.25x, 1.5x, 1.75x, 2x, 2.25x, 2.5x, 2.75x, 3x, 3.25x, 3.5x, 3.75x, 4x, 4.5x, 5x, 5.5x, 6x, 6.5x, 7x, 7.5x, 8x, 8.5x, 9x, 9.5x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x the level of wild-type CD47 protein in an unmodified cell. In some embodiments, CD47/TSP-1-mediated signaling and/or CD47/TSP-1-mediated exhaustion comprises autocrine signaling. In some embodiments, CD47/TSP-1-mediated signaling and/or CD47/TSP-1-mediated exhaustion comprises paracrine signaling. In some embodiments, CD47/TSP-1-mediated signaling and/or CD47/TSP-1-mediated exhaustion comprises both autocrine signaling and paracrine signaling. In some embodiments, increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via autocrine signaling. In some embodiments, increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via paracrine signaling. In some embodiments, increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via both autocrine signaling and paracrine signaling. In some embodiments, exhaustion comprises increased expression of PD1, LAG3, TIM3, CD39, CD101, or a combination thereof. In some embodiments, exhaustion comprises decreased or eliminated cytokine expression.

[0284] In some embodiments, genetically engineered cells of the present disclosure comprise an engineered CD47 protein of the present disclosure, wherein the engineered CD47 protein exhibits improved binding with SIRPα relative to a wild-type CD47 protein. In some embodiments, genetically engineered cells of the present disclosure

comprise an engineered CD47 protein of the present disclosure, wherein contacting a cell expressing SIRPa with a genetically engineered cell increases CD47/SIRPa-mediated signaling in the cell expressing SIRPa relative to contacting the cell expressing SIRPa with a control cell. In some embodiments, genetically engineered cells of the present disclosure comprise an engineered CD47 protein of the present disclosure, wherein contacting a cell expressing SIRPa with a genetically engineered cell increases CD47/SIRPa-mediated signaling in the cell expressing the engineered CD47 relative to contacting the cell expressing SIRPa with a control cell. In some embodiments, genetically engineered cells of the present disclosure comprise an engineered CD47 protein of the present disclosure, wherein contacting a cell expressing SIRPa with a genetically engineered cell increases CD47/SIRPa-mediated signaling in both the cell expressing SIRPa and the cell expressing CD47 relative to contacting the cell expressing SIRPa with a control cell. In some embodiments, genetically engineered cells of the present disclosure comprise an engineered CD47 protein of the present disclosure, wherein the genetically engineered cells: exhibit decreased CD47-mediated undesirable effects relative to a control cell; and/or exhibit increased CD47-mediated desirable effects relative to a control cell; and/or exhibit increased longevity relative to a control cell; and/or exhibit increased persistence relative to a control cell; and/or exhibit increased expansion relative to a control cell; and/or exhibit increased response to its intended therapeutic target, and/or exhibit increased hypoimmunity relative to a control cell; and/or exhibit improved immune evasion relative to a control cell; and/or exhibit increased ability to evade a host immune response relative to a control cell; and/or exhibit increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; and/or express the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would be killed by one or more immune cells. In some embodiments, a control cell comprises a wild-type cell, an unmodified cell, a cell that has not been engineered to express CD47 or a cell that has been engineered to express wild-type CD47. In some embodiments, a first expression level comprises 1.25x, 1.5x, 1.75x, 2x, 2.25x, 2.5x, 2.75x, 3x, 3.25x, 3.5x, 3.75x, 4x, 4.5x, 5x, 5.5x, 6x, 6.5x, 7x, 7.5x, 8x, 8.5x, 9x, 9.5x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x the level of wild-type CD47 protein in an unmodified cell.

[0285] In some embodiments, genetically engineered cells of the present disclosure comprise an engineered CD47 protein of the present disclosure, wherein the engineered CD47 protein exhibits: decreased binding with TSP-1 relative to a wild-type CD47 protein; decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein; and improved binding with SIRPa relative to a wild-type CD47 protein; and wherein the genetically engineered cells: exhibit decreased CD47/TSP-1-mediated undesirable effects relative to a control cell; exhibit decreased CD47-mediated undesirable effects relative to a control cell; exhibit decreased CD47/TSP-1-mediated exhaustion relative to a control cell; exhibit decreased CD47-mediated undesirable effects relative to a control cell; exhibit increased CD47-mediated desirable effects relative to a control cell; exhibit increased longevity relative to a control cell; exhibit increased persistence relative to a control cell; exhibit increased expansion relative to a control cell; exhibit increased response to its intended therapeutic target; exhibit increased hypoimmunity relative to a control cell; exhibit increased immune evasion relative to a control cell; exhibit increased ability to evade a host immune response relative to a control cell; exhibit increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; express the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would experience one or more unwanted effects and would be killed by one or more immune cells; and wherein contacting a cell expressing SIRPa with the genetically engineered cell increases CD47/SIRPa-mediated signaling in both the cell expressing SIRPa and the cell expressing CD47 relative to contacting the cell expressing SIRPa with a control cell. In some embodiments, a control cell comprises a wild-type cell, an unmodified cell, a cell that has not been engineered to express CD47 or a cell that has been engineered to express wild-type CD47.

In some embodiments, a first expression level comprises 1.25x, 1.5x, 1.75x, 2x, 2.25x, 2.5x, 2.75x, 3x, 3.25x, 3.5x, 3.75x, 4x, 4.5x, 5x, 5.5x, 6x, 6.5x, 7x, 7.5x, 8x, 8.5x, 9x, 9.5x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x the level of wild-type CD47 protein in an unmodified cell. In some embodiments, CD47/TSP-1-mediated signaling and/or CD47/TSP-1-mediated exhaustion comprises autocrine signaling. In some embodiments, CD47/TSP-1-mediated signaling and/or CD47/TSP-1-mediated exhaustion comprises paracrine signaling. In some embodiments, CD47/TSP-1-mediated signaling and/or CD47/TSP-1-mediated exhaustion comprises both autocrine signaling and paracrine signaling. In some embodiments, increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via autocrine signaling. In some embodiments, increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via paracrine signaling. In some embodiments, increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via both autocrine signaling and paracrine signaling. In some embodiments, exhaustion comprises increased expression of PD1, LAG3, TIM3, CD39, CD101, or a combination thereof. In some embodiments, exhaustion comprises decreased or eliminated cytokine expression.

Instant blood-mediated inflammatory reaction

[0286] In some embodiments, the modified cells provided herein evade an instant blood-mediated inflammatory reaction. A major contributor to the poor outcome of clinical islet transplantation is the occurrence of the destructive instant blood mediated inflammatory reaction (IBMIR), which leads to loss of transplanted tissue when the islets encounter the blood in the portal vein (Bennet et al., (1995) *Diabetes* 48:1907-1914; Moberg et al., (2002) *Lancet* 360:2039-2045). This reaction is triggered by tissue factor (TF) expression by the endocrine cells of the islets, combined with an array of other proinflammatory events, such as the expression of MCP-1 (Piemonti et al., (2002) *Diabetes* 51:55-65), IL-8, and MIF (Waeber et al., (1997) *Proc Natl Acad Sci USA* 94:4782-4787; Johansson et al., (2006) *Am J Transplantation* 6(2):305).

[0287] Instant blood-mediated inflammatory reaction (IBMIR) is a nonspecific inflammatory and thrombotic reaction that can occur when cells expressing CD142 come into contact with blood. IBMIR is initiated rapidly by exposure to human blood in the portal vein. It is characterized by activation of complement, platelets, and the coagulation pathway, which in turn leads to the recruitment of neutrophils. IBMIR causes significant loss of transplanted islets. In some embodiments, provided herein are compositions (e.g., modified cells comprising reduced expression of CD142 in combination with one or more of the other modifications described herein), combinations (e.g., a combination comprising any of the populations of modified cells described herein and an anti-coagulant agent that reduces coagulation), and methods (e.g., methods of treating a patient comprising administering any of the populations of modified cells described herein and anti-coagulant agent that reduces coagulation) that reduce an IBMIR associated with transplantation of the cells or exposure of the cells to blood.

[0288] In some embodiments, IBMIR can be assayed in vitro, for example, in an in vitro tubing loop model of IBMIR, which has been previously described in U.S. Pat. No. 7,045,502, which is herein incorporated by reference in its entirety.

[0289] In some embodiments, IBMIR can be assayed in vivo (e.g., in a mammal or in a human patient) by drawing blood samples during the peritransplant period and evaluating plasma levels of thrombin-anti-thrombin III complex (TAT), C-peptide, factor XIa-antithrombin (FXIa-AT), factor XIIa-antithrombin (FXIIa-AT), thrombin-antithrombin (TAT) plasmin-alpha 2 antiplasmin (PAP), and/or complement C3a. In some embodiments, IBMIR is associated with increased levels of TAT, C-peptide, FXIa-AT, FXIIa-AT, PAP, and/or complement C3a during infusion of transplanted cells and/or in a period of time following transplant (e.g., up to 3, 5, 10, or more than 10 hours after transplant). In some embodiments, IBMIR can be assayed by monitoring counts of free circulating platelets, wherein a decrease in the counts of platelets during or following transplantation is associated with IBMIR (e.g., with platelet consumption due to IBMIR).

Complement-dependent cytotoxicity

[0290] In some embodiments, the modified cells (e.g., beta islets) provided herein evade complement dependent cytotoxicity (CDC). In some embodiments, the CDC is secondary to a thrombotic reaction of IBMIR. In some embodiments, the CDC occurs independently of IBMIR.

[0291] In some embodiments, susceptibility of cells to CDC can be analyzed in vitro according to standard protocols understood by one of ordinary skill in the art. In some embodiments, CDC can be analyzed in vitro by mixing serum comprising the components of the complement system (e.g., human serum), with target cells bound by an antibody (e.g., an IgG or IgM antibody), and then to determine cell death. In some embodiments, susceptibility of cells to CDC can be analyzed in vitro by incubating cells in the presence of ABO-incompatible or Rh factor incompatible serum, comprising the components of the complement system and antibodies against ABO type A, ABO type B, and/or Rh factor antigens of the cells.

[0292] A common CDC assay determines cell death via pre-loading the target cells with a radioactive compound. As cells die, the radioactive compound is released from them. Hence, the efficacy of the antibody to mediate cell death is determined by the radioactivity level. Unlike radioactive CDC assays, non-radioactive CDC assays often determine the release of abundant cell components, such as GAPDH, with fluorescent or luminescent determination. In some embodiments, cell killing by CDC can be analyzed using a label-free platform such as xCELLigence™ (Agilent).

VI. Compositions

[0293] In another aspect, the present disclosure provides a composition comprising the engineered CD47 protein disclosed herein.

[0294] In another aspect, the present disclosure provides a composition comprising the cell that comprises a polynucleotide encoding the engineered CD47 protein disclosed herein, and/or a vector comprising the polynucleotide.

[0295] As used herein, the term "composition" includes, but is not limited to, a pharmaceutical composition. A "pharmaceutical composition" refers to an active pharmaceutical agent formulated in pharmaceutically acceptable or physiologically acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions of the invention may be administered in combination with other agents, such as, e.g., cytokines, growth factors, hormones, small molecules, chemotherapeutics, pro-drugs, drugs, antibodies, or other various pharmaceutically active agents. There is virtually no limit to other components that may also be included in the compositions, provided that the additional agents do not adversely affect the ability of the composition to deliver the intended therapy. The phrase "pharmaceutically acceptable" is used herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of

sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0296] The compositions may also comprise a pharmaceutically acceptable carrier, diluent, or excipient. As used herein "pharmaceutically acceptable carrier, diluent, or excipient" includes, without limitation, any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose, and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter; waxes; animal and vegetable fats; paraffins; silicones; bentonites; silicic acid; zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol, and polyethylene glycol; esters, such as ethyl oleate, and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations.

[0297] The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline; Ringers solution; isotonic sodium chloride; fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium; polyethylene glycols; glycerin; propylene glycol or other solvents; antibacterial agents, such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates, or phosphates; and agents for the adjustment of tonicity, such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

[0298] The composition may be suitably developed for intravenous, intratumoral, oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration.

VII. Methods of gene editing

[0299] In some embodiments, the methods for genetically modifying cells to knock out, knock down, or otherwise modify one or more genes comprise using a site-directed nuclease, including, for example, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases, transposases, and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas systems, as well as nickase systems, base editing systems, prime editing systems, and any other gene editing systems known in the art.

[0300] ZFNs are fusion proteins comprising an array of site-specific DNA binding domains adapted from zinc finger-containing transcription factors attached to the endonuclease domain of the bacterial FokI restriction enzyme. A ZFN may have one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) DNA binding domains or zinc finger domains. See, *e.g.*, Carroll *et al.*, *Genetics Society of America* (2011) 188:773-782; Kim *et al.*, *Proc. Natl. Acad. Sci. USA* (1996) 93:1156-1160. The individual DNA binding domains are typically referred to as "fingers" or "zinc fingers." Each zinc finger domain is a small protein structural motif stabilized by one or more zinc ions and usually recognizes a 3- to 4-bp

DNA sequence. Tandem domains can thus potentially bind to an extended nucleotide sequence that is unique within a cell's genome. Each finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. A DNA binding domain binds to a nucleic acid sequence called a target site or target segment. Each finger typically comprises an approximately 30 amino acid, zinc-chelating, DNA-binding subdomain. Studies have demonstrated that a single zinc finger of this class consists of an alpha helix containing the two invariant histidine residues coordinated with zinc along with the two cysteine residues of a single beta turn (see, e.g., Berg & Shi, *Science* 271:1081-1085 (1996)).

[0301] Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15, or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells. Zinc fingers can be engineered to bind a predetermined nucleic acid sequence. Criteria to engineer a zinc finger to bind to a predetermined nucleic acid sequence are known in the art. See, e.g., Sera *et al.*, *Biochemistry* (2002) 41:7074-7081; Liu *et al.*, *Bioinformatics* (2008) 24:1850-1857.

[0302] ZFNs containing FokI nuclease domains or other dimeric nuclease domains function as a dimer. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. See Bitinaite *et al.*, *Proc. Natl. Acad. Sci. USA* (1998) 95:10570-10575. To cleave a specific site in the genome, a pair of ZFNs are designed to recognize two sequences flanking the site, one on the forward strand and the other on the reverse strand. Upon binding of the ZFNs on either side of the site, the nuclease domains dimerize and cleave the DNA at the site, generating a DSB with 5' overhangs. HDR can then be utilized to introduce a specific mutation, with the help of a repair template containing the desired mutation flanked by homology arms. The repair template is usually an exogenous double-stranded DNA vector introduced to the cell. See Miller *et al.*, *Nat. Biotechnol.* (2011) 29:143-148; Hockemeyer *et al.*, *Nat. Biotechnol.* (2011) 29:731-734.

[0303] TALENs are another example of an artificial nuclease which can be used to edit a target gene. A "TALE-nuclease" (TALEN) is a fusion protein consisting of a nucleic acid-binding domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease catalytic domain to cleave a nucleic acid target sequence. The catalytic domain is preferably a nuclease domain and more preferably a domain having endonuclease activity, for instance I-TevI, CoIE7, NucA and Fok-I. In numerous embodiments, the TALE domain can be fused to a meganuclease, for instance I-CreI and I-OnuI or functional variant thereof. In a more preferred embodiment, said nuclease is a monomeric TALE-Nuclease. A monomeric TALE-Nuclease is a TALE-Nuclease that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927.

[0304] Transcription Activator like Effector (TALE) are proteins from the bacterial species *Xanthomonas* and comprise a plurality of repeated sequences. Each repeat is 33 to 35 amino acids in length, with two adjacent amino acids (termed the repeat-variable di-residue, or RVD) in position 12 and 13 that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from new modular proteins recently discovered in a different bacterial species. The new modular proteins have the advantage of displaying more sequence variability than TAL repeats. Preferably, RVDs associated with recognition of the different nucleotides are HD for recognizing C, NG for recognizing T, NI for recognizing A, NN for recognizing G or A, NS for recognizing A, C, G or T, HG for recognizing T, IG for recognizing T, NK for recognizing G, HA for recognizing C, ND for recognizing C, HI for recognizing C, HN for recognizing G, NA for recognizing G, SN for recognizing G or A and YG for recognizing T, TL for recognizing A, VT for recognizing A or G and SW for

recognizing A. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards nucleotides A, T, C and G and in particular to enhance this specificity. TALEN kits are sold commercially.

[0305] TALENs are produced artificially by fusing one or more TALE DNA binding domains (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) to a nuclease domain, for example, a FokI endonuclease domain. See Zhang, *Nature Biotech.* (2011) 29:149-153. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. See Cermak *et al.*, *Nucl. Acids Res.* (2011) 39:e82; Miller *et al.*, *Nature Biotech.* (2011) 29:143-148; Hockemeyer *et al.*, *Nature Biotech.* (2011) 29:731-734; Wood *et al.*, *Science* (2011) 333:307; Doyon *et al.*, *Nature Methods* (2010) 8:74-79; Szczepiek *et al.*, *Nature Biotech* (2007) 25:786-793; Guo *et al.*, *J. Mol. Biol.* (2010) 200:96. The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI nuclease domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller *et al.*, *Nature Biotech.* (2011) 29:143-148.

[0306] By combining engineered TALE repeats with a nuclease domain, a site-specific nuclease can be produced specific to any desired DNA sequence. Similar to ZFNs, TALENs can be introduced into a cell to generate DSBs at a desired target site in the genome, and so can be used to knock out genes or knock in mutations in similar, HDR-mediated pathways. See Boch, *Nature Biotech.* (2011) 29:135-136; Boch *et al.*, *Science* (2009) 326:1509-1512; Moscou *et al.*, *Science* (2009) 326:3501.

[0307] Meganucleases are sequence-specific enzymes in the endonuclease family which are characterized by their capacity to recognize and cut large DNA sequences (from 14 to 40 base pairs). (Chevalier, B. S. and B. L. Stoddard, *Nucleic Acids Res.*, 2001, 29, 3757-3774). They can cleave unique sites in living cells, thereby enhancing gene targeting by 1000-fold or more in the vicinity of the cleavage site (Puchta *et al.*, *Nucleic Acids Res.*, 1993, 21, 5034-5040; Rouet *et al.*, *Mol. Cell. Biol.*, 1994, 14, 8096-8106; Choulika *et al.*, *Mol. Cell. Biol.*, 1995, 15, 1968-1973; Puchta *et al.*, *Proc. Natl. Acad. Sci. USA*, 1996, 93, 5055-5060; Sargent *et al.*, *Mol. Cell. Biol.*, 1997, 17, 267-77; Donoho *et al.*, *Mol. Cell. Biol.*, 1998, 18, 4070-4078; Elliott *et al.*, *Mol. Cell. Biol.*, 1998, 18, 93-101; Cohen-Tannoudji *et al.*, *Mol. Cell. Biol.*, 1998, 18, 1444-1448).

[0308] Meganucleases are grouped into families based on their structural motifs which affect nuclease activity and/or DNA recognition. The most widespread and best known meganucleases are the proteins in the LAGLIDADG family, which owe their name to a conserved amino acid sequence. See Chevalier *et al.*, *Nucleic Acids Res.* (2001) 29(18): 3757-3774. On the other hand, the GIY-YIG family members have a GIY-YIG module, which is 70-100 residues long and includes four or five conserved sequence motifs with four invariant residues, two of which are required for activity. See Van Roey *et al.*, *Nature Struct. Biol.* (2002) 9:806-811. The His-Cys family meganucleases are characterized by a highly conserved series of histidines and cysteines over a region encompassing several hundred amino acid residues. See Chevalier *et al.*, *Nucleic Acids Res.* (2001) 29(18):3757-3774. Members of the NHN family are defined by motifs containing two pairs of conserved histidines surrounded by asparagine residues. See Chevalier *et al.*, *Nucleic Acids Res.* (2001) 29(18):3757-3774.

[0309] Because the chance of identifying a natural meganuclease for a particular target DNA sequence is low due to the high specificity requirement, various methods including mutagenesis and high throughput screening methods have been used to create meganuclease variants that recognize unique sequences. Strategies for engineering a meganuclease with altered DNA-binding specificity, *e.g.*, to bind to a predetermined nucleic acid sequence are known in the art. See, *e.g.*, Chevalier *et al.*, *Mol. Cell.* (2002) 10:895-905; Epinat *et al.*, *Nucleic Acids Res* (2003) 31:2952-2962;

Silva *et al.*, *J Mol. Biol.* (2006) 361:744-754; Seligman *et al.*, *Nucleic Acids Res* (2002) 30:3870-3879; Sussman *et al.*, *J Mol Biol* (2004) 342:31-41; Doyon *et al.*, *J Am Chem Soc* (2006) 128:2477-2484; Chen *et al.*, *Protein Eng Des Sel* (2009) 22:249-256; Arnould *et al.*, *J Mol Biol.* (2006) 355:443-458; Smith *et al.*, *Nucleic Acids Res.* (2006) 363(2):283-294.

[0310] Like ZFNs and TALENs, Meganucleases can create DSBs in the genomic DNA, which can create a frame-shift mutation if improperly repaired, *e.g.*, via NHEJ, leading to a decrease in the expression of a target gene in a cell. Alternatively, foreign DNA can be introduced into the cell along with the meganuclease. Depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to modify the target gene. See Silva *et al.*, *Current Gene Therapy* (2011) 11:11-27.

[0311] Transposases are enzymes that bind to the end of a transposon and catalyze its movement to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. By linking transposases to other systems such as the CRISPR/Cas system, new gene editing tools can be developed to enable site specific insertions or manipulations of the genomic DNA. There are two known DNA integration methods using transposons which use a catalytically inactive Cas effector protein and Tn7-like transposons. The transposase-dependent DNA integration does not provoke DSBs in the genome, which may guarantee safer and more specific DNA integration.

[0312] The CRISPR system was originally discovered in prokaryotic organisms (*e.g.*, bacteria and archaea) as a system involved in defense against invading phages and plasmids that provides a form of acquired immunity. Now it has been adapted and used as a popular gene editing tool in research and clinical applications.

[0313] CRISPR/Cas systems generally comprise at least two components: one or more guide RNAs (gRNAs) and a Cas protein. The Cas protein is a nuclease that introduces a DSB into the target site. CRISPR-Cas systems fall into two major classes: class 1 systems use a complex of multiple Cas proteins to degrade nucleic acids; class 2 systems use a single large Cas protein for the same purpose. Class 1 is divided into types I, III, and IV; class 2 is divided into types II, V, and VI. Different Cas proteins adapted for gene editing applications include, but are not limited to, Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr5, Cse1, Cse2, Csf1, Csm2, Csn2, Csx10, Csx11, Csy1, Csy2, Csy3, and Mad7. The most widely used Cas9 is described herein as illustrative. These Cas proteins may be originated from different source species. For example, Cas9 can be derived from *S. pyogenes* or *S. aureus*.

[0314] In the original microbial genome, the type II CRISPR system incorporates sequences from invading DNA between CRISPR repeat sequences encoded as arrays within the host genome. Transcripts from the CRISPR repeat arrays are processed into CRISPR RNAs (crRNAs) each harboring a variable sequence transcribed from the invading DNA, known as the "protospacer" sequence, as well as part of the CRISPR repeat. Each crRNA hybridizes with a second transactivating CRISPR RNA (tracrRNA), and these two RNAs form a complex with the Cas9 nuclease. The protospacer-encoded portion of the crRNA directs the Cas9 complex to cleave complementary target DNA sequences, provided that they are adjacent to short sequences known as "protospacer adjacent motifs" (PAMs).

[0315] Since its discovery, the CRISPR system has been adapted for inducing sequence specific DSBs and targeted genome editing in a wide range of cells and organisms spanning from bacteria to eukaryotic cells including human cells. In its use in gene editing applications, artificially designed, synthetic gRNAs have replaced the original crRNA:tracrRNA complex. For example, the gRNAs can be single guide RNAs (sgRNAs) composed of a crRNA, a tetraloop, and a tracrRNA. The crRNA usually comprises a complementary region (also called a spacer, usually about 20 nucleotides in length) that is user-designed to recognize a target DNA of interest. The tracrRNA sequence comprises a scaffold region for Cas nuclease binding. The crRNA sequence and the tracrRNA sequence are linked by the tetraloop

and each have a short repeat sequence for hybridization with each other, thus generating a chimeric sgRNA. One can change the genomic target of the Cas nuclease by simply changing the spacer or complementary region sequence present in the gRNA. The complementary region will direct the Cas nuclease to the target DNA site through standard RNA-DNA complementary base pairing rules.

[0316] In order for the Cas nuclease to function, there must be a PAM immediately downstream of the target sequence in the genomic DNA. Recognition of the PAM by the Cas protein is thought to destabilize the adjacent genomic sequence, allowing interrogation of the sequence by the gRNA and resulting in gRNA-DNA pairing when a matching sequence is present. The specific sequence of PAM varies depending on the species of the Cas gene. For example, the most commonly used Cas9 nuclease derived from *S. pyogenes* recognizes a PAM sequence of 5'-NGG-3' or, at less efficient rates, 5'-NAG-3', where "N" can be any nucleotide. Other Cas nuclease variants with alternative PAMs have also been characterized and successfully used for genome editing, which are summarized in **Table 3** below.

Table 3. Exemplary Cas nuclease variants and their PAM sequences

CRISPR Nuclease	Source Organism	PAM Sequence (5'→3')
SpCas9	<i>Streptococcus pyogenes</i>	NGG or NAG
SaCas9	<i>Staphylococcus aureus</i>	NGRRT or NGRRN
NmeCas9	<i>Neisseria meningitidis</i>	NNNGATT
CjCas9	<i>Campylobacter jejuni</i>	NNNNRYAC
StCas9	<i>Streptococcus thermophilus</i>	NNAGAAW
TdCas9	<i>Treponema denticola</i>	NAAAAC
LbCas12a (Cpf1)	<i>Lachnospiraceae bacterium</i>	TTTV
AsCas12a (Cpf1)	<i>Acidaminococcus sp.</i>	TTTV
AacCas12b	<i>Alicyclobacillus acidiphilus</i>	TTN
BhCas12b v4	<i>Bacillus hisashii</i>	ATTN, TTTN, or GTTN

R = A or G; Y = C or T; W = A or T; V = A or C or G; N = any base

[0317] In some embodiments, Cas nucleases may comprise one or more mutations to alter their activity, specificity, recognition, and/or other characteristics. For example, the Cas nuclease may have one or more mutations that alter its fidelity to mitigate off-target effects (*e.g.*, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9). For another example, the Cas nuclease may have one or more mutations that alter its PAM specificity.

[0318] Nuclease domains of the Cas, in particular the Cas9, nuclease can be mutated independently to generate enzymes referred to as DNA "nickases". Nickases are capable of introducing a single-strand cut with the same specificity as a regular CRISPR/Cas nuclease system, including for example CRISPR/Cas9. Nickases can be employed to generate double-strand breaks which can find use in gene editing systems (Mali *et al.*, *Nat Biotech*, 31(9):833-838 (2013); Mali *et al.*, *Nature Methods*, 10:957-963 (2013); Mali *et al.*, *Science*, 339(6121):823-826 (2013)). In some instances, when two Cas nickases are used, long overhangs are produced on each of the cleaved ends instead of blunt ends which allows for additional control over precise gene integration and insertion (Mali *et al.*, *Nat Biotech*, 31(9):833-838 (2013); Mali *et al.*, *Nature Methods*, 10:957-963 (2013); Mali *et al.*, *Science*, 339(6121):823-826 (2013)). As both nicking Cas enzymes must effectively nick their target DNA, paired nickases can have lower off-target effects compared to the double-strand-cleaving Cas-based systems (Ran *et al.*, *Cell*, 155(2):479-480(2013); Mali *et al.*, *Nat Biotech*, 31(9):833-838 (2013); Mali *et al.*, *Nature Methods*, 10:957-963 (2013); Mali *et al.*, *Science*, 339(6121):823-826 (2013)).

[0319] The molecular machinery of such Cas proteins that allows the CRISPR/Cas system to alter target polynucleotide sequences in cells include RNA binding proteins, endo- and exo-nucleases, helicases, and polymerases. In some embodiments, the CRISPR/Cas system includes a Cas protein and at least one to two ribonucleic acids that are

capable of directing the Cas protein to and hybridizing to a target motif of a target polynucleotide sequence. As used herein, "protein" and "polypeptide" are used interchangeably to refer to a series of amino acid residues joined by peptide bonds (i.e., a polymer of amino acids) and include modified amino acids (e.g., phosphorylated, glycosylated, etc.) and amino acid analogs. Exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, paralogs, fragments and other equivalents, variants, and analogs of the above.

[0320] In some embodiments, a Cas protein comprises one or more amino acid substitutions or modifications. In some embodiments, the one or more amino acid substitutions comprises a conservative amino acid substitution. In some instances, substitutions and/or modifications can prevent or reduce proteolytic degradation and/or extend the half-life of the polypeptide in a cell. In some embodiments, the Cas protein can comprise a peptide bond replacement (*e.g.*, urea, thiourea, carbamate, sulfonyl urea, etc.). In some embodiments, the Cas protein can comprise a naturally occurring amino acid. In some embodiments, the Cas protein can comprise an alternative amino acid (*e.g.*, D-amino acids, beta-amino acids, homocysteine, phosphoserine, etc.). In some embodiments, a Cas protein can comprise a modification to include a moiety (*e.g.*, PEGylation, glycosylation, lipidation, acetylation, end-capping, etc.).

[0321] In some embodiments, a Cas protein comprises a core Cas protein, isoform thereof, or any Cas-like protein with similar function or activity of any Cas protein or isoform thereof. In some embodiments, a Cas protein comprises a core Cas protein. Exemplary Cas core proteins include, but are not limited to Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 and Cas9. In some embodiments, a Cas protein comprises type V Cas protein. In some embodiments, a Cas protein comprises a Cas protein of an E. coli subtype (also known as CASS2). Exemplary Cas proteins of the E. Coli subtype include, but are not limited to Cse1, Cse2, Cse3, Cse4, and Cas5e. In some embodiments, a Cas protein comprises a Cas protein of the Ypest subtype (also known as CASS3). Exemplary Cas proteins of the Ypest subtype include, but are not limited to Csy1, Csy2, Csy3, and Csy4. In some embodiments, a Cas protein comprises a Cas protein of the Nmeni subtype (also known as CASS4). Exemplary Cas proteins of the Nmeni subtype include, but are not limited to Csn1 and Csn2. In some embodiments, a Cas protein comprises a Cas protein of the Dvulg subtype (also known as CASS1). Exemplary Cas proteins of the Dvulg subtype include Csd1, Csd2, and Cas5d. In some embodiments, a Cas protein comprises a Cas protein of the Tneap subtype (also known as CASS7). Exemplary Cas proteins of the Tneap subtype include, but are not limited to, Cst1, Cst2, Cas5t. In some embodiments, a Cas protein comprises a Cas protein of the Hmari subtype. Exemplary Cas proteins of the Hmari subtype include, but are not limited to Csh1, Csh2, and Cas5h. In some embodiments, a Cas protein comprises a Cas protein of the Apern subtype (also known as CASS5). Exemplary Cas proteins of the Apern subtype include, but are not limited to Csa1, Csa2, Csa3, Csa4, Csa5, and Cas5a. In some embodiments, a Cas protein comprises a Cas protein of the Mtube subtype (also known as CASS6). Exemplary Cas proteins of the Mtube subtype include, but are not limited to Csm1, Csm2, Csm3, Csm4, and Csm5. In some embodiments, a Cas protein comprises a RAMP module Cas protein. Exemplary RAMP module Cas proteins include, but are not limited to, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, and Cmr6. See, *e.g.*, Klompe et al., Nature 571, 219–225 (2019); Strecker et al., Science 365, 48–53 (2019). Examples of Cas proteins include, but are not limited to: Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, and/or GSU0054. In some embodiments, a Cas protein comprises Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, and/or GSU0054. Examples of Cas proteins include, but are not limited to: Cas9, Csn2, and/or Cas4. In some embodiments, a Cas protein comprises Cas9, Csn2, and/or Cas4. In some embodiments, Examples of Cas proteins include, but are not limited to: Cas10, Csm2, Cmr5, Cas10, Csx11, and/or Csx10. In some embodiments, a Cas protein comprises a Cas10, Csm2, Cmr5, Cas10, Csx11, and/or Csx10. In some embodiments, examples of Cas proteins include, but are not limited to: Csf1. In some embodiments, a Cas protein comprises Csf1. In some embodiments, examples of Cas proteins include, but are not limited

to: Cas12a, Cas12b, Cas12c, C2c4, C2c8, C2c5, C2c10, and C2c9; as well as CasX (Cas12e) and CasY (Cas12d). Also see, e.g., Koonin et al., *Curr Opin Microbiol.* 2017; 37:67-78: "Diversity, classification and evolution of CRISPR-Cas systems." In some embodiments, a Cas protein comprises Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, and/or Cas12f. In some embodiments, a Cas protein comprises Cas13, Cas13a, C2c2, Cas13b, Cas13c, and/or Cas13d. In some embodiments, the CRISPR/Cas system comprises a Cas effector protein selected from the group consisting of: a) Cas3, Cas8a, Cas5, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, and GSU0054; b) Cas9, Csn2, and Cas4; c) Cas10, Csm2, Cmr5, Cas10, Csx11, and Csx10; d) Csf1; e) Cas12a, Cas12b, Cas12c, C2c4, C2c8, C2c5, C2c10, C2c9, CasX (Cas12e), and CasY (Cas12d); and f) Cas13, Cas13a, C2c2, Cas13b, Cas13c, and Cas13d.

[0322] In some embodiments, a Cas protein comprises any one of the Cas proteins described herein or a functional portion thereof. As used herein, "functional portion" refers to a portion of a peptide which retains its ability to complex with at least one ribonucleic acid (*e.g.*, guide RNA (gRNA)) and cleave a target polynucleotide sequence. In some embodiments, the functional portion comprises a combination of operably linked Cas9 protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional portion comprises a combination of operably linked Cas12a (also known as Cpf1) protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional domains form a complex. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of a RuvC-like domain. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of the HNH nuclease domain. In some embodiments, a functional portion of the Cas12a protein comprises a functional portion of a RuvC-like domain.

[0323] In some embodiments, the exogenous Cas protein can be introduced into the cell in polypeptide form. In certain embodiments, the Cas proteins can be conjugated to or fused to a cell-penetrating polypeptide or cell-penetrating peptide. As used herein, "cell-penetrating polypeptide" and "cell-penetrating peptide" refers to a polypeptide or peptide, respectively, which facilitates the uptake of molecule into a cell. The cell-penetrating polypeptides can contain a detectable label.

[0324] In many embodiments, Cas proteins can be conjugated to or fused to a charged protein (*e.g.*, that carries a positive, negative or overall neutral electric charge). Such linkage may be covalent. In some embodiments, the Cas protein can be fused to a superpositively charged GFP to significantly increase the ability of the Cas protein to penetrate a cell (Cronican et al. *ACS Chem Biol.* 2010; 5(8):747-52). In certain embodiments, the Cas protein can be fused to a protein transduction domain (PTD) to facilitate its entry into a cell. Exemplary PTDs include Tat, oligoarginine, and penetratin. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a cell-penetrating peptide. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a PTD. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a tat domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to an oligoarginine domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a penetratin domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a superpositively charged GFP. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a cell-penetrating peptide. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a PTD. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a tat domain. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to an oligoarginine domain. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a penetratin domain. In some embodiments, the Cas12a protein comprises a Cas12a polypeptide fused to a superpositively charged GFP.

[0325] In some embodiments, the Cas protein can be introduced into a cell containing the target polynucleotide sequence in the form of a nucleic acid encoding the Cas protein. The process of introducing the nucleic acids into cells can be achieved by any suitable technique. Suitable techniques include those described herein.

[0326] In some embodiments, the Cas protein is complexed with one to two ribonucleic acids. In some embodiments, the Cas protein is complexed with two ribonucleic acids. In some embodiments, the Cas protein is complexed with one ribonucleic acid. In some embodiments, the Cas protein is encoded by a modified nucleic acid, as described herein (*e.g.*, a synthetic, modified mRNA).

[0327] The methods of the present disclosure contemplate the use of any ribonucleic acid that is capable of directing a Cas protein to and hybridizing to a target motif of a target polynucleotide sequence. In some embodiments, at least one of the ribonucleic acids comprises tracrRNA. In some embodiments, at least one of the ribonucleic acids comprises CRISPR RNA (crRNA). In some embodiments, a single ribonucleic acid comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, at least one of the ribonucleic acids comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, both of the one to two ribonucleic acids comprise a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. The ribonucleic acids of the present disclosure can be selected to hybridize to a variety of different target motifs, depending on the particular CRISPR/Cas system employed, and the sequence of the target polynucleotide, as will be appreciated by those skilled in the art. The one to two ribonucleic acids can also be selected to minimize hybridization with nucleic acid sequences other than the target polynucleotide sequence. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least two mismatches when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least one mismatch when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids are designed to hybridize to a target motif immediately adjacent to a deoxyribonucleic acid motif recognized by the Cas protein. In some embodiments, each of the one to two ribonucleic acids are designed to hybridize to target motifs immediately adjacent to deoxyribonucleic acid motifs recognized by the Cas protein which flank a mutant allele located between the target motifs.

[0328] In some embodiments, each of the one to two ribonucleic acids comprises guide RNAs that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell.

[0329] In some embodiments, one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to sequences on the same strand of a target polynucleotide sequence. In some embodiments, one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.*, guide RNAs) are not complementary to and/or do not hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to overlapping target motifs of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.*, guide RNAs) are complementary to and/or hybridize to offset target motifs of a target polynucleotide sequence.

VIII. Methods of expressing engineered CD47 proteins

[0330] The engineered CD47 protein provided herein can be produced by any method known to those of skill in the art including *in vivo* and *in vitro* methods. Desired proteins can be expressed in any organism suitable to produce

the required amounts and forms of the proteins. Expression hosts include prokaryotic and eukaryotic organisms such as *E. coli*, yeast, plants, insect cells, mammalian cells, including human cell lines and transgenic animals. Expression hosts can differ in their protein production levels as well as the types of post-translational modification that are present on the expressed proteins. The choice of expression host can be made based on these and other factors, such as regulatory and safety considerations, production costs and the need and methods for purification.

[0331] In some embodiments, introducing the polynucleotides encoding the engineered CD47 protein described herein into cells can be achieved by any suitable technique. Suitable techniques include calcium phosphate, lipid-mediated transfection, electroporation, fusogens, and transduction or infection using a viral vector, as discussed herein. In some embodiments, the polynucleotides are introduced into a cell via viral transduction (*e.g.*, AAV transduction, lentiviral transduction) or otherwise delivered on a viral vector (*e.g.*, fusogen-mediated delivery). In some embodiments, the polynucleotides are introduced into a cell via a fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mos1 transposons, and conditional or inducible Tol2 transposons.

[0332] Many expression vectors are available and known to those of skill in the art and can be used for expression of proteins. The choice of expression vector will be influenced by the choice of host expression system. In general, expression vectors can include transcriptional promoters and optionally enhancers, translational signals, and transcriptional and translational termination signals. Expression vectors that are used for stable transformation typically have a selectable marker which allows selection and maintenance of the transformed cells. In some cases, an origin of replication can be used to amplify the copy number of the vector.

[0333] Expression vectors can be introduced into host cells via, for example, transformation, transfection, transduction, infection, electroporation, and sonoporation. A skilled artisan is able to select methods and conditions suitable for introducing an expression vector into host cells.

[0334] In some embodiments, the engineered CD47 protein is delivered using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide.

[0335] In some embodiments, the engineered CD47 protein is delivered using one or more gene editing systems. In some embodiments, the gene editing system is CRISPR/Cas. In some embodiments, the gene editing system includes a TALEN. In some embodiments, the gene editing system includes a zinc finger nuclease. In some embodiments, the gene editing system includes a meganuclease.

[0336] Following the introduction of a vector comprising a selectable marker, cells can be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant cells of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell types. In some embodiments, the engineered CD47 protein is expressed in a mammalian expression system. Expression constructs can be transferred to mammalian cells by viral infection, such as by adenovirus constructs, or by direct DNA transfer, such as liposomes, calcium phosphate, DEAE-dextran, and by physical means such as electroporation and microinjection. In some embodiments, the engineered CD47 protein is delivered using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector

pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some embodiments, the engineered CD47 protein is delivered using one or more gene editing systems. In some embodiments, the gene editing system is CRISPR/Cas. In some embodiments, the gene editing system includes a TALEN. In some embodiments, the gene editing system includes a zinc finger nuclease. In some embodiments, the gene editing system includes a meganuclease.

[0337] In some embodiments, the expression system used in the context of this disclosure is disclosed in the US application 63/270,454.

[0338] Expression vectors for mammalian cells typically include an mRNA cap site, a TATA box, a translational initiation sequence (Kozak consensus sequence), and polyadenylation elements. IRES elements also can be added to permit bicistronic expression with another gene, such as a selectable marker. Such vectors often include transcriptional promoter-enhancers for high-level expression, for example the SV40 promoter-enhancer, the human cytomegalovirus (CMV) promoter and the long terminal repeat of Rous sarcoma virus (RSV). These promoter-enhancers are active in many cell types. Tissue and cell-type promoters and enhancer regions also can be used for expression. Exemplary promoter/enhancer regions include, but are not limited to, those from genes such as elastase I, insulin, immunoglobulin, mouse mammary tumor virus, albumin, alpha fetoprotein, alpha 1 antitrypsin, beta globin, myelin basic protein, myosin light chain 2, and gonadotrophic releasing hormone gene control. Selectable markers can be used to select for and maintain cells with the expression construct. Examples of selectable marker genes include, but are not limited to, hygromycin B phosphotransferase, adenosine deaminase, xanthine-guanine phosphoribosyl transferase, aminoglycoside phosphotransferase, dihydrofolate reductase (DHFR) and thymidine kinase. For example, expression can be performed in the presence of methotrexate to select for only those cells expressing the DHFR gene.

[0339] Once the vector has been incorporated into the appropriate host cell, the host cell is maintained under conditions suitable for expression of the engineered CD47 proteins encoded by the incorporated polynucleotides. A skilled artisan is able to select conditions suitable for expression of the engineered CD47 proteins.

IX. Other Features

[0340] It will be understood that, in the process of manufacturing a cell therapy, certain modifications may be introduced to the cell that are considered desirable for the cell therapy product. Cells that are profiled for donor capability can be edited or unedited cells. Profiling cells can take place before or after cell editing. Edited cells include one or more modifications such as HIP modifications (hypoimmune gene modifications that enable immune evasion). When transplanted in vivo without immunosuppression, HIP-modified cells have reduced expression of one or more molecules of the MHC class I and/or MHC class II molecules and thus there may be no evidence of a systemic immune response, such as no T cell activation, antibody production, or NK cell activity. Disclosure relating to edited cells is provided herein. Such disclosure is applicable to the methods, uses and cells described herein. The methods for profiling a population of cells for donor capability disclosed herein are advantageous for many cell types as described herein. In some embodiments, the cells are T cells (e.g., CAR-T cells).

Engineered Cells and Methods of Engineering Cells

[0341] One modification considered desirable for the cell therapy product is that the engineered cells and populations thereof exhibit reduced expression of one or more molecules of the MHC class I and/or MHC class II molecules. A further modification considered desirable for the cell therapy product is that the engineered cells and populations thereof exhibit increased expression of at least one tolerogenic factor, such as tolerogenic factors described herein.

[0342] Provided herein are methods and compositions for alleviating and/or evading the effects of immune system reactions to allogeneic transplants. To overcome the problem of immune rejection of cell-derived and/or tissue transplants, disclosed herein is an engineered immune-evasive cell (e.g., an engineered primary hypo-immunogenic cell), or population or pharmaceutical composition thereof, that represents a viable source for any transplantable cell type. The engineered cells disclosed herein provide for reduced recognition the recipient subject's immune system, regardless of the subject's genetic make-up, or any existing response within the subject to one or more previous allogeneic transplants, previous autologous chimeric antigen receptor (CAR) T rejection, and/or other autologous or allogeneic therapies wherein a transgene is expressed. The engineered cells may include, but are not limited to, beta islet cells, B cells, T cells, NK cells, retinal pigmented epithelium cells, glial progenitor cells, endothelial cells, hepatocytes, thyroid cells, skin cells, and blood cells (e.g., plasma cells or platelets).

[0343] In some embodiments, the engineered cells described herein further comprise increased expression and/or overexpression of one or more complement inhibitors. In some embodiments, the one or more complement inhibitors are selected from CD46, CD59, and DAF/CD55. In some embodiments, the engineered cells comprise increased expression of two or more complement inhibitors in combination, such as increased expression of CD46 and CD59 or increased expression of CD46, CD59, and CD55.

[0344] The engineered cells provided herein utilize expression of tolerogenic factors and can also modulate (e.g., reduce or eliminate) one or more MHC class I molecules and/or one or more MHC class II molecules expression (e.g., surface expression). In some embodiments, genome editing technologies utilizing rare-cutting endonucleases (e.g., the CRISPR/Cas, TALEN, zinc finger nuclease, meganuclease, and homing endonuclease systems) are also used to reduce or eliminate expression of critical immune genes (e.g., by deleting genomic DNA of critical immune genes) in human cells. In certain embodiments, genome editing technologies or other gene modulation technologies are used to insert tolerance-inducing (tolerogenic) factors in human cells, (e.g., CD47), thus producing engineered cells that can evade immune recognition upon engrafting into a recipient subject. Therefore, the engineered cells provided herein exhibit modulated expression (e.g., reduced or eliminated expression) of one or more genes and factors that affect one or more MHC class I molecules and/or one or more MHC class II molecules, modulated expression (e.g., reduced or and modulated expression (e.g., overexpression) of tolerogenic factors, such as CD47, and provide for reduced recognition by the recipient subject's immune system. In some embodiments, the engineered cells provided herein exhibit modulated expression (e.g., reduced expression) of CD142. In some embodiments, the engineered cells provided herein exhibit modulated expression (e.g., increased expression) of one or more complement inhibitors selected from CD46, CD59, and DAF/CD55.

[0345] In some aspects, engineered cells provided herein exhibit reduced innate immune cell rejection and/or adaptive immune cell rejection (e.g., hypo-immunogenic cells). For example, in some embodiments, the engineered cells exhibit reduced susceptibility to NK cell-mediated lysis and/or macrophage engulfment. In some embodiments, the engineered cells are useful as a source of universally compatible cells or tissues (e.g., universal donor cells or tissues) that are transplanted into a recipient subject with little to no immunosuppressant agent needed. Such hypo-immunogenic cells retain cell-specific characteristics and features upon transplantation.

[0346] Also provided herein are methods for treating a disorder comprising administering the engineered cells (e.g., engineered primary cells) that evade immune rejection in an MHC-mismatched allogeneic recipient. In some embodiments, the engineered cells produced from any one of the methods described herein evade immune rejection when repeatedly administered (e.g., transplanted or grafted) to MHC-mismatched allogeneic recipient.

[0347] The practice of the embodiments described herein will employ, unless indicated specifically to the contrary, conventional methods of chemistry, biochemistry, organic chemistry, molecular biology, microbiology, recombinant DNA techniques, genetics, immunology, and cell biology that are within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See e.g., Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); Maniatis et al., *Molecular Cloning: A Laboratory Manual* (1982); Ausubel et al., *Current Protocols in Molecular Biology* (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience; Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford, 1985); Anand, *Techniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); *Transcription and Translation* (B. Hames & S. Higgins, Eds., 1984); Perbal, *A Practical Guide to Molecular Cloning* (1984); Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998) *Current Protocols in Immunology* Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); *Annual Review of Immunology*; as well as monographs in journals such as *Advances in Immunology*.

[0348] All publications, including patent documents, scientific articles, and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

[0349] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of the present disclosure. The following description illustrates the disclosure and, of course, should not be construed in any way as limiting the scope of contemplated embodiments described herein.

[0350] Described here are engineered cells that comprise one or more modifications. In some embodiments, the provided engineered cells also contain a modification of one or more target polynucleotide sequences that regulates the expression of one or more MHC class I molecules, one or more MHC class II molecules, or one or more MHC class I molecules and one or more MHC class II molecules.

[0351] In some embodiments, the provided engineered cells also include a modification to increase expression of one or more tolerogenic factor. In some embodiments, the tolerogenic factor is one or more of A20/TNFAIP3, B2M-HLA-E, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, CD52, CD55, CD59, CD64, CD200, CCL21, CCL22, CTLA4-Ig, C1 inhibitor, CR1, DUX4, FASL, HLA-C, HLA-E, HLA-E heavy chain, HLA-F, HLA-G, H2-M3, IDO1, IL-10, IL15-RF, IL-35, IL-39, MANF, Mfge8, PD-L1, Serpinb9, or any combination thereof. In some embodiments, the modification to increase expression of one or more tolerogenic factor is or includes increased expression of CD47. In some embodiments, the modification to increase expression of one or more tolerogenic factor is or includes increased expression of PD-L1. In some embodiments, the modification to increase expression of one or more tolerogenic factor is or includes increased expression of HLA-E. In some embodiments, the modification to increase expression of one or more tolerogenic factor is or includes increased expression of HLA-G. In some embodiments, the modification to increase expression of one or more tolerogenic factor is or includes increased expression of CCL21, PD-L1, FasL, Serpinb9, H2-M3 (HLA-G), CD47, CD200, and Mfge8.

[0352] In some embodiments, the cells include one or more genomic modifications that reduce expression of one or more MHC class I molecules and a modification that increases expression of CD47. In other words, the engineered cells comprise exogenous CD47 proteins and exhibit reduced or silenced surface expression of one or more MHC class I molecules. In some embodiments, the cells include one or more genomic modifications that reduce expression of one or more MHC class II molecules and a modification that increases expression of CD47. In some instances, the engineered cells comprise exogenous CD47 nucleic acids and proteins, and exhibit reduced or silenced surface expression of one or more MHC class I molecules. In some embodiments, the cells include one or more genomic modifications that reduce or eliminate expression of one or more MHC class II molecules, one or more genomic modifications that reduce or eliminate expression of one or more MHC class II molecules, and a modification that increases expression of CD47. In some embodiments, the engineered cells comprise exogenous CD47 proteins, exhibit reduced or silenced surface expression of one or more MHC class I molecules and exhibit reduced or lack surface expression of one or more MHC class II molecules. In many embodiments, the cells are $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $CD47^{tg}$ cells.

1. Chimeric Antigen Receptor

[0353] In some embodiments, a provided engineered cell is further modified to express a chimeric antigen receptor (CAR). It will be understood that embodiments concerning CAR modified cells may be readily applied to any suitable cell type as described herein, as well as HIP cells, safety switches and other modified/ gene edited cells as described herein.

[0354] In some embodiments, a provided cell contains a genetic modification of one or more target polynucleotide sequences that regulates the expression of one or more MHC class I molecules, one or more MHC class II molecules, or one or more MHC class I molecules and one or more MHC class II molecules overexpresses a tolerogenic factor as described herein (e.g., CD47), and expresses a CAR. In some embodiments, the cell is one in which: $B2M$ is reduced or eliminated (e.g., knocked out), $CIITA$ is reduced or eliminated (e.g., knocked out), CD47 is overexpressed, and a CAR is expressed. In some embodiments, the cell is $B2M^{-/-}$, $CIITA^{-/-}$, $CD47^{tg}$, CAR+. In some embodiments, the cell (e.g., T cell) may additionally be one in which TRAC is reduced or eliminated (e.g., knocked out). In some embodiments, the cell is $B2^{-/-}$, $CIITA$, $CD47^{tg}$, $TRAC^{-/-}$ CAR+.

[0355] In some embodiments, a polynucleotide encoding a CAR is introduced into the cell. In some embodiments, the cell is a T cell, such as a primary T cell or a T cell differentiated from a pluripotent cell (e.g., iPSC). In some embodiments, the cell is a Natural Killer (NK) cell, such as a primary NK cell or an NK cell differentiated from a pluripotent cell (e.g., iPSC).

[0356] In some embodiments, the CAR is selected from the group consisting of a first generation CAR, a second generation CAR, a third generation CAR, and a fourth generation CAR. In some embodiments, the CAR is or comprises a first generation CAR comprising an antigen binding domain, a transmembrane domain, and at least one signaling domain (e.g., one, two or three signaling domains). In some embodiments, the CAR comprises a second generation CAR comprising an antigen binding domain, a transmembrane domain, and at least two signaling domains. In some embodiments, the CAR comprises a third generation CAR comprising an antigen binding domain, a transmembrane domain, and at least three signaling domains. In some embodiments, a fourth generation CAR comprising an antigen binding domain, a transmembrane domain, three or four signaling domains, and a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, the antigen binding domain is or comprises an antibody, an antibody fragment, an scFv or a Fab.

[0357] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a first generation CAR. In some embodiments, a first generation CAR comprises an antigen binding domain, a transmembrane domain, and signaling domain. In some embodiments, a signaling domain mediates downstream signaling during T cell activation.

[0358] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a second generation CAR. In some embodiments, a second generation CAR comprises an antigen binding domain, a transmembrane domain, and two signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T cell proliferation, and/or CAR T cell persistence during T cell activation.

[0359] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a third generation CAR. In some embodiments, a third generation CAR comprises an antigen binding domain, a transmembrane domain, and at least three signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T cell proliferation, and or CAR T cell persistence during T cell activation. In some embodiments, a third generation CAR comprises at least two costimulatory domains. In some embodiments, the at least two costimulatory domains are not the same.

[0360] In some embodiments, any one of the cells described herein comprises a nucleic acid encoding a CAR or a fourth generation CAR. In some embodiments, a fourth generation CAR comprises an antigen binding domain, a transmembrane domain, and at least two, three, or four signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T cell proliferation, and or CAR T cell persistence during T cell activation.

[0361] In some embodiments, an engineered cell provided herein (e.g., primary or iPSC-derived T cell or primary or iPSC-derived NK cell) includes a polynucleotide encoding a CAR, wherein the polynucleotide is inserted in a genomic locus. In some embodiments, the polynucleotide is inserted into a safe harbor locus, such as but not limited to, an AAVS1, CCR5, CLYBL, ROSA26, SHS231, F3 (also known as CD142), MICA, MICB, LRP1 (also known as CD91), HMGB1, ABO, RHD, FUT1, or KDM5D gene locus. In some embodiments, the polynucleotide is inserted in a B2M, CIITA, TRAC, TRB, PD1 or CTLA4 gene. Any suitable method can be used to insert the CAR into the genomic locus of the hypoinmunogenic cell including the gene editing methods described herein (e.g., a CRISPR/Cas system).

[0362] In some embodiments, a first, second, third, or fourth generation CAR further comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, a cytokine gene is endogenous or exogenous to a target cell comprising a CAR which comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, a cytokine gene encodes a pro-inflammatory cytokine. In some embodiments, a cytokine gene encodes IL-1, IL-2, IL-9, IL-12, IL-18, TNF, or IFN-gamma, or functional fragment thereof. In some embodiments, a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments, a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments, a transcription factor or functional domain or fragment thereof is or comprises a nuclear factor of activated T cells (NFAT), an NF- κ B, or functional domain or fragment

thereof. See, e.g., Zhang. C. et al., Engineering CAR-T cells. Biomarker Research. 5:22 (2017); WO 2016126608; Sha, H. et al. Chimaeric antigen receptor T-cell therapy for tumour immunotherapy. Bioscience Reports Jan 27, 2017, 37 (1).

[0363] A skilled artisan is familiar with CARs and different components and configurations of CARs. Any known CAR can be employed in connection with the provided embodiments. In addition to the CARs described herein, various CARs and nucleotide sequences encoding the same are known in the art and would be suitable for engineering cells as described herein. See, e.g., WO2013040557; WO2012079000; WO2016030414; Smith T, et al., Nature Nanotechnology. 2017. DOI: 10.1038/NNANO.2017.57, the disclosures of which are herein incorporated by reference. Exemplary features and components of a CAR are described in the following subsections.

a. Antigen Binding Domain

[0364] In some embodiments, a CAR antigen binding domain (ABD) is or comprises an antibody or antigen-binding portion thereof. In some embodiments, a CAR antigen binding domain is or comprises an scFv or Fab.

[0365] In some embodiments, an antigen binding domain binds to a cell surface antigen of a cell. In some embodiments, a cell surface antigen is characteristic of (e.g., expressed by) a particular or specific cell type. In some embodiments, a cell surface antigen is characteristic of more than one type of cell.

[0366] In some embodiments, the antigen may be an antigen that is exclusively or preferentially expressed on tumor cells, or an antigen that is characteristic of an autoimmune or inflammatory disease. In some embodiments, the antigen binding domain (ABD) targets an antigen characteristic of a neoplastic cell. For instance, the antigen binding domain targets an antigen expressed by a neoplastic or cancer cell. In some embodiments, the ABD binds a tumor associated antigen. In some embodiments, the antigen characteristic of a neoplastic cell (e.g., antigen associated with a neoplastic or cancer cell) or a tumor associated antigen is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor.

[0367] In some embodiments, the target antigen is an antigen that includes, but is not limited to, Epidermal Growth Factor Receptors (EGFR) (including ErbB1/EGFR, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4), Fibroblast Growth Factor Receptors (FGFR) (including FGF1, FGF2, FGF3, FGF4, FGF5, FGF6, FGF7, FGF18, and FGF21) Vascular Endothelial Growth Factor Receptors (VEGFR) (including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF), RET Receptor and the Eph Receptor Family (including EphA1, EphA2, EphA3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphA10, EphB1, EphB2, EphB3, EphB4, and EphB6), CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GABA receptor, glycine receptor, ABC transporters, NAV1.1, NAV1.2, NAV1.3, NAV1.4, NAV1.5, NAV1.6, NAV1.7, NAV1.8, NAV1.9, sphingosin-1-phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs; T-cell alpha chains; T-cell β chains; T-cell γ chains; T-cell δ chains, CCR7, CD3, CD4, CD5, CD7, CD8, CD11b, CD11c, CD16, CD19, CD20, CD21, CD22, CD25, CD28, CD34, CD35, CD40, CD45RA, CD45RO, CD52, CD56, CD62L, CD68, CD80, CD95, CD117, CD127, CD133, CD137 (4-1 BB), CD163, F4/80, IL-4Ra, Sca-1, CTLA-4, GITR, GARP, LAP, granzyme B, LFA-1, transferrin receptor, NKp46, perforin, CD4+, Th1, Th2, Th17, Th40, Th22, Th9, Tfh, Canonical Treg, FoxP3+, Tr1, Th3, Treg17, T_{REG}, CD137, CD138, CD139, CD140, CD141, CD142, CD143, CD144, CD145, CD146, CD147, CD148, CD149, CD150, CD151, CD152, CD153, CD154, CD155, CD156, CD157, CD158, CD159, CD160, CD161, CD162, CD163, CD164, CD165, CD166, CD167, CD168, CD169, CD170, CD171, CD172, CD173, CD174, CD175, CD176, CD177, CD178, CD179, CD180, CD181, CD182, CD183, CD184, CD185, CD186, CD187, CD188, CD189, CD190, CD191, CD192, CD193, CD194, CD195, CD196, CD197, CD198, CD199, CD200, CD201, CD202, CD203, CD204, CD205, CD206, CD207, CD208, CD209, CD210, CD211, 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CD1311, CD1312, CD1313, CD1314, CD1315, CD1316, CD1317, CD1318, CD1319, CD1320, CD1321, CD1322, CD1323, CD1324, CD1325, CD1326, CD1327, CD1328, CD1329, CD1330, CD1331, CD1332, CD1333, CD1334, CD1335, CD1336, CD1337, CD1338, CD1339, CD1340, CD1341, CD1342, CD1343, CD1344, CD1345, CD1346, CD1347, CD1348, CD1349, CD1350, CD1351, CD1352, CD1353, CD1354, CD1355, CD1356, CD1357, CD1358, CD1359, CD1360, CD1361, CD1362, CD1363, CD1364, CD1365, CD1366, CD1367, CD1368, CD1369, CD1370, CD1371, CD1372, CD1373, CD1374, CD1375, CD1376, CD1377, CD1378, CD1379, CD1380, CD1381, CD1382, CD1383, CD1384, CD1385, CD1386, CD1387, CD1388, CD1389, CD1390, CD1391, CD1392, CD1393, CD1394, CD1395, CD1396, CD1397, CD1398, CD1399, CD1400, CD1401, CD1402, CD1403, CD1404, CD1405, CD1406, CD1407, CD1408, CD1409, CD1410, CD1411, CD1412, CD1413, CD1414, CD1415, CD1416, CD1417, CD1418, CD1419, CD1420, CD1421, CD1422, CD1423, CD1424, CD1425, CD1426, CD1427, CD1428, CD1429, CD1430, CD1431, CD1432, CD1433, CD1434, CD1435, CD1436, CD1437, CD1438, CD1439, CD1440, CD1441, CD1442, CD1443, CD1444, CD1445, CD1446, CD1447, CD1448, CD1449, CD1450, CD1451, CD1452, CD1453, CD1454, CD1455, CD1456, CD1457, CD1458, CD1459, CD1460, CD1461, CD1462, CD1463, CD1464, CD1465, CD1466, CD1467, CD1468, CD1469, CD1470, CD1471, CD1472, CD1473, CD1474, CD1475, CD1476, CD1477, CD1478, CD1479, CD1480, CD1481, CD1482, CD1483, CD1484, CD1485, CD1486, CD1487, CD1488, CD1489, CD1490, CD1491, CD1492, CD1493, CD1494, CD1495, CD1496, CD1497, CD1498, CD1499, CD1500, CD1501, CD1502, CD1503, CD1504, CD1505, CD1506, CD1507, CD1508, CD1509, CD1510, CD1511, CD1512, CD1513, CD1514, CD1515, CD1516, CD1517, CD1518, CD1519, CD1520, CD1521, CD1522, CD1523, CD1524, CD1525, CD1526, CD1527, CD1528, CD1529, CD1530, CD1531, CD1532, CD1533, CD1534, CD1535, CD1536, CD1537, CD1538, CD1539, CD1540, CD1541, CD1542, CD1543, CD1544, CD1545, CD1546, CD1547, CD1548, CD1549, CD1550, CD1551, CD1552, CD1553, CD1554, CD1555, CD1556, CD1557, CD1558, CD1559, CD1560, 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CD2061, CD2062, CD2063, CD2064, CD2065, CD2066, CD2067, CD2068, CD2069, CD2070, CD2071, CD2072, CD

ANTXR1, Folate receptor alpha (FRa), ERBB2 (Her2/neu), EphA2, IL-13Ra2, epidermal growth factor receptor (EGFR), Mesothelin, TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII, GD2, GD3, BCMA, MUC16 (CA125), L1CAM, LeY, MSLN, IL13R α 1, L1-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, interleukin-11 receptor a (IL-11Ra), PSCA, PRSS21, VEGFR2, LewisY, CD24, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, CD20, MUC1, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-1a, MAGE-A1, legumain, HPV E6, E7, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Major histocompatibility complex class I-related gene protein (MR1), urokinase-type plasminogen activator receptor (uPAR), Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYP1B 1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, a neoantigen, CD133, CD15, CD184, CD24, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, or trkC, or an antigenic fragment or antigenic portion thereof.

[0368] In some embodiments, exemplary target antigens include, but are not limited to, CDS, CD19, CD20, CD22, CD23, CD30, CD70, Kappa, Lambda, and B cell maturation agent (BCMA) (associated with leukemias); CS1/SLAMF7, CD38, CD138, GPRC5D, TACI, and BCMA (associated with myelomas); GD2, HER2, EGFR, EGFRvIII, B7H3, PSMA, PSCA, CAIX, CD171, CEA, CSPG4, EPHA2, FAP, FRa, IL-13Ra, Mesothelin, MUC1, MUC16, and ROR1 (associated with solid tumors).

[0369] In some embodiments, the CAR is a CD19 CAR. In some embodiments, the extracellular binding domain of the CD19 CAR comprises an antibody that specifically binds to CD19, for example, human CD19. In some embodiments, the extracellular binding domain of the CD19 CAR comprises an scFv antibody fragment derived from the FMC63 monoclonal antibody (FMC63), which comprises the heavy chain variable region (VH) and the light chain variable region (VL) of FMC63 connected by a linker peptide. In some embodiments, the linker peptide is a "Whitlow" linker peptide. FMC63 and the derived scFv have been described in Nicholson et al., *Mal. Immun.* 34(16-17):1157-1165 (1997) and PCT Application Publication No. WO2018/213337 A 1, the entire content of each of which is incorporated by reference herein.

[0370] In some embodiments, the extracellular binding domain of the CD19 CAR comprises an antibody derived from one of the CD19-specific antibodies including, for example, SJ25C1 (Bejcek et al., *Cancer Res.* 55:2346-2351 (1995)), HD37 (Pezutto et al., *J. Immunol.* 138(9):2793-2799 (1987)), 4G7 (Meeker et al., *Hybridoma* 3:305-320 (1984)), B43 (Bejcek (1995)), BLY3 (Bejcek (1995)), B4 (Freedman et al., 70:418-427 (1987)), B4 HB12b (Kansas & Tedder, *J. Immunol.* 147:4094-4102 (1991); Yazawa et al., *Proc. Natl. Acad. Sci. USA* 102:15178-15183 (2005); Herbst et al., *J. Pharmacol. Exp. Ther.* 335:213-222 (2010)), BU12 (Gallard et al., *J. Immunology*, 148(10): 2983-2987 (1992)), and CLB-CD19 (De Rie Cell. Immunol. 118:368-381(1989)).

[0371] In some embodiments, the CAR is CD22 CAR. CD22, which is a transmembrane protein found mostly on the surface of mature B cells that functions as an inhibitory receptor for B cell receptor (BCR) signaling. CD22 is expressed in 60-70% of B cell lymphomas and leukemias (e.g., B-chronic lymphocytic leukemia, hairy cell leukemia, acute lymphocytic leukemia (ALL), and Burkitt's lymphoma) and is not present on the cell surface in early stages of B cell development or on stem cells. In some embodiments, the CD22 CAR comprises an extracellular binding domain that specifically binds CD22, a transmembrane domain, an intracellular signaling domain, and/or an intracellular costimulatory

domain. In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv antibody fragment derived from the m971 monoclonal antibody (m971), which comprises the heavy chain variable region (VH) and the light chain variable region (VL) of m971 connected by a linker. In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv antibody fragment derived from m971-L7, which is an affinity matured variant of m971 with significantly improved CD22 binding affinity compared to the parental antibody m971 (improved from about 2 nM to less than 50 pM). In some embodiments, the scFv antibody fragment derived from m971-L7 comprises the VH and the VL of m971-L7 connected by a 3xG4S linker. In some embodiments, the extracellular binding domain of the CD22 CAR comprises immunotoxins HA22 or BL22. Immunotoxins BL22 and HA22 are therapeutic agents that comprise an scFv specific for CD22 fused to a bacterial toxin, and thus can bind to the surface of the cancer cells that express CD22 and kill the cancer cells. BL22 comprises a dsFv of an anti-CD22 antibody, RFB4, fused to a 38-kDa truncated form of *Pseudomonas* exotoxin A (Bang et al., Clin. Cancer Res., 11:1545-50 (2005)). HA22 (CAT8015, moxetumomab pasudotox) is a mutated, higher affinity version of BL22 (Ho et al., J. Biol. Chem., 280(1): 607-17 (2005)). Suitable sequences of antigen binding domains of HA22 and BL22 specific to CD22 are disclosed in, for example, U.S. Patent Nos. 7,541,034; 7,355,012; and 7,982,011, which are hereby incorporated by reference in their entirety.

[0372] In some embodiments, the CAR is BCMA CAR. BCMA is a tumor necrosis family receptor (TNFR) member expressed on cells of the B cell lineage, with the highest expression on terminally differentiated B cells or mature B lymphocytes. BCMA is involved in mediating the survival of plasma cells for maintaining long-term humoral immunity. The expression of BCMA has been recently linked to a number of cancers, such as multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, various leukemias, and glioblastoma. In some embodiments, the BCMA CAR comprises an extracellular binding domain that specifically binds BCMA, a transmembrane domain, an intracellular signaling domain, and/or an intracellular costimulatory domain. In some embodiments, the extracellular binding domain of the BCMA CAR comprises an antibody that specifically binds to BCMA, for example, human BCMA. CARs directed to BCMA have been described in PCT Application Publication Nos. WO2016/014789, WO2016/014565, WO2013/154760, and WO 2015/128653. BCMA-binding antibodies are also disclosed in PCT Application Publication Nos. WO2015/166073 and WO2014/068079. In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv antibody fragment derived from a murine monoclonal antibody as described in Carpenter et al., Clin. Cancer Res. 19(8):2048-2060 (2013). In some embodiments, the scFv antibody fragment is a humanized version of the murine monoclonal antibody (Sommermeyer et al., Leukemia 31:2191-2199 (2017)). In some embodiments, the extracellular binding domain of the BCMA CAR comprises single variable fragments of two heavy chains (VHH) that can bind to two epitopes of BCMA as described in Zhao et al., J. Hematol. Oncol. 11(1):141 (2018). In some embodiments, the extracellular binding domain of the BCMA CAR comprises a fully human heavy-chain variable domain (FHVH) as described in Lam et al., Nat. Commun. 11(1):283 (2020).

[0373] In some embodiments, the antigen binding domain targets an antigen characteristic of an autoimmune or inflammatory disorder. In some embodiments, the ABD binds an antigen associated with an autoimmune or inflammatory disorder. In some instances, the antigen is expressed by a cell associated with an autoimmune or inflammatory disorder. In some embodiments, the autoimmune or inflammatory disorder is selected from chronic graft-vs-host disease (GVHD), lupus, arthritis, immune complex glomerulonephritis, goodpasture, uveitis, hepatitis, systemic sclerosis or scleroderma, type I diabetes, multiple sclerosis, cold agglutinin disease, Pemphigus vulgaris, Grave's disease, autoimmune hemolytic anemia, Hemophilia A, Primary Sjogren's Syndrome, thrombotic thrombocytopenia purpura, neuromyelitis optica, Evan's syndrome, IgM mediated neuropathy, cryoglobulinemia, dermatomyositis, idiopathic thrombocytopenia, ankylosing spondylitis, bullous pemphigoid, acquired angioedema, chronic urticarial, antiphospholipid demyelinating polyneuropathy, and autoimmune thrombocytopenia or neutropenia or pure red cell aplasia, while

exemplary non-limiting examples of alloimmune diseases include allosensitization (see, for example, Blazar et al., 2015, *Am. J. Transplant*, 15(4):931-41) or xenosensitization from hematopoietic or solid organ transplantation, blood transfusions, pregnancy with fetal allosensitization, neonatal alloimmune thrombocytopenia, hemolytic disease of the newborn, sensitization to foreign antigens such as can occur with replacement of inherited or acquired deficiency disorders treated with enzyme or protein replacement therapy, blood products, and gene therapy. Allosensitization, in some instances, refers to the development of an immune response (such as circulating antibodies) against human leukocyte antigens that the immune system of the recipient subject or pregnant subject considers to be non-self antigens. In some embodiments, the antigen characteristic of an autoimmune or inflammatory disorder is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor.

[0374] In some embodiments, an antigen binding domain of a CAR binds to a ligand expressed on B cells, plasma cells, or plasmablasts. In some embodiments, an antigen binding domain of a CAR binds to CD10, CD19, CD20, CD22, CD24, CD27, CD38, CD45R, CD138, CD319, BCMA, GPRC5D, CD28, TNF, interferon receptors, GM-CSF, ZAP-70, LFA-1, CD3 gamma, CD5 or CD2. See, US 2003/0077249; WO 2017/058753; WO 2017/058850, the contents of which are herein incorporated by reference. In some embodiments, the CAR is an anti-CD19 CAR. In some embodiments, the CAR is an anti-BCMA CAR.

[0375] In some embodiments, the antigen binding domain targets an antigen characteristic of senescent cells, e.g., urokinase-type plasminogen activator receptor (uPAR). In some embodiments, the ABD binds an antigen associated with a senescent cell. In some instances, the antigen is expressed by a senescent cell. In some embodiments, the CAR may be used for treatment or prophylaxis of disorders characterized by the aberrant accumulation of senescent cells, e.g., liver and lung fibrosis, atherosclerosis, diabetes and osteoarthritis.

[0376] In some embodiments, the antigen binding domain targets an antigen characteristic of an infectious disease. In some embodiments, the ABD binds an antigen associated with an infectious disease. In some instances, the antigen is expressed by a cell affected by an infectious disease. In some embodiments, wherein the infectious disease is selected from HIV, hepatitis B virus, hepatitis C virus, Human herpes virus, Human herpes virus 8 (HHV-8, Kaposi sarcoma-associated herpes virus (KSHV)), Human T-lymphotrophic virus-1 (HTLV-1), Merkel cell polyomavirus (MCV), Simian virus 40 (SV40), Epstein-Barr virus, CMV, human papillomavirus. In some embodiments, the antigen characteristic of an infectious disease is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, HIV Env, gp120, or CD4-induced epitope on HIV-1 Env.

[0377] In any of these embodiments, the extracellular binding domain of the CAR can be codon-optimized for expression in a host cell or have variant sequences to increase functions of the extracellular binding domain.

[0378] In some embodiments, the CAR is bispecific to two target antigens. In some embodiments, the target antigens are different target antigens. In some of any such embodiments, the two different target antigens are any two different antigens described above. In some embodiments, the extracellular binding domains are different and bind two different antigens from (i) CD19 and CD20, (ii) CD20 and L1-CAM, (iii) L1-CAM and GD2, (iv) EGFR and L1-CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1. In some embodiments, each of the two different antigen binding domains is an scFv. In some embodiments, the C-terminus of one variable domain (VH or VL) of a first scFv is tethered to the N-terminus of the second scFv (VL or VH, respectively)

via a polypeptide linker. In some embodiments, the linker connects the N-terminus of the VH with the C-terminus of VL or the C-terminus of VH with the N-terminus of VL. These scFvs lack the constant regions (Fc) present in the heavy and light chains of the native antibody. The scFvs, specific for at least two different antigens, are arranged in tandem and linked to the co-stimulatory domain and the intracellular signaling domain via a transmembrane domain. In some embodiments, an extracellular spacer domain may be linked between the antigen-specific binding region and the transmembrane domain.

[0379] In some embodiments, each antigen-specific targeting region of the CAR comprises a divalent (or bivalent) single-chain variable fragment (di-scFvs, bi-scFvs). In CARs comprising di-scFVs, two scFvs specific for each antigen are linked together by producing a single peptide chain with two VH and two VL regions, yielding tandem scFvs. (Xiong, Cheng-Yi; Natarajan, A; Shi, X B; Denardo, G L; Denardo, S J (2006). "Development of tumor targeting anti-MUC-1 multimer: effects of di-scFv unpaired cysteine location on PEGylation and tumor binding". Protein Engineering Design and Selection 19 (8): 359-367; Kufer, Peter; Lutterbüse, Ralf; Baeuerle, Patrick A. (2004). "A revival of bispecific antibodies". Trends in Biotechnology 22 (5): 238-244). CARs comprising at least two antigen-specific targeting regions would express two scFvs specific for each of the two antigens. The resulting antigen-specific targeting region, specific for at least two different antigens, is joined to the co-stimulatory domain and the intracellular signaling domain via a transmembrane domain. In some embodiments, an extracellular spacer domain may be linked between the antigen-specific binding domain and the transmembrane domain.

[0380] In some embodiments, each antigen-specific targeting region of the CAR comprises a diabody. In a diabody, the scFvs are created with linker peptides that are too short for the two variable regions to fold together, driving the scFvs to dimerize. Still shorter linkers (one or two amino acids) lead to the formation of trimers, the so-called triabodies or tribodies. Tetrabodies may also be used.

[0381] In some embodiments, the cell is engineered to express more than one CAR, such as two different CARs, in which each CAR has an antigen-binding domain directed to a different target antigen. In some of any such embodiments, the two different target antigens are any two different antigens described above. In some embodiments, the extracellular binding domains are different and bind two different antigens from (i) CD19 and CD20, (ii) CD20 and L1-CAM, (iii) L1-CAM and GD2, (iv) EGFR and L1-CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1.

[0382] In some embodiments, two different engineered cells are prepared that contain the provided modifications with each engineered with a different CAR. In some embodiments, each of the two different CARs has an antigen-binding domain directed to a different target antigen. In some of any such embodiments, the two different target antigens are any two different antigens described above. In some embodiments, the extracellular binding domains are different and bind two different antigens from (i) CD19 and CD20, (ii) CD20 and L1-CAM, (iii) L1-CAM and GD2, (iv) EGFR and L1-CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1. In some embodiments, a population of engineered cells (e.g., hypoinmunogenic) expressing a first CAR directed against a first target antigen and a population of engineered cells (e.g., hypoinmunogenic) expressing a second CAR directed against a second target antigen are separately administered to the subject. In some embodiments, the first and second population of cells are administered sequentially in any order. For instance, the population of cells expressing the second CAR is administered after administration of the population of cells expressing the first CAR.

b. Spacer

[0383] In some embodiments, the CAR further comprises one or more spacers, e.g., wherein the spacer is a first spacer between the antigen binding domain and the transmembrane domain. In some embodiments, the first spacer

includes at least a portion of an immunoglobulin constant region or variant or modified version thereof. In some embodiments, the spacer is a second spacer between the transmembrane domain and a signaling domain. In some embodiments, the second spacer is an oligopeptide, e.g., wherein the oligopeptide comprises glycine and serine residues such as but not limited to glycine-serine doublets. In some embodiments, the CAR comprises two or more spacers, e.g., a spacer between the antigen binding domain and the transmembrane domain and a spacer between the transmembrane domain and a signaling domain.

c. Transmembrane Domain

[0384] In some embodiments, the CAR transmembrane domain comprises at least a transmembrane region of the alpha, beta or zeta chain of a T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD28, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or functional variant thereof. In some embodiments, the transmembrane domain comprises at least a transmembrane region(s) of CD8 α , CD8 β , 4-1BB/CD137, CD28, CD34, CD4, Fc ϵ RI γ , CD16, OX40/CD134, CD3 ζ , CD3 ϵ , CD3 γ , CD3 δ , TCR α , TCR β , TCR ζ , CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or functional variant thereof.

d. Signaling Domain(s)

[0385] In some embodiments, a CAR described herein comprises one or at least one signaling domain selected from one or more of B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6; 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFSF13C; CD27/TNFSF7; CD27 Ligand/TNFSF7; CD30/TNFSF8; CD30 Ligand/TNFSF8; CD40/TNFSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFSF25; GITR/TNFSF18; GITR Ligand/TNFSF18; HVEM/TNFSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFSF4; OX40 Ligand/TNFSF4; RELT/TNFSF19L; TACI/TNFSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFSF1B; 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB-A/SLAMF6; SLAM/CD150; CD2; CD7; CD53; CD82/Kai-1; CD90/Thy1; CD96; CD160; CD200; CD300a/LMIR1; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-1; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin-1/CLEC7A; DPPIV/CD26; EphB6; TIM-1/KIM-1/HAVCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, a CD3 zeta domain, an immunoreceptor tyrosine-based activation motif (ITAM), CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, or functional fragment thereof.

[0386] In some embodiments, the at least one signaling domain comprises a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof.

[0387] In some embodiments, a CAR comprises a signaling domain which is a costimulatory domain. In some embodiments, a CAR comprises a second costimulatory domain. In some embodiments, a CAR comprises at least two costimulatory domains. In some embodiments, a CAR comprises at least three costimulatory domains. In some embodiments, a CAR comprises a costimulatory domain selected from one or more of CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83. In some embodiments, if a CAR comprises two or more costimulatory domains, two costimulatory domains are different. In some embodiments, if a CAR comprises two or more costimulatory domains, two costimulatory domains are the same.

[0388] In other embodiments, the at least one signaling domain comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB

domain, or functional variant thereof. In yet other embodiments, the at least one signaling domain comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In some embodiments, the at least one signaling domain comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

[0389] In some embodiments, the at least two signaling domains comprise a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In other embodiments, the at least two signaling domains comprise (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof. In yet other embodiments, the at least one signaling domain comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In some embodiments, the at least two signaling domains comprise a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

[0390] In some embodiments, the at least three signaling domains comprise a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In other embodiments, the at least three signaling domains comprise (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof. In yet other embodiments, the at least three signaling domains comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof. In some embodiments, the at least three signaling domains comprise a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

[0391] In some embodiments, the CAR comprises a CD3 zeta domain or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof. In some embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; and (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof.

[0392] In some embodiments, the CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; and (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof.

[0393] In some embodiments, the CAR comprises (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain, or a 4-1BB domain, or functional variant thereof, and/or (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof.

[0394] In some embodiments, the CAR comprises a (i) a CD3 zeta domain, or an immunoreceptor tyrosine-based activation motif (ITAM), or functional variant thereof; (ii) a CD28 domain or functional variant thereof; (iii) a 4-1BB domain, or a CD134 domain, or functional variant thereof; and (iv) a cytokine or costimulatory ligand transgene.

e. Exemplary CARs

[0395] In some embodiments, the CAR comprises an extracellular antigen binding domain (e.g., antibody or antibody fragment, such as an scFv) that binds to an antigen (e.g., tumor antigen), a spacer (e.g., containing a hinge domain, such as any as described herein), a transmembrane domain (e.g., any as described herein), and an intracellular signaling domain (e.g., any intracellular signaling domain, such as a primary signaling domain or costimulatory signaling domain as described herein). In some embodiments, the intracellular signaling domain is or includes a primary cytoplasmic signaling domain. In some embodiments, the intracellular signaling domain additionally includes an intracellular signaling domain of a costimulatory molecule (e.g., a costimulatory domain). Any of such components can be any as described above.

[0396] Examples of exemplary components of a CAR are described in **Table 4**. In provided aspects, the sequences of each component in a CAR can include any combination listed in **Table 4**.

Table 4. CAR Components and Exemplary Sequences

Component	Sequence	SEQ ID NO
Extracellular binding domain		
Anti-CD19 scFv (FMC63)	DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYH TSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGG GTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVS GVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSK SQVFLKMNSLQTDDTAIYYCAKHYGGSYAMDYWGQGTSTVTVSS	208
Anti-CD19 scFv (FMC63)	DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYH TSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGG GTKLEITGGGSGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGV SLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQ VFLKMNSLQTDDTAIYYCAKHYGGSYAMDYWGQGTSTVTVSS	209
Spacer (e.g., hinge)		
IgG4 Hinge	ESKYGPCCPCP	210
CD8 Hinge	TTTPAPRPPTPAPTASQPLSLRPE	211
CD28	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP	212
Transmembrane		
CD8	ACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYC	213
CD28	FWVLVVGGVLACYSLLVTVAFIIFWV	214
Costimulatory domain		
CD28	RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	215
4-1BB	KRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	216
Primary Signaling Domain		
CD3zeta	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGDGLYQGLSTA TKDITYDALHMQALPPR	217
CD3zeta	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGDGLYQGLSTA TKDITYDALHMQALPPR	218

[0397] In certain embodiments, the CAR may comprise a signal peptide at the N-terminus. Non-limiting examples of signal peptides include CD8 α signal peptide, IgK signal peptide, and granulocyte-macrophage colony-stimulating factor receptor subunit alpha (GMCSFR- α , also known as colony stimulating factor 2 receptor subunit alpha (CSF2RA)) signal peptide, and variants thereof, the amino acid sequences of which are provided in **Table 5** below.

Table 5. Exemplary Sequences of Signal Peptides

SEQ ID NO:	Sequence	Description
219	MALPVTALLPLALLHAARP	CD8a signal peptide
220	METDTLLLVVLLWVPGSTG	IgK signal peptide
221	MLLVTSLLLCELPHAPALLIP	GMCSFR- α (CSF2RA) signal peptide

[0398] In certain embodiments, the extracellular binding domain of the CAR may comprise one or more antibodies specific to one target antigen or multiple target antigens. The antibody may be an antibody fragment, for example, an scFv, or a single-domain antibody fragment, for example, a VHH. In certain embodiments, the scFv may comprise a heavy chain variable region (V_H) and a light chain variable region (V_L) of an antibody connected by a linker. The V_H and the V_L may be connected in either order, i.e., V_H -linker- V_L or V_L -linker- V_H . Non-limiting examples of linkers include Whitlow linker, $(G_4S)_n$ (n can be a positive integer, e.g., 1, 2, 3, 4, 5, 6, etc.) linker, and variants thereof. In certain embodiments, the antigen may be an antigen that is exclusively or preferentially expressed on tumor cells, or an antigen that is characteristic of an autoimmune or inflammatory disease. Exemplary target antigens include, but are not limited to, CD5, CD19, CD20, CD22, CD23, CD30, CD33, CD70, Kappa, Lambda, B cell maturation agent (BCMA), and G-protein coupled receptor family C group 5 member D (GPC5D) (associated with leukemias); CS1/SLAMF7, CD38, CD138, GPRC5D, TACI, and BCMA (associated with myelomas); CD123, LeY, NKG2D ligand, and WT1 (associated with other hematological cancers); GD2, HER2, EGFR, EGFRvIII, B7H3, PSMA, PSCA, CAIX, CD171, CEA, CSPG4, EPHA2, FAP, FR α , IL-13R α , Mesothelin, MUC1, MUC16, ROR1, C-Met, CD133, Ep-CAM, GPC3, HPV16-E6, IL13R α 2, MAGEA3, MAGEA4, MART1, NY-ESO-1, VEGFR2, α -Folate receptor, CD24, CD44v7/8, EGP-2, EGP-40, erb-B2, erb-B 2,3,4, FBP, Fetal acetylcholine e receptor, G D_2 , G D_3 , HMW-MAA, IL-11R α , KDR, Lewis Y, L1-cell adhesion molecule, MAGE-A1, Oncofetal antigen (h5T4), and TAG-72 (associated with solid tumors); A*02 (associated with organ transplantation); fibroblast activation protein (FAP)(associated with fibrosis); urokinase-type plasminogen activator receptor (uPAR) (associated with senescence). In certain embodiments, the CAR can be re-engineered as a chimeric autoantibody receptor (CAAR) to selectively deplete autoreactive immune cells. In certain embodiments, CAARs are engineered to target autoantibodies present on immune cells. Exemplary target antigens for CAARs include, but are not limited to, DSG3 (associated with pemphigus vulgaris); factor VIII (FVIII)(associated with haemophilia). In any of these embodiments, the extracellular binding domain of the CAR can be codon-optimized for expression in a host cell or have variant sequences to increase functions of the extracellular binding domain.

[0399] In certain embodiments, the CAR may comprise a hinge domain, also referred to as a spacer. The terms "hinge" and "spacer" may be used interchangeably in the present disclosure. Non-limiting examples of hinge domains include CD8a hinge domain, CD28 hinge domain, IgG4 hinge domain, IgG4 hinge-CH2-CH3 domain, and variants thereof, the amino acid sequences of which are provided in **Table 6** below.

Table 6. Exemplary Sequences of Hinge Domains

SEQ ID NO:	Sequence	Description
222	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	CD8a hinge domain
223	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP	CD28 hinge domain
224	AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP	CD28 hinge domain
225	ESKYGPPCP	IgG4 hinge domain
226	ESKYGPPCPSCP	IgG4 hinge domain

227	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVHLQDVLNGKEYKCKVSNKGLPSSIEKTKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK	IgG4 hinge-CH2-CH3 domain
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[0400] In certain embodiments, the transmembrane domain of the CAR may comprise a transmembrane region of the alpha, beta, or zeta chain of a T cell receptor, CD28, CD3 ϵ , CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or a functional variant thereof, including the human versions of each of these sequences. In other embodiments, the transmembrane domain may comprise a transmembrane region of CD8 α , CD8 β , 4-1BB/CD137, CD28, CD34, CD4, Fc ϵ RI γ , CD16, OX40/CD134, CD3 ζ , CD3 ϵ , CD3 γ , CD3 δ , TCR α , TCR β , TCR ζ , CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or a functional variant thereof, including the human versions of each of these sequences. **Table 7** provides the amino acid sequences of a few exemplary transmembrane domains.

Table 7. Exemplary Sequences of Transmembrane Domains

SEQ ID NO:	Sequence	Description
228	IYIWAPLAGTCGVLLSLVITLYC	CD8 α transmembrane domain
229	FWVLVVGGVLACYSLLVTVAFIIFWV	CD28 transmembrane domain
230	MFWVLVVGGVLACYSLLVTVAFIIFWV	CD28 transmembrane domain

[0401] In certain embodiments, the intracellular signaling domain and/or intracellular costimulatory domain of the CAR may comprise one or more signaling domains selected from B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2, B7-H3, B7-H4, B7-H6, B7-H7, BTLA/CD272, CD28, CTLA-4, Gi24/VISTA/B7-H5, ICOS/CD278, PD-1, PD-L2/B7-DC, PDCD6, 4-1BB/TNFSF9/CD137, 4-1BB Ligand/TNFSF9, BAFF/BLyS/TNFSF13B, BAFF R/TNFSF13C, CD27/TNFSF7, CD27 Ligand/TNFSF7, CD30/TNFSF8, CD30 Ligand/TNFSF8, CD40/TNFSF5, CD40/TNFSF5, CD40 Ligand/TNFSF5, DR3/TNFSF25, GITR/TNFSF18, GITR Ligand/TNFSF18, HVEM/TNFSF14, LIGHT/TNFSF14, Lymphotoxin- α /TNF β , OX40/TNFSF4, OX40 Ligand/TNFSF4, RELT/TNFSF19L, TACI/TNFSF13B, TL1A/TNFSF15, TNF α , TNF RII/TNFSF1B, 2B4/CD244/SLAMF4, BLAME/SLAMF8, CD2, CD2F-10/SLAMF9, CD48/SLAMF2, CD58/LFA-3, CD84/SLAMF5, CD229/SLAMF3, CRACC/SLAMF7, NTB-A/SLAMF6, SLAM/CD150, CD2, CD7, CD53, CD82/Kai-1, CD90/Thy1, CD96, CD160, CD200, CD300a/LMIR1, HLA Class I, HLA-DR, Ikaros, Integrin alpha 4/CD49d, Integrin alpha 4 beta 1, Integrin alpha 4 beta 7/LPAM-1, LAG-3, TCL1A, TCL1B, CRTAM, DAP12, Dectin-1/CLEC7A, DPPIV/CD26, EphB6, TIM-1/KIM-1/HAVCR, TIM-4, TSLP, TSLP R, lymphocyte function associated antigen-1 (LFA-1), NKG2C, CD3 ζ , an immunoreceptor tyrosine-based activation motif (ITAM), CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and a functional variant thereof including the human versions of each of these sequences. In some embodiments, the intracellular signaling domain and/or intracellular costimulatory domain comprises one or more signaling domains selected from a CD3 ζ domain, an ITAM, a CD28 domain, 4-1BB domain, or a functional variant thereof. **Table 8** provides the amino acid sequences of a few exemplary intracellular costimulatory and/or signaling domains. In certain embodiments, as in the case of tisagenlecleucel as described below, the CD3 ζ signaling domain of SEQ ID NO:233 may have a mutation, e.g., a glutamine (Q) to lysine (K) mutation, at amino acid position 14 (see SEQ ID NO:234).

Table 8. Exemplary Sequences of Intracellular Co-Stimulatory and/or Signaling Domains

SEQ ID NO:	Sequence	Description
231	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL	4-1BB costimulatory domain
232	RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS	CD28 costimulatory domain
233	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLS TATKDTYDALHMQALPPR	CD3ζ signaling domain
234	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLS TATKDTYDALHMQALPPR	CD3ζ signaling domain (with Q to K mutation at position 14)

f. CD19 CAR

[0402] In some embodiments, the CAR is a CD19 CAR, and in these embodiments, the second transgene comprises a nucleotide sequence encoding a CD19 CAR. In some embodiments, the CD19 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD19, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[0403] In some embodiments, the signal peptide of the CD19 CAR comprises a CD8α signal peptide. In some embodiments, the CD8α signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:219 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:219. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:220 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:220. In some embodiments, the signal peptide comprises a GMCSFR-α or CSF2RA signal peptide. In some embodiments, the GMCSFR-α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:221 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:221.

[0404] In some embodiments, the extracellular binding domain of the CD19 CAR is specific to CD19, for example, human CD19. The extracellular binding domain of the CD19 CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain. In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv.

[0405] In some embodiments, the extracellular binding domain of the CD19 CAR comprises an scFv derived from the FMC63 monoclonal antibody (FMC63), which comprises the heavy chain variable region (V_H) and the light chain variable region (V_L) of FMC63 connected by a linker. FMC63 and the derived scFv have been described in Nicholson et al., Mol. Immun. 34(16-17):1157-1165 (1997) and PCT Application Publication No. WO2018/213337, the entire contents of each of which are incorporated by reference herein. In some embodiments, the amino acid sequences of the entire FMC63-derived scFv (also referred to as FMC63 scFv) and its different portions are provided in **Table 9** below. In some embodiments, the CD19-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO: 235, 236, or 241, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO: 235, 236, or 241. In some embodiments, the CD19-specific scFv may comprise one or more CDRs having

amino acid sequences set forth in SEQ ID NOs: 237, 238, 239 and 241, 243, 244. In some embodiments, the CD19-specific scFv may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 237, 238, 239. In some embodiments, the CD19-specific scFv may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 242, 243, 244. In any of these embodiments, the CD19-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD19 CAR comprises or consists of the one or more CDRs as described herein.

[0406] In some embodiments, the linker linking the V_H and the V_L portions of the scFv is a Whitlow linker having an amino acid sequence set forth in SEQ ID NO:240. In some embodiments, the Whitlow linker may be replaced by a different linker, for example, a 3xG₄S linker having an amino acid sequence set forth in SEQ ID NO:246, which gives rise to a different FMC63-derived scFv having an amino acid sequence set forth in SEQ ID NO:245. In certain of these embodiments, the CD19-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO:245 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:245.

Table 9. Exemplary Sequences of anti-CD19 scFv and Components

SEQ ID NO:	Amino Acid Sequence	Description
235	DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKL LIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDITYFCQQGNT LPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPS QSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYA MDYWGGQTSVTVSS	Anti-CD19 FMC63 scFv entire sequence, with Whitlow linker
236	DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKL LIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDITYFCQQGNT LPYTFGGGKLEIT	Anti-CD19 FMC63 scFv light chain variable region
237	QDISKY	Anti-CD19 FMC63 scFv light chain CDR1
238	HTS	Anti-CD19 FMC63 scFv light chain CDR2
239	QQGNTLPYT	Anti-CD19 FMC63 scFv light chain CDR3
240	GSTSGSGKPGSGEGSTKG	Whitlow linker
241	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLE WLGVIWGSETTYYN SALKSRLTIKDNSKSQVFLKMNSLQTDDTAI YYCAKHYYYGGSYAMDYWGGQTSVTVSS	Anti-CD19 FMC63 scFv heavy chain variable region
242	GVSLPDYG	Anti-CD19 FMC63 scFv heavy chain CDR1
243	IWGSETT	Anti-CD19 FMC63 scFv heavy chain CDR2
244	AKHYYYGGSYAMDY	Anti-CD19 FMC63 scFv heavy chain CDR3
245	DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKL LIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDITYFCQQGNT LPYTFGGGKLEITGGGGSGGGSGGGSEVKLQESGPGLVAPSQS LSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDY WGQTSVTVSS	Anti-CD19 FMC63 scFv entire sequence, with 3xG ₄ S linker
246	GGGGSGGGSGGGGS	3xG ₄ S linker

[0407] In some embodiments, the extracellular binding domain of the CD19 CAR is derived from an antibody specific to CD19, including, for example, SJ25C1 (Bejcek et al., Cancer Res. 55:2346-2351 (1995)), HD37 (Pezutto et al., J. Immunol. 138(9):2793-2799 (1987)), 4G7 (Meeker et al., Hybridoma 3:305-320 (1984)), B43 (Bejcek (1995)), BLY3 (Bejcek (1995)), B4 (Freedman et al., 70:418-427 (1987)), B4 HB12b (Kansas & Tedder, J. Immunol. 147:4094-4102 (1991); Yazawa et al., Proc. Natl. Acad. Sci. USA 102:15178-15183 (2005); Herbst et al., J. Pharmacol. Exp. Ther. 335:213-222 (2010)), BU12 (Callard et al., J. Immunology, 148(10): 2983-2987 (1992)), and CLB-CD19 (De Rie Cell. Immunol. 118:368-381(1989)). In any of these embodiments, the extracellular binding domain of the CD19 CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies.

[0408] In some embodiments, the hinge domain of the CD19 CAR comprises a CD8α hinge domain, for example, a human CD8α hinge domain. In some embodiments, the CD8α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:222 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:222. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:223 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:223. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:225 or SEQ ID NO:226, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:225 or SEQ ID NO:226. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:227 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:227.

[0409] In some embodiments, the transmembrane domain of the CD19 CAR comprises a CD8α transmembrane domain, for example, a human CD8α transmembrane domain. In some embodiments, the CD8α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:228 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:228. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:229 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:229.

[0410] In some embodiments, the intracellular costimulatory domain of the CD19 CAR comprises a 4-1BB costimulatory domain. 4-1BB, also known as CD137, transmits a potent costimulatory signal to T cells, promoting differentiation and enhancing long-term survival of T lymphocytes. In some embodiments, the 4-1BB costimulatory domain is human. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:231 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least

90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:231. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory domain. CD28 is another co-stimulatory molecule on T cells. In some embodiments, the CD28 costimulatory domain is human. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:232 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:232. In some embodiments, the intracellular costimulatory domain of the CD19 CAR comprises a 4-1BB costimulatory domain and a CD28 costimulatory domain as described.

[0411] In some embodiments, the intracellular signaling domain of the CD19 CAR comprises a CD3 zeta (ζ) signaling domain. CD3 ζ associates with T cell receptors (TCRs) to produce a signal and contains immunoreceptor tyrosine-based activation motifs (ITAMs). The CD3 ζ signaling domain refers to amino acid residues from the cytoplasmic domain of the zeta chain that are sufficient to functionally transmit an initial signal necessary for T cell activation. In some embodiments, the CD3 ζ signaling domain is human. In some embodiments, the CD3 ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:233 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:233.

[0412] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD19 CAR, including, for example, a CD19 CAR comprising the CD19-specific scFv having sequences set forth in SEQ ID NO:235 or SEQ ID NO:245, the CD8 α hinge domain of SEQ ID NO:222, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the disclosed sequence) thereof. In any of these embodiments, the CD19 CAR may additionally comprise a signal peptide (e.g., a CD8 α signal peptide) as described.

[0413] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD19 CAR, including, for example, a CD19 CAR comprising the CD19-specific scFv having sequences set forth in SEQ ID NO:235 or SEQ ID NO:245, the IgG4 hinge domain of SEQ ID NO:225 or SEQ ID NO:226, the CD28 transmembrane domain of SEQ ID NO:229, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the disclosed sequence) thereof. In any of these embodiments, the CD19 CAR may additionally comprise a signal peptide (e.g., a CD8 α signal peptide) as described.

[0414] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD19 CAR, including, for example, a CD19 CAR comprising the CD19-specific scFv having sequences set forth in SEQ ID NO:235 or SEQ ID NO:245, the CD28 hinge domain of SEQ ID NO:223, the CD28 transmembrane domain of SEQ ID NO:229, the CD28 costimulatory domain of SEQ ID NO:232, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the disclosed sequence) thereof. In any of these embodiments, the CD19 CAR may additionally comprise a signal peptide (e.g., a CD8 α signal peptide) as described.

[0415] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD19 CAR as set forth in SEQ ID NO:247 or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO:247 (see **Table 10**). The encoded CD19 CAR has a corresponding amino acid sequence set forth in SEQ ID NO:248

or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:248, with the following components: CD8 α signal peptide, FMC63 scFv (V_L-Whitlow linker-V_H), CD8 α hinge domain, CD8 α transmembrane domain, 4-1BB costimulatory domain, and CD3 ζ signaling domain.

[0416] In some embodiments, the second transgene comprises a nucleotide sequence encoding a commercially available embodiment of CD19 CAR. Non-limiting examples of commercially available embodiments of CD19 CARs expressed and/or encoded by T cells include tisagenlecleucel, lisocabtagene maraleucel, axicabtagene ciloleucel, and brexucabtagene autoleucel.

[0417] In some embodiments, the second transgene comprises a nucleotide sequence encoding tisagenlecleucel or portions thereof. Tisagenlecleucel comprises a CD19 CAR with the following components: CD8 α signal peptide, FMC63 scFv (V_L-3xG₄S linker-V_H), CD8 α hinge domain, CD8 α transmembrane domain, 4-1BB costimulatory domain, and CD3 ζ signaling domain. The nucleotide and amino acid sequence of the CD19 CAR in tisagenlecleucel are provided in **Table 10**, with annotations of the sequences provided in **Table 11**.

[0418] In some embodiments, the second transgene comprises a nucleotide sequence encoding lisocabtagene maraleucel or portions thereof. Lisocabtagene maraleucel comprises a CD19 CAR with the following components: GMCSFR- α or CSF2RA signal peptide, FMC63 scFv (V_L-Whitlow linker-V_H), IgG4 hinge domain, CD28 transmembrane domain, 4-1BB costimulatory domain, and CD3 ζ signaling domain. The nucleotide and amino acid sequence of the CD19 CAR in lisocabtagene maraleucel are provided in **Table 10**, with annotations of the sequences provided in **Table 12**.

[0419] In some embodiments, the second transgene comprises a nucleotide sequence encoding axicabtagene ciloleucel or portions thereof. Axicabtagene ciloleucel comprises a CD19 CAR with the following components: GMCSFR- α or CSF2RA signal peptide, FMC63 scFv (V_L-Whitlow linker-V_H), CD28 hinge domain, CD28 transmembrane domain, CD28 costimulatory domain, and CD3 ζ signaling domain. The nucleotide and amino acid sequence of the CD19 CAR in axicabtagene ciloleucel are provided in **Table 10**, with annotations of the sequences provided in **Table 13**.

[0420] In some embodiments, the second transgene comprises a nucleotide sequence encoding brexucabtagene autoleucel or portions thereof. Brexucabtagene autoleucel comprises a CD19 CAR with the following components: GMCSFR- α signal peptide, FMC63 scFv, CD28 hinge domain, CD28 transmembrane domain, CD28 costimulatory domain, and CD3 ζ signaling domain.

[0421] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD19 CAR as set forth in SEQ ID NO: 249, 251, or 253, or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO: 249, 251, or 253. The encoded CD19 CAR has a corresponding amino acid sequence set forth in SEQ ID NO: 250, 252, or 254, respectively, or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO: 250, 252, or 254, respectively.

Table 10. Exemplary Sequences of CD19 CARs

SEQ ID NO:	Sequence	Description
247	atggccttaccagtgaccgccttgctcctgctggccttgctgctccacgccgccaggccg gacatccagatgacacagactacatcctcctgctgctcctcctggagacagagtcaccatc agttgcagggaagtcaggacattagtaaatatttaattggtatcagcagaaaccagatg gaactgttaactcctgatctaccatacatcaagattacactcaggagtcctcaagggtca gtggcagtggtctggaacagattattctctaccattagcaacctggagcaagaagatatt gccacttacttttgccaacagggaataacgcttcctacacgttcggaggggggaccaagct	Exemplary CD19 CAR nucleotide sequence

	ggagatcacaggctccacctctggatccggaagcccggatctggcaggagatccaccaa ggcgagggtgaactgcaggagtcaggacctggcctggcggccctcacagagcctgtc cgatcacatgcactgtctcaggggtctcattaccgactatggtgtaagctggattccagcc tccacgaagggtctggagtggctgggagtaatatgggtagtgaaccacatacataat tcagctctcaaatccagactgacctatcaaggacaactccaagagccaagttttctaaaa atgaacagctgcaaaactgatgacacagccatttactactgtgcaaacattattactacggt ggtagctatgctatggactactgggccaaggaacctcagtcaccgtctcctcaaccagac ggcagcgccgaccaccaacacggcgccaccatcgctgcagccctgtccctgcgc ccagaggcgctccgcccagcgccggggggcgagtgacacgagggggctggacttcg cctgtgatatctacatctggcgccctggcgggactgtggggctcttctctgtcactggt atcaccccttactgcaaacggggcagaagaactcctgtatatattcaaacaccattatg agaccagtacaaactactcaagaggaagatggctgtagctgccgatttccagaagaaga gaaggaggatgtgaactgagagtgaagttcagcaggagcgcagacgcccccgctacca gcagggccagaaaccagctctataacgagctcaatctaggacgaagagaggagtacgatgt tttgacaagagacgtggccgggaccctgagatgggggaaagccgagaaggaagaac cctcaggaaggcctgtacaatgaactgcagaagataagatggcggaggcctacagtga attgggatgaaggcgagcgccggaggggcaaggggcacgatggcctttaccagggtct cagtacagccaccaaggacacctacgacgccttcacatgcaggccctgccccctgc	
248	MALPVTALLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDIS KYLNWYQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLE QEDIATYFCQQGNTLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVK LQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIW GSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYG GSYAMDYWGQTSVTVSSTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQ PFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNL LYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRRGKHDGLYQGLSTATKDTYDALHMQALPPR	Exemplary CD19 CAR amino acid sequence
249	atggccttaccagtgaccgccttgcctcctggcctgtgctccacgcgcaggccg gacatccagatgacacagactacatcctcctgtctgcctctctggagacagagtcaccatc agttgcaggcgaagtgcagacattagtaaatattaaattggtatcagcagaaaccagatg gaactgttaactcctgatctaccatacatcaagattacactcaggagtcctcatcaaggttca gtggcagtggtgtggaacagattattctcaccattagcaacctggagcaagaagataatt gccacttacttttccaacagggtataacgcttccgtacacgttcggaggggggaccaagct ggagatcacagggtggcgtggctcggcggtggtgggtgggtggcggtggatctgagg tgaaactgcaggagtcaggacctggcctggcgccctcacagagcctgtccgtcacatg cactgtctcaggggtctcattaccgactatggtgtaagctggattcgccagcctccacgaaa gggtctggagtggctgggagtaatatgggtagtgaaaccacatacataattcagctctca aatccagactgacctatcaaggacaactccaagagccaagttttctaaaaaagaacagt ctgcaaacctgatgacacagccatttactactgtgccaacattattactcgggtgtagctatg ctatggactactgggccaaggaacctcagtcaccgtctcctcaaccacgacgccagcgcc gcgaccaccaacacggcgccaccatcgctgcagccctgtccctgcgccagagggc gtgccggccagcgccggggggcgagtgacacgaggggggctggacttcgctgtgat ctacatctggcgcccttggccgggactgtggggctctctcctgtcactggttatcaccctt actgcaaacggggcagaagaactcctgtatatattcaaacaccatttatgagaccagta caaactactcaagaggaagatggctgtagctgccgatttccagaagaagaagaaggagg atgtgaactgagagtgaagttcagcaggagcgcagacgcccccgctacaagcaggggc agaaccagctctataacgagctcaatctaggacgaagagagagtagatggttttgacaa gagacgtggccgggaccctgagatggggggaagccgagaaggaagaacccctcaggaa ggcctgtacaatgaactgcagaaagataagatggcggaggcctacagtgaattgggatg aaaggcgagcgccggagggggcaaggggcacgatggcctttaccagggtcctcagtagc accaaggacacctacgacgccttcacatgcaggccctgccccctgc	Tisagenlecleucel CD19 CAR nucleotide sequence
250	MALPVTALLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDIS KYLNWYQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLE QEDIATYFCQQGNTLPYTFGGGKLEITGGGSGGGGGSGGGSEVKLQ ESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGS ETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYGGS YAMDYWGQTSVTVSSTTPAPRPPTPAPTIASQPLSLRPEACRPAAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYKQGQNLQY	Tisagenlecleucel CD19 CAR amino acid sequence

	NELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKHDGLYQGLSTATKDTYDALHMQALPPR	
251	atgctgctgctggtgaccagcctgctgctgctgagctgccccacccgccttctgctgatcccgacatccagatgaccagaccacctccagcctgagcgcagcctggcgaccgggtgacccatcagctgccccgagcaggacatcagcaagctgaactggtatcagcagaagccgacggcaccgtcaagctgctgatctaccacaccagcggctgcacagcggcgtgccagccggtttagcggcagcggctccggcaccgactacagcctgacatctccaactggaacaggaagatatcgccactacttttgccagcagggcaacacactgccctacaccttggcgggcggaacaaagctggaatcaccggcagcacctcggcagcggcaagcctggcagcggcgaggcgagcaccaaggcgagggtgaagctgcaggaagcgccctggcctggtgccccagccagagcctgagcgtgacctgcaccgtgagcggcgtgagcctgcccactacggcgtgagctggatccggcagccccaggaaggcctggaatggctggcgctgattctggggcagcagagaccactactacaacagcgcctgaagagccggctgacctatcaaggacaaacagcaagagccaggtgttctgaagatgaacagcctgcagaccgacgacaccgcatctactactcgcgcaagcactactactacggcggcagctacgcatggtacttggggccagggcaccagcgtgacgtgagcagaatctaagtagcagcgcctgcccccttgcctatgttctgggtgctgggtgggtgctggaggcgtgctggcctgctacagcctgctggctaccgtggcctcatcatcttttgggtgaaacggggcagaaagaaactcctgtatatattcaacaaccatttatgagaccagtaaaactactcaagaggaagatggctgttagctgccgatttccagaagaagaagaaggaggatgtgaactcgggtgaagttcagcagaagcggcagccccctgctaccagcaggccagaatcagctgtacaacgagctgaacctgggcagaaagggaagtagcagctcctgataagcggagagccgggaccctgagatggcgccaagcctggcggaagaacccccaggaaggcctgtataacgaactgcagaagacaagatggcggaggcctacagcgagatcgcatgaaggcgagcggaggcggggcaaggggcacgagcgtgtatcagggcctgtccaccgccaaaggatacctacgacgcctgcacatgcaggccctgcccccaagg	Lisocabtagene maraleucl CD19 CAR nucleotide sequence
252	MLLLVTSLLLCELPHPAFLIPDIQMTQTSSLSASLGDRVTISCRASQDISKYNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNLTPYTFGGGTKEITGSTSGSGKPGSGEGSTKGEVQLQESGPGLVAPQSLSVCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLLTIKDNSKSQVFLKMNSLQTDITAIYYCAKHHYYGGSYAMDYWGQGTSTVTSSESKYGPCCPPCFMFVVLVVVGVLACYSLLVTVAFIIFVWKRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKHDGLYQGLSTATKDTYDALHMQALPPR	Lisocabtagene maraleucl CD19 CAR amino acid sequence
253	atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattcctcctgatccagacatccagatgacacagactacatcctcctgctcctcctggtgagacagagtcacatcagttgcaggcgaagtcaggacattagtaaatattaaattggtatcagcagaaaccagatggaactgttaaaactcctgatctaccatacatcaagattacactcaggagtcctcatcaagggtcagtggcagtggtctggaacagattattctctaccattagcaacctggagcaagaagatatgtccactacttttgccaacagggtataacgctccgtacacgttcggaggggggactaagttggaaataacaggctccacctctggtatccgcaagcccgtatcggcagggatccacaaaggcgagggtgaactgcaggagtcaggacctggcctggtggcgccctcacagagcctgtcgtcacatgcactgtctcagggtctcattaccgactatggtgtaagctggattcggcagcctccacgaaagggtctggagtggctgggagtaatatgggtagtgaaccacatactataatcagctcctcaaatccagactgacctatcaaggacaactccaagagccaagttttcttaaaatgaacagctctgcaactgatgacacagccattactactgtgccaacattattactacggtggtagctatgctatggactactgggtcaaggaaacctcagtcaccgtctcctcagcggccgc aattgaagttatgtatcctcctcttaccagacaatgagaagagcaatggaaccattatccatgtgaagggaacacacctttgtcaagtccttattccggaccttctaagccctttgggtgc tgggtggtggtggggagtcctggtgctatagctgtagtaacagtggttattattttctgggtgaggagtaagaggagcaggctctgcacagtactacatgaacatgactccccgcgccccggggccacccgcaagcattaccagccctatccccaccacgagcacttcgagcctatcgctccagagtgaagttcagcaggagcgcagacgccccgcgtaccagcaggggccagaaccagctctataacgagctcaatctaggacgaagagaggagtagatgtttggacaagagacgtggccgggacccctgagatggggggaaagccgagagaaggaaacccctcaggaaggcctgtacaatgaactgcagaagataagatggcgaggcctacagtgagattgggatgaaagcgagcgcggaggggcaaggggcagcagtggttaccagggtcagtagacgccacaaggacacctacgacgccttcacatgcaggccctgccccctgc	Axicabtagene ciloleucl CD19 CAR nucleotide sequence

254	MLLLVTSLLLCELPHPAFLIPDIQMTQTSSLSASLGDRVTISCRASQDIS KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLE QEDIATYFCQQGNTLPYTFGGGKLEITGSTSGSGKPGSGEGSTKGEVK LQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIW GSETYYNSALKSRLTIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYG GSYAMDYWGQGTSTVSSAAAEVMPYPYLDNEKSNGTIIHVKGKHLG PSPLFPGPSKPFWVLVVGGLACYSLLVTVAIFVVRSKRSRLHSDY MNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNNQ LYNELNLGRREYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYDALHMQALPPR	Axicabtagene ciloleucel CD19 CAR amino acid sequence
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Table 11. Annotation of Tisagenlecleucel CD19 CAR sequences

Feature	Nucleotide Sequence Position	Amino Acid Sequence Position
CD8α signal peptide	1-63	1-21
FMC63 scFv (V _L -3xG ₄ S linker-V _H)	64-789	22-263
CD8α hinge domain	790-924	264-308
CD8α transmembrane domain	925-996	309-332
4-1BB costimulatory domain	997-1122	333-374
CD3ζ signaling domain	1123-1458	375-486

Table 12. Annotation of Lisocabtagene Maraleucel CD19 CAR Sequences

Feature	Nucleotide Sequence Position	Amino Acid Sequence Position
GMCSFR-α signal peptide	1-66	1-22
FMC63 scFv (V _L -Whitlow linker-V _H)	67-801	23-267
IgG4 hinge domain	802-837	268-279
CD28 transmembrane domain	838-921	280-307
4-1BB costimulatory domain	922-1047	308-349
CD3ζ signaling domain	1048-1383	350-461

Table 13. Annotation of Axicabtagene Ciloleucel CD19 CAR Sequences

Feature	Nucleotide Sequence Position	Amino Acid Sequence Position
CSF2RA signal peptide	1-66	1-22
FMC63 scFv (V _L -Whitlow linker-V _H)	67-801	23-267
CD28 hinge domain	802-927	268-309
CD28 transmembrane domain	928-1008	310-336
CD28 costimulatory domain	1009-1131	337-377
CD3ζ signaling domain	1132-1467	378-489

[0422] In some embodiments, the second transgene comprises a nucleotide sequence encoding CD19 CAR as set forth in SEQ ID NO: 244, 246, or 248, or at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO: 244, 246, or 248. The encoded CD19 CAR has a corresponding amino acid sequence set forth in SEQ ID NO: 245, 247, or 249, respectively, is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO: 245, 247, or 249, respectively.

g. CD20 CAR

[0423] In some embodiments, the CAR is a CD20 CAR, and in these embodiments, the second transgene comprises a nucleotide sequence encoding a CD20 CAR. CD20 is an antigen found on the surface of B cells as early as the pro-B phase and progressively at increasing levels until B cell maturity, as well as on the cells of most B-cell neoplasms. CD20 positive cells are also sometimes found in cases of Hodgkin's disease, myeloma, and thymoma. In some embodiments, the CD20 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD20, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[0424] In some embodiments, the signal peptide of the CD20 CAR comprises a CD8a signal peptide. In some embodiments, the CD8a signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:219 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:219. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:220 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:220. In some embodiments, the signal peptide comprises a GMCSFR- α or CSF2RA signal peptide. In some embodiments, the GMCSFR- α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:221 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:221.

[0425] In some embodiments, the extracellular binding domain of the CD20 CAR is specific to CD20, for example, human CD20. The extracellular binding domain of the CD20 CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain. In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv.

[0426] In some embodiments, the extracellular binding domain of the CD20 CAR is derived from an antibody specific to CD20, including, for example, Leu16, IF5, 1.5.3, rituximab, obinutuzumab, ibritumomab, ofatumumab, tositumumab, odronextamab, veltuzumab, ublituximab, and ocrelizumab. In any of these embodiments, the extracellular binding domain of the CD20 CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies.

[0427] In some embodiments, the extracellular binding domain of the CD20 CAR comprises an scFv derived from the Leu16 monoclonal antibody, which comprises the heavy chain variable region (V_H) and the light chain variable region (V_L) of Leu16 connected by a linker. See Wu et al., Protein Engineering. 14(12):1025-1033 (2001). In some embodiments, the linker is a 3xG₄S linker. In other embodiments, the linker is a Whitlow linker as described herein. In some embodiments, the amino acid sequences of different portions of the entire Leu16-derived scFv (also referred to as Leu16 scFv) and its different portions are provided in **Table 14** below. In some embodiments, the CD20-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO: 255, 256, or 260, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO: 255, 256, or 260. In some embodiments, the CD20-specific scFv may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 257, 258, 259, 261, and 262. In some embodiments, the CD20-specific scFv may comprise a light chain with

one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 257, 258, 259. In some embodiments, the CD20-specific scFv may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 261, 262. In any of these embodiments, the CD20-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD20 CAR comprises or consists of the one or more CDRs as described herein.

Table 14. Exemplary Sequences of anti-CD20 scFv and Components

SEQ ID NO:	Amino Acid Sequence	Description
255	DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWI YATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNP PTFGGGTKLEIKGSTSGSGKPGSGEGSTKGEVQLQQSGAELVKPGAS VKMSCKASGYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQ KFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFF DVWGAGTTTVTVSS	Anti-CD20 Leu16 scFv entire sequence, with Whitlow linker
256	DIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWYQKKPGSSPKPWI YATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNP PTFGGGTKLEIK	Anti-CD20 Leu16 scFv light chain variable region
257	RASSSVNYMD	Anti-CD20 Leu16 scFv light chain CDR1
258	ATSNLAS	Anti-CD20 Leu16 scFv light chain CDR2
259	QQWSFNPT	Anti-CD20 Leu16 scFv light chain CDR3
260	EVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGL EWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSA DYYCARSNYYGSSYWFFDVWGAGTTTVTVSS	Anti-CD20 Leu16 scFv heavy chain
261	SYNMH	Anti-CD20 Leu16 scFv heavy chain CDR1
262	AIYPGNGDTSYNQKFKG	Anti-CD20 Leu16 scFv heavy chain CDR2

[0428] In some embodiments, the hinge domain of the CD20 CAR comprises a CD8a hinge domain, for example, a human CD8a hinge domain. In some embodiments, the CD8a hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:222 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:222. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:223 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:223. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:225 or SEQ ID NO:226, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:225 or SEQ ID NO:226. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence

set forth in SEQ ID NO:227 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:227.

[0429] In some embodiments, the transmembrane domain of the CD20 CAR comprises a CD8 α transmembrane domain, for example, a human CD8 α transmembrane domain. In some embodiments, the CD8 α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:228 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:228. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:229 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:229.

[0430] In some embodiments, the intracellular costimulatory domain of the CD20 CAR comprises a 4-1BB costimulatory domain, for example, a human 4-1BB costimulatory domain. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:231 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:231. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory domain, for example, a human CD28 costimulatory domain. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:232 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:232.

[0431] In some embodiments, the intracellular signaling domain of the CD20 CAR comprises a CD3 zeta (ζ) signaling domain, for example, a human CD3 ζ signaling domain. In some embodiments, the CD3 ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:233 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:233.

[0432] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:255, the CD8 α hinge domain of SEQ ID NO:222, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0433] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:255, the CD28 hinge domain of SEQ ID NO:223, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0434] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:255, the IgG4 hinge domain of SEQ ID NO:225 or SEQ ID NO:226, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0435] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:255, the CD8 α hinge domain of SEQ ID NO:222, the CD28 transmembrane domain of SEQ ID NO:229, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0436] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:255, the CD28 hinge domain of SEQ ID NO:223, the CD28 transmembrane domain of SEQ ID NO:229, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0437] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD20 CAR, including, for example, a CD20 CAR comprising the CD20-specific scFv having sequences set forth in SEQ ID NO:255, the IgG4 hinge domain of SEQ ID NO:225 or SEQ ID NO:226, the CD28 transmembrane domain of SEQ ID NO:229, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

h. CD22 CAR

[0438] In some embodiments, the CAR is a CD22 CAR, and in these embodiments, the second transgene comprises a nucleotide sequence encoding a CD22 CAR. CD22, which is a transmembrane protein found mostly on the surface of mature B cells that functions as an inhibitory receptor for B cell receptor (BCR) signaling. CD22 is expressed in 60-70% of B cell lymphomas and leukemias (e.g., B-chronic lymphocytic leukemia, hairy cell leukemia, acute lymphocytic leukemia (ALL), and Burkitt's lymphoma) and is not present on the cell surface in early stages of B cell development or on stem cells. In some embodiments, the CD22 CAR may comprise a signal peptide, an extracellular binding domain that specifically binds CD22, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[0439] In some embodiments, the signal peptide of the CD22 CAR comprises a CD8 α signal peptide. In some embodiments, the CD8 α signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:219 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:219. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:220 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least

98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:220. In some embodiments, the signal peptide comprises a GMCSFR- α or CSF2RA signal peptide. In some embodiments, the GMCSFR- α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:221 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:221.

[0440] In some embodiments, the extracellular binding domain of the CD22 CAR is specific to CD22, for example, human CD22. The extracellular binding domain of the CD22 CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain. In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv.

[0441] In some embodiments, the extracellular binding domain of the CD22 CAR is derived from an antibody specific to CD22, including, for example, SM03, inotuzumab, epratuzumab, moxetumomab, and pinatuzumab. In any of these embodiments, the extracellular binding domain of the CD22 CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies.

[0442] In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv derived from the m971 monoclonal antibody (m971), which comprises the heavy chain variable region (V_H) and the light chain variable region (V_L) of m971 connected by a linker. In some embodiments, the linker is a 3xG₄S linker. In other embodiments, the Whitlow linker may be used instead. In some embodiments, the amino acid sequences of the entire m971-derived scFv (also referred to as m971 scFv) and its different portions are provided in **Table 15** below. In some embodiments, the CD22-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO: 263, 264, or 268, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO: 263, 264, or 268. In some embodiments, the CD22-specific scFv may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 265, 266, 267 and 269, 270, 271. In some embodiments, the CD22-specific scFv may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 265, 266, 267. In some embodiments, the CD22-specific scFv may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 269, 270, 271. In any of these embodiments, the CD22-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD22 CAR comprises or consists of the one or more CDRs as described herein.

[0443] In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv derived from m971-L7, which is an affinity matured variant of m971 with significantly improved CD22 binding affinity compared to the parental antibody m971 (improved from about 2 nM to less than 50 pM). In some embodiments, the scFv derived from m971-L7 comprises the V_H and the V_L of m971-L7 connected by a 3xG₄S linker. In other embodiments, the Whitlow linker may be used instead. In some embodiments, the amino acid sequences of the entire m971-L7-derived scFv (also referred to as m971-L7 scFv) and its different portions are provided in **Table 15** below. In some embodiments, the CD22-specific scFv comprises or consists of an amino acid sequence set forth in SEQ ID NO: 272, 273, or 277, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO: 272, 273, or 277. In some embodiments, the CD22-specific scFv may comprise one or more CDRs having amino acid sequences

set forth in SEQ ID NOs: 274, 275, 276 and 278, 279, 280. In some embodiments, the CD22-specific scFv may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 274, 275, 276. In some embodiments, the CD22-specific scFv may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 278, 279, 280. In any of these embodiments, the CD22-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the CD22 CAR comprises or consists of the one or more CDRs as described herein.

Table 15. Exemplary Sequences of anti-CD22 scFv and Components

SEQ ID NO:	Amino Acid Sequence	Description
263	QVQLQQSGPGLVKPSQTLTLTCAISGDSVSSNSAAWNWIRQSPSRGLE WLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAV YYCAREVTGDLEDAFDIWGQGTMTVSSGGGGSGGGGSGGGGSDIQ MTQSPSSLSASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLIYAA SSLQSGVPSRFSGRGSGTDFTLTISLQAEDFATYYCQSYSIPQTFGQ GTKLEIK	Anti-CD22 m971 scFv entire sequence, with 3xG ₄ S linker
264	QVQLQQSGPGLVKPSQTLTLTCAISGDSVSSNSAAWNWIRQSPSRGLE WLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAV YYCAREVTGDLEDAFDIWGQGTMTVSS	Anti-CD22 m971 scFv heavy chain variable region
265	GDSVSSNSAA	Anti-CD22 m971 scFv heavy chain CDR1
266	YYRSKWYN	Anti-CD22 m971 scFv heavy chain CDR2
267	AREVTGDLEDAFDI	Anti-CD22 m971 scFv heavy chain CDR3
268	DIQMTQSPSSLSASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLI YAASSLQSGVPSRFSGRGSGTDFTLTISLQAEDFATYYCQSYSIPQT FGQGTKLEIK	Anti-CD22 m971 scFv light chain
269	QTIWSY	Anti-CD22 m971 scFv light chain CDR1
270	AAS	Anti-CD22 m971 scFv light chain CDR2
271	QSYSIPQT	Anti-CD22 m971 scFv light chain CDR3
272	QVQLQQSGPGMVKPSQTLTLTCAISGDSVSSNSVAWNWIRQSPSRGL EWLGRTYYRSTWYNDYAVSMKSRITINPDTSKNQFSLQLNSVTPEDTA VYYCAREVTGDLEDAFDIWGQGTMTVSSGGGGSGGGGSGGGGSDI QMIQSPSSLSASVGDRVTITCRASQTIWSYLNWYRQRPGEAPNLLIYAA SSLQSGVPSRFSGRGSGTDFTLTISLQAEDFATYYCQSYSIPQTFGQ GTKLEIK	Anti-CD22 m971-L7 scFv entire sequence, with 3xG ₄ S linker
273	QVQLQQSGPGMVKPSQTLTLTCAISGDSVSSNSVAWNWIRQSPSRGL EWLGRTYYRSTWYNDYAVSMKSRITINPDTSKNQFSLQLNSVTPEDTA VYYCAREVTGDLEDAFDIWGQGTMTVSS	Anti-CD22 m971-L7 scFv heavy chain variable region
274	GDSVSSNSVA	Anti-CD22 m971-L7 scFv heavy chain CDR1
275	YYRSTWYN	Anti-CD22 m971-L7 scFv heavy chain CDR2
276	AREVTGDLEDAFDI	Anti-CD22 m971-L7 scFv heavy chain CDR3
277	DIQMIQSPSSLSASVGDRVTITCRASQTIWSYLNWYRQRPGEAPNLLIY AASSLQSGVPSRFSGRGSGTDFTLTISLQAEDFATYYCQSYSIPQTF GQGTKLEIK	Anti-CD22 m971-L7 scFv light chain variable region

278	QTIWSY	Anti-CD22 m971-L7 scFv light chain CDR1
279	AAS	Anti-CD22 m971-L7 scFv light chain CDR2
280	QQSYSIPQT	Anti-CD22 m971-L7 scFv light chain CDR3

[0444] In some embodiments, the extracellular binding domain of the CD22 CAR comprises immunotoxins HA22 or BL22. Immunotoxins BL22 and HA22 are therapeutic agents that comprise an scFv specific for CD22 fused to a bacterial toxin, and thus can bind to the surface of the cancer cells that express CD22 and kill the cancer cells. BL22 comprises a dsFv of an anti-CD22 antibody, RFB4, fused to a 38-kDa truncated form of *Pseudomonas* exotoxin A (Bang et al., Clin. Cancer Res., 11:1545-50 (2005)). HA22 (CAT8015, moxetumomab pasudotox) is a mutated, higher affinity version of BL22 (Ho et al., J. Biol. Chem., 280(1): 607-17 (2005)). Suitable sequences of antigen binding domains of HA22 and BL22 specific to CD22 are disclosed in, for example, U.S. Patent Nos. 7,541,034; 7,355,012; and 7,982,011, which are hereby incorporated by reference in their entirety.

[0445] In some embodiments, the hinge domain of the CD22 CAR comprises a CD8α hinge domain, for example, a human CD8α hinge domain. In some embodiments, the CD8α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:222 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:222. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:223 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:223. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:225 or SEQ ID NO:226, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:225 or SEQ ID NO:226. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:227 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:227.

[0446] In some embodiments, the transmembrane domain of the CD22 CAR comprises a CD8α transmembrane domain, for example, a human CD8α transmembrane domain. In some embodiments, the CD8α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:228 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:228. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:229 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:229.

[0447] In some embodiments, the intracellular costimulatory domain of the CD22 CAR comprises a 4-1BB costimulatory domain, for example, a human 4-1BB costimulatory domain. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:231 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:231. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory domain, for example, a human CD28 costimulatory domain. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:232 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:232.

[0448] In some embodiments, the intracellular signaling domain of the CD22 CAR comprises a CD3 zeta (ζ) signaling domain, for example, a human CD3 ζ signaling domain. In some embodiments, the CD3 ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:233 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:233.

[0449] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:263 or SEQ ID NO:272, the CD8 α hinge domain of SEQ ID NO:222, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0450] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:263 or SEQ ID NO:272, the CD28 hinge domain of SEQ ID NO:223, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0451] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:263 or SEQ ID NO:272, the IgG4 hinge domain of SEQ ID NO:225 or SEQ ID NO:226, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0452] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:263 or SEQ ID NO:272, the CD8 α hinge domain of SEQ ID NO:222, the CD28 transmembrane domain of SEQ ID NO:229, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0453] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:263 or

SEQ ID NO:272, the CD28 hinge domain of SEQ ID NO:223, the CD28 transmembrane domain of SEQ ID NO:229, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

[0454] In some embodiments, the second transgene comprises a nucleotide sequence encoding a CD22 CAR, including, for example, a CD22 CAR comprising the CD22-specific scFv having sequences set forth in SEQ ID NO:263 or SEQ ID NO:272, the IgG4 hinge domain of SEQ ID NO:225 or SEQ ID NO:226, the CD28 transmembrane domain of SEQ ID NO:229, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof.

i. BCMA CAR

[0455] In some embodiments, the CAR is a BCMA CAR, and in these embodiments, the second transgene comprises a nucleotide sequence encoding a BCMA CAR. BCMA is a tumor necrosis family receptor (TNFR) member expressed on cells of the B cell lineage, with the highest expression on terminally differentiated B cells or mature B lymphocytes. BCMA is involved in mediating the survival of plasma cells for maintaining long-term humoral immunity. The expression of BCMA has been recently linked to a number of cancers, such as multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, various leukemias, and glioblastoma. In some embodiments, the BCMA CAR may comprise a signal peptide, an extracellular binding domain that specifically binds BCMA, a hinge domain, a transmembrane domain, an intracellular costimulatory domain, and/or an intracellular signaling domain in tandem.

[0456] In some embodiments, the signal peptide of the BCMA CAR comprises a CD8 α signal peptide. In some embodiments, the CD8 α signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:219 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:219. In some embodiments, the signal peptide comprises an IgK signal peptide. In some embodiments, the IgK signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:220 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:220. In some embodiments, the signal peptide comprises a GMCSFR- α or CSF2RA signal peptide. In some embodiments, the GMCSFR- α or CSF2RA signal peptide comprises or consists of an amino acid sequence set forth in SEQ ID NO:221 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:221.

[0457] In some embodiments, the extracellular binding domain of the BCMA CAR is specific to BCMA, for example, human BCMA. The extracellular binding domain of the BCMA CAR can be codon-optimized for expression in a host cell or to have variant sequences to increase functions of the extracellular binding domain.

[0458] In some embodiments, the extracellular binding domain comprises an immunogenically active portion of an immunoglobulin molecule, for example, an scFv. In some embodiments, the extracellular binding domain of the BCMA CAR is derived from an antibody specific to BCMA, including, for example, belantamab, erlanatamab, teclistamab, LCAR-B38M, and ciltacabtagene. In any of these embodiments, the extracellular binding domain of the BCMA CAR can comprise or consist of the V_H, the V_L, and/or one or more CDRs of any of the antibodies.

[0459] In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from C11D5.3, a murine monoclonal antibody as described in Carpenter et al., Clin. Cancer Res. 19(8):2048-2060 (2013). See also PCT Application Publication No. WO2010/104949. The C11D5.3-derived scFv may comprise the heavy chain variable region (V_H) and the light chain variable region (V_L) of C11D5.3 connected by the Whitlow linker, the amino acid sequences of which is provided in **Table 16** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:281, 282, or 286, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:281, 282, or 286. In some embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 283, 284, 285 and 287, 288, 289. In some embodiments, the BCMA-specific extracellular binding domain may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 283, 284, 285. In some embodiments, the BCMA-specific extracellular binding domain may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 287, 288, 289. In any of these embodiments, the BCMA-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[0460] In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from another murine monoclonal antibody, C12A3.2, as described in Carpenter et al., Clin. Cancer Res. 19(8):2048-2060 (2013) and PCT Application Publication No. WO2010/104949, the amino acid sequence of which is also provided in **Table 16** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:290, 291, or 295, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:290, 291, or 295. In some embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 292, 293, 294 and 296, 297, 298. In some embodiments, the BCMA-specific extracellular binding domain may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 292, 293, 294. In some embodiments, the BCMA-specific extracellular binding domain may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 296, 297, 298. In any of these embodiments, the BCMA-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[0461] In some embodiments, the extracellular binding domain of the BCMA CAR comprises a murine monoclonal antibody with high specificity to human BCMA, referred to as BB2121 in Friedman et al., Hum. Gene Ther. 29(5):585-601 (2018)). See also, PCT Application Publication No. WO2012163805.

[0462] In some embodiments, the extracellular binding domain of the BCMA CAR comprises single variable fragments of two heavy chains (VHH) that can bind to two epitopes of BCMA as described in Zhao et al., J. Hematol. Oncol. 11(1):141 (2018), also referred to as LCAR-B38M. See also, PCT Application Publication No. WO2018/028647.

[0463] In some embodiments, the extracellular binding domain of the BCMA CAR comprises a fully human heavy-chain variable domain (FHVH) as described in Lam et al., Nat. Commun. 11(1):283 (2020), also referred to as FHVH33. See also, PCT Application Publication No. WO2019/006072. The amino acid sequences of FHVH33 and its CDRs are provided in **Table 16** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:299 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:299. In some embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 300, 301, 302. In any of these embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[0464] In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from CT103A (or CAR0085) as described in U.S. Patent No. 11,026,975 B2, the amino acid sequence of which is provided in **Table 16** below. In some embodiments, the BCMA-specific extracellular binding domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:303, 304, or 308, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO: 303, 304, or 308. In some embodiments, the BCMA-specific extracellular binding domain may comprise one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 305, 306, 307 and 309, 310, 311. In some embodiments, the BCMA-specific extracellular binding domain may comprise a light chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 305, 306, 307. In some embodiments, the BCMA-specific extracellular binding domain may comprise a heavy chain with one or more CDRs having amino acid sequences set forth in SEQ ID NOs: 309, 310, 311. In any of these embodiments, the BCMA-specific scFv may comprise one or more CDRs comprising one or more amino acid substitutions, or comprising a sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical), to any of the sequences identified. In some embodiments, the extracellular binding domain of the BCMA CAR comprises or consists of the one or more CDRs as described herein.

[0465] Additionally, CARs and binders directed to BCMA have been described in U.S. Application Publication Nos. 2020/0246381 A1 and 2020/0339699 A1, the entire contents of each of which are incorporated by reference herein.

Table 16. Exemplary Sequences of anti-BCMA Binder and Components

SEQ ID NO:	Amino Acid Sequence	Description
281	DIVLTQSPASLAMS LGKRATISCRASESVSVIGAHLIHWYQQKPGQPP KLLIYLASNLETGVPARFSGSGSGTDFTLTIDPVEEDDAIYSCLSRIF PRTFGGGTKLEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGET VKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTETREPAYAYDF RGRFAFSLETSASTAYLQINNLYEDTATYFCALDYSYAMDYWGQGT SVTVSS	Anti-BCMA C11D5.3 scFv entire sequence, with Whitlow linker
282	DIVLTQSPASLAMS LGKRATISCRASESVSVIGAHLIHWYQQKPGQPP KLLIYLASNLETGVPARFSGSGSGTDFTLTIDPVEEDDAIYSCLSRIF PRTFGGGTKLEIK	Anti-BCMA C11D5.3 scFv light chain variable region
283	RASESVSVIGAHLIH	Anti-BCMA C11D5.3 scFv light chain CDR1

284	LASNLET	Anti-BCMA C11D5.3 scFv light chain CDR2
285	LQSRIFPRT	Anti-BCMA C11D5.3 scFv light chain CDR3
286	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKW MGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLYEDTATYF CALDYSYAMDYWGQGTSTVTVSS	Anti-BCMA C11D5.3 scFv heavy chain variable region
287	DYSIN	Anti-BCMA C11D5.3 scFv heavy chain CDR1
288	WINTETREPAYAYDFRG	Anti-BCMA C11D5.3 scFv heavy chain CDR2
289	DYSYAMDY	Anti-BCMA C11D5.3 scFv heavy chain CDR3
290	DIVLTQSPPSLAMSGLKRTISCRASESVTILGSHLIYWYQQKPGQPPT LLIQLASNVQTVGPARGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTI PRTFGGGTKLEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGET VKISCKASGYTFRHYSMNWVKQAPGKGLKWMGRINTESGVPIYADDF KGRFAFSVETSASTAYLVINNLKDEDTASYFCSNDYLSLDFWGQGTAL TVSS	Anti-BCMA C12A3.2 scFv entire sequence, with Whitlow linker
291	DIVLTQSPPSLAMSGLKRTISCRASESVTILGSHLIYWYQQKPGQPPT LLIQLASNVQTVGPARGSGSRTDFTLTIDPVEEDDVAVYYCLQSRTI PRTFGGGTKLEIK	Anti-BCMA C12A3.2 scFv light chain variable region
292	RASESVTILGSHLIY	Anti-BCMA C12A3.2 scFv light chain CDR1
293	LASNVQT	Anti-BCMA C12A3.2 scFv light chain CDR2
294	LQSRITPRT	Anti-BCMA C12A3.2 scFv light chain CDR3
295	QIQLVQSGPELKKPGETVKISCKASGYTFRHYSMNWVKQAPGKGLKW MGRINTESGVPIYADDFKGRFAFSVETSASTAYLVINNLKDEDTASYFC SNDYLSLDFWGQGTALTIVSS	Anti-BCMA C12A3.2 scFv heavy chain variable region
296	HYSMN	Anti-BCMA C12A3.2 scFv heavy chain CDR1
297	RINTESGVPIYADDFKG	Anti-BCMA C12A3.2 scFv heavy chain CDR2
298	DYLSLDF	Anti-BCMA C12A3.2 scFv heavy chain CDR3
299	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEW VSSISGSGDYIYYADSVKGRFTISRDISKNLTLYLQMNSLRAEDTAVYYC AKEGTGANSSLADYRGQGTALTIVSS	Anti-BCMA FHVH33 entire sequence
300	GFTFSSYA	Anti-BCMA FHVH33 CDR1
301	ISGSGDYI	Anti-BCMA FHVH33 CDR2
302	AKEGTGANSSLADY	Anti-BCMA FHVH33 CDR3
303	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQKYDLLTF GGGKVEIKGSTSGSGKPGSGEGSTKGQLQLQESGPGLVKPSSETLSLT CTVSGGSISSSSYYWGWRQPPGKGLEWIGSISYSGSTYYNPSLKSRV TISVDTSKNQFSLKLSSVTAADTAVYYCARDRGDTILDVWGQGTMTVT VSS	Anti-BCMA CT103A scFv entire sequence, with Whitlow linker
304	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQKYDLLTF GGGKVEIK	Anti-BCMA CT103A scFv light chain variable region
305	QSISSY	Anti-BCMA CT103A scFv light chain CDR1
306	AAS	Anti-BCMA CT103A scFv light chain CDR2

307	QQKYDLLT	Anti-BCMA CT103A scFv light chain CDR3
308	QLQLQESGPGLVKPSSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLE WIGSISYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC ARDRGDTILDVWGQGTMTVTVSS	Anti-BCMA CT103A scFv heavy chain variable region
309	GGSISSSSYY	Anti-BCMA CT103A scFv heavy chain CDR1
310	ISYSGST	Anti-BCMA CT103A scFv heavy chain CDR2
311	ARDRGDTILDV	Anti-BCMA CT103A scFv heavy chain CDR3

[0466] In some embodiments, the hinge domain of the BCMA CAR comprises a CD8 α hinge domain, for example, a human CD8 α hinge domain. In some embodiments, the CD8 α hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:222 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:222. In some embodiments, the hinge domain comprises a CD28 hinge domain, for example, a human CD28 hinge domain. In some embodiments, the CD28 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:223 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:223. In some embodiments, the hinge domain comprises an IgG4 hinge domain, for example, a human IgG4 hinge domain. In some embodiments, the IgG4 hinge domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:225 or SEQ ID NO:226, or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:225 or SEQ ID NO:226. In some embodiments, the hinge domain comprises a IgG4 hinge-Ch2-Ch3 domain, for example, a human IgG4 hinge-Ch2-Ch3 domain. In some embodiments, the IgG4 hinge-Ch2-Ch3 domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:227 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:227.

[0467] In some embodiments, the transmembrane domain of the BCMA CAR comprises a CD8 α transmembrane domain, for example, a human CD8 α transmembrane domain. In some embodiments, the CD8 α transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:228 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:228. In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, for example, a human CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:229 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:229.

[0468] In some embodiments, the intracellular costimulatory domain of the BCMA CAR comprises a 4-1BB costimulatory domain, for example, a human 4-1BB costimulatory domain. In some embodiments, the 4-1BB costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:231 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least

98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:231. In some embodiments, the intracellular costimulatory domain comprises a CD28 costimulatory domain, for example, a human CD28 costimulatory domain. In some embodiments, the CD28 costimulatory domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:232 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:232.

[0469] In some embodiments, the intracellular signaling domain of the BCMA CAR comprises a CD3 zeta (ζ) signaling domain, for example, a human CD3 ζ signaling domain. In some embodiments, the CD3 ζ signaling domain comprises or consists of an amino acid sequence set forth in SEQ ID NO:233 or an amino acid sequence that is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in SEQ ID NO:233.

[0470] In some embodiments, the second transgene comprises a nucleotide sequence encoding a BCMA CAR, including, for example, a BCMA CAR comprising any of the BCMA-specific extracellular binding domains as described, the CD8 α hinge domain of SEQ ID NO:222, the CD8 α transmembrane domain of SEQ ID NO:228, the 4-1BB costimulatory domain of SEQ ID NO:231, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof. In any of these embodiments, the BCMA CAR may additionally comprise a signal peptide (e.g., a CD8 α signal peptide) as described.

[0471] In some embodiments, the second transgene comprises a nucleotide sequence encoding a BCMA CAR, including, for example, a BCMA CAR comprising any of the BCMA-specific extracellular binding domains as described, the CD8 α hinge domain of SEQ ID NO:222, the CD8 α transmembrane domain of SEQ ID NO:228, the CD28 costimulatory domain of SEQ ID NO:232, the CD3 ζ signaling domain of SEQ ID NO:233, and/or variants (i.e., having a sequence that is at least 80% identical, for example, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99 identical to the disclosed sequence) thereof. In any of these embodiments, the BCMA CAR may additionally comprise a signal peptide as described.

[0472] In some embodiments, the second transgene comprises a nucleotide sequence encoding a BCMA CAR as set forth in SEQ ID NO:312 or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the nucleotide sequence set forth in SEQ ID NO:312 (see **Table 17**). The encoded BCMA CAR has a corresponding amino acid sequence set forth in SEQ ID NO:313 or is at least 80% identical (e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to the amino acid sequence set forth in of SEQ ID NO:313, with the following components: CD8 α signal peptide, CT103A scFv (V_L-Whitlow linker-V_H), CD8 α hinge domain, CD8 α transmembrane domain, 4-1BB costimulatory domain, and CD3 ζ signaling domain.

[0473] In some embodiments, the second transgene comprises a nucleotide sequence encoding a commercially available embodiment of BCMA CAR, including, for example, idecabtagene vicleucel (ide-cel, also called bb2121). In some embodiments, the second transgene comprises a nucleotide sequence encoding idecabtagene vicleucel or portions thereof. Idecabtagene vicleucel comprises a BCMA CAR with the following components: the BB2121 binder, CD8 α hinge domain, CD8 α transmembrane domain, 4-1BB costimulatory domain, and CD3 ζ signaling domain.

Table 17. Exemplary Sequences of BCMA CARs

SEQ ID NO:	Sequence	Description
312	atggccttaccagtgaccgccttgctcctgccgctggccttgctgctccacgccgagcc ggacatccagatgaccagctctccatcctcctgctgcatctgtaggagacagagtcacca tcacttgcgggcaagtcagagcattagcagctattaaattggatcagcagaaaccagg gaaagccctaaagctcctgatctatgctgcatccagtttgcaagtggtggtccatcaaggt tcagtggcagtgatctgggacagatttcaactctaccatcagcagctgcaacctgaagat tttgcaacttactactgtcagcaaaaatacgaacctcctcacttttgccgagggaccaaggt tgagatcaaaggcagcaccagcggtcgcgcaagcctggctcggcagggcagcaca aagggacagctgcagctgcaggagtcgggccaggactggtgaagcctcggagaccct gtcctcacctgcactgtctggtggtccatcagcagtagtagttactactggggtggat ccgagcccccagggaaggggctggagtggtgggagtagtctctatagtgaggagca cctactacaacccgtccctcaagagtcgagtcaccatatccgtagacagtcacaagaacca gttctcctgaagctgagttctgtgaccgcccagacacggcggtgtactactgcggcaga gatcgtggagacaccatactagacgtatggggtcagggtaaatggtcaccgtcagctca ttcgtgccgtgttctgcccgcgaacactaccaccaccctgcccctagacctccacccca gcccacaatcgcagcagcctctgtctctgcggccgaagcctgtagacctgtgctcgg gaggagcgtgcaaccagagggcctgacttcgctgcgacatctacatctggccctct ggccggcactgtggcgtgctgctgagcctggtgacacctgtactgtcaaccaccgg aacaacggggcagaaagaaactcctgtatatattcaacaaccattatgagaccagta caactactcaagagggaagatggctgtagctgccgatttccagaagaagaaggagg atgtgaactgagagtgaaagtcagcagatccgcgacgccccgcctaccagcagggaca gaaccagctgtacaacgagctgaacctgggcagacgggaagagtacgacgtgctggac aagcggagagggcgggaccccgagatgggcggaaagccagacggaagaaccccca ggaaggcctgtataacgaactgcagaagacaagatggccgaggcctacagcgagatc ggcatgaaggcgagcggagggcgcggaaggccacgatggcctgtaccagggcctg agcaccgccaccaaggacacctacgacgccctgcacatgcaggccctgccccccaga	Exemplary BCMA CAR nucleotide sequence
313	MALPVTALLPLALLHAARPDIMTQSPSSLSASVGDRTTTCRASQSI SSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFLTISL QPEDFATYYCQKYDLLTFGGGTKVEIKGSTSGSGKPGSGEGSTKGQL QLQESGPGLVKPSSETLSLTCTVSGGSISSSYWGWIRQPPGKGLEWI GSISYSGSTYYNPSLKSRTISVDTSKNQFSLKSSVTAADTAVYYCARD RGDTILDVWGQTMVTVSSFPVFLPAKPTTTPAPRPPTPAPTIASQPL SLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCN HRNKRGRKKLLYIFKQPFMRPVQTTEEDGCSCRFEEEEGGCELRVKF SRADAPAYQQGNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR	Exemplary BCMA CAR amino acid sequence

j. Multiple CARs

[0474] In some embodiments, the second transgene comprises two or more nucleotide sequences, each encoding a CAR targeting a specific target antigen. In these embodiments, the second transgene encodes two or more different CARs specific to different target antigens (e.g., a CD19 CAR and a CD22 CAR). The two or more CARs may each comprise an extracellular binding domain specific to a specific target antigen, and may comprise the same, or one or more different, non-antigen binding domains. For example, the two or more CARs may comprise different signal peptides, hinge domains, transmembrane domains, costimulatory domains, and/or intracellular signaling domains, in order to minimize the risk of recombination due to sequence similarities. Or, alternatively, the two or more CARs may comprise the same non-antigen binding domains. In the cases where the same non-antigen binding domain(s) and/or backbone are used, it is optional to introduce codon divergence at the nucleotide sequence level to minimize the risk of recombination. As one non-limiting example, the second transgene may comprise a nucleotide sequence encoding a CD19 CAR and a nucleotide sequence encoding a CD22 CAR. The CD19 CAR may comprise one transmembrane domain (e.g., CD28 transmembrane domain) while the CD22 CAR comprises a different transmembrane domain (e.g., CD8a transmembrane domain), or vice versa. As another non-limiting example, the CD19 CAR may comprise one

costimulatory domain (e.g., 4-1BB costimulatory domain) while the CD22 CAR comprises a different costimulatory domain (e.g., CD28 costimulatory domain), or vice versa. Or, alternatively, the CD22 CAR and the CD19 CARs may comprise the same non-antigen binding domains but have codon divergence introduced at the nucleotide sequence level to minimize the risk of recombination. In any of these embodiments, the two or more nucleotide sequences of the second transgene may be connected by one or more cleavage sites as described (e.g., a 2A site and/or a furin site), in the form of polycistronic constructs as described herein.

2. Regulatory Elements

[0475] In some embodiments, the second transgene encoding a CAR may comprise additional regulatory elements operatively linked to the CAR encoding sequence as described, including, for example, promoters, insulators, enhancers, polyadenylation (poly(A)) tails, and/or ubiquitous chromatin opening elements.

3. Genomic Insertion

[0476] In some embodiments, the second transgene encoding a CAR may be delivered into a host cell in the form of a vector for insertion into the host genome. The insertion may be random (i.e., insertion into a random genomic locus of the host cell) or targeted (i.e., insertion into a specific genomic locus of the host cell), using any of the random or site-directed insertion methods described herein.

[0477] In some embodiments, the first transgene encoding a tolerogenic factor and the second transgene encoding a CAR may be introduced into a host for genomic insertion separately. In some embodiments, the first transgene encoding a tolerogenic factor and the second transgene encoding a CAR may be introduced into a host for genomic insertion at the same time, via a single vector or multiple vectors. In cases where the first and the second transgene are delivered into a host cell together in a single vector, the first and the second transgene may be designed as a polycistronic construct as described below.

4. Polycistronic Constructs

[0478] In some embodiments, the first transgene encoding a tolerogenic factor and the second transgene encoding a CAR, and/or the multiple CAR encoding sequences of the second transgene, may be in the form of polycistronic constructs. Polycistronic constructs have two or more expression cassettes for co-expression of two or more proteins of interest in a host cell. In some embodiments, the polycistronic construct comprises two expression cassettes, i.e., is bicistronic. In some embodiments, the polycistronic construct comprises three expression cassettes, i.e., is tricistronic. In some embodiments, the polycistronic construct comprises four expression cassettes, i.e., is quadricistronic. In some embodiments, the polycistronic construct comprises more than four expression cassettes. In any of these embodiments, each of the expression cassettes comprises a nucleotide sequence encoding a protein of interest (e.g., a tolerogenic or a CAR). In certain embodiments, the two or more genes being expressed are under the control of a single promoter and are separated from one another by one or more cleavage sites to achieve co-expression of the proteins of interest from one transcript. In other embodiments, the two or more genes may be under the control of separate promoters.

[0479] In some embodiments, the two or more expression cassettes of the polycistronic construct may be separated by one or more cleavage sites. As the name suggests, a polycistronic construct allows simultaneous expression of two or more separate proteins from one mRNA transcript in a host cell. Cleavage sites can be used in the design of a polycistronic construct to achieve such co-expression of multiple genes.

[0480] In some embodiments, the one or more cleavage sites comprise one or more self-cleaving sites. In some embodiments, the self-cleaving site comprises a 2A site. 2A peptides are a class of 18-22 amino acid-long peptides first discovered in picornaviruses and can induce ribosomal skipping during translation of a protein, thus producing equal amounts of multiple genes from the same mRNA transcript. 2A peptides function to "cleave" an mRNA

transcript by making the ribosome skip the synthesis of a peptide bond at the C-terminus, between the glycine (G) and proline (P) residues, leading to separation between the end of the 2A sequence and the next peptide downstream. There are four 2A peptides commonly employed in molecular biology, T2A, P2A, E2A, and F2A, the sequences of which are summarized in **Table 18**. A glycine-serine-glycine (GSG) linker is optionally added to the N-terminal of a 2A peptide to increase cleavage efficiency. The use of “()” around a sequence in the present disclosure means that the enclosed sequence is optional.

Table 18. Sequences of 2A Peptides

SEQ ID NO:	Amino Acid Sequence	2A Peptide
314	(GSG) EGRGSLTTCGDVEENPGP	T2A
315	(GSG) ATNFSLLKQAGDVEENPGP	P2A
316	(GSG) QCTNYALLKLAGDVESNPGP	E2A
317	(GSG) VKQTLNFDLLKLAGDVESNPGP	F2A

[0481] In some embodiments, the one or more cleavage sites additionally comprise one or more protease sites. The one or more protease sites can either precede or follow the self-cleavage sites (e.g., 2A sites) in the 5' to 3' order. The protease site may be cleaved by a protease after translation of the full transcript or after translation of each expression cassette such that the first expression product is released prior to translation of the next expression cassette. In these embodiments, having a protease site in addition to the 2A site, especially preceding the 2A site in the 5' to 3' order, may reduce the number of extra amino acid residues attached to the expressed proteins of interest. In some embodiments, the protease site comprises a furin site, also known as a Paired basic Amino acid Cleaving Enzyme (PACE) site. There are at least three furin cleavage sequences, FC1, FC2, and FC3, the amino acid sequences of which are summarized in **Table 19**. Similar to the 2A sites, one or more optional glycine-serine-glycine (GSG) sequences can be included for cleavage efficiency.

Table 19. Sequences of Furin Sites

SEQ ID NO:	Amino Acid Sequence	Furin site
318	RRRR (GSG)	FC1
319	RKRR (GSG)	FC2
320	RKRR (GSG) TPD PW (GSG)	FC3

[0482] In some embodiments, the one or more cleavage sites comprise one or more self-cleaving sites, one or more protease sites, and/or any combination thereof. For example, the cleavage site can include a 2A site alone. For another example, the cleavage site can include a FC2 or FC3 site, followed by a 2A site. In these embodiments, the one or more self-cleaving sites may be the same or different. Similarly, the one or more protease sites may be the same or different.

[0483] In some embodiments, the polycistronic construct may be in the form of a vector. Any type of vector suitable for introduction of nucleotide sequences into a host cell can be used, including, for example, plasmids, adenoviral vectors, adenoviral-associated vectors, retroviral vectors, lentiviral vectors, phages, and homology-directed repair (HDR)-based donor vectors.

X. Populations of Engineered Cells and Pharmaceutical Compositions

[0484] As described elsewhere herein, in the process of manufacturing a cell therapy, certain modifications may be introduced to the cell. Provided herein are populations of cells containing a plurality of the provided engineered cells. Provided herein are also populations of cells containing a plurality of engineered cells.

[0485] Also provided herein are compositions comprising the engineered cells or populations of engineered cells. In some embodiments, the compositions are pharmaceutical compositions.

[0486] In some embodiments, the pharmaceutical composition provided herein further include a pharmaceutically acceptable excipient or carrier. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as polysorbates (TWEEN™), poloxamers (PLURONICS™) or polyethylene glycol (PEG). In some embodiments, the pharmaceutical composition includes a pharmaceutically acceptable buffer (e.g., neutral buffer saline or phosphate buffered saline). In some embodiments, the pharmaceutical composition can contain one or more excipients for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. In some aspects, a skilled artisan understands that a pharmaceutical composition containing cells may differ from a pharmaceutical composition containing a protein.

[0487] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0488] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0489] The pharmaceutical composition in some embodiments contains engineered cells as described herein in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. In some embodiments, the pharmaceutical composition contains engineered cells as described herein in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is monitored by periodic assessment of treated subjects. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and can be determined. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0490] In some embodiments, engineered cells as described herein are administered using standard administration techniques, formulations, and/or devices. In some embodiments, engineered cells as described herein are administered using standard administration techniques, formulations, and/or devices. Provided are formulations and

devices, such as syringes and vials, for storage and administration of the compositions. Engineered cells can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition (e.g., a pharmaceutical composition containing an engineered cell), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

[0491] Formulations include those for intravenous, intraperitoneal, or subcutaneous, administration. In some embodiments, the cell populations are administered parenterally. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. In some embodiments, the cell populations are administered to a subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0492] Compositions in some embodiments are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, or dispersions, which may in some aspects be buffered to a selected pH. Liquid compositions are somewhat more convenient to administer, especially by injection. Liquid compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof. Sterile injectable solutions can be prepared by incorporating the cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like.

[0493] In some embodiments, a pharmaceutically acceptable carrier can include all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration (Gennaro, 2000, Remington: The science and practice of pharmacy, Lippincott, Williams & Wilkins, Philadelphia, PA). Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. Supplementary active compounds can also be incorporated into the compositions. The pharmaceutical carrier should be one that is suitable for the engineered cells, such as a saline solution, a dextrose solution or a solution comprising human serum albumin. In some embodiments, the pharmaceutically acceptable carrier or vehicle for such compositions is any non-toxic aqueous solution in which the engineered cells can be maintained, or remain viable, for a time sufficient to allow administration of live cells. For example, the pharmaceutically acceptable carrier or vehicle can be a saline solution or buffered saline solution.

[0494] In some embodiments, the composition, including pharmaceutical composition, is sterile. In some embodiments, isolation, enrichment, or culturing of the cells is carried out in a closed or sterile environment, for example and for instance in a sterile culture bag, to minimize error, user handling and/or contamination. In some embodiments, sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. In some embodiments, culturing is carried out using a gas permeable culture vessel. In some embodiments, culturing is carried out using a bioreactor.

[0495] In some embodiments, the cells and compositions provided herein may be stored. In some embodiments, the cells and compositions provided herein may be stored for 1-72 hours. In some embodiments, the cells and compositions provided herein may be stored for 1-7 days. In some embodiments, the cells and compositions provided herein may be stored for 1-5 weeks. In some embodiments, the cells and compositions provided herein may be stored for 1-12 months. In some embodiments, the cells and compositions provided herein may be stored for 1-30 years.

[0496] In some embodiments, the cells and compositions provided herein may be stored after they have been collected from a donor or pool of donors. In some embodiments, the cells and compositions provided herein may be stored

before manufacturing. In some embodiments, the cells and compositions provided herein may be stored after starting manufacturing. In some embodiments, the cells and compositions provided herein may be stored after completing 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 steps of the manufacturing process. In some embodiments, the cells and compositions provided herein may be stored after completing 1 or more steps of the manufacturing process. In some embodiments, the cells and compositions provided herein may be stored after completing the manufacturing process. In some embodiments, the cells and compositions provided herein may be stored before modification. In some embodiments, the cells and compositions provided herein may be stored after starting modification. In some embodiments, the cells and compositions provided herein may be stored after completing 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 modifications. In some embodiments, the cells and compositions provided herein may be stored after completing 1 or more modifications. In some embodiments, the cells and compositions provided herein may be stored after completing modification. In some embodiments, the cells and compositions provided herein may be stored before gene-editing. In some embodiments, the cells and compositions provided herein may be stored after starting gene-editing. In some embodiments, the cells and compositions provided herein may be stored after completing 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 gene-edits. In some embodiments, the cells and compositions provided herein may be stored after completing 1 or more gene-edits. In some embodiments, the cells and compositions provided herein may be stored after completing gene-editing. In some embodiments, the cells and compositions provided herein may be stored before viral transduction. In some embodiments, the cells and compositions provided herein may be stored after starting viral transduction. In some embodiments, the cells and compositions provided herein may be stored after completing 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 viral transductions. In some embodiments, the cells and compositions provided herein may be stored after completing 1 or more viral transductions. In some embodiments, the cells and compositions provided herein may be stored after completing viral transduction.

[0497] In some embodiments, the cells and compositions may be stored in liquid nitrogen. In some embodiments, the cells and compositions may be stored in a freezer at -80 °C. In some embodiments, the cells and compositions may be stored in a freezer at -20 °C. In some embodiments, the cells and compositions may be stored on ice. In some embodiments, the cells and compositions may be stored on dry ice. In some embodiments, the cells and compositions may be stored in refrigerator at 4 °C.

[0498] Also provided herein are compositions that are suitable for cryopreserving the provided engineered cells. In some embodiments, the provided engineered cells are cryopreserved in a cryopreservation medium. In some embodiments, the cryopreservation medium is a serum free cryopreservation medium. In some embodiments, the composition comprises a cryoprotectant. In some embodiments, the cryoprotectant is or comprises DMSO and/or s glycerol. In some embodiments, the cryopreservation medium is between at or about 5% and at or about 10% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 5% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 6% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 7% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 7.5% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 8% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 9% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 10% DMSO (v/v). In some embodiments, the cryopreservation medium contains a commercially available cryopreservation solution (CryoStor™ CS10). CryoStor™ CS10 is a cryopreservation medium containing 10% dimethyl sulfoxide (DMSO). In some embodiments, compositions formulated for cryopreservation can be stored at low temperatures, such as ultra-low temperatures, for example, storage with temperature ranges from -40 °C to -150 °C, such as or about 80 °C ± 6.0 °C.

[0499] It will be understood that following cryo preservation some cells in the population may be dead, such as up to 10%, up to 5%, up to 1%, up to 0.5% or up to 0.1% of cells. Alternatively, cell recovers after cryo preservation may be up to 20% or up to 10%. In some embodiments, the pharmaceutical composition comprises engineered cells described herein and a pharmaceutically acceptable carrier comprising 31.25 % (v/v) Plasma-Lyte A, 31.25 % (v/v) of 5% dextrose/0.45% sodium chloride, 10% dextran 40 (LMD)/5% dextrose, 20% (v/v) of 25% human serum albumin (HSA), and 7.5% (v/v) dimethylsulfoxide (DMSO).

[0500] In some embodiments, the cryopreserved engineered cells are prepared for administration by thawing. In some cases, the engineered cells can be administered to a subject immediately after thawing. In some such embodiments, the composition is ready-to-use without any further processing. In other cases, the engineered cells are further processed after thawing, such as by resuspension with a pharmaceutically acceptable carrier, incubation with an activating or stimulating agent, or are activated washed and resuspended in a pharmaceutically acceptable buffer prior to administration to a subject.

XI. Kits, Components, and Articles of Manufacture

[0501] In some aspects, provided herein are kits, components, and compositions (such as consumables) of the methods, devices, and systems described herein. In some embodiments, the kit comprises instructions for use according to the disclosure herein.

[0502] In some embodiments, provided herein is a kit comprising a population of engineered cells described herein. In some embodiments, provided herein is a kit comprising: (a) a population of cells comprising a plurality of engineered cells, , and (ii) reduce expression of one or more MHC class I molecules and/or one or more MHC class II molecules (e.g., one or more MHC class I human leukocyte antigens and/or one or more MHC class II human leukocyte antigens), wherein the increased expression of (i) and the reduced expression of (ii) is relative to a cell of the same cell type that does not comprise the modifications. In some embodiments, provided herein is a kit or combination, comprising a population of cells comprising a plurality of engineered cells,; (ii) increase expression of CD47, and (iii) reduce expression of one or more MHC class I molecules and/or one or more MHC class II molecules (e.g., one or more MHC class I human leukocyte antigens and/or one or more MHC class II human leukocyte antigens), wherein the increased expression of (i) and (ii) and the reduced expression of (iii) is relative to a cell of the same cell type that does not comprise the modifications.

[0503] In some embodiments, there is provided an article of manufacture containing materials useful for clinical transplantation therapies, including cell therapies. In some embodiments, the articles of manufacture contain material useful for the treatment of cellular deficiencies, such as but not limited to diabetes (e.g., Type I diabetes), vascular conditions or disease, autoimmune thyroiditis, liver disease (e.g., cirrhosis of the liver), corneal disease (e.g., Fuchs dystrophy or congenital hereditary endothelial dystrophy), kidney disease, and cancer (e.g., B cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma, liver cancer, pancreatic cancer, breast cancer, ovarian cancer, colorectal cancer, lung cancer, non-small cell lung cancer, acute myeloid lymphoid leukemia, multiple myeloma, gastric cancer, gastric adenocarcinoma, pancreatic adenocarcinoma, glioblastoma, neuroblastoma, lung squamous cell carcinoma, hepatocellular carcinoma, and bladder cancer). The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. (e.g., glass or plastic containers) Generally, the container holds a composition which is effective for allogenic cell therapy and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle).

[0504] In some aspects, a kit or article of manufacture provided herein comprises a population of engineered cells, such as any of the engineered cells provided herein. In some embodiments, a kit or article of manufacture comprises a composition comprising a population of engineered cells, wherein the engineered cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding an engineered CD47, (ii) a, and (iii) inactivation or disruption of both alleles of a *B2M* gene. In some embodiments, the engineered beta cells further comprise inactivation or disruption of both alleles of a *CIITA* gene.

[0505] The label or package insert indicates that the composition is used for treating the particular condition. The label or package insert will further comprise instructions for administering the pharmaceutical composition to the patient. In some embodiments, the article of manufacture comprises a combination treatment.

[0506] The article of manufacture and/or kit may further comprise a package insert. The insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

XII. Methods of Treatment

[0507] Provided herein are compositions and methods relating to the provided cell compositions comprising a population of engineered cells described herein for use in treating diseases or conditions in a subject. Provided herein is a method of treating a patient by administering a population engineered cells described herein. In some embodiments, the population of cells are formulated for administration in a pharmaceutical composition, such as any described here. Such methods and uses include therapeutic methods and uses, for example, involving administration of the population of engineered cells, or compositions containing the same, to a subject having a disease, condition, or disorder. It is within the level of a skilled artisan to choose the appropriate engineered cells as provided herein for a particular disease indication. In some embodiments, the cells or pharmaceutical composition thereof is administered in an effective amount to effect treatment of the disease or disorder. Uses include uses of the engineered cells or pharmaceutical compositions thereof in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject.

[0508] The engineered cells provided herein can be administered to any suitable patients including, for example, a candidate for a cellular therapy for the treatment of a disease or disorder. Candidates for cellular therapy include any patient having a disease or condition that may potentially benefit from the therapeutic effects of the subject engineered cells provided herein. In some embodiments, the patient is an allogeneic recipient of the administered cells. In some embodiments, the provided engineered cells are effective for use in allogeneic cell therapy. A candidate who benefits from the therapeutic effects of the subject engineered cells provided herein exhibit an elimination, reduction or amelioration of a disease or condition.

[0509] In some embodiments, the engineered cells as provided herein, including those produced by any of the methods provided herein, can be used in cell therapy. Therapeutic cells outlined herein are useful to treat a disorder such as, but not limited to, a cancer, a genetic disorder, a chronic infectious disease, an autoimmune disorder, a neurological disorder, and the like.

[0510] In some embodiments, the patient has a cellular deficiency. As used herein, a "cellular deficiency" refers to any disease or condition that causes a dysfunction or loss of a population of cells in the patient, wherein the patient is unable to naturally replace or regenerate the population of cells. Exemplary cellular deficiencies include, but are not limited

to, autoimmune diseases (e.g., multiple sclerosis, myasthenia gravis, rheumatoid arthritis, diabetes, systemic lupus and erythematosus), neurodegenerative diseases (e.g., Huntington's disease and Parkinson's disease), cardiovascular conditions and diseases, vascular conditions and diseases, corneal conditions and diseases, liver conditions and diseases, thyroid conditions and diseases, and kidney conditions and diseases. In some embodiments, the patient administered the engineered cells has a cancer. Exemplary cancers that can be treated by the engineered cells provided herein include, but are not limited to, B cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma, liver cancer, pancreatic cancer, breast cancer, ovarian cancer, colorectal cancer, lung cancer, non-small cell lung cancer, acute myeloid lymphoid leukemia, multiple myeloma, gastric cancer, gastric adenocarcinoma, pancreatic adenocarcinoma, glioblastoma, neuroblastoma, lung squamous cell carcinoma, hepatocellular carcinoma, and bladder cancer. In certain embodiments, the cancer patient is treated by administration of an engineered CAR T-cell provided herein.

[0511] In some embodiments, provided herein is a method of administering a population of engineered cells to a patient in need thereof, wherein the engineered cells come into contact with the blood during or after administration, and wherein the engineered cells comprise modifications that prevent or attenuate an IBMIR when the engineered cells come into contact with the blood. In some embodiments, the engineered cells are administered intravenously or via intramuscular injection. In some embodiments, the engineered cells overexpress a tolerogenic factor (e.g., CD47), have reduced expression or lack expression of one or more MHC class I molecules. In some embodiments, the engineered cells are beta islet cells or hepatocytes. In some embodiments, the engineered cells further comprise overexpression of one or more complement inhibitor.

[0512] In some embodiments, the cellular deficiency is associated with diabetes or the cellular therapy is for the treatment of diabetes, optionally wherein the diabetes is Type I diabetes. In some embodiments, the population of engineered cells is a population of islet cells, including beta islet cells. In some embodiments, the islet cells are selected from the group consisting of an islet progenitor cell, an immature islet cell, and a mature islet cell. In some embodiments, the method comprises administering to the patient a composition comprising a population of engineered beta islet cells, wherein the engineered cells comprise: (i) a multicistronic vector comprising an exogenous polynucleotide encoding an engineered CD47, and (ii) inactivation or disruption of both alleles of a *B2M* gene. In some embodiments, the method comprises administering to the patient a composition comprising a population of engineered beta islet cells, wherein the engineered beta islet cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding an engineered CD47. In some embodiments, the engineered beta cells comprise inactivation or disruption of both alleles of a *CIITA* gene. In some embodiments, the transgene comprising the polynucleotide encoding an engineered CD47 is the transgene is a multicistronic vector.

[0513] In some embodiments, the cellular deficiency is associated with a liver disease or the cellular therapy is for the treatment of liver disease. In some embodiments, the liver disease comprises cirrhosis of the liver. In some embodiments, the population of cells is a population of hepatocytes or hepatic progenitor cells. In some embodiments, the method comprises administering to the patient a composition comprising a population of engineered hepatocyte cells, wherein the engineered hepatocyte cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding an engineered CD47, and (iii) inactivation or disruption of both alleles of a *B2M* gene. In some embodiments, the method comprises administering to the patient a composition comprising a population of engineered hepatocyte cells, wherein the engineered hepatocyte cells comprise: (i) a transgene comprising an exogenous polynucleotide encoding an engineered CD47 and (ii) inactivation or disruption of both alleles of a *B2M* gene. In some embodiments, the engineered hepatocyte cells comprise inactivation or disruption of both alleles of a *CIITA* gene. In some embodiments, the transgene comprising the polynucleotide encoding an engineered CD47 is the transgene is a multicistronic vector.

[0514] In some embodiments, the engineered cells, or a composition containing the same, provided herein are useful for the treatment of a patient sensitized from one or more antigens present in a previous transplant such as, for example, a cell transplant, a blood transfusion, a tissue transplant, or an organ transplant. In certain embodiments, the previous transplant is an allogeneic transplant and the patient is sensitized against one or more alloantigens from the allogeneic transplant. Allogeneic transplants include, but are not limited to, allogeneic cell transplants, allogeneic blood transfusions, allogeneic tissue transplants, or allogeneic organ transplants. In some embodiments, the patient is sensitized patient who is or has been pregnant (e.g., having or having had alloimmunization in pregnancy). In certain embodiments, the patient is sensitized from one or more antigens included in a previous transplant, wherein the previous transplant is a modified human cell, tissue or organ. In some embodiments, the modified human cell, tissue or organ is a modified autologous human cell, tissue or organ. In some embodiments, the previous transplant is a non-human cell, tissue or organ. In exemplary embodiments, the previous transplant is a modified non-human cell, tissue, or organ. In certain embodiments, the previous transplant is a chimera that includes a human component. In certain embodiments, the previous transplant is a CAR T-cell. In certain embodiments, the previous transplant is an autologous transplant and the patient is sensitized against one or more autologous antigens from the autologous transplant. In certain embodiments, the previous transplant is an autologous cell, tissue or organ. In certain embodiments, the sensitized patient has an allergy and is sensitized to one or more allergens. In exemplary embodiments, the patient has a hay fever, a food allergy, an insect allergy, a drug allergy or atopic dermatitis.

[0515] In some embodiments, the patient undergoing a treatment using the provided engineered cells, or a composition containing the same, received a previous treatment. In some embodiments, the engineered cells, or a composition containing the same, are used to treat the same condition as the previous treatment. In certain embodiments, the engineered cells, or a composition containing the same, are used to treat a different condition from the previous treatment. In some embodiments, the engineered cells, or a composition containing the same, administered to the patient exhibit an enhanced therapeutic effect for the treatment of the same condition or disease treated by the previous treatment. In certain embodiments, the administered engineered cells, or a composition containing the same, exhibit a longer therapeutic effect for the treatment of the condition or disease in the patient as compared to the previous treatment. In exemplary embodiments, the administered cells exhibit an enhanced potency, efficacy and/or specificity against the cancer cells as compared to the previous treatment. In embodiments, the engineered cells are CAR T-cells for the treatment of a cancer.

[0516] The methods provided herein can be used as a second-line treatment for a particular condition or disease after a failed first line treatment. In some embodiments, the previous treatment is a therapeutically ineffective treatment. As used herein, a "therapeutically ineffective" treatment refers to a treatment that produces a less than desired clinical outcome in a patient. For example, with respect to a treatment for a cellular deficiency, a therapeutically ineffective treatment may refer to a treatment that does not achieve a desired level of functional cells and/or cellular activity to replace the deficient cells in a patient, and/or lacks therapeutic durability. With respect to a cancer treatment, a therapeutically ineffective treatment refers to a treatment that does not achieve a desired level of potency, efficacy and/or specificity. Therapeutic effectiveness can be measured using any suitable technique known in the art. In some embodiments, the patient produces an immune response to the previous treatment. In some embodiments, the previous treatment is a cell, tissue or organ graft that is rejected by the patient. In some embodiments, the previous treatment included a mechanically assisted treatment. In some embodiments, the mechanically assisted treatment included a hemodialysis or a ventricle assist device. In some embodiments, the patient produced an immune response to the mechanically assisted treatment. In some embodiments, the previous treatment included a population of therapeutic cells

that include a safety switch that can cause the death of the therapeutic cells should they grow and divide in an undesired manner. In certain embodiments, the patient produces an immune response as a result of the safety switch induced death of therapeutic cells. In certain embodiments, the patient is sensitized from the previous treatment. In exemplary embodiments, the patient is not sensitized by the administered engineered cells as provided herein.

[0517] In some embodiments, the provided engineered cells, or compositions containing the same, are administered prior to providing a tissue, organ or partial organ transplant to a patient in need thereof. In embodiments, the patient does not exhibit an immune response to the engineered cells. In certain embodiments, the engineered cells are administered to the patient for the treatment of a cellular deficiency in a particular tissue or organ and the patient subsequently receives a tissue or organ transplant for the same particular tissue or organ. In such embodiments, the engineered cell treatment functions as a bridge therapy to the eventual tissue or organ replacement. For example, in some embodiments, the patient has a liver disorder and receives an engineered hepatocyte treatment as provided herein, prior to receiving a liver transplant. In certain embodiments, the engineered cells are administered to the patient for the treatment of a cellular deficiency in a particular tissue or organ and the patient subsequently receives a tissue or organ transplant for a different tissue or organ. For example, in some embodiments, the patient is a diabetes patient who is treated with engineered pancreatic beta cells as provided herein prior to receiving a kidney transplant. In some embodiments, the method is for the treatment of a cellular deficiency. In exemplary embodiments, the tissue or organ transplant is a heart transplant, a lung transplant, a kidney transplant, a liver transplant, a pancreas transplant, an intestine transplant, a stomach transplant, a cornea transplant, a bone marrow transplant, a blood vessel transplant, a heart valve transplant, or a bone transplant.

[0518] The methods of treating a patient are generally through administrations of engineered cells, or a composition containing the same, as provided herein. As will be appreciated, for all the multiple embodiments described herein related to the cells and/or the timing of therapies, the administering of the cells is accomplished by a method or route that results in at least partial localization of the introduced cells at a desired site. The cells can be implanted directly to the desired site, or alternatively be administered by any appropriate route which results in delivery to a desired location in the subject where at least a portion of the implanted cells or components of the cells remain viable. In some embodiments, the cells are administered to treat a disease or disorder, such as any disease, disorder, condition, or symptom thereof that can be alleviated by cell therapy.

[0519] In some embodiments, the population of engineered cells, or a composition containing the same, is administered at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5, days, at least 6 days, at least 1 week, or at least 1 month or more after the patient is sensitized. In some embodiments, the population of engineered cells, or a composition containing the same, is administered at least 1 week (e.g., 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, or more) or more after the patient is sensitized or exhibits characteristics or features of sensitization. In some embodiments, the population of engineered cells, or a composition containing the same, is administered at least 1 month (e.g., 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, or more) or more after the patient has received the transplant (e.g., an allogeneic transplant), has been pregnant (e.g., having or having had alloimmunization in pregnancy) or is sensitized or exhibits characteristics or features of sensitization.

[0520] In some embodiments, the patient who has received a transplant, who has been pregnant (e.g., having or having had alloimmunization in pregnancy), and/or who is sensitized against an antigen (e.g., alloantigens) is

administered a dosing regimen comprising a first dose administration of a population of engineered cells described herein, a recovery period after the first dose, and a second dose administration of a population of engineered cells described. In some embodiments, the composite of cell types present in the first population of cells and the second population of cells are different. In certain embodiments, the composite of cell types present in the first population of engineered cells and the second population of engineered cells are the same or substantially equivalent. In many embodiments, the first population of engineered cells and the second population of engineered cells comprises the same cell types. In some embodiments, the first population of engineered cells and the second population of engineered cells comprises different cell types. In some embodiments, the first population of engineered cells and the second population of engineered cells comprises the same percentages of cell types. In other embodiments, the first population of engineered cells and the second population of cells comprises different percentages of cell types.

[0521] In some embodiments, the recovery period begins following the first administration of the population of engineered cells or a composition containing the same, and ends when such cells are no longer present or detectable in the patient. In some embodiments, the duration of the recovery period is at least 1 week (e.g., 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks, or more) or more after the initial administration of the cells. In some embodiments, the duration of the recovery period is at least 1 month (e.g., 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, or more) or more after the initial administration of the cells.

[0522] In some embodiments, the administered population of engineered cells, or a composition containing the same, is hypoimmunogenic when administered to the subject. In some embodiments, the engineered cells are hypimmune. In some embodiments, an immune response against the engineered cells is reduced or lower by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of the immune response produced by the administration of immunogenic cells (e.g., a population of cells of the same or similar cell type or phenotype but that do not contain the modifications, e.g., genetic modifications, of the engineered cells). In some embodiments, the administered population of engineered cells, or a composition containing the same, fails to elicit an immune response against the engineered cells in the patient.

[0523] In some embodiments, the administered population of engineered cells, or a composition containing the same, elicits a decreased or lower level of systemic TH1 activation in the patient. In some instances, the level of systemic TH1 activation elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of systemic TH1 activation produced by the administration of immunogenic cells (e.g., a population of cells of the same or similar cell type or phenotype but that do not contain the modifications, e.g., genetic modifications, of the engineered cells). In some embodiments, the administered population of engineered cells, or a composition containing the same, fails to elicit systemic TH1 activation in the patient.

[0524] In some embodiments, the administered population of engineered cells, or a composition containing the same, elicits a decreased or lower level of immune activation of peripheral blood mononuclear cells (PBMCs) in the patient. In some instances, the level of immune activation of PBMCs elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of immune activation of PBMCs produced by the administration of immunogenic cells (e.g., a population of cells of the same or similar cell type or phenotype but that do not contain the modifications,

e.g., genetic modifications, of the engineered cells). In some embodiments, the administered population of engineered cells, or a composition containing the same, fails to elicit immune activation of PBMCs in the patient.

[0525] In some embodiments, the administered population of engineered cells, or a composition containing the same, elicits a decreased or lower level of donor-specific IgG antibodies in the patient. In some instances, the level of donor-specific IgG antibodies elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of donor-specific IgG antibodies produced by the administration of immunogenic cells (e.g., a population of cells of the same or similar cell type or phenotype but that do not contain the modifications, e.g., genetic modifications, of the engineered cells). In some embodiments, the administered population of engineered cells fails to elicit donor-specific IgG antibodies in the patient.

[0526] In some embodiments, the administered population of engineered cells, or a composition containing the same, elicits a decreased or lower level of IgM and IgG antibody production in the patient. In some instances, the level of IgM and IgG antibody production elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of IgM and IgG antibody production produced by the administration of immunogenic cells (e.g., a population of cells of the same or similar cell type or phenotype but that do not contain the modifications, e.g., genetic modifications, of the engineered cells). In some embodiments, the administered population of engineered cells, or a composition containing the same, fails to elicit IgM and IgG antibody production in the patient.

[0527] In some embodiments, the administered population of engineered cells, or a composition containing the same, elicits a decreased or lower level of cytotoxic T cell killing in the patient. In some instances, the level of cytotoxic T cell killing elicited by the cells is at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% lower compared to the level of cytotoxic T cell killing produced by the administration of immunogenic cells (e.g., a population of cells of the same or similar cell type or phenotype but that do not contain the modifications, e.g., genetic modifications, of the engineered cells). In some embodiments, the administered population of engineered cells, or a composition containing the same, fails to elicit cytotoxic T cell killing in the patient.

[0528] As discussed above, provided herein are cells that in certain embodiments can be administered to a patient sensitized against alloantigens such as human leukocyte antigens. In some embodiments, the patient is or has been pregnant, e.g., with alloimmunization in pregnancy (e.g., hemolytic disease of the fetus and newborn (HDFN), neonatal alloimmune neutropenia (NAN) or fetal and neonatal alloimmune thrombocytopenia (FNAIT)). In other words, the patient has or has had a disorder or condition associated with alloimmunization in pregnancy such as, but not limited to, hemolytic disease of the fetus and newborn (HDFN), neonatal alloimmune neutropenia (NAN), and fetal and neonatal alloimmune thrombocytopenia (FNAIT). In some embodiments, the patient has received an allogeneic transplant such as, but not limited to, an allogeneic cell transplant, an allogeneic blood transfusion, an allogeneic tissue transplant, or an allogeneic organ transplant. In some embodiments, the patient exhibits memory B cells against alloantigens. In some embodiments, the patient exhibits memory T cells against alloantigens. Such patients can exhibit both memory B and memory T cells against alloantigens.

[0529] Upon administration of the cells described, the patient exhibits no systemic immune response or a reduced level of systemic immune response compared to responses to cells that are not hypoimmunogenic. In some embodiments, the patient exhibits no adaptive immune response or a reduced level of adaptive immune response compared to responses to cells that are not hypoimmunogenic. In some embodiments, the patient exhibits no innate

immune response or a reduced level of innate immune response compared to responses to cells that are not hypoimmunogenic. In some embodiments, the patient exhibits no T cell response or a reduced level of T cell response compared to responses to cells that are not hypoimmunogenic. In some embodiments, the patient exhibits no B cell response or a reduced level of B cell response compared to responses to cells that are not hypoimmunogenic.

A. Dose and Dosage Regimen

[0530] Any therapeutically effective amount of cells described herein can be included in the pharmaceutical composition, depending on the indication being treated. Non-limiting examples of the cells include primary cells (e.g., primary beta islet cells) and cells differentiated from engineered induced pluripotent stem cells as described (e.g., beta islet cells or hepatocytes differentiated from iPSCs). In some embodiments, the pharmaceutical composition includes at least about 1×10^2 , 5×10^2 , 1×10^3 , 5×10^3 , 1×10^4 , 5×10^4 , 1×10^5 , 5×10^5 , 1×10^6 , 5×10^6 , 1×10^7 , 5×10^7 , 1×10^8 , 5×10^8 , 1×10^9 , 5×10^9 , 1×10^{10} , or 5×10^{10} cells. In some embodiments, the pharmaceutical composition includes up to about 1×10^2 , 5×10^2 , 1×10^3 , 5×10^3 , 1×10^4 , 5×10^4 , 1×10^5 , 5×10^5 , 1×10^6 , 5×10^6 , 1×10^7 , 5×10^7 , 1×10^8 , 5×10^8 , 1×10^9 , 5×10^9 , 1×10^{10} , or 5×10^{10} cells. In some embodiments, the pharmaceutical composition includes up to about 6.0×10^8 cells. In some embodiments, the pharmaceutical composition includes up to about 8.0×10^8 cells. In some embodiments, the pharmaceutical composition includes at least about 1×10^2 - 5×10^2 , 5×10^2 - 1×10^3 , 1×10^3 - 5×10^3 , 5×10^3 - 1×10^4 , 1×10^4 - 5×10^4 , 5×10^4 - 1×10^5 , 1×10^5 - 5×10^5 , 5×10^5 - 1×10^6 , 1×10^6 - 5×10^6 , 5×10^6 - 1×10^7 , 1×10^7 - 5×10^7 , 5×10^7 - 1×10^8 , 1×10^8 - 5×10^8 , 5×10^8 - 1×10^9 , 1×10^9 - 5×10^9 , 5×10^9 - 1×10^{10} , or 1×10^{10} - 5×10^{10} cells. In exemplary embodiments, the pharmaceutical composition includes from about 1.0×10^6 to about 2.5×10^8 cells.

[0531] In some embodiments, the pharmaceutical composition has a volume of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, or 500 ml. In exemplary embodiments, the pharmaceutical composition has a volume of up to about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, or 500 ml. In exemplary embodiments, the pharmaceutical composition has a volume of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, or 500 ml. In some embodiments, the pharmaceutical composition has a volume of from about 1-50 ml, 50-100 ml, 100-150 ml, 150-200 ml, 200-250 ml, 250-300 ml, 300-350 ml, 350-400 ml, 400-450 ml, or 450-500 ml. In some embodiments, the pharmaceutical composition has a volume of from about 1-50 ml, 50-100 ml, 100-150 ml, 150-200 ml, 200-250 ml, 250-300 ml, 300-350 ml, 350-400 ml, 400-450 ml, or 450-500 ml. In some embodiments, the pharmaceutical composition has a volume of from about 1-10 ml, 10-20 ml, 20-30 ml, 30-40 ml, 40-50 ml, 50-60 ml, 60-70 ml, 70-80 ml, 70-80 ml, 80-90 ml, or 90-100 ml. In some embodiments, the pharmaceutical composition has a volume that ranges from about 5 ml to about 80 ml. In exemplary embodiments, the pharmaceutical composition has a volume that ranges from about 10 ml to about 70 ml. In many embodiments, the pharmaceutical composition has a volume that ranges from about 10 ml to about 50 ml.

[0532] The specific amount/dosage regimen will vary depending on the weight, gender, age and health of the individual; the formulation, the biochemical nature, bioactivity, bioavailability and the side effects of the cells and the number and identity of the cells in the complete therapeutic regimen.

[0533] In some embodiments, a dose of the pharmaceutical composition includes about 1.0×10^5 to about 2.5×10^8 cells at a volume of about 10 mL to 50 mL and the pharmaceutical composition is administered as a single dose.

[0534] In many embodiments, the cells are T cells and the pharmaceutical composition includes from about 2.0×10^6 to about 2.0×10^8 cells, such as but not limited to, primary T cells, T cells differentiated from engineered induced pluripotent stem cells. In some cases, the dose includes about 1.0×10^5 to about 2.5×10^8 primary T cells described herein

at a volume of about 10 ml to 50 ml. In several cases, the dose includes about 1.0×10^5 to about 2.5×10^8 primary T cells that have been described above at a volume of about 10 ml to 50 ml. In various cases, the dose includes about 1.0×10^5 to about 2.5×10^8 T cells differentiated from engineered induced pluripotent stem cells described herein at a volume of about 10 ml to 50 ml. In other cases, the dose is at a range that is lower than about 1.0×10^5 to about 2.5×10^8 T cells, including primary T cells or T cells differentiated from engineered induced pluripotent stem cells. In yet other cases, the dose is at a range that is higher than about 1.0×10^5 to about 2.5×10^8 T cells, including primary T cells and T cells differentiated from engineered induced pluripotent stem cells.

[0535] In some embodiments, the pharmaceutical composition is administered as a single dose of from about 1.0×10^5 to about 1.0×10^7 engineered cells (such as primary cells or cells differentiated from engineered induced pluripotent stem cells) per kg body weight for subjects 50 kg or less. In some embodiments, the pharmaceutical composition is administered as a single dose of from about 0.5×10^5 to about 1.0×10^7 , about 1.0×10^5 to about 1.0×10^7 , about 1.0×10^5 to about 1.0×10^7 , about 5.0×10^5 to about 1×10^7 , about 1.0×10^6 to about 1×10^7 , about 5.0×10^6 to about 1.0×10^7 , about 1.0×10^5 to about 5.0×10^6 , about 1.0×10^5 to about 1.0×10^6 , about 1.0×10^5 to about 5.0×10^5 , about 1.0×10^5 to about 5.0×10^6 , about 2.0×10^5 to about 5.0×10^6 , about 3.0×10^5 to about 5.0×10^6 , about 4.0×10^5 to about 5.0×10^6 , about 5.0×10^5 to about 5.0×10^6 , about 6.0×10^5 to about 5.0×10^6 , about 7.0×10^5 to about 5.0×10^6 , about 8.0×10^5 to about 5.0×10^6 , or about 9.0×10^5 to about 5.0×10^6 cells per kg body weight for subjects 50 kg or less. In some embodiments, the dose is from about 0.2×10^6 to about 5.0×10^6 cells per kg body weight for subjects 50 kg or less. In many embodiments, the dose is at a range that is lower than from about 0.2×10^6 to about 5.0×10^6 cells per kg body weight for subjects 50 kg or less. In many embodiments, the dose is at a range that is higher than from about 0.2×10^6 to about 5.0×10^6 cells per kg body weight for subjects 50 kg or less. In exemplary embodiments, the single dose is at a volume of about 10 ml to 50 ml. In some embodiments, the dose is administered intravenously.

[0536] In exemplary embodiments, the cells are administered in a single dose of from about 1.0×10^6 to about 5.0×10^8 cells (such as primary cells and cells differentiated from engineered induced pluripotent stem cells) for subjects above 50 kg. In some embodiments, the pharmaceutical composition is administered as a single dose of from about 0.5×10^6 to about 1.0×10^9 , about 1.0×10^6 to about 1.0×10^9 , about 1.0×10^6 to about 1.0×10^9 , about 5.0×10^6 to about 1.0×10^9 , about 1.0×10^7 to about 1.0×10^9 , about 5.0×10^7 to about 1.0×10^9 , about 1.0×10^6 to about 5.0×10^7 , about 1.0×10^6 to about 1.0×10^7 , about 1.0×10^6 to about 5.0×10^7 , about 1.0×10^7 to about 5.0×10^8 , about 2.0×10^7 to about 5.0×10^8 , about 3.0×10^7 to about 5.0×10^8 , about 4.0×10^7 to about 5.0×10^8 , about 5.0×10^7 to about 5.0×10^8 , about 6.0×10^7 to about 5.0×10^8 , about 7.0×10^7 to about 5.0×10^8 , about 8.0×10^7 to about 5.0×10^8 , or about 9.0×10^7 to about 5.0×10^8 cells per kg body weight for subjects 50 kg or less. In many embodiments, the cells are administered in a single dose of about 1.0×10^7 to about 2.5×10^8 cells for subjects above 50 kg. In some embodiments, the cells are administered in a single dose of a range that is less than about 1.0×10^7 to about 2.5×10^8 cells for subjects above 50 kg. In some embodiments, the cells are administered in a single dose of a range that is higher than about 1.0×10^7 to about 2.5×10^8 cells for subjects above 50 kg. In some embodiments, the dose is administered intravenously. In exemplary embodiments, the single dose is at a volume of about 10 ml to 50 ml. In some embodiments, the dose is administered intravenously.

[0537] In exemplary embodiments, the dose is administered intravenously at a rate of about 1 to 50 ml per minute, 1 to 40 ml per minute, 1 to 30 ml per minute, 1 to 20 ml per minute, 10 to 20 ml per minute, 10 to 30 ml per minute, 10 to 40 ml per minute, 10 to 50 ml per minute, 20 to 50 ml per minute, 30 to 50 ml per minute, 40 to 50 ml per minute. In numerous embodiments, the pharmaceutical composition is stored in one or more infusion bags for intravenous administration. In some embodiments, the dose is administered completely at no more than 10 minutes, 15 minutes, 20

minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 60 minutes, 70 minutes, 80 minutes, 90 minutes, 120 minutes, 150 minutes, 180 minutes, 240 minutes, or 300 minutes.

[0538] In some embodiments, a single dose of the pharmaceutical composition is present in a single infusion bag. In other embodiments, a single dose of the pharmaceutical composition is divided into 2, 3, 4 or 5 separate infusion bags.

[0539] In some embodiments, the cells described herein are administered in a plurality of doses such as 2, 3, 4, 5, 6 or more doses. In some embodiments, each dose of the plurality of doses is administered to the subject ranging from 1 to 24 hours apart. In some instances, a subsequent dose is administered from about 1 hour to about 24 hours (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or about 24 hours) after an initial or preceding dose. In some embodiments, each dose of the plurality of doses is administered to the subject ranging from about 1 day to 28 days apart. In some instances, a subsequent dose is administered from about 1 day to about 28 days (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or about 28 days) after an initial or preceding dose. In many embodiments, each dose of the plurality of doses is administered to the subject ranging from 1 week to about 6 weeks apart. In certain instances, a subsequent dose is administered from about 1 week to about 6 weeks (e.g., about 1, 2, 3, 4, 5, or 6 weeks) after an initial or preceding dose. In several embodiments, each dose of the plurality of doses is administered to the subject ranging from about 1 month to about 12 months apart. In several instances, a subsequent dose is administered from about 1 month to about 12 months (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months) after an initial or preceding dose.

[0540] In some embodiments, a subject is administered a first dosage regimen at a first timepoint, and then subsequently administered a second dosage regimen at a second timepoint. In some embodiments, the first dosage regimen is the same as the second dosage regimen. In other embodiments, the first dosage regimen is different than the second dosage regimen. In some instances, the number of cells in the first dosage regimen and the second dosage regimen are the same. In some instances, the number of cells in the first dosage regimen and the second dosage regimen are different. In some cases, the number of doses of the first dosage regimen and the second dosage regimen are the same. In some cases, the number of doses of the first dosage regimen and the second dosage regimen are different.

[0541] In some embodiments, the cells are engineered T cells (e.g., primary T cells or T cells differentiated from engineered induced pluripotent stem cells) and the first dosage regimen includes engineered T cells expressing a first CAR and the second dosage regimen includes engineered T cells expressing a second CAR such that the first CAR and the second CAR are different. For instance, the first CAR and second CAR bind different target antigens. In some cases, the first CAR includes an scFv that binds an antigen and the second CAR includes an scFv that binds a different antigen. In some embodiments, the first dosage regimen includes engineered T cells expressing a first CAR and the second dosage regimen includes engineered T cells or primary T cells expressing a second CAR such that the first CAR and the second CAR are the same. The first dosage regimen can be administered to the subject at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1-3 months, 1-6 months, 4-6 months, 3-9 months, 3-12 months, or more months apart from the second dosage regimen. In some embodiments, a subject is administered a plurality of dosage regimens during the course of a disease (e.g., cancer) and at least two of the dosage regimens comprise the same type of engineered T cells described herein. In other embodiments, at least two of the plurality of dosage regimens comprise different types of engineered T cells described herein.

B. Immunosuppressive Agent

[0542] In some embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient before the first administration of the population of engineered cells, or in a composition containing the same.

[0543] In some embodiments, an immunosuppressive and/or immunomodulatory agent may be administered to a patient received administration of engineered cells. In some embodiments, the immunosuppressive and/or immunomodulatory agent is administered prior to administration of the engineered cells. In some embodiments, the immunosuppressive and/or immunomodulatory agent is administered prior to administration of a first and/or second administration of engineered cells.

[0544] Non-limiting examples of an immunosuppressive and/or immunomodulatory agent include cyclosporine, azathioprine, mycophenolic acid, mycophenolate mofetil, corticosteroids such as prednisone, methotrexate, gold salts, sulfasalazine, antimalarials, brequinar, leflunomide, mizoribine, 15-deoxyspergualine, 6-mercaptopurine, cyclophosphamide, rapamycin, tacrolimus (FK-506), OKT3, anti-thymocyte globulin, thymopentin, thymosin- α and similar agents. In some embodiments, the immunosuppressive and/or immunomodulatory agent is selected from a group of immunosuppressive antibodies consisting of antibodies binding to p75 of the IL-2 receptor, antibodies binding to, for instance, MHC, CD2, CD3, CD4, CD7, CD28, B7, CD40, CD45, IFN- γ , TNF- α , IL-4, IL-5, IL-6R, IL-6, IGF, IGFR1, IL-7, IL-8, IL-10, CD11a, or CD58, and antibodies binding to any of their ligands. In some embodiments where an immunosuppressive and/or immunomodulatory agent is administered to the patient before or after the first administration of the cells, the administration is at a lower dosage than would be required for cells with MHC class I and/or MHC class II expression and without exogenous expression of CD47.

[0545] In some embodiments, such an immunosuppressive and/or immunomodulatory agent may be selected from soluble IL-15R, IL-10, B7 molecules (e.g., B7-1, B7-2, variants thereof, and fragments thereof), ICOS, and OX40, an inhibitor of a negative T cell regulator (such as an antibody against CTLA-4) and similar agents.

[0546] In some embodiments, an immunosuppressive and/or immunomodulatory agent can be administered to the patient before the first administration of the population of engineered cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more before the first administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more before the first administration of the cells.

[0547] In embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient after the first administration of the cells, or is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more after the first administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more after the first administration of the cells.

[0548] In some embodiments, an immunosuppressive and/or immunomodulatory agent is not administered to the patient before the administration of the population of engineered cells. In many embodiments, an immunosuppressive and/or immunomodulatory agent is administered to the patient before the first and/or second administration of the population of engineered cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more before the administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more before the first and/or second administration of the cells. In embodiments, an immunosuppressive and/or immunomodulatory agent is administered at

least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days or more after the administration of the cells. In some embodiments, an immunosuppressive and/or immunomodulatory agent is administered at least 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks or more after the first and/or second administration of the cells.

[0549] In some embodiments where an immunosuppressive and/or immunomodulatory agent is administered to the patient before or after the administration of the cells, the administration is at a lower dosage than would be required for immunogenic cells (e.g., a population of cells of the same or similar cell type or phenotype but that do not contain the modifications, e.g., genetic modifications, of the engineered cells, e.g., with endogenous levels of, MHC class I, and/or MHC class II expression and without increased (e.g., exogenous) expression of CD47).

XIII. Conclusion

[0550] The above detailed description of embodiments of the technology are not intended to be exhaustive or to limit the technology to the precise forms disclosed above. Although specific embodiments of, and examples for, the technology are disclosed herein for illustrative purposes, various equivalent modifications are possible within the scope of the technology as those skilled in the relevant art will recognize. For example, although steps are presented in a given order, alternative embodiments may perform steps in a different order. The various embodiments disclosed herein may also be combined to provide further embodiments.

[0551] From the foregoing, it will be appreciated that specific embodiments of the technology have been disclosed herein for purposes of illustration, but well-known components and functions have not been shown or disclosed in detail to avoid unnecessarily obscuring the description of the embodiments of the technology. Where the context permits, singular or plural terms may also include the plural or singular term, respectively. Further, while advantages associated with some embodiments of the technology have been disclosed in the context of those embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the technology. Accordingly, the disclosure and associated technology can encompass other embodiments not expressly shown or disclosed herein.

EXAMPLES

[0552] The present disclosure may be further described by the following non-limiting examples, in which standard techniques known to the skilled artisan and techniques analogous to those described in these examples may be used where appropriate. It is understood that the skilled artisan will envision additional embodiments consistent with the disclosure provided herein.

Example 1. Measurement of CD47 Isoform Expression with RNA-seq

[0553] Expression of each CD47 isoform was measured by RNA-seq in two human stem cell samples, Gibco WT and RUES2 WT. Specifically, RNA was extracted from 1×10^6 cells per sample using the Qiagen AllPrep DNA/RNA isolation kit. Qias shredder was used to homogenize the lysates during the extraction process. Bioanalyzer was used to quantify RNA and assess RNA quality. 500ng of RNA from the RUES2 WT sample, and 100ng of RNA from the Gibco WT sample was used for library generation (because less than 500ng of RNA was extracted from the Gibco WT sample). Libraries were generated using the Illumina TruSeq Stranded mRNA kit. The manufacturer's protocol was used. IDT for Illumina Truseq Indices (96 samples, 96 indices) plate was used for adapters. Libraries were run on the Bioanalyzer to check for quality and were quantified using qPCR (Kappa quantification kit).

[0554] The RNA-seq results were summarized in **Table 20**. According to the RNA-seq results, CD47-202 and CD47-201 were expressed at relatively equal levels in Gibco and Rues2 human stem cell lines. CD47-206, 205, 204 also appeared to be highly expressed in these stem cell lines. No evidence of CD47-203 expression was detected in these stem cell lines.

Table 20. RNA-seq Results

Transcript Name	Transcript ID (Length in bp)	TPM* of Gibco WT (SSAM000366)	TPM of RUES2 WT (SSAM000361)
CD47-202	ENST00000361309 (5292)	0.21	1.07
CD47-201	ENST00000355354 (5228)	1.90	0.84
CD47-206	ENST00000644850 (766)	3.38	5.96
CD47-205	ENST00000517766 (538)	4.68	2.81
CD47-204	ENST00000471694 (6796)	0.90	2.46
* TPM refers to transcripts per million. **CD47-203 had no read support			

Example 2. Expressing Truncated CD47 Variants in CD47KO Cells

[0555] The overall goal of these experiments was to design truncation variants of CD47 that could be delivered to target cells and achieve functional interaction with SIRPa. **Table 21** below shows the truncated CD47 variants that were designed as part of this study.

Table 21. Truncated CD47 Variants

Truncated CD47 Variants Designed	Nucleic Acid SEQ ID NO:	Amino Acid SEQ ID NO:
Wild-type hCD47	19	N/A
CD47-202	14	2
CD47-201	13	1
CD47-206 w/o N-Terminus	133	80
CD47-206 w/ restored N-Terminus	134	81
deleted TMD3	135	82
deleted EC + TMD3	136	83
deleted TMD2 + EC + TMD3	137	84
deleted last IC	138	85
deleted TMD5 + last IC	139	86
deleted EC + TMD5 + Last IC	140	87
deleted TMD4	141	88
CD47 ECD w/ DAF/CD55 GPI Anchor	142	89
CD47 ECD w/ Long TRAILR3- GPI Anchor	143	90
CD47 ECD w/ Short TRAILR3- GPI Anchor	144	91
CD47 ECD with CD8a TMD and Short ICD (9aa)	145	92
CD47 ECD with CD28 TMD and Short ICD (9aa)	146	93
CD47 ECD with PDGFR TMD and Short ICD (9aa)	147	94

CD47 IgV, 3aa from Hinge Region, with PDGFR Hinge, TMD and Short ICD (9aa)	148	95
CD47 IgV, 3aa from Hinge Region, IgG4 Hinge - CD28 TM	149	96
CD47 IgV, 3aa from Hinge Region, IgG4 Hinge - CD8a TM	150	97
CD47 IgV, 3aa from Hinge Region, CD28 Hinge - CD28 TM	151	98
CD47 IgV, 3aa from Hinge Region, CD28 Hinge - CD8a TM	152	99
CD47 IgV, 3aa from Hinge Region, CD8a Hinge - CD28 TM	153	100
CD47 IgV, 3aa from Hinge Region, CD8a Hinge - CD8a TM	154	101
Cod Opt CD47 derived from CDA Bicistronic	200	199
hCD47 N361 Mutant	171	118
hCD47 N362 Mutant	172	119
hCD47 N363 Mutant	173	120
hCD47 N364 Mutant	174	121
hCD47 N366 Mutant	175	122
hCD47 N368 Mutant	176	123
hCD47 N369 Mutant	177	124
hCD47 N3610 Mutant	178	125
hCD47 N3612 Mutant	179	126
hCD47 N3614 Mutant	180	127
hCD47 N3617 Mutant	181	128
hCD47 N3618 Mutant	182	129
hCD47 N3621 Mutant	183	130
hCD47 N3623 Mutant	184	131
hCD47 N3624 Mutant	185	132

[0556] The following experiments were conducted to generate LVV for each of the truncated CD47 variants (described in **Table 21** above) and determine if there are differences in CD47 extra cellular domain (ECD) expression as measured by anti-CD47 antibody staining and SIRPa-Fc staining.

Methods

Lentiviral vector (LVV) production

[0557] In the present example, lentiviral vector (LVV) was produced using a 3rd generation lentiviral production method. One of ordinary skill would understand that other methods known in the art for producing LVV could be used with the methods described herein. Exemplary methods are available through the world wide web at addgene.org/guides/lentivirus. Briefly, on day 1, 293T were plated in tissue cultures plates. The next day, the cells were transfected with a lentiviral transfer vector encoding one of the CD47 variants described in **Table 29** (see above), a lentiviral envelope plasmid, and lentiviral packaging plasmid. 2 days later, the cell media containing the LVV was harvested and frozen for later use.

LVV titer via p24 ELISA

[0558] In the present example, LVV titer was assessed via p24 ELISA on the Ella automated immunoassay system. One of ordinary skill would understand that other methods known in the art for assessing LVV titer could be used with the methods described herein. Briefly, LVV samples were thawed and aliquots of each were added to wells of

a 96-well plate. Next, an equal volume of lysis buffer (protease inhibitor diluted in RIPA buffer) was added to each well and the mixture was incubated on a shaker at 4°C for 30 minutes. After the incubation, the mixtures were diluted and added to a p24 Ella cartridge and run on the Ella automated immunoassay system.

Infecting CD47KO HEK293T with truncated CD47 LVV

[0559] HEK293T cells that have been modified such that they no longer express CD47 (CD47KO HEK293T) were plated on tissue culture plates and incubated at 37°C and 5% CO₂. Next, LVV samples were thawed, mixed via gentle pipetting, and applied to the wells containing CD47KO HEK293T cells and spinoculate plate at 1000xg for 30 minutes at room temperature. Next, all recipient cell plates were placed into a cell culture incubator 37°C and 5% CO₂ to grow for 3 days.

Staining CD47KO HEK293T with anti-CD47

[0560] CD47KO HEK293T cells treated with truncated CD47 LVV were harvested and re-suspended in cell staining buffer (BioLegend catalog number 420201). Cells were then centrifuged and re-suspended in cell staining buffer with cell viability dye (eBiosciences catalog number 65-0865-14), incubated on ice for 30 minutes, washed, and re-suspended in cell staining buffer. Next, cells were centrifuged and re-suspended in cell staining buffer with either PE anti-human CD47 antibody clone CC2C6 (BioLegend catalog number 323108) or FITC anti-human CD47 antibody clone CC2C6 (BioLegend catalog number 323106), incubated on ice for 30 minutes, washed, and re-suspended in cell staining buffer. Finally, CD47 expression was assessed via flow cytometry.

Staining CD47KO HEK293T with SIRPa-FC

[0561] CD47KO HEK293T cells treated with truncated CD47 LVV were harvested and re-suspended in cell staining buffer (BioLegend catalog number 420201). Cells were then centrifuged and re-suspended in cell staining buffer with cell viability dye (eBiosciences catalog number 65-0865-14), incubated on ice for 30 minutes, washed, and re-suspended in cell staining buffer. Next, cells were centrifuged and re-suspended in cell staining buffer with PE SIRPa-Fc-PE (AcroBiosystems catalog number SIA-HP252), incubated on ice for 30 minutes, washed, and re-suspended in cell staining buffer. Finally, CD47 expression was assessed via flow cytometry.

Results

[0562] **Figure 6** shows viral titers, as assessed via the Ella automated immunoassay system, of LVV comprising exemplary CD47 truncated variants.

[0563] **Figures 7-14** and **Figure 18A** show the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by anti-CD47 flow cytometry). The data demonstrate that many truncated CD47 variants (e.g., CAG-CD47 ECD - CD59 GPI and CAG-CD47 ECD - DAF/CD55 GPI) are expressed at similar levels to an overexpressed WT CD47.

[0564] **Figures 15-17** and **Figure 18B** show the percentage of CD47KO HEK293T cells expressing CD47 after treatment with truncated CD47 LVV (as assessed by SIRPa-FC flow cytometry). The data demonstrate that many truncated CD47 variants (e.g., CAG-CD47 ECD - CD59 GPI and CAG-CD47 ECD - DAF/CD55 GPI) are expressed at similar levels to overexpressed WT CD47.

Example 3. Determining Expression Levels of Truncated CD47 Variants

[0565] The following experiments are conducted to generate LVV for each of the truncated CD47 variants (described in **Table 21** above) and determine the relative exogenous expression levels of the CD47 variants compared to exogenous wild-type CD47 expression in different cell types, as measure by anti-CD47 antibody staining and SIRPa-Fc staining.

[0566] These experiments can include any cell types disclosed herein, including (but not limited to) islet cells, beta islet cells, pancreatic islet cells, immune cells, B cells, T cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophage cells, endothelial cells, muscle cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, dopaminergic neurons, retinal pigmented epithelium cells, optic cells, hepatocytes, thyroid cells, skin cells, glial progenitor cells, neural cells, cardiac cells, stem cells, hematopoietic stem cells, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), pluripotent stem cell (PSCs), and/or blood cells.

[0567] Exemplary methods include those described in Example 2, including transfecting cells with a lentiviral transfer vector encoding one of the CD47 variants described in **Table 21** (see above), a lentiviral envelope plasmid, and lentiviral packaging plasmid.

[0568] Exemplary results include achieving exogenous expression levels at or above the exogenous expression levels achieved with wild-type CD47 in a cell after delivering (e.g., transfecting) cells with a 0.25x, 0.5x, 0.75x, 1x, 1.25x, 1.5x, 1.75x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, or 10x dose (e.g., lentiviral titer) of a truncated CD47 variant.

Example 4. Determining Expression Levels of Truncated CD47 Variants to Achieve Hypoimmunity

[0569] The following experiments are conducted to determine the level of truncated CD47 variant (described in **Table 21** above) expression required to achieve hypoimmunity.

[0570] These experiments can include any cell types disclosed herein, including (but not limited to) islet cells, beta islet cells, pancreatic islet cells, immune cells, B cells, T cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophage cells, endothelial cells, muscle cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, dopaminergic neurons, retinal pigmented epithelium cells, optic cells, hepatocytes, thyroid cells, skin cells, glial progenitor cells, neural cells, cardiac cells, stem cells, hematopoietic stem cells, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), pluripotent stem cell (PSCs), and/or blood cells.

[0571] NK cell killing assays and macrophage killing assays are performed on the XCELLIGENCE SP platform and MP platform (ACEA BioSciences, San Diego, CA.). 96-well E-plates (ACEA BioSciences) are coated with collagen (Sigma-Aldrich) and 4×10^5 MHC-I knockout, MHC-II knockout, or MHC-I and MHC-II knockout cells expressing exogenous wild type CD47 or truncated CD47 are plated in 100 μ L cell specific media. After the Cell Index value reaches 0.7, human NK cells or human macrophages are added with an effector cell to target cell (E:T) ratio of 0.5:1, 0.8:1 or 1:1 with or without 1 ng/ml human IL-2 or human IL-15 (both Peprotech). As a negative control, cells are treated with 2% Triton™ X-100. Data are standardized and analyzed with the RTCA software (ACEA). Using both NK cells and macrophages, MHC-I knockout, MHC-II knockout, or MHC-I and MHC-II knockout cells without exogenously expressed CD47 are rapidly killed. If successful, exogenous expression of truncated CD47 reverses the killing effect. Exemplary results include achieving hypoimmunity levels at 0.5x, 0.75x, 1x, 1.25x, 1.5x, 1.75x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, or 10x the level achieved with exogenous expression of wild-type.

[0572] In vivo killing of cells by NK cells and macrophages is measured by adoptive transfer. 5×10^6 wild-type cells are mixed with 5×10^6 truncated CD47 expressing cells and the mixture is stained with 5 μ M CFSE (ThermoFisher). Cells in saline with human IL-2 (1 ng/mL, Peprotech) and 2.5×10^6 human primary NK cells (StemCell

Technologies) or 2.5×10^6 human macrophages (differentiated from PBMCs) are injected i.p. into immunodeficient NSG-SGM3 mice (013062, Jackson Laboratory). Human primary NK cells are pretreated with human IL-2 in vitro 12 hours before injection. After 48 hours, cells are collected from the abdomen and stained with APC-conjugated anti-HLA-A,B,C antibody (clone G46_2.6, BD Biosciences) for 45 minutes at 4 °C. The CFSE-positive and HLA-A,B,C-negative population is analyzed by flow cytometry (FACS Calibur, BD Bioscience) and compared between the wild-type cells and cells expressing exogenous truncated CD47. If successful, no reduction in the CFSE+/HLA- population is seen for the truncated CD47 cells.

[0573] Macrophage phagocytosis is also measured by BLI. Luciferase-expressing cells that express exogenous truncated CD47 cells or wild-type cells are counted and plated at a concentration of 1×10^5 cells per 24-well. After 16 hours, human macrophages are added to at an E:T ratio of 1:1. After 120 minutes, luciferase expression is confirmed by adding D-luciferin (Promega, Madison, WI). As controls, target cells are untreated or treated with 2% TRITON X100. Signals are quantified with Ami HT (Spectral Instruments Imaging, Tucson, AZ) in maximum photons per second per centimeter square per steradian (p/s/cm²/sr). If successful, phagocytosis levels will be similar for wild-type cells and cells expressing exogenous truncated CD47.

[0574] For NK cell-specific Elispot assays, human primary NK cells are co-cultured with wild-type cells and cells expressing exogenous truncated CD47 and their IFN- γ release is measured. K562 cells (Sigma-Aldrich) are used as positive control. Mitomycin-treated (50 μ g/mL for 30 minutes) stimulator cells are incubated with NK cells (stimulated with 1 ng/mL human IL-2) at an E:T ratio of 1:1 for 24 hours and IFN- γ spot frequencies are enumerated using an Elispot plate reader. If successful, NK cell activation levels will be similar for wild-type cells and cells expressing exogenous truncated CD47.

[0575] Transplant studies are performed in humanized CD34+ hematopoietic stem cell-engrafted NSG-SGM3 mice (Wunderlich et al., 2010, Leukemia 24:1785-88), which are allogeneic to the test cells. Since no syngeneic controls are available in this humanized mouse model, background measurements are collected in naïve mice. If successful, after 5 days, recipients of wild-type cells show a high splenocyte IFN- γ spot frequency and elevated IgM levels whereas recipients of cells expressing exogenous truncated CD47 do not mount a detectable cellular IFN- γ response or antibody response or mount a significantly lesser cellular IFN- γ response or antibody response.

Example 5. Reduction of Binding Between Mutant CD47 Protein and TSP-1

[0576] The present Example demonstrates that exemplary cells as described herein comprising an engineered CD47 protein have decreased TSP-1 binding relative to a control cell.

[0577] SUPT1 cell lines with endogenous CD47 knocked out will be genetically engineered to over-express either wild-type CD47 protein or engineered CD47 protein comprising an S64A mutation. SUPT1 CD47 KO cells will be transduced with VSV-G viral vector encoding either wild-type CD47 or the CD47-S64A variant.

[0578] Tested cell lines will be co-cultured with recombinant TSP-1 protein comprising a HIS tag. TSP-1 binding will be determined via flow staining of CD47 and anti-HIS. Signal in a gate of CD47+ > anti HIS (TSP-1) will indicate if TSP-1 is bound to CD47.

[0579] If the S64A mutation in the engineered CD47 protein disrupts CD47 binding to TSP-1, then the cells expressing the engineered CD47 protein will exhibit decreased TSP-1 binding relative to control cells over expressing wild-type CD47.

Example 6. Immune Evasion by T Cells Comprising Mutant CD47 Protein

[0580] The present example demonstrates that exemplary cells as described herein comprising an engineered CD47 protein (e.g., comprising an S64A variant) can evade cells of the innate immune system.

[0581] In vitro assays involving killing by natural killer (NK) cells and macrophages will be performed with the Xcelligence system to measure killing of engineered cells comprising an engineered CD47 protein (e.g., comprising an S64A variant). The Xcelligence system measures electrical impedance changes as adherent target cells detach following cell death. A drop in electrical impedance below an initial starting cell index will be used as an indicator of innate immune cell-mediated killing of target T cells by NK cells and macrophages after an extended co-culture.

[0582] If the S64A mutation in the engineered CD47 protein supports immune evasion, then the cells expressing the engineered CD47 protein will exhibit similar levels of killing by NK cells and macrophages relative to control cells over-expressing wild-type CD47.

Example 7. Enhanced Function of T cells Comprising Mutant CD47 Protein

[0583] The present Example demonstrates that engineered T cells as described herein with reduced binding to TSP-1 (e.g., comprising a CD47 S64A variant protein) may have enhanced function as compared to a wild-type T cell.

[0584] T cell exhaustion and cytolytic activity will be tested via a serial tumor challenge assay. Donor-matched Mock (untransduced) T cells, EGFRt and CD19CAR transduced, CD47 and CD19CAR transduced, or CD47 S64A and CD19CAR transduced T cells will be generated. CD4 and CD8 T cells are stimulated with CD3/CD28 beads, transduced with one of the above constructs, and expanded.

[0585] T cells, as described above, will be co-cultured with NALM6 or RAJI iRFP713 cells at various Effector (E) to Target (T) ratios (e.g. 1:1, 1:4, 1:8), in the presence or absence of soluble TSP-1. Then, co-cultures will be placed inside an IncuCyte SX5 Imaging system for serial imaging analysis every 4-hours. On Day 1, an aliquot of culture supernatant will be harvested for cytokine analysis. On Day 5, co-cultures will be divided into two groups and tested. Group 1 will be analyzed for tumor re-challenge and Group 2 will be analyzed with flow cytometry using a T cell exhaustion panel. In this panel, differences in phenotypic markers (CD45RA, CD62L) and exhaustion markers (PD1, LAG3, TIM3, CD39, CD101) will be analyzed to demonstrate differences in T cell phenotype (e.g. Tn, Tcm, Tem) and exhaustion marker expression between the various T cell conditions. Tumor re-challenge will be performed on Day 5, Day 8, and Day 11 in the presence or absence of TSP-1. Correlate exhaustion panel flow analysis will also be performed on the same days of tumor re-challenge, and culture supernatant will be harvested for future analysis 24 hours after each tumor re-challenge.

[0586] To identify differences in cytolytic activity, tTumor iRFP713 fluorescence will be analyzed throughout a serial tumor challenge assay. Correlate supernatant analysis by Meso-scale discovery cytokine analysis will be analyzed to determine if there are differences in cytokine production (e.g. IL2, IFN γ , TNF α , Granzyme B) between T cells that overexpress wild-type CD47 and T cells that express a CD47 S64A variant protein, in the presence or absence of TSP-1. Furthermore, phenotypic and exhaustion panel analysis will be compared between treatment groups. Improved cytolytic activity will be identified by the control of tumor fluorescence at low E:T ratios by the S64A/CD19CAR T cells comprising a CD47 S64A variant protein relative to the wtCD47/CD19CAR T cells.

[0587] This Example demonstrates that a T cell comprising a CD47-S64A mutation may have reduced binding to TSP-1 and enhanced function (relative to a T cell engineered to overexpress wild-type CD47). In some embodiments, a T cell comprising a CD47-S64A mutation may demonstrate differences in T cell phenotype (e.g. Tn, Tcm, Tem) and decreased expression of one or more exhaustion markers as compared to a control T cell engineered to overexpress

wild-type CD47. In some embodiments, a T cell comprising a CD47-S64A mutation may have decreased T cell exhaustion and/or improved cancer killing ability (e.g., cytolytic activity) as compared to a control T cell engineered to overexpress wild-type CD47. In some embodiments, a T cell comprising a CD47-S64A mutation may have decreased T cell exhaustion and/or improved cancer killing ability as compared to a control T cell engineered to overexpress wild-type CD47. In some embodiments, a T cell comprising a CD47-S64A mutation may have differences in cytolytic activity over time (e.g., demonstrated using the IncuCyte SX5 imaging system) as compared to a control T cell engineered to overexpress wild-type CD47.

[0588] In addition, the present Example demonstrates that a T cell comprising a CD47-S64A mutation may overexpress a CD47 variant comprising a S64A mutation. In some embodiments, a T cell comprising a CD47-S64A mutation may have differences in cytokine production (e.g. IL2, IFN γ , TNF α , Granzyme B) as compared to a control T cell (e.g., a T cell engineered to overexpress wild-type CD47) in the presence or absence of TSP-1.

[0589] Thus, the present Example demonstrates that S64A/CD19CAR T cells may be less susceptible to activity inhibition by TSP-1. Without wishing to be bound by any particular theory, CD19CAR T cells comprising a CD47 S64A variant protein may have a reduction to cytokine responses in the presence of TSP-1 as compared to a control T cell (e.g., a T cell engineered to overexpress wild-type CD47).

Example 8. Increased CD47 Expression In T Cells without Increasing T Cell Exhaustion

[0590] The present Example demonstrates that T cells with reduced binding to TSP-1 may have higher exogenous CD47 expression without increasing T cell exhaustion.

[0591] T cell exhaustion will be measured using an IncuCyte serial tumor challenge assay. CD47-overexpressing population of cells will be gated by flow analysis during different tumor re-challenges. Then, CD47-overexpressing populations will be sub-gated for expression of exhaustion markers PD1, LAG3, TIM3, CD39, and CD101. Exhaustion marker expression will be compared between a CAR+ population of the EGFRt and CD19CAR transduced cells, a wt CD47 and CD19CAR overexpressing population of cells, and/or a CD47 S64A and CD19CAR overexpressing population of cells.

[0592] In addition, donor-matched Mock (untransduced) T cells, EGFRt and CD19CAR transduced, CD47 and CD19CAR transduced, and/or CD47 S64A and CD19CAR transduced T cells will be co-cultured with K562 tumor cells that express a membrane tethered OKT3 scFv. OKT3scFv is an anti CD3 epsilon scFv able to non-specifically stimulate T cells. In this assay, T cells and tumor cells will undergo co-culture similar to the IncuCyte serial tumor challenge assay described above. For flow analysis, wt CD47/CD19CAR transduced cells and CD47 S64A/CD19CAR transduced cells will be gated for CD47-overexpression and endogenous CD47 expression. These gated populations will then be analyzed for differences in phenotypic and exhaustion marker profiles.

[0593] This Example demonstrates that a T cell comprising a CD47-S64A mutation may have higher exogenous CD47 expression without increasing T cell exhaustion as compared to a control T cell (e.g., WT T cell). In some embodiments, a T cell comprising a CD47-S64A mutation may have higher exogenous CD47 expression without increasing T cell exhaustion as determined by an IncuCyte serial tumor challenge assay.

Example 9. Improved Binding Between CD47 Protein Variant and SIRPa

[0594] The present Example demonstrates that cells comprising a CD47 protein variant (e.g., comprising a WQP insertion) have may have improved SIRPa binding.

[0595] SUPT1 cell lines with endogenous CD47 knocked out will be genetically engineered to over-express either wild-type CD47 protein or engineered CD47 protein comprising a WQP insertion. SUPT1 CD47 KO cells will be transduced with VSV-G viral vector encoding either wild-type CD47 or the CD47 WQP insertion variant. SUPT1 cell lines expressing a CD47 WQP insertion variant are stained with a soluble SIRP α protein conjugated with PE. Flow staining will be done via a titration protocol using flow cytometry.

[0596] If the CD47 WQP insertion variant improves CD47 binding to SIRP α , then the cells expressing the CD47 WQP insertion variant will exhibit improved SIRP α binding relative to control cells over expressing wild-type CD47.

Example 10. Immune Evasion by T Cells Comprising CD47 Protein Variant

[0597] The present example demonstrates that exemplary cells as described herein comprising a CD47 protein variant (e.g., comprising a WQP insertion) can evade cells of the innate immune system.

[0598] In vitro assays involving killing by natural killer (NK) cells and macrophages will be performed with the xCelligence system to measure killing of engineered T cells comprising a CD47 protein variant (e.g., comprising a WQP insertion). The xCelligence system measures electrical impedance changes as adherent target cells detach following cell death. A drop in electrical impedance below an initial starting cell index will be used as an indicator of innate immune cell-mediated killing of target T cells by NK cells and macrophages after an extended co-culture.

[0599] If the CD47 WQP insertion variant supports immune evasion, then the cells expressing the CD47 WQP insertion variant will exhibit similar levels of killing by NK cells and macrophages relative to control cells over expressing wild-type CD47. Alternatively, if the CD47 WQP insertion variant improves immune evasion, then the cells expressing the CD47 WQP insertion variant will exhibit decreased levels of killing by NK cells and macrophages relative to control cells over expressing wild-type CD47.

Example 11. Functionality of T Cells Comprising CD47 Protein Variant

[0600] The present Example demonstrates that engineered cells as described herein comprising a CD47 protein variant (e.g., comprising a WQP insertion) can exhibit immune evasion.

[0601] Engineered T cells will be generated and tested for their ability to evade allogeneic human immune cells. In order to generate the engineered T cells, T cells transduced with wtCD47 or CD47 WQP insertion variant will be engineered to knock-out B2M, and CIITA genes. Transduced T cells will be further sorted by flow cytometry for HLA-ABC and HLA-DR knock-out and different levels of CD47 expression (e.g., creating T cell populations with low, medium, or high CD47 expression). Unedited, Mock T cells and/or control EGFRt engineered T cells will be used as controls in the assay. Controls and the sorted populations of interest will then be tested using an xCelligence NK and macrophage protection assay. In this assay, T cells are initially plated as adherent target cells in xCelligence 96-well plates using a specialized coating mix to allow for cell adhesion to 96-well plates. After T cell adherence is demonstrated by increased electrical impedance using xCelligence equipment, human allogeneic NK cells or macrophages are then added as non-adherent effector cells. Real time monitoring of T cell viability will then be measured by changes in electrical impedance over time. Maintenance or improvement of immune evasion by the engineered T cells comprising a CD47 protein variant (e.g., comprising a WQP insertion) will be evaluated.

This Example demonstrates that engineered T cells comprising a CD47 protein variant (e.g., comprising a WQP insertion) can have maintained or improved T cell viability and/or immune evasion. In some embodiments, engineered T cells comprising a CD47 protein variant (e.g., comprising a WQP insertion) can have maintained or improved T cell viability and/or immune evasion as compared to a control T cell (e.g., Mock T cell and/or control EGFRt engineered T cell). In some embodiments, engineered T cells comprising a CD47 protein variant (e.g., comprising a WQP insertion) can have

changes in electrical impedance over time during a xCelligence NK and macrophage protection assay as compared to a control T cell (e.g., Mock T cell and/or control EGFRt engineered T cell). Without wishing to be bound by any particular theory, impedance loss may indicate innate immune mediated killing of the target T cell populations. In some embodiments, engineered T cells comprising a CD47 protein variant (e.g., comprising a WQP insertion) can maintain impedance signal during an xCelligence NK and macrophage protection assay. In some embodiments, engineered T cells comprising a CD47 protein variant (e.g., comprising a WQP insertion) can maintain impedance signal during an xCelligence NK and macrophage protection assay at low CD47 expression levels as compared to a control T cell (e.g., Mock T cell and/or control EGFRt engineered T cell).

Example 12. Expression Levels of Truncated CD47 Variants and Resulting Immune Evasion

[0602] This Example describes *in vitro* expression of truncated CD47 variants in hypimmune iPSCs after treatment with lentiviral vectors (LVV) and resulting evasion of killing by natural-killer cells.

Methods

[0603] Human Gibco iPSCs were treated with truncated CD47 lentiviral vectors (LVV) encoding the CD47 variants described in **Figure 19** and **Table 21**. Control cells were treated with LVV encoding wild-type (WT) human CD47 (LV1) or with HDR knock-in of WT CD47 (HDR1). Resulting CD47 overexpression was assessed with flow cytometry as described herein. CD47 (staining CC2C6; 20ug/mL) fluorescence (FITC-A) was quantified and compared to (i) isotype control expression fluorescence (staining MOPC-21; 20ug/mL) to calculate fold change over isotype or (ii) to a double (HLA-I and HLA-II) knockout control (dKO) without a CD47 transgene to calculate fold change over dKO.

[0604] Expression of each of HLA-I, HLA-II, and CD47 relative to isotype control was assessed with flow cytometry as described herein. Cells were plated in 6-well plates with a density of 100,000 per well. IFN γ and TNF α (both 100ng/ml) were used for cell stimulation for 48h. Cells were harvested and labeled with a mouse anti-human antibody (HLA-I: 562006, APC clone G46_2.6; HLA-II: 563591, AF647 clone Tu3a; CD47: 561261, PerCP-Cy5.5 clone B6H12; all BD Biosciences) and κ isotype control (HLA-I: APC Mouse IgG1, 555751; HLA-II: AF647 Mouse IgG2a, 557715; CD47: PerCP-Cy5.5 Mouse IgG1, 550795; all BD Biosciences). CD47 expression was further assessed using BDQuantibrite™ Beads (Cat. No. 340495) with a mouse anti-human CD47 antibody (Biolegend, 323108), according to manufacturer instructions.

[0605] NK cell killing assays and macrophage killing assays were performed on the XCELLIGENCE MP platform (ACEA BioSciences, San Diego, CA.). 96-well E-plates (ACEA BioSciences) were coated with laminin (Gibco), and 4×10^4 MHC-I and MHC-II knockout cells (i) without a CD47 transgene ("dKO"), (ii) with exogenous WT CD47 or (iii) with exemplary truncated CD47 variants were plated in 100 μ L cell specific media. After the Cell Index value reached 0.7, human NK cells or human macrophages were added with an effector cell to target cell (E:T) ratio of 1:1 with 1 ng/ml human IL-2 (Peprotech). As controls, cells were untreated or treated with 2% Triton™ X-100. All assays were performed for 50h. Data were standardized and analyzed with the RTCA software (ACEA).

Results

Table 22. CD47 Overexpression

LV ID	SEQ ID NO:	Description	%CD47 Overexpression	Fold Change over dKO	Fold Change over Isotype
dKO	-	dKO	0%	1	3.9
LV1	19	CAG-hCD47	100%	45	186

HDR1	19	CAG-CD47_CLYBL	99.9%	131	555
LV2	14	CAG-CD47(202)	99.9	245	829
LV3	13	CAG-CD47(201)	100%	153	564
LV4	133	CAG-CD47(206) – w/o N-Terminus	0%	1	24
LV5	142	CAG-CD47 ECD w/ DAF/CD55 GPI Anchor	99.7%	60	190
LV6	144	CAG-CD47 w/ Short TRAILR3-GPI Anchor	99.9%	271	892
LV7	341	CAG CD47 ECD w/ CD59 GPI Anchor	99.9%	112	340
LV8	134	CD47-206 w/ restored N-Terminus	99.9%	176	518
LV9	200	Cod Opt CD47 derived from CDA Bicistronic	99.8%	300	786
LV10	145	CD47 ECD with CD8a TMD and Short ICD (9aa)	99.9%	283	932

[0606] **Figure 20** provides graphs showing Gibco iPSC expression of CD47 (left) and isotype control (right) after treatment with truncated CD47 LVV, as assessed by flow cytometry. These data demonstrate that the majority of the truncated CD47 variants tested (e.g., LV2, LV3, LV5-LV10) are expressed at a similar level to overexpressed WT CD47 (LV1, HDR1) and at a higher level than a control not expressing a CD47 transgene (dKO) and a truncated CD47 variant lacking an N-terminus (LV4). By contrast, expression of isotype control was similar across all CD47 variants tested. **Table 22** provides further quantification of CD47 surface expression across CD47 variants, including percent CD47 overexpression and computed fold change relative to dKO or to isotype control. Some truncated CD47 variants (e.g., LV2, LV9, LV10) demonstrated fold change over dKO or isotype greater than that of the WT CD47 variants tested (LV1 and HDR1). Amino acid sequences corresponding to the variants in **Figure 19** and **Table 22** and expressed with LVV are described in **Table 23**.

[0607] **Figure 21A** and **Figure 21B** provide exemplary graphs showing Gibco iPSC surface expression of HLA-I (top row), HLA-II (middle row), and CD47 (bottom row) after treatment with control or truncated CD47 LVV (as assessed by anti-HLA-I, anti-HLA-II, or anti-CD47 flow cytometry, respectively, relative to isotype control). Surface expression of HLA-I and HLA-II was similar for treated cells compared to isotype controls, demonstrating successful knock-out of HLA-I and HLA-II. These data further demonstrate that surface expression of CD47 was increased for the truncated CD47 variants compared to isotype controls at levels ranging from 62-fold to 300-fold.

[0608] **Figure 22A** and **Figure 22B** provide exemplary graphs showing killing of Gibco iPSC target cells expressing CD47 LVV by natural-killer (NK) effector cells (top row), in the absence of effector cells (middle row), or after treatment with TritonX (bottom row), as assessed by XCELLIGENCE assay. These data demonstrate that the majority of the clones exhibit sufficient CD47 expression to protect from NK-cell killing. By contrast, a dKO control lacking CD47 transgene and a truncated CD47 lacking an N-terminus (LV4) did not exhibit sufficient CD47 expression to evade NK cell killing.

EXEMPLARY SEQUENCES

Table 23. Exemplary Amino Acid Sequences for CD47 Proteins

Description	SEQ ID NO:	Amino Acid Sequence
CD47-201	SEQ ID NO: 1	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAAC IPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRNN
CD47-202	SEQ ID NO: 2	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAAC IPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
CD47-202 without signal peptide	SEQ ID NO: 3	QLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGRDIYTFDGLNKTSTVPTDF SSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETIIEELKYRVVSWFSPNENILIV IFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGAILFVPGEYSLKNATGLGLI VTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQ LLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
CD47-203	SEQ ID NO: 4	XWTESLYCGVYTNAWPSSDFRFEYLSSTITWTSLYEICGVQRDTYSQLKGKKRQASNQKTIQ PPRKAVEEPLNAFKESKGMNDE
CD47-205	SEQ ID NO: 5	XKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLI SGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNE
CD47-206	SEQ ID NO: 6	MEAQNTTEVYVKWKFGRDIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSH TGNYTCEVTELTREGETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDE KTIALLVAGLVITVIVIGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVF
CD47t-1	SEQ ID NO: 7	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTL
CD47t-2	SEQ ID NO: 8	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPG
CD47t-3	SEQ ID NO: 9	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTA
CD47t-4	SEQ ID NO: 10	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLC
CD47t-5	SEQ ID NO: 11	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAAC IPMHGPLLISGLSILALAQLLGLVYMKFV
CD47t-6	SEQ ID NO: 12	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPGEYSLKNATGLGLIVTSTGILILLHYVVF
CD47-206 w/o N-Terminus	SEQ ID NO: 80	MEAQNTTEVYVKWKFGRDIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSH TGNYTCEVTELTREGETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDE KTIALLVAGLVITVIVIGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVF
CD47-206 w/ restored N- Terminus	SEQ ID NO: 81	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIGA ILFVPGEYSLKNATGLGLIVTSTGILILLHYVVF
Deleted TMD3	SEQ ID NO: 82	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGLNKTSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII

		ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVVGA ILFVPGEYSLKNA
Deleted EC + TMD3	SEQ ID NO:83	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVVGA ILF
Deleted TMD2 + EC + TMD3	SEQ ID NO:84	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKT
Deleted last IC	SEQ ID NO:85	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVVGA ILFVPGEYSLKNATGLGLIVTSTGILLHHYVVFSTAIGLTSFVAILVIQVIAYILAVVGLSLCIAAC IPMHGPLLISGLSILALQLLGLVYM
Deleted TMD5 + last IC	SEQ ID NO:86	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVVGA ILFVPGEYSLKNATGLGLIVTSTGILLHHYVVFSTAIGLTSFVAILVIQVIAYILAVVGLSLCIAAC IPMHG
Deleted EC + TMD5 + Last IC	SEQ ID NO:87	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVVGA ILFVPGEYSLKNATGLGLIVTSTGILLHHYVVFSTAIGLTSFVAILVIQVIAYILAVVGL
Deleted TMD4	SEQ ID NO:88	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVVGA ILFVPGEYSLKNATGLGLIVTSTGILLHHYVVFSTAIGLT
CD47 ECD w/ DAF/CD55 GPI Anchor	SEQ ID NO:89	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNEPNKSGTTS GTTRLLSGHTCFTLTGLLGLVTMGLLT
CD47 ECD w/ Long TRAILR3- GPI Anchor	SEQ ID NO:90	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNEATVETPAAEETMNTSPGTPAPAAEETMNTSPGTPAPAAEETMTTSPGTP APAAEETMTTSPGTPAPAAEETMITSPGTPASSHYLSCTIVGIIVLIVLLIVFV
CD47 ECD w/ Short TRAILR3- GPI Anchor	SEQ ID NO:91	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNEMITSPGTPASSHYLSCTIVGIIVLIVLLIVFV
CD47 ECD with CD8a TMD and Short ICD (9aa)	SEQ ID NO:92	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNEIYIWA PLAGTCGVLLLSLVITLYCNHRNR
CD47 ECD with CD28 TMD and Short ICD (9aa)	SEQ ID NO:93	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNEFWLVVVGVLACYSLLVTVAFIIFWVRSKRSLHH
CD47 ECD with PDGFR TMD and Short ICD (9aa)	SEQ ID NO:94	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKYRVVSWFSPNEVISAILALVLTIIISLIILMLWQKKPRY
CD47 IgV, 3aa from Hinge Region, with PDGFR Hinge, TMD and Short ICD (9aa)	SEQ ID NO:95	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKAVGQDTQEIVVPHSLPFKVVVISAILALVLTIIISLIILMLWQKKPRY
CD47 IgV, 3aa from Hinge Region, IgG4	SEQ ID NO:96	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKESKYGPCPPCFWVLVVGVLACYSLLVTVAFIIFWVRSKRSLHH

Hinge - CD28 TM		
CD47 IgV, 3aa from Hinge Region, IgG4 Hinge - CD8a TM	SEQ ID NO:97	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKESKYGPPCPPCIYIWAPLAGTCGVLLLSLVITLYCNHRNR
CD47 IgV, 3aa from Hinge Region, CD28 Hinge - CD28 TM	SEQ ID NO:98	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFWVLVVVGGVLACYSLLVTVAFII FWVRSKRSRLH
CD47 IgV, 3aa from Hinge Region, CD28 Hinge - CD8a TM	SEQ ID NO:99	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPIYIWAPLAGTCGVLLLSLVITLYC NHRNR
CD47 IgV, 3aa from Hinge Region, CD8a Hinge - CD28 TM	SEQ ID NO:100	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACYSL LTVAFIIFWVRSKRSRLH
CD47 IgV, 3aa from Hinge Region, CD8a Hinge - CD8a TM	SEQ ID NO:101	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGR DIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETII ELKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLYCNHRNR
WT, C15G	SEQ ID NO:102	MWPLVAALLGSACCGSAQLLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKWKFGR RDIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETI IELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVG AILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIA ACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
N361, C15G	SEQ ID NO:103	MWPLVAALLGSACCGSAWQRPLRFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGA WANKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
N362, C15G	SEQ ID NO:104	MWPLVAALLGSACCGSAWAPPLRFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGYTNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
N363, C15G	SEQ ID NO:105	MWPLVAALLGSACCGSAWQLPLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGDKNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
N364, C15G	SEQ ID NO:106	MWPLVAALLGSACCGSAWQPLAFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGA MNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
N366, C15G	SEQ ID NO:107	MWPLVAALLGSACCGSAWQTPLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTRE ETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
N368, C15G	SEQ ID NO:108	MWPLVAALLGSACCGSAWQPLRFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGA ENKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI

		VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N369, C15G	SEQ ID NO:109	MWPLVAALLGSACCGSAWQIPLNFKNKTKEVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAWNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3610, C15G	SEQ ID NO:110	MWPLVAALLGSACCGSAWQPLLLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3612, C15G	SEQ ID NO:111	MWPLVAALLGSACCGSAWQPLLLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3614, C15G	SEQ ID NO:112	MWPLVAALLGSACCGSAWQPLLLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3617, C15G	SEQ ID NO:113	MWPLVAALLGSACCGSAWQPLLLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE ETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3618, C15G	SEQ ID NO:114	MWPLVAALLGSACCGSAWQPLLLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE ETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3621, C15G	SEQ ID NO:115	MWPLVAALLGSACCGSAWQIPLFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE ETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3623, C15G	SEQ ID NO:116	MWPLVAALLGSACCGSAWQPLVFNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGVNKNSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
N3624, C15G	SEQ ID NO:117	MWPLVAALLGSACCGSAWQIPLRNKTKSVEFTFGNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGVNKNSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
hCD47 N361 Mutant	SEQ ID NO:118	MWPLVAALLGSACCGSAWQPLRNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
hCD47 N362 Mutant	SEQ ID NO:119	MWPLVAALLGSACCGSAWQPLRNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGYTNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFESKSGMMNDE
hCD47 N363 Mutant	SEQ ID NO:120	MWPLVAALLGSACCGSAWQPLLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKKW FKGRDIYTFDGDKNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI

		VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N364 Mutant	SEQ ID NO:121	MWPLVAALLLSACCGSAWQPLAFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGMNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N366 Mutant	SEQ ID NO:122	MWPLVAALLLSACCGSAWQTPLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE ETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N368 Mutant	SEQ ID NO:123	MWPLVAALLLSACCGSAWQPLRFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N369 Mutant	SEQ ID NO:124	MWPLVAALLLSACCGSAWQIPLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3610 Mutant	SEQ ID NO:125	MWPLVAALLLSACCGSAWQPLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3612 Mutant	SEQ ID NO:126	MWPLVAALLLSACCGSAWQPLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3614 Mutant	SEQ ID NO:127	MWPLVAALLLSACCGSAWQPLLFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3617 Mutant	SEQ ID NO:128	MWPLVAALLLSACCGSAWQPLRFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE ETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3618 Mutant	SEQ ID NO:129	MWPLVAALLLSACCGSAWQIPLEFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW KGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE ETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3621 Mutant	SEQ ID NO:130	MWPLVAALLLSACCGSAWQIPLEFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW KGRDIYTFDGAENKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE ETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3623 Mutant	SEQ ID NO:131	MWPLVAALLLSACCGSAWQMPVFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGVNKTSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSL CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
hCD47 N3624 Mutant	SEQ ID NO:132	MWPLVAALLLSACCGSAWQPLRFNKTKSVEFTFCNDTVVIPCFTVNMEAQNTTEVYVKKW FKGRDIYTFDGVNKTSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVELTRE GETIIEKRYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVI

		VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
CAG-CD47 (202) – CodOpt	SEQ ID NO:199	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWFKGRDIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETIIELK YRVVSWFSPNENILVIFPIFAILLFWGQFGIKTLKYRSGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNMNDE
CD47 ECD-CD59 GPI	SEQ ID NO:340	MWPLVAALLLSACCGSAQLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWFKGRDIYTFDGA LNKSTVPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREGETIIELK YRVVSWFSPNEQLNGGTSLSSEKTVLLLVTPFLAAAWSLHP*

Table 24. Exemplary Nucleotide Sequences for CD47 Proteins

Description	SEQ ID NO:	Nucleotide Sequence
CD47-201 CDS	SEQ ID NO:13	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAAATTAACAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTCTGCCAGGTGAATATTCAATAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTTCACTACTA TGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTATTCAGGTGA TAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATACCAATGCA TGGCCCTCTTCTGATTTCAAGTTTGAATATCTAGCTCTAGCACAATTACTTGGACTAGTTT ATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAATAACTGA
CD47-202 CDS	SEQ ID NO:14	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAAATTAACAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTCTGCCAGGTGAATATTCAATAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTTCACTACTA TGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTATTCAGGTGA TAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATACCAATGCA TGGCCCTCTTCTGATTTCAAGTTTGAATATCTAGCTCTAGCACAATTACTTGGACTAGTTT ATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAGCTGTAGAGGA ACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
CD47-203 CDS	SEQ ID NO:15	TGGACTGAGTCTGTGATTGCGGCGTGATACCAATGCATGGCCCTCTTCTGATTTCAAGT TTGAGTATCTAGCTCTAGCACAATTACTTGGACTAGTTTATATGAAATTTGTGGGGTTCA AGGGACACCTACAGTCAGTTGAAAGGCAAGAAGAGACAAGCTTCCAATCAGAAGACTATAC AACCTCCTAGGAAGCTGTAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGAT GAATGATGAATAA

CD47-204 CDS	SEQ ID NO:16	AGATATTTTCTTGTTC AATTTAAGGAGAGGTAAATTTGGTATCAATAGAAAAATGTTTCTG AAAAATTTAAACCCTGGAAATGTATTTATGGCATGGAGTCAGATGTTTCAGGGAGAGAAGA ACAAATCAAGAAGCATTGCAAGTATGCTCATATGGAATGCTTAAGGCTTGTGGTTAAAAAA TATATATATATGGCTGTCAATGTCTTAGGCTCATGGTAGCAGCAGAAATCGTAATAATTCTT TTGTCACATGGGTTATATCCATATTGGAGAGAATTAAGTCAAGGTGAAATTAAGTGTACACT GTTTGGTTTTATAATTTAGAGGGATCACAAGTACTGATGTCCCTTTGAAGTACCAATCT TCATAAATCTTTTTTTTTCAGAATGGGCCAGCCAAGTGTGACATCCCTTGGATCGGAGATTT AGAACTAGAAAGTATTCTTTCTACATTATTAGGGAAGAAAAGGAGTTACTTGGCGGTTAGC AATATTCTATTTTGTGTTTTGTTTTGTTTTAGAGACAGGGTCTCATTATGTTGACCAGGCTGG CCTCGAGCTCCTGGGCTCAAGCAATGCTCCCACTCAGCCTCCCAAGTAGCTGGGACTACA GGCATGTGCCACTACACCTGGCAGTGTATTCTGATAAATACATTTATGAGCTCAAAAATG TAACTCTAAAACCTTATCTCTGAAGTCCATATTACCATCAGAAATTTAGATAGTTGTTTAG TTCTCTTTTTCTTTGTAGAACATAGATATAAGGCATGGTTTCATTGAAGTCAGTTGTATATA CATGTAAGTATCCTGATGTTCCCAATAAAGCTCTGTATTTCTGCTTAGTTTATTGGGGAGG CTGCTAAATGTAGTGCATCCCAACCCATTTTACCCTGTTCTACTTTAAAAAGAGGTTGGCTT CTTGTTTGGATACAAGGACCAAGTCACTCCCCCAGGTTCTCCACAGTAACGGGAGGCTAT TTAAAGCCGCCATGGCACTAACAGAACTGGACTCCTATGAGCTCAGATACATAACTGGG CCTCACAGGGGTGGGACAGATGTAGTCTAGGAATTGGAAGGATCCATTCCATATCAAGA ACTGAAGCATCGTGTGCCCTCTCAGCAGCAAGAGTAAGGTGATGCCCTGTGAGTTATAG TTCTGAGTTCCTCTGTCTTTGATTCTTTGCCTATTAGCCAGTACCTCACCCTCTGTTTA TGCCACTGTTTTTATCCTATTTCATGCCTTCTCAGACAACTTTTCTTACCTACAGCTTTG GACTCATCCTTGTCTCCTTTCTGTTTCTTTTCTTCTTCCCTTCCCATCACCCTTTCTGGG TTTTTTCTGTTTCTTCTTAGAGTCCAGTGGCAGGGAGAACTTGTGAGTCCAGTCTGTTG CCATTTTCTGTTTGTAGAAAGACTCACCAGCTTTTGGCTGGCTCAGATTTGGCTTTCCTT GGGTGAGGACCCACCTTTTCCCTGCCAGCTTTGGAAGCTTGACAGAAATTCAGTGTTCAG TGGTGGTAAATAAATAGTAAGGAACACAGAGCAGTCTGGAGGCGTGCTCCATCTGCTGA TGAGAAAATCCAGTGCTGTATCCAGCCAGGTCACGCGGAATGGGCTCTCTGTTCAAGT AGGATCCCCCTCTGCTGAGTGGTTCATGGCATGTTCTGTTCAACGCTTTTCCATCTGTAG GATCTTATCTGATTTTATTTGTTTTTGGGTTTTTTTTATTTTGTAGATGGAGTCTCGC TCTGTGCCCAGGCTGGAGTGCAGTGGCACGACCCAGCTCGCTGCAGCCTCTGCCTCCCA GGACGAGGGAGATCCTCCACCTCAGCCTTCCAGTAGCTGGGACTACAGGCATGCACCAC AGGCATGCACCACCGCCAGCTAATTTTTGTATTTTGGTAGAGACAGGGTTGCATCATG TTGCCCAGGCTGGTCTTGAATGCCTGAGCTCAAGCAATCTATTGCTTGGCTGCCCTCCAAAG TGCTGGGATTACAGGCATGAGCCACCGCCAGCCTTCTCATTTGTTTTTTTATAAGGA AGCTATCTCTTCTTCCCTCCCAACTAGGGTATTCTTTTCCCTTTCGTCACTTTGCTCATG TACTGTATTCCTTCAACTTCATTAAATGAATCCATTTGGAAGCAGTGAAAAAGGCAACTCAGA AAGCTAAGAAGAAATAGATAGAGGAATACTCAGAGCTATCTGAGTATTTCTTTAGTTTGT AGCTCTTTGGAGCTTTGAAACTGGAAAGACCCAGGGAGTGATGTGGAGAAAGAGACTGAG CTTGTAAAGACACAGGAGCAGTGAAGTGAAGGAGAGTGGAGTGGGAGCAAAATCTGGCA CATTCTGTCTACACTCTGGGTAGATAGAGGAGGAGGATGGAGCACCCATGGTGGGGGTA TGTTGGTGACAGCATTTTCCACAGCCAGTGAACAAGTGGCTGATTTGGGGGAAAGATG GCATAAACAAATGAGAGAATGTGTTTACTATTTGATGTAGATGGGTTATTTGCTTCATTTT CAAATCAGTGTATATAATCAAGAATATTGAGCATGTTTGAATAGACTGTCAGAGCTGGAAGT CTTTCATTAACATCTCTGGCACCTTTAGTTTTAGCCCTGAACATTTATCTTAAAAATTAACA TTACCAATGCCTTAGTTTATTTTATTTAATTTATATTCTTATTTGTTATTTATATCAG CTTCCAATCAGAAGACTATACAACTCCTAGGAATACTGAAGTGAAGTATGGACTCCGAT TTGGAGAGTAGTAAGACGTGAAAGGAATACACTTGTGTTTAAAGCACCATGGCCTTGATGAT TCACTGTTGGGAGAGAAGAAACAAGAAAAGTAACTGGTTGTCACCTATGAGACCTTACGTG ATTGTTAGTTAAGTTTTTATTCAAAGCAGCTGTAATTTAGTTAATAAAATAATATGATCTA TGTTGTTGCCCAATTGAGATCCAGTTTTTGTGTTATTTTTAATCAATTAGGGGCAATAG TAGAATGGCAATTTCCAAGAATGATGCCTTTCAGGTCCTAGGGCCTCTGGCCTTAGGTA ACCAGTTTAAATTGGTTTCAAGGTGATAACTACTTAGCACTGCCCTGGTGATTACCCAGAGA TATCTATGAAAACAGTGGCTTCCATCAAACCTTTGCCAACTCAGGTTTACAGCAGCTTTGG GCAGTTATGGCAGTATGGCATTAGCTGAGAGGTGTCTGCCACTTCTGGGTCAATGGAATAA TAAATTAAGTACAGGACAGGAATTTGGTTGGGAGCATCTTGATGATCTCCGTATGATGTGA TATTGATGGAGATAGTGGTCTCTATTCTTGGGGTTGCCATTCCACATTCCCCCTTCAACA AACAGTGTAAACAGTCTTCCAGATTTAGGGTACTTTTATTGATGGATATGTTTTCTTTT ATTACATAACCCCTTGAAACCTGTCTTGTCTCTCTGTTACTTGTCTGCTGTACAAGAT GTAGCACCTTTTCTCTCTTGAACATGGTCTAGTGACACGGTAGCACCAGTTGCAGGAAG GAGCCAGACTTGTCTCAGAGCACTGTGTTTCAACATTTTCAAGCAAAATAGCTATGGTTGTA ACATATGTATTCCTTCTCTGATTTGAAGGCAAAATCTACAGTGTCTTCTCACTCTTTTT
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		CTGATCTGGGGCATGAAAAAGCAAGATTGAAATTTGAACTATGAGTCTCCTGCATGGCAA CAAAATGTGTGTACCATCAGGCCAACAGGCCAGCCCTTGAATGGGGATTTATTAAGTGTG TATCTATGTTGCATGATAAACATTTCATCACCTTCCTCCTGTAGTCTGCCTCGTACTCCCT TCCCTATGATTGAAAAGTAAACAAAACCCACATTTCTATCCTGGTTAGAAGAAAAATTAAT GTTCTGACAGTTGTGATCGCCTGGAGTACTTTTAGACTTTTAGCATTCGTTTTTACCTGTT TGTGGATGTGTGTTGTATGTGCATACGTATGAGATAGGCACATGCATCTTCTGTATGGAC AAAGGTGGGGTACCTACAGGAGAGCAAAGGTTAATTTTGTGCTTTTAGTAAAAACATTTAA ATACAAAGTTCTTTATGGGTGGAATTATATTTGATGCAAAATTTTGATCACTTAAAACCTTT TAAAACCTCTAGGTAATTTGCCACGCTTTTGTACTGCTCACCATACCTGTAAAAATACGT AATCTTCTCTGTTTGTGAATAAGATATTCATATTTGTAGTTGCATTAATAATAGTTATTTT TTAGTCCATCAGATGTTCCCGTGTGCCTCTTTTATGCCAAATTGATTGTATATTTTCATGTT GGGACCAAGTAGTTTGGCCATGGCAAACCTAAATTTATGACCTGCTGAGGCCTCTCAGAAA ACTGAGCATACTAGCAAGACAGCTCTTCTTGAAAAAATAATGTATACACAAATATATAC GTATATCTATATACGTATGTATATACACACATGTATATTTCTTCTTGATTGTAGCTGT CAAAATAATAACATATATAGAGGGAGCTGTATTCCTTTATACAAATCTGATGGCTCCTGCA GCACTTTTCTTCTGAAAAATTTTACATTTTGCTAACCTAGTTTGTACTTTTGGAAATCAGT TTTGATGAAAGGAGGAAAAAGCAGATGGACTTGAAAAAGATCCAAGCTCCTATTAGAAAAAG GTATGAAAACTTTTATAGTAAATTTTTTATAAACTAAAGTTGTACCTTTTAAATATGTAGTA AACTCTCATTTATTTGGGGTTCGCTCTTGGATCTCATCCATCCATTGTGTTCTCTTAAATGC TGCCTGACCTTTTGAGGCATTCACTGCCCTAGACAATGCCACAGAGATAGGGGAAATG CCAGATGAAACCAACTCTTGCTCTCACTAGTTGTGACGCTTCTCTGGATAAGTGACCACAGAA GCAGGAGTCTCCTGCTTGGGCATCATTGGGCCAGTTCTTCTCTTTAAATCAGATTGTGTA ATGGCTCCCAAATTCATCACATCACATTTAAATTTGCAGACAGTGTTTTGACATCATGTAT CTGTTTTGTCCATAATATGCTTTTTACTCCCTGATCCAGTTTCTGCTGTTGACTCTTCCA TTCAGTTTTATTTATTTGTGTGTTCTCACAGTGACACCATTTGTCTTTTCTGCAACAACTT TCCAGCTACTTTTGCCAAATCTATTTGTCTTCTCTTCAAACATTTCTCTTTGCAAGTCTCT CTTTCATCTGTGTAGCTGCTTTTTGTCTCTTAACCTACCATTCCTATAGTACTTTATGCATC TCTGCTTAGTTCTATTAGTTTTTGGCCTTGCTCTTCTCTTGAATTTAAATTCCTCTATA GCTAGAGCTTTTCTTTCTTCTTCTCTCTTCTGCAAGTGTGTCATCAGACAGAGCTAG GTACATAAGTTAAATGATTGAGAGTTGGCTGTATTTAGATTTATCACTTTTTAATAGGGTGA GCTTGAGAGTTTTCTTTCTTTCTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTGACTAATTCACATGCTCTAAAAACCTTCAAAGGTGATTATTTTTCTCCTGGAAAC TCCAGGTCCATTCTGTTTAAATCCCTAAGAATGTCAGAAATTAATAACAGGGCTATCCCGT AATTGAAATATTTCTTTTTTTCAGGATGCTATAGTCAATTTAGTAAGTGACCACCAATTTGT TATTTGCACTAACAAGCTCAAAACACGATAAGTTTACTCCTCCATCTCAGTAATAAAAAAT AAGCTGTAATCAACCTTCTAGGTTTCTCTTGCTTAAATGGGTATTCAAAAATGGGGATCT GTGGTGTATGTATGAAACACATACTCCTTAATTTACCTGTTGTTGAAACTGGAGAAATG ATTGTCGGGCAACCGTTTATTTTTTATTGTATTTTATTTGGTTGAGGATTTTTTTATAAAC AGTTTTACTTTGTGCATATTTAAATTAATACTGATCCTGCTGGGGTCTTTGTGTA GGTCATTTTCAGTGACTAATAGGATAATCCAGGTAACCTTTGAAGAGATGAGCAGTGAGTG ACCAGGCAGTTTTCTGCCTTTAGCTTTGACAGTTCTTAATTAAGATCATTGAAGACCAGCT TTCTCATAAATTTCTCTTTTTGAAAAAAGAAAGCATTTGTACTAAGCTCCTCTGTAAGACA ACATCTTAAATCTTAAAGTGTGTTATCATGACTGGTGAGAGAAGAAAACATTTTGTTTTT ATTAATGAGCATTATTTACAAAAAGCCATTGTTGAGAATTAGATCCCATCGTATAAAT ATCTATTAACCATTTCTAAATAAGAGAACTCCAGTGTGTATGTGCAAGATCCTCTCTTGG AGCTTTTTTGCATAGCAATTAAGGTGTGCTATTTGTCAGTAGCCATTTTTTTCAGATGATT TGAAGACCAAAGTTGTTTACAGCTGTGTTACCGTTAAAGGTTTTTTTTTATATGTATTA AATCAATTTATCACTGTTTAAAGCTTTGAATATCTGCAATCTTGCCAAGGTACTTTTTTAT TTAACAAAAAACATAACTTTGTAAATATTACCCTGTAATATTATATATACTTAATAAAACATT TTAAGCTAT
CD47-205 CDS	SEQ ID NO: 17	AAGAATGCTACTGGCCTTGGTTTAAATGTGACTTCTACAGGGATATTAATATTACTTCACTA CTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTATTCAGG TGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATACCAAT GCATGGCCCTCTTCTGATTTACAGTTTGTAGTATCTTAGCTCTAGCACAATTAAGTGGACTAG TTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTGTAGAG GAACCCCTTAATGAATAA
CD47-206 CDS	SEQ ID NO: 18	ATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGAATTTAAAGGAAGAGATATTT ACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGTAGTGCAAAAAAT GAAGTCTCACAATTAATAAAGGAGATGCCTCTTTGAAGATGGATAAGAGTGATGCTGTCT CACACACAGGAAACTACACTTGTGAAGTAACAGAAATTAACAGAGAGGTAAGGTAACGATCAT CGAGCTAAATATCGTGTGTTTCATGGTTTTCTCCAATGAAAAATTTCTATTGTTATTT

		TCCCAATTTTGTCTATACTCTGTTCTGGGGACAGTTTGGTATTAACAACTTAAATATAGA TCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAGTGATCACTGTCA TTGTCAATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATTCATTAAAGAATGCTACTGGC CTTGGTTAATTGTGACTTCTACAGGGATATTAATATTACTTCACTACTATGTGTTT
Human CD47 gene	SEQ ID NO: 19	CTCATTCTATTTATTCTTTTAAACAAGATTGTTGAGTACATACTATGTGTACAGGCAAAGGG GCTAGCAGTAAAGAAGACTCATATGGTCCCTCCCCTCATTGAGCTTACAGTATAGGAAACA GTTATTACTGAACACGAAGACAGCTATATAACATAATTTCAATAAAAGATGGTAACTGATA TGCACCAAGTAAGACTAAAAACACAGAGAAGTGACCTTAACTTATCTGGCATATCATGGG CCTTGATTTACTGTGGGATCCTTTGCTGTTATGTTCCCTTACGTTGAAAGATGCATCTGGCC CTACACAAAGCACAGACTATGAGTAGGTAGGCACATACAGTTCTAAGCCAACCCCTTGGGG CACCAGGAGACAGCACTGTCTCATGTCGTTGCATGACACACCACCATGGGGCAAAACATTT GCTGAATGAAGTTACAATAAGGATACAATTGTATCTACATAAACATTTAAAGATACTCAT ATCAATACCTACTATGTGGCAGGATCTTGATGTTGTGTGGGGAGGAAGATAAAAGTAGATG TATCGTTGAGACAGAAATGAGGGCAAGGGAAATTTTTATGCATTGAGAGAGGAAGTTTGT GAATTTTTTCGCTGGCCATGGAGTCAGGGAGACTTCAGTCCAACCACCGTCCCTCCCTGAG CTTGAGCAAGGCTCACTTTCTTATCTATAAAGGTGACAAGAGTATTTTTTAAGTTTA TTTATCTGTAAAGGGGAACTGTAGCAACTTTGCTATTATAGGGTAAAAAGAAAAAGGAT GAAAACCAGAACAGGGGAGAGAGACAAGAGAGAGAGCAGTGTCACTTATACTGTGGGCAA AGTGCTCACCATTCCTCTTTCCCTTTGTTTCCCTCATCTCAAAAAGGGAGATCAACATTTTA TCCTTTCTGAGGTTGTTATGAAGTAACTTAGGTGATATAGCTAATGTGTACTTCTGTGT TCAAAAACATTTAATATGTATTATCCCTCCCTCTCTACCCAAGAACTCCTTACATACAGAT AAGATGAGCTCAACATCCAATTAATGCTTTGAAAAGTTAAAAGGCAACCTTTAAATG ATTAAATCTGAAAAGAGCTTAAGGTTATCTCAGAGAGAGGATAATCAAACTAGGCAGGC TGCTTTGGACTGTCTCTCTGGGATATGCCTGCTTTTGCCCCACCACCAAAACATACCCAG CTAGATTCCCAAGAGCAGCAGTGGTCACCCAGGTGGCCTCTCATTTCCATTTTCTGCACAAAA AGACGTTAAGCATATAGCTCAGTAGCTCCAGTGTAACAGAACATCACCCGATCTCCACC TCTGAAGGTTGGGGACAGTCTTTCTAGTCACTCTCAGCTTAGGGGGGTTGGTTGGTAT CAGGGACAGCTGACAGCCTTATTTAGCCAACCAGCTGCTGTGTATACAAATGTCCTAAT GCTCAAAGTCTGGTGGATGATTCTTTGTATCCACATAAGCAGCAGTGGAGGAGAATCGA TGAGTCCCTTTGGTTGCTCGGGGTGTCAAGGCTGCCATTCAATCATTTACCACTAATTA TTAACCATTGTTTTAGTGAGATGTCTGCCAGGCAATTGTTTTATTTTTCAATCATTAAAAA AGCAATCAAATTTCACTAGAGCAATGCCTGCCTCACGCCGATCAACACATTTATTAAACCC CTTCTGTTTGCCGAGCTCAATGGAAGTCTTGAGGAGGGAATACTTAAATGCTCTCGGA TTTTAAAGAATTGATAATGCTGTGGGGAAGAGGTTACACAAAAAGCAATTACAGGAAGA AGCTCGATAGTATACAATACACTGGTACAGAGTGTATAGACAGTGCCACTTTCATACGCT GGATTTTATTTCTGTGGCAATGGGATGCTTGGGAGGAGCGCACTGTGTAGAGGATTTGG AGAAGTGGGGTATTGTGGTGGGAATTGCTTTCTTTCCAGGAGGTAGGAGGAAAAACAATC AAGGAGGTGGACAGGATGTGCACTCCATTAGAGCAGCCACGAGCTGACTTTTGTATAA GAGAGTACATCAGTTAGGATAACGGTTAAAAGTATCTTTAAAAGACTTTTGCTTCAGGATG AATGATGTGCCTGTGTGATTGAGCGATAAATCAAAAGCCTTGTCCTATTGTGGCTTGC GGCCACATTTGAAACCCATTTTCAAGCATGTTAAACCCAGCGCAGCGCAGAGGGCTGCA CATGGGGCAGTCACAAACCAAGCTCAATAACCTTGCTGGTGGGATGTGTTGGATACGCTG CTAATGCCTGTTTGGCACAATGCTCGCTAGTCCCGGTGGTGGCGGTGTTACAGGTAACAA TGTTTACCACCGTGAATGGAATGTTTGATTAAACCTGATCAGAGGATGAAACACTAAA GAACCAAGTGAGAAAGAGGGGAAGAGAACCGCATAGGGAAGAGCAGAGCGAGTAGACGAGC CGAACGCAGAGCCCGCAGGGGCGAGTGGAAGCTCCCTGCGGGCAGGTACCCGACCAACCG CCCTGCCCTGGGCGTGGCGGCTCGGGCTCAGGGACCGCTTCGGCGCTAGACGGCCGCT CCGGAGGAAACGGGCGCTGGTGAAAGCCTAGGTGTCTGCTGCCACGCGCGCAGCCGACG TCGGGTCCAGGGAGAGACGCGGGCTGGGGCGGGACGGGACCCGCCCTGAAGCGCGAG GGTGGGAGTGAAAGCAAGAGGAGAAAAGTATAGAGAGAGAGGACAGTGGGGCCAGCGCCG CGCGAAAGGCAGGAACCGACCGGACAGGAACGGGTGCAATGAGTCCCTCCCGGAGCG TGGGAACACAGGGTTACAGCTCCTGCGGGCGGCGAGCACGCGGACCCAGGGCGGGCGG GTGCGACAGGACGTGACCTGGAAGCGCGGCGGTGCCACCGCCTGGAGCAGGCATCCGG CCTCCGTGGAGCGGGCAGGCGGGCCCGGGTCTGGAGCCTGCGACTGGGGAGGGCGCGG CGTCAACAGCAGCGGTTGCGGGGCGGGGCCGAGTGCGCGTGCAGCGGCTCTCGGGGCGG GGAGCAGGCGGGGAGCGGGCGGGAAGCAGTGGGAGCGCGCTGCGCGCGGCGCTGCAG CCTGGGCAGTGGGTCTGCTGTGACGCGCGGCGGCGGTGCGTCTGCTGTAACGCGCG CGGCGGCTGCTGCTCCGACACCTGCGGCGGCGGCGGCGGACCCCGCGGCGGGCGCGGAG ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGTGCGGTGAGTGGCTCCTC GCTCCAGCCCTGCGGCTGCTGCTGCTTCCGCCCGCGGCGGTGTGGGCTGCGCCGACG CAGCCCGGCGGCGCCTGAAGAGGTTGGCCGGGGCGCAGAACACTCGGGCCCTGAGCGCC

		CGAAGTGCAGACGTGGGAGGGCCCCACGGGGAATCGGGCGCCCCCTTCTTCTCCCTTCC TTTCCCTGGTCGTCTTCTTCCCTGGGTGAGAGCGGGGCTCATCTCTCCACCCGGTTCT CCATCTCCAAATGCACACACACAGGAAGACTCCAAACCGCGCACCTCGCGCAAAATTA AGCAAGAAAGGGCGGATCAGAGGCAAGGGGCTGCAGCTGTAGGGATCCCTTCTTTG CACCCCTCTCCCTTCTCAGTGTCTAGACGCATTTGGGGTTGAGGGAGGAGAGTGGGAGG GCCCGGGCCTCTTGCGATGGAAGTGCGCATTTTGGCGAAGTCGGTGAGAAGGGGTTTCTG CCGTTTGCCTCCACATGAACATAGGAGCGAAGAGGACGTAAAGGACACAATTAAGTTTCA TTTTCAACTCAGCATTCAATCAGAAGAATCTTCCGGCCGTGAATTTTGTCTGCTGTTTTA ATCCTAAAAATAAGCTTGTCTGGAAGTCTTCTTCTCTGACCTCACGCCCATCAGCCA CTTGATACATTCTAAATGTACGTTGAAGTTTTTCCACTTTATTTGGGGGAACCGTTTTA AAAGTAATCTGGTTTTTCGCTGAAATAGGAAGACAGTAACCTCCAGTCAAAACATGTGCAG CAGAATGGGATTTGGTGTTTTTGACGCCAAAGAACCTCCACCCCCCACCACCCCGA GCTTTTGAATCCATTTTCGCTTTGTAAAACGTGTGCTTCTGTAAAACTGAGCAAGG AATAGAAACATTCTAGCTCTACAGTGAGTGCCTGTCACTTCACATCCATAGAGCCTTTT GTGAACTTTTATAAGATTCAGAATTCAGCTTGAGCACCAGTGACAGCAATTTGACATTTAT TCTAGAATGCTAAATTAATTCGATTTTAACTGATTATTAGCCTCGGTGTCCTTTCTAAA GGCTTGACTTTAGGTATTTCCAAGATGCCCTTAAGTCTGCTTACCCTTTCTCCCTC CCCAGCATCCTGGATGTTGGGACTGTGAATCCAGGTCTCCGTTATCTAAATGGTGTATGT TAGTGTTCCTGTCATCACGTTTAGTATGCTTGTTCCTTTACTATCATTATAGCTAAATA ATACTGCTTTCAGAGATGTTGTATATAATCGCATAATAATTCAGAACGCCCTTCATATTGAA CCAGATATGAAGTGATACAGTATCATTATTCAAACCTGCCTAAAAAGTAAAAAGTATAAAC ATATACTATTTTAAAAAGGTAGGTTAGATTTAAATCACGTAGTTTAGAACTGTTGGAATG GTACTTTGAGTGATAGGATTTATGTTAGGCCCTCTTTAGTAATAGTTAATATCATGTAATTA GCACTCGTTTACACACAATTTAATGTGTTTGAATCCCTAGAGTCATCGAATTCAGAATTTA TAGTATTTTATTTTACTTAGTTAATTTAACCTAAAAAAGCAAAATACAGGCATTATGC AGTTGAGTTGTGTTAAGTGTGGACTGTAAACAGGATAGTATTTGTTATAAAATATGTTTT GTATGTGTTTAAATATAGCTTCAAAAGGACATGTATGAAGAAAGATGTCTCAGCACATAG GTAGTTATAACCAAGGGTTTGAAGACTGACTTTAGAATCTAGAATGAGTAAGAAAGTGA TGGATCTGTACATTCATTTGATTGAAGTTTGATTTTGCCTTTCGTTAATTAAAGCCC CGGGAATCAGGGACCTTTCTGGCACCCTCTACAGTGTTAGTGGCCTTTGTGGTAAAGAA GTTATCTCAGATACTCATTTTCAATGCAATTACAGGCAAACTGGAAGGCACCTTAATGGTATGC AAGATGTGAAATGAATTAATGCTTGCATTCCTCTGCCCCACTCATGTACAATTAATCT TTGTTTAAAGTGCAATGTTTCAAGTGGTATTTATTAGCTAGCAAAATAAACTTTAAATAAAT AATGATGGTGTGGAAGTGTCTTTTCAAGTGGTGAATGATTCTCTTTGTTCAAAATGAGT TGTTTTTTTTTAAATCAGGGTACATATGATTAAATAAAATTTTTTCCATCGTCTAAGTCT AGTAGTAATTTGGCTTGTTTAAATAAAATGTTTTCTCTTAAAGAATATATTATTTTAAATTA CAAGTTGCCATTTAAGGAAGTAAATGCCTATTTAAAGTACGTATTTATGGGGCCGCCCA GCAGTCTAGAGGCAGTGTTTTTAAAGCATTACACTAAATGCCTACCATTAGGACACTTGCT CATGCTGTCATCTGTGTTTTGGCAGATGTTTTATCTTTGTCAGTGAGTTGTTTATGGAGATT TGAAAGGCCAAGTTCTAAAGATGAGAGATAGATGACTTTCCCCCAAATTGATACATATTTCT AAATCCAAAATAATCACGCATATTCGAAAACCTAGATTTGCATTTTATAGTATGAATTCAGC TATAGCTAGTATATAGGCATTGCTTTTAAATATAGTAATGTTCTTTTGTGGCATAATTTTCA ATCACTTTCCCTGTCTTAGGTTTTGCCTACCTTTTTATACAAGATAAACATTTCTATTTTCA TATCTTATCTCTCAGTCTGATTTTAAATATCGTCTTGGGTTATCATAAAGTTACTTTCAC TTTGTAATAGTGTAGATATTTCTCTGTGATAAAAGAACAAAGATAATAGTGTAGGATC AGTTTTGTAAACTCATTGTAGTACTAAAGTAGAAAATACAGAGCACCCAGGAAATTTGATT CTGATATACCTAGACTAAGACAATAGAATCTGGGTTTTCTCTTTACGATTATACAGTGTT GAAACAGAAATCAAGCTACACCCACCTCTGAATATTTTCAATCCAAATCCATGGAGCAGTTGC ATAAGACTCAGAATCTGGTCAATCTCATGGTTTGAGATTTCAGATTAGGAGAAATTAATGTGC ATATTTGAATATATATCACACTTGTAGGTTAAAGGTAAGCAAGGGGTTTGGCTAGTTCA AGGCCTGTCAAAACCACTTTTTTCCATTTCAAGCAAGTATGAGCCTATACACACCAAGC AGTTTGCAAGCCCTCTTTGGCATATAGACAAATTCAGACAGTATGTAGTATGTCTCTTA ATGACTTAAGATGTAGTATATAGACAGTAGGGATATCTTGTTTTTATTCCTCATGGTACTT AGGTGCTTGTTAAGGCCATAATCTGGATGTTATTGACTTTAACTGCCCTGCCCTTTTTAA TTCTAAGTTGGCCTCTTCCATTTCTATAAGGCCACTTTTAAATTCATTAGGGGATTCGCTT GGGTCAGAACTAAGTACAACCTGTGGACACCCCACTTACCCAGACCTTTATTTAGTGTCT CATTGAGGAGAGATCTGACTTCATCCTTTTTGTACGTAACCCAGTGTAGCTAGAGACTCCA GTTCTTCAAGCCAGACACTAGTTCTAAGGGGCAAAAGTAGGTAGGATTGCTTTCTGGCATG TGCTTTACTGATGATCTTTGCTTCTTACCTAAGAGGCTGCTGAGACTTCTTACTCCATTC CTGAACATCCATTCCAGCAGATAATTAACCTTCTACTCTACTGCAAAAAATAGTGGTGTG GGTTTGGACTCCCTCTGCTTCCCTCCACCATTAACCTAGTCTATAACATCTTAGATAA
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		CCTTCGTCTCATTCTCTCCGTCATCCAGCTTCAGAAGAAAACCTACTCACTTTTCCAACGA AAGGACAACCTGTCTACCACCTTTTCACTTCTACCATCTTGGAGACCTGCTCAGTCAGCATA ATTCTTCAGTCTTGCACTCTTAGATCTTTCCCTCTCCATTGACTCCTTCCCGTAGCTTGCAC ACATGATCAAAATTTACCCCATATTAATAAATAAAACAAAAACCTCAAGAACCAAAATGTCC TTGCCCTTGTCTGCCTGCACATTGTCAAGTTGTTGCCTTCTCTGCTTTCTTACACTGCCAA ATTTATTGTAAAAGAATCACTGCCACTTAATATATTTAAGTGCTTGATAAAGCCATCTTGT TTCTCTCTCATTCTCAAAACATAGTGTGCATCAGATCTCACTAGTCCATTGACAGATGGAAA GATATTCTCCCAAAATATTTAAGCCTCTTTTCTCTATAATTAGCCTACCATCTAAGCTAC ACATACTATTTTGGTTCACAATTTTAATTTTCCCTCTTTATCTCCCTGCTTCCCAACATA ACTTCTGTTGATTACTGTAAGTTTATAGGTCTTCTCTCCAGTTGTTCTTTACCTGGACTATTG CAGTAGTCTGTTAACGGATCTCCCTTTCTCTTTTCCCTTGTGCGCTCAGTCCCTCTCTTAC ACTGCTGTCAATGTTTTTTTTTAAATGAAATTAATCTGAGTGGTTTACAGTTGTTTATGTT ATCAAGTCTAAACTCAGCATGACATTCAAGGAAGCCCTTTAGAATTTTCTTATTCATCTTT TCCAACCTCATTCTAGAGCTCACCTAAATAATCTCTGCTTTCAGTTCTTCACTTTCTTTA CTTGCTGTACAGTTACTCTGCCTAGAAAGCCCTCTTTCCCAATAACATTCTTACTCATC CTTTAAGACCCAGTTTAAACATCACCTCTAGGAAGATCATTTAGACACATCTGTGAT TTTTAAGGAACCTTTCTATCCAACCATAACAAGTGACTTGAGATTTTCCATGAAAATATGCAG CTTCATGATTTCAATCAAGTCTATACAAGTGAGAAGCAGTGGCAGGTTCTCTTGAAATAC AGAAGAAAAATCTATCTTCCCATGATTTTATAGATTTTCTTCTATGGAATTTTACTTT AACTTTTTTACATTCAAGGAAGGTATTGAATCCTACTTTCTGAGCCCTGTGTTAGATTCT TAGGATACCAATGAAGACAGTGTCTTACTTTCTGAGCCTGGAGAGATCAGTTAAGTAAAA TAGTAATTACACAGTAGTAGGTACAGTGACAACATTTGAACACAAGGCAATGGGAGCAATA AGGAGGGGAAGAATACAATTGAGTAATGGTAGGGAAGGGGAACAAGATTGAACACTCAAC TGTGCTTTAGTAGGAAGAGCAAAAGTTAGCAAACTGTAGCTGCCATTTATTAGTACTTGC TGATATGTGAAAGGTGATACAAAAACATGTTATCTTATTAAATCTTAACAATATCTCTATG AGGTATATACCATCACTATGCACATTTATAGATGAGGAACTGAGGAGCAGAGGTAAGTA ACTTGCCTAAGGTTATACAGCTAAGAAGTACATGAGGTGAATCTTAACTAGGGTCGACCC CTTAATGTGTCACTTAACTATTATACACCCTATGTACTCAGTGGGGTAATAGTGATAACA GTTAAGAGGCTATTTAGGTTTGAGGAACAGTATGTGCTAAGGCATGGGGATGAGGTGCA TTTGCATAGTCATGGAATTGCAATAACGTCATTATGATTGGAGCTTAGTGTGGAATGAGG GAGTACAGGAGGTGGGCTGGAAAGGATCTTGAAGGACCTGTTTGTATTTCCATGCTAATG TACTTAGAATTTAGCCTGAAAAGGGTTGAGGGTGTGTTAAAGTATCTTAGGCAAAGGAGT GCACATTTGCATTTAGAACAGTTATTTTGGGAAGTGTAGAAAATACATCAGGGCCAGGATG GAGAAACCTTGAGGATGGAGGCAGGGAGCTGGTTAGAAGGGTATGGCAGTAGTTCGAGGG GGGAGGATGAGTGTCTAATTCATTCAACAGACATTGGATTTAGGAACTATTAATTAG GTTGAACCTACTAAGAATTCTCCATGGCTAAGGAGAGGGAGTAATCTAATGTGATTCTTAG GTCCAGGTCTGGTTCACTGGTAGATGATAGAAAATACTTTAGCAAAGTATTAGGCTGAG GGTAAGAAGTGGGGTACGTAAGTTTTTGGACATACTGACTTTTGAAGTAACTGATTACAT CCAAGTGAAGTGACAGCAGACAGTTGGCTGTCCAACATCTGCAGTTTAGAAGAAAGGCA GTGCATGATTCAGTTTTTATTTAAAGTTTGCAGGTGAAGAAAACCATGGGAGTCAGGGA AAATACTAGGGGCGAGAGGTAAGTGAATCTGAAGCCCTTAGGGACACTAACATTTAGTGTG AGTCAAGGAATAGATTTCAACAAAGAAAGCAAACTAGGATGGGGTGGCAAAAGCAGGAGGA AATTCGTAATCAGGAGATACCATGGAGGCCAAGAGAGAAGAGCAAGAAAGAGACTGCCAC TGGCTCAAGAACTGTGAAAAGTCAAGACAGAAAGTGAACCATGCATATTTGATTTTTAC AAGGGATTGGTGGTGACTTTGGCAAGAGCTGTAGTAATGAAGAAGTAGGGGTGAATGGGT GGAGGCCACGTAACAGATTTAAGGAGTGAATTGGAAGTGAGGAAATGCTTATAGATTGTTA ATTTGGGATGCTAGGGAGAGAGAAAGTATGAGGGATTTTTTTTAAAGTTGGCAAATTTCAA GCAATTTTTAAAGACACACAAAAACAACACCTTGTAATGGAATGGGGATGTGGGAGG CTGATAATCAACTAAGGAAAGTCGTTGAGGAGACTGATGGGTATGGAGGCAGAAATGGAAA CTAGCTTTCAGGTGATGGGATTCATTTAGAAAAGGAAGCAAAAAAAAAAAGGTGTGG ATAGTTGGGGTTACAGGTAGGTTTATAGGGAAGGAGTATTGGAAGCTTCAGGATTTCTCTC TGAAGGTTTCAGTTTGATTAAGAAATAGGGAGAGAGGACTGTTGGAGAGTATGGGATAAA GGGCAGAAGGAGTAATTTAAGAGTTGTTAAAAAGAAAGCAACATTTACATGAAATATGTA AAATGGTAAAGATTGAAGGCCGAGGAGCAGGGACTATGACATTTCTTCTGTTTTGCTTA TTAGCAACATGAATTTTCTTAGAAATCTGTAAGACCAATTACTCTTGCCTCCATCCATAAGG ACGTTGTCCATGCAGAAAAGATAACACTTGTAACCTTGATTATATACTTATCATCGCCCAT TTGAGAATTGTAAGCTCTAAAAGATAATAACTATATTTTTTATCACTATATCCCCACATCC TATCACAATATTTATCACAGGTAATTTCTTGACAAATGTTGATTGCATTTTAAAAATTTCTAA CCTGAACCTGTGTGCTGTGACCACCATGGATTGAGTCTTCTGCCACTACAAAGCTCTTT CTAGACTATGATATGAGATGGTTTGGGCTGATAGTCTATATTACCAATACTTGTACAGTTT CAATGAAGGTTTCAAGTCTAATACTTTTGGCATTTGATATAAAATCATTTTCCATTTTATT
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		TGCTAATTTATCTATAACTCTGGCATTACTCTTGGTTACATTTGTCTGCTGCTATCTGATTT AGACTGCTATAAGCATATCTTGTGTTTATTTCAGAGTTTTCTTATTCATGTTAATTATCTGTGTT TCTTTATTTGCTCTCTACATTTTAAATAGTTTCTTCACTTTGCTGTTTTATGAGAAGGGAGT AGGCAAAAGGAAAAACCCCAATCAGACAGTTTTACTACTTAATAGTTTTTAAATGCATCT TTATAGAGATTGAAGTTGTAGTTAACAGCTAGAGTGATATTTTGGCCTGCCCTTATTTATT AATTACTAGTGCAAAGGTTATTCAAATTGTGGTTTATCCAGGTCAATTTTACTGTTATTTTT ACTAATAGCATTTATTTCTACTTAAATTGCTTCAGCTATAAAATGTTTTTATTGTAACAAATAA TGCAGTATAATTATTGCTTTCTGTATTCTTTGAAAATTCATTCTCTAAACATATGTTATGA AATGGTGGATTGTTCCCAACTACTTCTTACTTACTTAATTAAAGCCTTTGCAAAAAGTTTC ATAGGATGACTGAGTTCTTCATTCGTACCTCTTTTTTTTAAAGAATAATCTCTTGAAAGTCAA ACCATGATTCATACAAATATAAAATGAACATGTGTCAAAGATTTTATTTCACTAATTAATTA CAAGCAACTAGCAAGAAGGCAAAACCTTTTTAAAGAAAATTGGAAAAACAGACACATTTA TAGGCAATCAAGAATGCCTAAATGAATTTGCTAATAAATGCAAAATTGGTCAATATCCTCAG GGCAGTAAATGTAGTCTTTTGGAAATCCTCTCTCCACCAGGTGGAAATCAATTGCAGAAC AAGATTTATTTTATCTGTATGGACAGCAATCCATTTGAATTACTCTCAGTTTTCTACAAC TATGAAACTGTAAACAGCCCACTCAAAGTCAGTGAAAGGCACAGGCTCTATAGAGTCAG ATAATCCAAAGCGTCTGTTAGACATTGCCAGCACTTGACTGAAAGGATACCCAGTAGTCT TTGTATCCCTTTCAAAATCTTCACCATATTTCTGATACTTTCTTCTTTATAATTTGAACA TCTTCACCCCTTTATTTCTCTGGTATACTATGCTCTCTCCCTCAGTGTTTTCTCTCTCAG GGGAAATAATTCACAATTCCAAAGTTTTAAAGAATGCAACGGAAGTCCAGGTTTTGCTTGG GGCTTCCTAGTATTTGGGTGGCTAGAAATGTAGAAAATGGGAAGAGGTGAGCTGTGGAT GCCCAATATAGGTTGAGACTGCAATTTCCAGAAATATAACAATTTGGACTAGTCAAAGA GGGCCCATATCATTACATTAATGCCAGATTATCTAGTTTTATAGTATCACCTACATTT TTACAGCATAGTATTGTTAGAGAGCACTTGCCGCTATGTTTTCCATGTTAATGCTCAACAC AGTTCTGTGAAGTAGCCCGGTGTTTTGTTTTGATCCTCCTACTTAAAGAGCTGCTCCTATTTT AGAGAGTAAACCAAGGCATAGAAAAGTGAGTTGCTTAAAGCAGCATATTAAGAGGGGCAG AAATAGTATTTTACTCAGGTGTTTTGAACATTGTCCAGTACCTATATCCAGATGCAATCC TTGCATTCAATTCATGGGGTATTTATGAGTAAACATAGTGTCTTACAATAGAAACATTT ATGCTCTGATTTTTTATTCAGTTTTATGTAGAAGTAAATCTTAAAGTGTGAACATTAACAAA GTTGATATTTATTTATATTGTTAGTAATTTGTGTTTTGTTTTGTTATGTTTTGAGGGG AAGGCCAAGTAGCTACTTAGGTAAAGAGTTGCTGAGTGGCTGAAGAATATGGAAGACAAC TACAATTCCTACACATTTCTGTACATTTTAGTTGAACAATGAGTGATTACATTTATTTACCC AGTGCTCTCTCTATAAGGCAACAACCTGGTAGTTTATCCTATTGAGAAGCGTAGATAGGATG CTTATTAGCAGTAACCTGCTCTTGGTTTCAACTTGCATCTTACTTACTGTTTTTACCCTTTTG TGGTTTCTTGGAGGAAGAATACCCATAATATACATTTGGAGACTGTTGTTCTGTAGTGCA ATGAAATGTGGGGGTGGGAAATGTCAATCAAGACTCCCATACAAAATGTCTATTGCTGCC TATATTTGCTATGGGAAAGTAGCCACAGATAATGTTTTTTTTTCTCATTAGTATTTTAA GATTTTCCATCCTGATGGAAAGATATGATTTGATTATCCTATTTACTTTGTATTTAAAGT ACAGTAGAACCTGCCACTTTTTTGGAAATGCAGCATAAGGATAAAGATAAATTCATATCA GTTGAGCAAGTTCTATTTAGCAGTGTGTTGAAGTTGAGACTGAATAAAATATTTGGTTTGG TTTTCTGTTCAAAATTTTACCTTGATAAGGACAATATTTTCTACATATATCAGTAGGCACT AATGATTACTTCAAAGCTTCCAAAGCCAGATACTACACCTGCATTTCCAAACATAGTTGCTG AATTTATCCCAAGATGCATGTAATGTATACTTTGTATTATTGAGAATGAATAAGAAAAGT CATAATGATGCCTTCCAGCTGTGCAAGTTAATATTAATAATAATTTGTTGCAATTTTAC CTAATAGGTCTTCTTATTGCTATACTGTTTACTTAAGTGAACAATGGAATGTTGCTGTTT ATCTTAAGGATTTGTAACATGCCTAAGATCTTACAGTACAGACTTCTATAATTAATGAAACA TTTTCTTTTTCTTTCCAGGATCAGCTCAGCTACTATTTAATAAAACAAAATCTGTAGAATT CACGTTTTGTAATGACACTGTCGTATTCCATGCTTTGTTACTAATATGGAGGCACAAAACA CTACTGAAGTATACGTAAGTGGAAATTTAAAGGAAGAGATATTTACACCTTTGATGGAGC TCTAAACAAGTCCACTGTCCCCACTGACTTTAGTAGTGCAAAAATTGAAGTCTCACAAATAC TAAAGGAGATGCCTCTTTGAAGATGGATAAGAGTGATGCTGTCTCACACACAGGAACTA CACTTGGAAGTAACAGAAATTAACCAGAGAAGGTGAACGATCATCGAGCTAAAAATATCGT GTTGGTAAGACTTCTATGAAAGCTTCTTTTTTATTGTCCTGGTGCAACCTGATCCTCTTT CGAGAGGAGGCCAAATGGGGTAGGTACTCTTTGAATCAAAAAGCAGGCTGTTATTAATA AATGTTGATGTAATGTTTAGCAAGTTTAAAGATTGTGATTTCTATCCTATTTGTTAATCTACT CTTCATGGTAAACACATTTACTATTTAATTTGTATTACTTGTAAATTTGTATATTGTGTGG TTCTCAAACTTTTGGTCTAAGGACCATTTTATTTGGCTGATTGGTCTGGGGTAATGTACTC TTCATTTCTGTTTGTGCTGCCATCCAAGTGCTTTCTTCTGTGACTAATTTTCAAGTCTCTTT CCAATTATTCAGCCTTCTGTTTTTGTGTTTTTTTTTTTTTTTTTTTTTTTTTAAACCTTA CACTTTGACCAAGGGATGACAAGTCCAGCCATGCTCCCTCGGAGAGAAATAGAGGAGAGT CAGGAGATTAATCACGTTATCATGGATATTTCTTCAGATTTTCTGTGCTTAGCCTTACTT
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		TGGTCTTCTTTTGTGGCCAAAATATGAGAGAACTAGAGATTCAAATTCATTTATTCAAAT TCAGACTTGATGATAGCACCATGATTCTGCCCTTTTATCACAACTTGGGTGCTGAGGT GTAGAAGGTAGTAAAATTAGCTGAAGTAAATAGTCTCCTCTCCTGCTGTTTCTGCTGTG CTAGCTGAGTCTTCAACCCCATATGAGTATGTTCTTCTGTTTTGAAATCTCTATTTAGAA TTACCTGTCATATTACCGATCATGGCTTAACCACATTTTGAGAACCTTAACCTAGATAGGT TCAGTCTGTTCAAGTTGGATCACTATTTGCCTAGCTTTTATAAAATAATCTACTACCAGAT TAGGAATGGGGTGTGTTGGTTTGATGAAATGTTTGACATTACTTAGGATTGAGAATCAGGA GGTCTGAGTTACAGCCCTGTTACGAACTTACTATTCACCTGTGTGATCTTGAGAAGGTG CTTAACCGAGTCCAAGTTACCTCATGTCCGAAATGGAGATGACAATAGATACTTGCTTTTAT GTATTCTACAGTCTAATGAGCATAAACCAAAGTGTGTTGTAATGTTAGTTGTTTCTGGG ATGTTTATTTTATGAAGTAAGGTATCAAGTTGTTGAAGTTAGCCTAGAGCATTGTGAGAGG GATTGTGTGCACTGAGGAATGGGCAAGGGATGGCTCCGAGTAGGCAAGGAGAATTTGAA AGGTCTTTCTGCTCAGAAATCTCTCTTACATTGTAGCTTTTACTATTGATATTAATTTGGGC AAAGGAGCTTTTTTGTGTTGTTAAGTATACCTTTAATGAAGGGTGAATCAGTCAGTCAT ATCCACTGCACTAACTGGTATTAGATCATCTAATACCTAGTTGAAATTTTCTGAGGTAAT GTACAATTTGTGAAATGAAAGAATAAGATATGGGGGAACAGTAACTTTGGGGGGTAAAT GCAGGTCAGTGAAGGTAACCTGGAAAACCTCAAATATGAGATTTGAAAGGCCAGAAGATG AAATGAAGATGGAAGAGAAAGGAATGCCAGGATCTGAGATAAAACAGTTTATTATTTTGC GTGTGATTGAAAGACTGTTTTATCTTTGTTTGCAAAATACCTATAAAATAAAACTAGAC TTATTTTGGGAGCCATTAAAAGGAGAAAGTATTGTTTTATTATAGAAATCTGAGAAATCT ACCAGAGGTTTGAACATTTGGCTTTCATCCATTTTAAATTTTGAATAATTTATTGATTT ACAGGACGATTCAAAGATAGAGAGCTTCCATGTGCCCTTACCCAGTCCCCCAATGGTTTC CTCTATGTGACACAATATGAAATCAGGAAGTTCACAATGGTGAGTGTGTGTTAATGGT TCGCTCTCATTTTATCACGTGTTTATTGCGTAACTACCTACACAATCAGGACATAGACCTA TCCATCATAACAAACATCTCCCTCATGCTTATTCTTACTAGTCATACCGCCACTCCCACTGT CCCTAACCTGGCAAGCACCAATTTGTCTCCATATCTATAATTTGTCTATTCACAATGCT ATATAAATGGAATGATACAAGATGTGACCTTTTGAGAAGGGCTTTTCTACTAAGCACAAATGC CCTTGAGATCTATCCAAGTTTTGCATCTATCAATAGTTCTTTTTCTTACTTTTTTTTTG CTGAGTAGTAGTCCATAGTATGAATATATCACAGTTTGTTTAACCACTTACCTATAGGA CATTTTGGTTGTTTCCAAGTTTGGGCTGTTACAAATGAACTGCTCTGAACAATTTGTGTACA GGTCTTGTGTAGACACAGTTTTCTTCTCTGGGATAAATGCTCAGAAATATGGTTACTG GGCTGTATGGTAAGTATATGCTTAGTTTTTAAAGAACTGCCAGACTTTCCAGAGTGGCT ATTTCAATTTACATTTCCATCAACAATGTAAGAGTGATCTGGTTTCTCCACATCTCCACAG TATCTGGTGCCACTATGTTGTAACCATTTTTAACTGAAAAAACAACAACAACAACAACC AGTTAAAAAGTTATCTGCACAAAAAGGTTAAATGGGGCAGGACTCTTACTATGGAGTATAA GTTCTTTTCTAATAAGAATACTTACTTGTACGGACTTTGAGGATTTAAGCATTAGAAACCA TTTTTACTATGTCGTGATTTTTGCAAAATACAGGCATAAATGAGAAACAGAATTTGCTCAATA GAGAAGTGAATTTCTACTTAATAGAATGCAGATAAATAGTGACCATTTTGTGCTTTTTAAA TTTTTGGAGGACTAGGACTGAATACATGGAACACTATAAGATATAGTTTTATATACCTCT GTGCCATGTATGATATAGTCCATTAGAAGGAGCACTGGACTTGAAATTAGAAAGCTGTTT TCTAGACTTCACTGTTTAAATAGCTTAAAGAAAGTTGGATAAAAACTCAACCTATCTGTCCC TCAAATTTCTTGTAGTACAGTAAAGATGGCATCACCATCATGGAATGTTTGAGATATAAAT AATACCTCTTACTTAACAAAAATTTTAACTAAGTGCCATCATGTGCCAGTCAACGTACTAG GCATCACAGATTCTGTGATGAATAAGACATTATTGCTGATTTCAAGGAAGTGGTTGCAGTA CAAATATGAAGAGTGAAGTGACCTAACATTTATTGACATGTGCCACATACTTTATGTAAG TCGTGTCCTGTTATCCTCTCAAGAACTTAGGAGATAGATGGAGTTCTTACCATTAAACAAA TAGGGAAGCAAAAGAGATTCAAGGTATTGTTCAAGGTCAGAGTAATAGCCAAGAAATTAAC CTAGATCTCTTTGGCATCAAAACCCAGTATTTTACCACCGTAATATGTGGTTCATGTGAAA ACACCTTGTAATAAATACTGAATGCAATACTAGTAATCAATAAATTTATCATTAATA TTTCTGTTCTATTAGCAATACAAAACTTGAAAACTTAAAGTTTATTTTTCTTAAGCTATTA GAGTTTTGTTTGAAGAGGTGAGTTCAAGTTCCGCAAGTGTGCCAATCCATTATTATCAACA GGACATATGGTCTGTTTATAATGAAGACATGAAGGCATTGCAAGATCTTTGTATTAGTTTTC TAGTGCTGCATAACAATAACAATATAGCAGCTTAAACACTACCCATTTTTTGTCTCAAAG TTTTGTAGGTGGCAAGCCTGGTCATGGCATGACTGGGTTCTCTGATCAAAGTCTTAAAGA CTAAAACTAAGGTGTTGCCAGGCATACCTTTATTTGGAGTTGGGGTCTTGTCTAGGCT CACATAATTGTGACAGAATTCATTTCTTGTGATTGTAAGACTGGGGTCCCTGTTTCTTGTGC TAAGTATCTGCCAAGGAATTGTAGCAGCTCCTAGAGATTGCCCTCATTTCTTGCCCCATGGC CCCTTGATATTTAAAGCCAGCAAGGAGAATCTTCTCTTGTGTAATCCCTCTCACATATT GAGTTTATTTGCCAGGAAGAACCCAGACCTTTTAAAGAGACCACTTGGTTAGGTCAGCTC TCTCTCCCTCAAAAATAATCTCTCTTTCTTAAAGTCAGCTGATTTGGGATCTTAAATTACATC AGCAAACTCCCTTTTGTGGGTGATATAATCATGAAATGAAATCCAATACATTCACAGTCC
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		TAGTCTCCACACACAAGGGGAGGGAATTATAAAAGATGCATGGCTAGGGCAGGGGTTTTG GGACCACCTTAGAATTCTGGGTTTGATTAAATAAATAATGGGACTGTAGCCTAGCAAGTCG ACACATCAGAAAAGCCATCACAATCCTGAAACATCAAGTAAAAATTTGGTTTGCAATTTTAGG ATTGTAATGTTACTGTGGTGTATGCGTGTGTGTGTGTGTGTAACTGGTGTCCCTATGT TTGTGGGAGTTTGAAAGGAGACACTTTGTTGATAGGAATGGTGTCTTCACTTTTTAGGTTG TCTCATTTTTGTACAGTGATAAGACAAATGAGGTCTGGGTTTTAATAACACTTCAGCTTGA AAGCAAAATTAACACTTATTCAATTGACTACACCCTTGTAATATCACTTCTTTGCCCTTTCCA CTAGCAAGAAGTTCATTTTCTGGAAGCCATTCTGCCTGTCCAGGAATTGGAGGAGAGTGA TAGACACAGTTGTGAGCCATAGCTTGGGTAGAATAAGGATGTGAATGTCCTTGGCTTATCT TTATTAATCTTGTGATGGAAAAATATCTGACATTGTTCTTAGTCCATTTAAGCTTAATTTA TGTTCTTAGTGGCATAGAAATTCAGAGCTGAAAGAAGTATCATGTCTCACTCTCCCTTGAG GAACAAGAGTTAACGTCATCTGACAGTACTACCATAACAAATCAATAGCTTAATAGGCATAT AAGTGGCTTTATATAAAATGTTGGTTTTTCCCCAGCATCTCAGTTGGTTCTTAAATATC TAATTCATGATCCTCAAATTTTTCCCACTGTAACAAATTAGAGAGAGGAGGAACATGTTCA TCAGTGACTGTAGTTCAAATACAGCAGAAATGTGCAGCAGTGATTTCAAAGTGAAGAAATC CTGGATGCCCTTCTCATTTGTGTGCCCCCCCCCCCCACCCGCCCATATACAGGGAACAAAT TTAAATCCTGGCACAATAATGAGAAGGGAGAGTGACAACTGATAAGTCCAGTTAAGAA TCACCTAGACAGGCCAGGTGTGGCAGCCACGCCTGTAATACCAGCACTTTGGGAGGGCTGA GGCAGGTGGATCACCTGAGGTCAGGAATTTGAGACCAGCCTGGCCAACATGATAAAACCT GTCTCTACCAAAATTACAAAATTTAGCTGGGCATGGTGGCGCTCCATATACGGCACTAC TTGGGAGGCTGAGACAGGAGAATTGCTTGAACCCGGGAGGTGGAGGTTGCAGTGAGCTGA GACCACACCACTGCACTCCAGCCTGGGCAACAGAGTGAGACTCCATCTCAAAAAATAAAAAA AAAGAACTCACTTAGACAGTATTTTTGTTCTGTAATCTTCTCTCTGTCAATTGAATGTCTC ATATTTCTGTTTCACATAGAATCTGGGAGTCATCAAGTAGTCATCTAGTCTATCTCATCAT CTAATTTACGAATTTATTTCTATCACACCCCAAGTAGCTGGCGATTTCAGATTTTTTACTGAAA GCTTGACATGATGAGACATTATTACTCCCAGAGGATCTTGGTCTGTTATCAGGCAATTTCA GTTCTTCTTAAATCAAGCTAAACTTGCTACTATTCTCACTCACTGGGAGGGCTGTAGTAT TTCTCTCAAGTTTATACCTCTCTGTGATGAAAATCTCTGTTCTTTAGCCATTGCTCATGGT TTCCGGATCCCCATTCTAGTCATCTTCTTTGGAGCAATTCCTGTTAGATCTTCAAATGTG ATGAGTAAACTGAGCTCAGGACTGCGTTTGTGGTCAACCCCTTCTCCCTTCCAAATGACT GCTGCTGTAACCTTCTGACAGGTCCTGCTTCCCTGTAATCCATTCTCCATGTGGCTGCCAAA GAAGATTTGAAATAAGTCAGGTATTTTTCTTCCCTATTTAAAGCCTTCCAGCCGCTTGCA TTGCTCTTAGAATAAAATCCAACTCTCCCAACAAGCTTTTAAGACTCTACATGGTCTTTGCC CCAGCAACTCTTCCCATTTGATCTCATCACTGAATTCAGTCACAATGGCCATCCTTTGGT TTCTCAAAGTTGCCGTTCTTTGGCATCTTCTCTCCCTGTCTTCAATGTAGGCTCCTTC CCATCCTAGGCTTAGTTTCGTTCTCTCCCTAGAGGCCCTTCTTACTGCCTCAGCTTCTCC CACTCTGCACACTCAGGGTCTCTGCATTGTTCACTGCTGGCCTTCAAGAGCCTAGAGGAG TTCTCCCCATGGTGGGCTTCAATAAGTGTTTGTGGAATAAGTGAAATGAGTGGTCAC ACCAGGATCGAATGCACCTCTTACTTTTGTTAATATATTCAAATTAATATTAATATTAATA TTCTATTAATATATCTAAATTTTTGTTGTGTATAGGGGCAAGGACCAATGAAAGAACATTT CATTGTGAGCTCATCTTGACTATGAGATCAGATATTGGCTATATTTGAAGGTAGAGTCAG CAGGATTTGCTGTTTAACTAGAGTGGGATGTGGGAGAAATCAGGGAGTCAGGTATAACTCC AAGGTTTTCAAGCCTCAAGCAACTGAAGAAATGGGGTCAACGACTGAAATGAGGAAATG AGAGGGGGAGTTAGTGGGGGATGGGGTGATGAAAATATCAGGATTCATTGTGGAACATG AACAGTTAAGATTAAGTGAAGAGCCTGAATTCAGTGTCTCAGTTTCTACAACATAAGGA GGGGTTAATAATTTCTCACATCATAATTGTGAGGATTTGAGGAGTTGAGGTACACAATTA AAACAAAAGCACAGGGGAAGTAAGGAAAAATACATCAATTAAGCATGGCTATTAGACCATA CAGCCTTACCAATCCATTGGGGATTAGATACAAAAATCCAAGCATGATCTCCAAATTTTT TCCTGGTAGGGTTTTAGGCTATACTGAAGTGACTTTTCTTTGGCATAAGAAGATATTCAGT TATACAGTTGAAATAAAGGTATTGATTTGGAGTATCCAAAAACATCTCTCAGTAGAGATCC ACAACCAAGAGCATAAAAAAAGTCTTCCATTCACTCGCAAACTGTCTTATGACCATTG CAACCTCAACAGCAACATATGGAGTCTCTACTACATATAGAGGAAAATGCTAGATTCTGA AAAGCTAATATCTATAAACAGAGTTTATGATGTTGTTATATTCTGGGGATTGATGTAAGCA TTTTACCAGGACTTATTATAGCATTATAAGCTTAACACAAGAAATATGTGGCTATCATTATG GAGTGAAAGGCTGGAAAAATCCTCACCTGTGACCTGAGAGATAGTGCCTGTAGAAATTTGA AAGAAGTGTTGATTTAGAGGAATGCTAGTGCTTGGTGGCAGTATTCAGAAGAACTCT TCCAATCACACAGCCCCTTGTACAGGCAAACTCCGGAATACTTTCACTACATTTACTTGGCT TTATTGTTAGAGCAGAACTTCACAGCTAAAATATGGTCTTAGGGGTTTATAAGATGCAAT TAACTTAATTTTTAAAGTTCTCACAAATCTTATTTGAAGTCTTAATATCTTAATTTCTTT ATATAGGATATGTGGATATATTTTATGATAAAAGTAACATGTGACCAACTTAATAACAGCT TCGTTAATATCTAAAGCCACTCATCTTACTTTACATATAACAGTCTTCTGAGCCTAATT
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		ATGCATGGATAATTGCAGAGTGAGTTTGGGAAAAGACCAACCGGGCACATCTCTCCATT TTAACCACAATGAGAAGAGAGTACCTAGTGGTGCATCTTCCTCGCTTAGGTTCCCTGAGTC TGCTTTTACAGGAAGACTTCATTGTTACTTGAAGGTACATTCTTGGAACCTTTACACACCCAG CCACTCAACACATGAATACATACTTATTTAGTTAACTGAGTACTTAGTGAGTGCTAAGCTT TGTTTTAAGCCCTTTGCATGTAGTTTATTAACCTTTGTTAATAGTTACAACAGTCCTTCAAGA TACATGTACTGTGTATACTAGTGTACAGTTGAGGACACCAAGAACAAAGAGGTGTTGA CACGTGGTCACGGTTCCATAGAGTGTAAAGTTAGAGTTGGGTTCAAAACCTGGCAGATGTGG CCACAGAGCCTTGTCTCAGCCACTGCACTGCACTACCTCCTCCCGTGAACATAAGAAAAA TGTGAGAAATGCTTAAGTAAGTGTAGTTTTTATTCATAAAATAAAATTTACATAAGTACATTA TGTGTAATTTGTTTTATGTATATGTGTGTATGTATAAAATAATACATGTAAAAATAAGGCCA CAGTTTTAATTTTTTCCATCTCTATAATAAAGCATGTATTATAGACCATTAGCAGAATTTAA AGTGTTATAGTAATATTAATTGTGACTTTTGTCTTCTTCCCGAGTTTCATGGTTTTCT CCAAATGAAAAATATTCTTATTGTTATTTTCCCAATTTTTGCTATACTCCTGTTCTGGGGACA GTTTTGGTATTAAAGTAAGTATTATTTCTACTTTTCAITTTATGTTTCACTGATGATATAGTT ATTTCTACGAGACATTGTCAGCGAAATATTTAAAGTTGTACTAGGAAAAGTGCTATTATGAT AAATATGAGTATGTAATTTGAATACTACTAGTCTCCTTGAAAGTATATGTTGTCGCCACATT TTGCTGCAGTTCACTTTAATTCCTAAGAAGGTTGTTTTCACTTGGTGTTTTTAACTCT TAAGAATGAATAGTAGGAATATTAGTACCAACACCTTAAACTCATGTACATTTTAATATTC ACAGAACATCTACACACATTTATGTTATTAGGTAACAGGTGGTGACAGCTGCATTAGTT TTAAGGTAGGACGTTTATACTTTGGAGCATTTAGATTCCCTCTTTTTATTGTCAGTTTGA TTTTCTCTGTGTACACGTGTTCAACCTTGGAAAAGTCCAGTCGGAACATGTTTTGTATCC TCTCGGTGAGTTCTGCAGCCTCTAAAGAAGCAGCCACCAGAGAGTTAGGTTCTTTGATCT TGCTTTCTATAATAGTAACGTAACAGACTTCTGAAGGCAGATCTTGATGCTGCATTAGAT TTAGCTTCAACAACACAGAATTGTCATTACTAGGCAATAGGTAATGCAATACGGTTAAT GTTTAAATCAACCATATTTTATATTTTGGTAAAGAAAAATTTACAAAATTTAATGAAGTTGA GGTGACAGTCTAACTTTTAAGCTTTTTAAATACAAGATTTATTCCTTCTTCTTGTAGCA TCCTGAAGACCAGTAAACATTTATATAAGCAAGGAATAAACTAGCTTATTTAATTTATTCT GCAACCTTTAAACACACAAAAGCTAGTAACTATTGCAGTGGATTGCCCTGTTGTATATTT ATGAATTTTACTTTTACTCAGCAGTTTAAGCTGTCTATATCTATGGTGGTGATTAACATGG AAGGGAGATGACTGATTGATTATATGTTTTAAGCGCTTTTCTCAGTGTATGGCATCTCGGA AATGCTTAGTGATTTACAGAAATGTTCTCAACTTTTGTCTGAAAGGAAAAAGGGGGAAGAA AGGGGTGGCAGTGGCAACTGTCAAGACATTTTATAACTTTTACTTTCAAGATAGTGTCTAG ACTTCTTTTGGAAATTTTCTTATACTCTTAGTTTTTATGTCAAGAAAAGGACTGGTGTA GCATTAAGACCTATTCTGGCATCAATGATATTAGGGAAGCTTTTAACTATTGGCAGAGC CAGACTTTAAGGGCTAAGTCATATGACTTGGGCAGGGAACAGCCTTTTCAAAGATCATGA AATTTATTTACATAGTGCAGATTTTGTAGTTTGGCTTGATGGATGTTTGTGATCCAGACGT TATCATTGGTGACATTATTTTGTAGTAAATGAACAGGAGTGAGTTTGTCTACTGTTGGCT GTATTTTTTACAAAATGAGCTTTTACAATATTTTTTCTGACTTAAAAAGTCATACATCACTT TAGAACACCTCGTACAAAGTAGGGAGTTATTTAAAAAAGGTAACCTATCTGTAGT CCCCAAACCTAGAAATAAACTATTGGTATATTTCTGGTGAATTTCTGTTTCCCTTTCTGTCT TTTTCTATTCTTTTTGTTATCTTTTTTAAAAAAGGTAACCATAAATGGACTCGTACAG CTTATATGCTTGTACGTTCTGATGTTCCCCCATGTCTTCAGAAATCTTGTATAGCTGCCTG TGTTCTACTTTTGTGGATGCACTATAATTTACTTAACTCTCATCTTGATGGATTTTAAAGGAT GTTTCCAATTTTTTGTACTATGAAAATAACTCTGCTAAAGATATGAAGCCTGGAAATTGGC AGAGATATTTTAAAGACTCTAGATACATACTGGCAAAATGTTTTCCAGAAACGTTTGTATCAA CTACTATTGCAGATGAGAATACCTGTCTTTACCTCAGTTATAATTTCTGATATTATAACGCT AAAATCTCTGCAAAATTTGATGGGTGAAAAAGCATCTTATTGTAATTTGTAATTTTTGTGTTAG TAAGGTGGAATGTTCTTTTTATATTTTTTGTACTGGTCATATTTAAGGAACGTGCTTATA AACCTAAAGAAATATTTTGGTGGGAATTTTTTGTGTTGGCTCATCTTGAACAGGTAGATGT GTGTGTATGTGCATGGAAGAGGTATGTCTATACATGATTCACCCAGCCTGCGCTCACATTT AAAGGTGTTGATGATAATAGTAGCTAACCATTTGTGGAGCTTGTCTGCTGTTGACAGGTT CTGTGCAAAGTACTCTATATCTGAAATGACATTTATTTCTCCAGAACTCTATGGGCATAG ACACTGTTGTTATTTCCCGTTTTGTAGATAAGAAACAGGCACAGATAGATTAGGCAATTTGCC CGCAATCACCCAGCCGTTTCTGATAGTACTGGGATTTGAAGTAGTACTGTTTACCACTGCA CTGACTGCCTCCCGTTTTGCAATTTATTTTGAAGGATTTTATTTCCATGAAGGGTGAACCTA TATCTAAGCACATAACTAGTAGTAAAACTTATTAGGAGAGCAGAAATGTTGACCTGATT TGTTTACTTATTCTGCAAAATACCTAGTGACAGGGTGCCTACTGATTGCTGGACACCAAGCTA CATGCCTGAAATGTGGGTGTGATGTGCATATTGCCCTGGTTTTGTAGACCCACATTTAAG CCAGGGAGACAGAAATGTATGTGCATCCCATGCCCTGTGCATCTTTGAGATAGTCATGGA TCCAAATCTAACTTTTCTGTTAGTGCCCTGTTGATGGCCTGAGCATTCACCAATAGAAA TAGAAAGCACCTCTATTCTCCACCTGCTTAGATGTATTTTTTGAATCCAGTATGGTAAA
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		TCTTCTAAAGCATTATAAATACTCAGACCCATGACTTTTATTTTATAGATTCTAGTTCTGC TTAACTTTTCCCTGATTACCATTTATGGTCTGTACTTGGCTACAGGGGATTCTCTCAGTGGT TTTTCCAGCTCTGCATGTGAGTCTTTGTGGTCCAAGACAAACCTGGACCTTTACCAGGCTAA ACTTTCAGTAAAGACAGCAGGTTATGCCCTCATTTGCTCACTCTGAGGAGAAAGAGTTTCTT TATACCAGAGCTGTATCTTGAAGATGTCTCAGGATGCCATTGGTCTACTGAGGAAGAAG CCTGAGAAGACTCTTAACTCCCAGAGCCCAGCCAGCACTTGGTGAGCCCTGGACCACGTT TCAAAGATAAAGGCCCTTTACAGGGAATGTTCTTAAATACCTTCACCTTTAGCTTAGCTTT AACTTAGGAACCTTTAAGCAGAATCTCTATGCTTAGCAAACAGCTCAGAGATTGCTAGAATC AAACACCAAGGCTTAACTGATAGTATTGAATTCAGACGCTTTGTTTGTGTTCTGAAAACCTA CACCAACTCACAGTTTGGCACTCTACTGACAATTAAGTTCTGGCTGATTTTCAGGATTCTT CTTTCTCACTCTGATATCATTAAAGTGCTGTCCACCTAGTCTCCAGTCTCCGAGGATTA AGGTCCAAGTGTATGTAAGAAGTGGCTAACATTTTGAAATCTTTGAGATAGGCCTATCTG TTCTTTCTTTGCCTTTGTAACCTTTGTTTATACAGGCAACTACTGGCTTCGCACAAAACCGCA AGACCATAAACATGACCATGTAGCTGAAATTTCTGCAAAACAATCTTCATGATGAATGGGAA AACTATTATTATATTGTTCCATGACCTTTAAATTTTTTTGTTGTTAAACCTTAAAACT CTGTTATTATCAATAACAACGGCATTGGGAAATGAAAAATAGTAAAGCTAGTATTTAGTATG TGGTAAATTAATCATTAGAAACATCGAGAATGAAAGTGTTATCAAGAGTAGTTTGAACA ACACTTGTGTGTTCTTCTGCAATACTTTGGATACAGAGCAAGCATCTTCTCTATGGCTTGG TGAATGTGATACCCCTTTCTAAGTTTGGATCGGTTTCTACCGTTTTATCCTTTGCACTCTC AATGTCATGAAAGAATTCTTTAATGTTTTTTGAAATGTTTAAAGTTTATTTATGGCAGT CACTTCTCTGGGACATCTTTATTCTTTTGTACATCCTCCTCACTTTCTCATTATATCA GTAAGTCCACCTTCGCTGAGTTCTTCTGGCTTCATCTCTAGAGTTTCTTGCAATGCAAGTAA GTCGACATTCGCACAATCAGCTATTTCTTCTTACTCCATTTATGGTCTATTCAAATTTCA CTCCTAGCATTTGTCAGTTTTATTCTTTTACTCTTTTCTGCTTTCTGCTAGTTTCTCCTTT TGATTTATCCATTATAAAATGTCACATGGGTTTATCACTGGGAAACAAGGAGGTAACAAAAC TCCATACTTTGCTGTCTGTGCATGACCTAAATATCAGATGTACAGTGAAGTACCAACAG TCTTCAAAGAAGTGACATGGTTGATCACTGATCATGATGGGACATCTGCTATTTACATAGT TATTTTGAAGTGAAGAATAGCAGTGAAGTTGTACTTTATGCAGTTACTCAGTTAATATAT TGTTGTAATTGAAATTTGGACTGTTTATGAGGGATTTATTTGATTAACCATGGTAACTGG AATTCATATCAGAATAGTGAAAGTGAGGACTGCTGTACTTGATTCCAAATTTAAACTGTA TTCTAGGCATCTTTAATTTTTTTTTTCAACTTTCCCTCCCTGCTCAAAATTAAGTCCACAGA GATTTTGGTTACGGCTCAGATGTCTTTGAGTAGATGTTCTGTAGAAATCATTGTTAGAAA ATTCAAGGAAAAAAGTTCTCATTACACTACCTTGAGATCCTACCAAGTCTTGAGTTTGGAC TTGAGGACAGCTTAATAATGAAAGTAATTCAGCCATGAAAGCATATTTAAATAAGTAATGG AACAGGAAATTTCTTCATAGATTAGAAAAATTATCTGAAAAAGTGAAGACATAGCCTATCT TGGACTAGGAAATTTCCATCCAAGAAAGTAAATAACAATAATATATCACAAAGAAATCA TTCTGTGACCTGGTTGATGGCCCTGGTAATAAGACTTTGTTATTAAGTTTACTGTGAATTC TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAGACGGAGTCTCGCTCTGTTGCCAGGC TGGAGTGCAGTGGCGTGATCTCGGCTCACTGCAACCTCCGCTCCCAGGTTCAAGCGATTCT TTCTGTCTCAGCCTCCGAGTAGCTACAGGCTACTGGCGTGCAACCATGCTGGGTAAT TTTTGTATTTTAGTAGTGATGGGGTTTACCATATTGGCCAGGCTGGTCTCGAACTCCTG ACCTCGTGATCCGTCTGTCTCGGCTCCCAAAGTGCTGAGATTACAGGCATTAGCCACCAT GCATGGCCCTTACAGTGAATTTCTGTTCAATAATCTTGAAGTCCAGTGTCTTCCCTTGGTC CTGTCCATATAATGATCACATTTCTGTTATTAATAATGTGCTTATGTGCTGATTTATTGTA GGAGGAAATGAACAAATCTATAGCTCAGCATTTTACTCTTTTGTAGAAATCCCTTGACCCCT TCCAAATCCAGACATCACAACTTCTCCATATACTCAAAATTTGTGAATCCACAAGGCAGG GAAAGTTGATAATGAAAAAAGGAAGTTTGGTCTGTTGGACATCCTCTTCTTCTATCTTTTA TGTTCAAAGTACTTGATGCACAAAGCCTGGACTCTTTATCTCTGTCTATAGACTGATGTC TAGTCAGTATTCATTGTGACAAATTTGTTTATTAGAGCAAGTTGGATAGTGATGGAGAT TATTATAAATGATTTAACTTGTCTTCTGTCAAGTAAATTTATAATGGTATTAATGTTTATT TTTTTCCAGTACCAGTTTCAAGGCTTTTATTGTTGATGATGCTGTGTGTTTAAATAAGGT ATTAGATACTTGAAAAATAGTAATTTAAATTTAAACAATTTAAAAATGAAATTTGTAATTTTA TGTGTAAAGGTGTCTGATGTGCTTTATTCTGCACTGAAACAAATATTCAATTTACAGCTCAAC AGACACCAATATATTCTAAATTAATTTCTAGAGTTATCAGAAAACAGCACTGTAATAAT GAAATCAAACCATCTTTCTTTATGATTTATTCTTAGTCTGATACGCATCAGCCTGGTAAGC CTTCTGTCTCTGCTTACTTACCAATCACTCCAATGTCATGTCTTTGGGAGGCATTAA ATTCTTGGGTTTTGGGTTTTGTTGGATGGACTGCAGTCTGTGTGAGCCTATATGGGTGTGT CAATCCAGTCTTTGGGTTGTCATGGAACCTTAGCATGATAGACTTGATTTTATCCCAAGT TGACTTGGTAATTTCAATAGATTTATCAGTCACAACCTGCATTTTATCTGTATGTGCTGT CTATTGGTCACAAAATCAGAAAACCTTCTTGTCCATTCAACATAGCTGTCTTTTCAGGT GGCTAGAGGACATGTCATTGCTTCATCTGCATGAATTTGAAAGATTAATGCATAAAGGA
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		ATTTTCTTAGAGTAGAGTAGGCCCTTACCATCTCTTTAACTGGGAAAGAAGTTTGGGAGT AACATACTCATCACTCACACCCCTCCCTCAAACACACACACTCACCCATTAGAATGTAA GGGCCTTGAGGCAGGCCCTTGTTTGTCTTTTCGTGTTCACTGTCAGCATTTAGAATAGTGA TAGTCATATAGATGGATAGTGCTCAATCAATTATTGTCAAATAAAGTAATCTACTTGTCTCT TGATTTAGACTAGCAAAAGGGGCTGGTACATTGTAGGTACACAGTAAATATTTGTAGAACA GATGAATGAACCAACCAACAGATTTCTTAGAAGTAGCCTGCTTTTGTTACTAATTATTTT ATAGAACATATAAAAGAAAATTTAGAATACCTAATTTGTTCACAAAAATGTTATATTGTCT CCCCTGATACTTGGTATTCCTGTGTGACGGCTGAGCACTAAGGTTATACATTTTGTAAAGC ATGTAATTACTGTTATTTCTCATGTACTTTATTTTGTGTATTGATTTCAGTAGTTGTAGCTG AGGGTAATCCTTAAGAGCATGCAGATTTTAAAAATTACATAGATTGCCTTGCCAGGCCGTCT GAAAGTCATACTAGAAAATTTGATAAGAGCCTGCTGTTAACAACACATTGAGTTATTTTAT CTTAATGCTAAGTGGAGAATTACTTGAATTTATTTTGTCTGCATTTAATGTTTGTATAC ATTCAATAAATAAGGATAAATCCACTTACTGAAGGACAAGAAAAGATCAATAAGAGATCCTT GAGGTTATTTGGGTTCTATGGCATCTATTATATCAAATTCCTTGCCCTTTCACTATACTGAGA AATTTGACGACTGGGCTAGCAACAAGATACCTACCCACATTAGCTGTGGTACCTGCTGAA ATGTGACCATGTGAGCAAAAGAAAATGAGCCAAAAGAACTCTTTCTGAAGATTCACTGAAG AAAACCTGGAGATACTCTTTCATTAGTAGACCCACAGAGACAGGTGGAGTGTCCGTTATC CAGAATGCCTGGGACCAGAAGTGTGTTGATTTCAGATTTTTCACACTTGGGATCCTCAA CATGTGTATTTGTTGGAATGGCTACTTAATTTAAGGAAAAGTTAAGGTGGCCTGGAAAA TAAGTAGTAGTTGAACATAACACAGGAACCAAACTACTTTCAATATATAGTTACTATATA CTCAAAGAGAGACGGACCATTTTATGGTGAACCTGTCTATGGTAGTATTTGTGATGTT TTATTTTGTGCTATTGTTTGCAGTGTCTCTCTATAACAGCTCTTCTAAGCCTTAAGAG GATAAATTTTATATGAGATTCAACTCTATTTTGTGAAGAGGATGAAGTACCAGGCA TGGTGGCTCACACCTGTAATCCAGCACTTTGGGAGGCCGTGGCAGGTGGATCATGAGGTA AGGAGCTCGAGATCAGCTTGGCCAACAATGTGAAACCCGCTCTCTACTAAAAAAGAAAAA AAGAATTCAAAATAGCCAGGCTGGTGGCGCATGCCTGTAGTCCAGCTACTCGGAGG CTGAGGCAGGAGAATCATTGAACCTGGGAGGCAGAGTTTGCAGTGAGCCGAGATTGTGCC ACTGCACTCCAGCTGGGTAACGGAGTGAGAGACTCTGTCTCAAAAAAAGAAAAAGAA AAGAAAAAGAAAAATATAGAAGAAGAACATAAGTCAACCTTTTCCATGAAATTATAGTGA CTCTAAGGCAAAATCTTGTGCTTGATCTTAGTAGCTTTTTATGCATTGACTTAAACCTGG AAGAGTTTCTTATGAATGTTTGATTGACTGTGAGGTGCATTTGAAACAGCTTCTACTTTATA ACCCTTTGCAGAACTTTCAAGCTCTATTTAGATAACAATATATATGGGATTAATGAAAA TGTGACATATGTCTAGAGAAAGTTCCCTTTTCTGTGTATGTGCCTTTTGTGACTTATACACT CAGTTTCATTCTAGCCAGCATTTGAGCCTAGAAAATAGGGAAGTTATGAAAACCTGTGCTCT TATAAGAAGGCAAGGCTATGACTGAGTATTAGACACATAAGTCCAGGCTGGGCGGAAGTA ATGAATCAAATAAAACTTGGGGAACCTTGCCATAGGATGTTTCTGATTAAATATGGGTTTCT CTTCTCTGATTAATGTGGATTTCAGTTACTCAACTGTGTAAGTAAATCCACATTAATAAAT TGGTTATTTATTGCTTAGGACTTATCTCCCATAAAGAGACTAAATTTGAGTTATAAATTA TGGGTAGATAGAAATCCAGAAATTTAGGTACCTGTCATATACCTTAAACATAGATTTT ATCACAATCATTTAGGGGCTTATTTATTGCTTTTACTTTTAAATGTTTGTCACTGTAGAA GAAAAAACTAAATGCTAAATATAACATTTAAAAATTTTCCCTTCATGACAGCCACCAAT TGATTACTGTCTAGGAGACTATCTCTATGTGGATGACACACAGCAGCATTTCAATCACACGC TGTTTCTTCCGCTACTCAGTTTGTCTTAGGTTGCCTTAAACAACCTTCTTGGTAAAAA TCAACTTCAATATTAACCAAAATTTAAAAGATTATATATAGTAAAAGAACTAATATTGCA GATTGACCCATTCCATTCTTTCAGAGAAGGTATGAGATACTTGACAGTGGAGCTAGCAGCA GAAACAATAACATGTATGATGAATCCATTGAGTCTTGTGAGTTTATTTTATTAATAATTT TTAATTGAACAATTTAAGCTTTTTTCTTCATCAGGATTTCAAGGGTGAATCTGCCAG TTTTATTCTGTCATTTTTCAAAACAA
CD47-206 w/o N-Terminus	SEQ ID NO:133	ATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGAATTTAAAGGAAGAGATATTT ACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGTAGTGCAAAATTT GAAGTCTCACAATTAATAAGGAGATGCCTCTTTGAAGATGGATAAGAGTGATGCTGTCT CACACACAGGAACTACACTTGTGAAGTAACAGAAATTAACCAGAGAAGGTGAAACGATCAT CGAGCTAAATATCGTGTTGTTTCATGGTTTTCTCCAATGAAAATATTCTATTGTTATTT TCCCAATTTTGTCTACTCTGTTCTGGGACAGTTTGGTATTAACAACTTAAATATAGA TCCGGTGGTATGGATGAGAAAACAAATTGCTTTACTTGTGCTGAGACTAGTATCACTGTCA TTGTCAATTGTTGGAGCCATTCTTTCTGCCAGGTGAATATTCATTAAAGAATGCTACTGGC CTTGGTTAATTGTGACTTCTACAGGGATTAATATTACTTCACTACTATGTGTTT
CD47-206 w/ restored N- Terminus	SEQ ID NO:134	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATCCATG CTTTGTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT

		AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATTCATTAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATTAATATTACTTCACTACTA TGTGTTT
Deleted TMD3	SEQ ID NO:135	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATTCATTAAG AATGCT
Deleted EC + TMD3	SEQ ID NO:136	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTC
Deleted TMD2 + EC + TMD3	SEQ ID NO:137	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACA
Deleted last IC	SEQ ID NO:138	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATTCATTAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATTAATATTACTTCACTACTA TGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTATTCAGGTGA TAGCCTATATCCTCGCTGTGTTGGACTGAGTCTCTGATTGCGGCGTGTATACCAATGTA TGGCCCTCTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTAAGTACTAGTTT ATATGTAA
Deleted TMD5 + last IC	SEQ ID NO:139	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA

		CTTAAATATAGATCCGGTGGTATGGATGAGAAAAAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATTCATTAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTTCACTACTA TGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCACTTCCATATTGGTTATTAGGTGA TAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATACCAATGCA TGGCTAA
Deleted EC + TMD5 + Last IC	SEQ ID NO:140	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATATCCTGTTCTGGGGACAGTTTGGTATTAAAAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAAAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATTCAATTAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTTCACTACTA TGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCACTTCCATATTGGTTATTAGGTGA TAGCCTATATCCTCGCTGTGGTTGGACTGTAA
Deleted TMD4	SEQ ID NO:141	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCAAATGAAAATATTC TTATTGTTATTTTCCCAATTTTGTATATCCTGTTCTGGGGACAGTTTGGTATTAAAAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAAAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATTCAATTAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTTCACTACTA TGTGTTTAGTACAGCGATTGGATTAACCTAA
CD47 ECD w/ DAF/CD55 GPI Anchor	SEQ ID NO:142	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCAAATGAAAGCCACTG AAGGAAGTGGAACCACTTCAGGTACTACCCGCTCTTCTATCTGGGCACACGTGTTTCACGTT GACAGGTTTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACTTAA
CD47 ECD w/ Long TRAILR3- GPI Anchor	SEQ ID NO:143	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCAAATGAAAGCCACTG TGGAACcccgactgctgaagagacaatgaacaccagcccggggactcctgcccagctgctgaagagacaatga acaccagcccggggactcctgcccagctgctgaagagacaatgaacaccagcccggggactcctgcccagctgctg aagagacaatgaacaccagcccggggactcctgcccagctgctgaagagacaatgaacaccagcccggggactcct gccTCTTCTCATTACCTCTCATGCACCATCGTAGGGATCATAGTTCTAATTGTGCTTCTGATT GTGTTTGTTTAA
CD47 ECD w/ Short TRAILR3- GPI Anchor	SEQ ID NO:144	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCAAATGAAatgacacc agccgggggactcctgccTCTTCTCATTACCTCTCATGCACCATCGTAGGGATCATAGTTCTAATT GTGCTTCTGATTGTGTTTGTTTAA

CD47 ECD with CD8a TMD and Short ICD (9aa)	SEQ ID NO:145	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCTCAATGAAATCTACA TCTGGGCGCCCTGGCCGGGACTTGTGGGGTCTTCTCCTGTCACTGGTTATCACCTTTTA CTGCAACCACAGGAACCGAAGATAA
CD47 ECD with CD28 TMD and Short ICD (9aa)	SEQ ID NO:146	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCTCAATGAAATTTGGG TGCTGGTGGTGGTGGTGGAGTCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTAT TATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACTAA
CD47 ECD with PDGFR TMD and Short ICD (9aa)	SEQ ID NO:147	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCTCAATGAAATTTGGG tcagccatcctggccctgggtgctcaccatcatctccctTATCATCCTCATCATGCTTTGGCAGAAGAAG CCACGTTACTAA
CD47 IgV, 3aa from Hinge Region, with PDGFR Hinge, TMD and Short ICD (9aa)	SEQ ID NO:148	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAAGCTGTGGGCCAGGACACGAGGATCATCGTGGTGCCA CACTCCTTGCCCTTTAAGTgtggtggtgatctcagccatcctggccctgggtggtgctcaccatcatctccctTATC ATCCTCATCATGCTTTGGCAGAAGAAGCCACGTTACTAA
CD47 IgV, 3aa from Hinge Region, IgG4 Hinge - CD28 TM	SEQ ID NO:149	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAAGaattcgaagtagcgaccgacctggcccccttgccctTTTTGGGTGCTG GTGGTGGTGGTGGAGTCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTT TCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACTAA
CD47 IgV, 3aa from Hinge Region, IgG4 Hinge - CD8a TM	SEQ ID NO:150	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAAGaattcgaagtagcgaccgacctggcccccttgccctATCTACATCTGG GCGCCCTTGCCCGGACTTGTGGGGTCTTCTCCTGTCACTGGTTATCACCTTTACTGCA ACCACAGGAACCGAAGATAA
CD47 IgV, 3aa from Hinge Region, CD28 Hinge - CD28 TM	SEQ ID NO:151	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGA

		AGAGCAATGGAACCATTTCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCTATTTCCC GGACCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTGGTGGAGTCTGGCTTGCTATAGCT TGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCA CTAA
CD47 IgV, 3aa from Hinge Region, CD28 Hinge - CD8a TM	SEQ ID NO:152	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAAATTGAAGTTATGTATCCTCCTCTTACCTAGACAATGAGA AGAGCAATGGAACCATTTCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCTATTTCCC GGACCTTCTAAGCCCATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCTTCTCC TGCTACTGGTTATCACCTTTTACTGCAACCACAGGAACCGAAGATAA
CD47 IgV, 3aa from Hinge Region, CD8a Hinge - CD28 TM	SEQ ID NO:153	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAAACCACGACGCGCAGCGCCGCGACCAACACCGGCGCCC ACCATCGCGTCGACGCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAgcgcgggggcgca gTGACACAGAGGGGGCTGGACTTCGCCTGTGATTTTGGGTGCTGGTGGTGGTGGTGGGA GTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTA AGAGGAGCAGGCTCCTGCACTAA
CD47 IgV, 3aa from Hinge Region, CD8a Hinge - CD8a TM	SEQ ID NO:154	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAAACCACGACGCGCAGCGCCGCGACCAACACCGGCGCCC ACCATCGCGTCGACGCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAgcgcgggggcgca gTGACACAGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGA CTTGTGGGTCTTCTCCTGTCTACTGGTTATCACCTTTTACTGCAACCACAGGAACCGAAG ATAA
WT, C15G	SEQ ID NO:155	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAATCTGTAGAATTCACGTTTTGAAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAAATATCGTGTGTTTCATGGTTTTCTCAAATGAAAATATT TTATTGTTATTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGTATTAACA CTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGCTGGACTAG TGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTCGTCAGGTGAATATTCATTAAG AATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGATATTAATATTACTTCACTACTA TGTGTTTAGTACAGCATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTATTCAGGTGA TAGCCTATATCCTCGCTGTGGTTGGAGTGAAGTCTGTATTGCGGCGTGATACCAATGCA TGGCCCTCTTCTGATTTAGGTTTGAAGTATCTAGCTCTAGCACAATTACTTGGACTAGTTT ATATGAAATTTGTGCTTCCAATCAGAAGACTATACAACCTCTAGGAAAGCTGTAGAGGA ACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N361, C15G	SEQ ID NO:156	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTTGGCAG AGACCACTAAGATTTAATAAAACAAATCTGTAGAATTCACGTTTTGAAATGACACTGTCG CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGATGGGCTAACAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTTGC

		TGGACTAGTGATCACTGTCATTGTGCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N362, C15G	SEQ ID NO:157	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGGCT CCACCCTAAGATTTAATAAAACAAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGATACACAAACAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAACTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTGCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CACTACTATGTGTTTAGTACAGCGATTGGATTAAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N363, C15G	SEQ ID NO:158	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CTACCACTACTATTTAATAAAACAAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGACAAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAACTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTGCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N364, C15G	SEQ ID NO:159	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CCACTACTAGCTTTTAATAAAACAAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTATGAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAACTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTCCCAATTTTGTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTGCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CACTACTATGTGTTTAGTACAGCGATTGGATTAAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N366, C15G	SEQ ID NO:160	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ACACCCTAATAATTTAATAAAACAAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTTAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAACTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC

		AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N368, C15G	SEQ ID NO:161	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG TTCCCACTAAGATTTAATAAAAAACAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTGAGAACAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAACTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCATTGTTGGAGCCATTCTTTTCGTCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N369, C15G	SEQ ID NO:162	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ATTCCACTAACTTTAATAAAAAACAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTTGAAGAACAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAACTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N3610, C15G	SEQ ID NO:163	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CCACTACTACTATTTAATAAAAAACAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTTCTAACAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAACTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N3612, C15G	SEQ ID NO:164	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CCACCCTACTATTTAATAAAAAACAAATCTGTAGAATTCACGTTTGGAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA

		AATTTAAAGGAAGAGATATTTACACCTTTGATGGACAGGCTAACAAGTCCACTGTCCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAAGTTTGAGTATCTTAGCTCTAGCACAAATTAAGTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N3614, C15G	SEQ ID NO:165	ATGTGGCCCTGAGTACGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CCACTACTAAAAATTAATAAAACAAAATCTGTAGAATTCACGTTTGAAATGACACTGTGCT CATTCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTGCTAACAAGTCCACTGTCCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTGTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAAGTTTGAGTATCTTAGCTCTAGCACAAATTAAGTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N3617, C15G	SEQ ID NO:166	ATGTGGCCCTGAGTACGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG AGACCACTAAGATTTAATAAAACAAAATCTGTAGAATTCACGTTTGAAATGACACTGTGCT CATTCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTGTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAAGTTTGAGTATCTTAGCTCTAGCACAAATTAAGTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N3618, C15G	SEQ ID NO:167	ATGTGGCCCTGAGTACGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CTACCACTACTATTTAATAAAACAAAATCTGTAGAATTCACGTTTGAAATGACACTGTGCT CATTCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTATTAACAAGTCCACTGTCCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTGTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTCTATTGTTGGAGCCATTCTTTTCGTCCCAGGTGAATATT CATTAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAAGTTTGAGTATCTTAGCTCTAGCACAAATTAAGTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA

N3621, C15G	SEQ ID NO:168	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTTGGCAG ATTCCACTAGAGTTTAAATAAAACAAAATCTGTAGAATTCACGTTTGAAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N3623, C15G	SEQ ID NO:169	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTTGGCAG ATGCCACTAGTTTTTAAATAAAACAAAATCTGTAGAATTCACGTTTGAAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGTTACAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
N3624, C15G	SEQ ID NO:170	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTTGGCAG ATTCCACTAAGATTTTAAATAAAACAAAATCTGTAGAATTCACGTTTGAAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGACAGGTTAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N361 Mutant	SEQ ID NO:171	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTTGGCAG AGACCCTAAGATTTTAAATAAAACAAAATCTGTAGAATTCACGTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGATGGGCTAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAACTTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA

		CCAATGCATGGCCCTCTTCTGATTTAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N362 Mutant	SEQ ID NO:172	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGGCT CCACCACTAAGATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGATACACAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N363 Mutant	SEQ ID NO:173	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CTACCACTACTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGACAAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N364 Mutant	SEQ ID NO:174	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CCACTACTAGCTTTTAAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTATGAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N366 Mutant	SEQ ID NO:175	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ACACCACTAAAATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA

		CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTACAGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N368 Mutant	SEQ ID NO:176	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG TTCCCACTAAGATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTGAGAACAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCACAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTACAGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N369 Mutant	SEQ ID NO:177	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ATTCCACTAACTTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTTGAACAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCACAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTACAGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3610 Mutant	SEQ ID NO:178	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CACTACTACTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTTCTAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTGTC TGGACTAGTGATCACTGTCATTGTCATTGTTGGAGCCATTCTTTTCGTCACAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACTCCTTCGTCATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTACAGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3612 Mutant	SEQ ID NO:179	ATGTGGCCCTGGTAGCGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CCACCACTACTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGACAGGCTAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG

		AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACCTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCTGTTGGAGCCATTCTTTTCGTCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3614 Mutant	SEQ ID NO:180	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG CCACTACTAAAATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTGCTAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACCTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCTGTTGGAGCCATTCTTTTCGTCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3617 Mutant	SEQ ID NO:181	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG AGACCACTAAGATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACCTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCTGTTGGAGCCATTCTTTTCGTCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3618 Mutant	SEQ ID NO:182	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ATTCCACTAGAGTTTAAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACCTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCTGTTGGAGCCATTCTTTTCGTCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3621 Mutant	SEQ ID NO:183	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ATTCCACTAGAGTTTAAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACCTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCATTGTCTGTTGGAGCCATTCTTTTCGTCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCAATTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTTCAGGTTTGAGTATCTTAGCTCTAGCACAAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA

		GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCTTGTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGTTTAAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCTTGCATTTGCCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3623 Mutant	SEQ ID NO:184	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ATGCCACTAGTTTTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGAGTTACAACAAGTCCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCTTGTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGTTTAAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCTTGCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
hCD47 N3624 Mutant	SEQ ID NO:185	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTCTGCGGATCAGCTTGGCAG ATTCCAATAAGATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGT CATTCCATGCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAGTGGA AATTTAAAGGAAGAGATATTTACACCTTTGATGGACAGGTTAACAAGTCCCACTGTCCCACT GACTTTAGTAGTGCAAAAATTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGAT GGATAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACC AGAGAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATG AAAATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGT ATTAACACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACAATTGCTTTACTTGTTGC TGGACTAGTGATCACTGTCAATTGTCTTGTGGAGCCATTCTTTTCGTCGCCAGGTGAATATT CATTAAAGAATGCTACTGGCCTTGTTTAAATTGTGACTTCTACAGGGATATTAATATTACTT CACTACTATGTGTTTAGTACAGCGATTGGATTAACCTCCTTCGTCTTGCATATTGGTTAT TCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTGCGGCGTGTATA CCAATGCATGGCCCTCTTCTGATTTCAGGTTTGAGTATCTTAGCTCTAGCACAATTACTTGG ACTAGTTTATATGAAATTTGTGGCTTCCAATCAGAAGACTATACACCTCCTAGGAAAGCTG TAGAGGAACCCCTTAATGCATTCAAAGAATCAAAGGAATGATGAATGATGAATAA
CAG-CD47 (202) – CodOpt	SEQ ID NO:200	ATGTGGCCCTGGTCGCCGCCCTGTTGCTGGGCTCGGCTGCTGCGGATCAGCTCAGCTAC TGTTTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAACGACACTGTCGTGATCCCATGC TTTTGTTACTAATATGGAGGCACAAAACCACTGAAGTGTACGTGAAGTGGAATTCAAAG GCAGAGACATTTACACCTTTGACGGCGCCCTCAACAAGTCCACCGTGCCCACTGACTTTAGT AGCGCAAAAATTGAGGTACGCCAATTACTAAAAGGAGATGCCTCTTTGAAGATGGACAAGA GCGATGCTGTCAGCCACACAGGGAACACTTGTGAAGTAACAGAGTTAACCCGCGAAGG TGAACGATCATCGAGCTGAAGTATCGAGTGGTGTCTGTTTCTCCGAACGAGAATATC CTTATCGTAATTTTCCCAATTTTGTCTATCCTCCTGTTCTGGGGCCAGTTTGGTATCAAGAC ACTCAAATATCGGTCCGGTGGGATGGATGAGAAGACAATTGCCCTGCTTGTGCTGGACTC GTGATCACCGTCATCGTGATTGTTGGGGCCATCCTTTTCGTCGCCAGGGGAGTACAGCCTGA AGAATGCTACGGGCTGGGATTAATTGTGACCTCTACAGGGATACTCATCCTGCTTCACTA CTATGTGTTTCAGTACCGGATTTGAGTACCTCCTTCGTCTTGCATATTGGTGATTTCAG GTGATAGCCTACATCCTCGCCGTGGTTGGCCTGAGTCTCTGTATCGCGGCGTGCATACCCA TGATGGCCCTCTTCTGATTTCAGGTTTGAGTATCCTCGCACTAGCAGAGTTGCTGGGACT GGTTTATATGAAATTTGTGGCCTCAACCAAGAACTATACAGCCTCCTAGGAAGGCTGTA GAGGAGCCCTGAATGCATTCAAGGAATCAAAGGCATGATGAATGATGAATAA

CD47 ECD- CD59 GPI	SEQ ID NO:341	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCTA CTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCATG CTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAAAG GAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAGT AGTGCAAAATTTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAGA GTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAGG TGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATGAACAGCTTG AGAACGGGGGCACATCTCTCTCCGAAAAGACTGTCCTGTTACTGGTGACCCCTTTCTTGGC TGCCGCATGGAGCCTGCACCCCTAA
CAG-CD47 ECD-CD59 GPI	SEQ ID NO:342	ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGG CGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTG ACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATG GGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGA CCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTC GAGGTGAGCCCCACGTTCTGCTTCACTCTCCCATCTCCCCCTCCCACTCCCACTTTT GTATTTATTTATTTTAAATTTTGTGACGCGATGGGGGCGGGGGGGGGGGGGGGGGCG CGCGCCAGGCGGGGCGGGGCGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCG GCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGGCGGG CGGCGGCCCTATAAAAGCGAAGCGCGCGGGCGGGGAGTGCCTGCGCGCTTCCG CCCGTGCCCGCTCCGCGCGCGCTCGCGCCCGCCCGCCCGCTCTGACTGACCGCGTTAC TCCACAGGTGAGCGGGCGGGACGGCCCTTCTCTCCGGGCTGTAATTAGCGCTTGGTTTA ATGACGGCTTGTTCCTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCC TTTGTGCGGGGGAGCGGCTCGGGGGTGCGTGCGTGTGTGTGCGTGCGGGAGCGCCG CGTGCGGCTCCGCGCTGCCGCGGCTGTGAGCGCTGCGGGCGCGGGCGGGGCTTTGT GCGCTCCGAGTGTGCGGAGGGGAGCGCGGGCGGGGCGGTGCCCGCGGTGCGGGGG GGGCTGCGAGGGGAACAAAGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGG GTGTGGGCGGTGCGTGCGGCTGCAACCCCCCTGCACCCCCCTCCCGAGTTGCTGAGCA CGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGCGCGGGGCTCGCCGTGCCCG GCGGGGGTGCGGCGAGGTGGGGGTGCCGGGCGGGGCGGGGCGGCTCGGGCGGGGA GGGCTCGGGGAGGGGCGCGGCGGCCCGGAGCGCGCGGCTGTGAGGCGCGGCGGA GCCGAGCCATTGCCTTTATGTAATCGTGCGAGAGGGCGCAGGACTTCTTTGTCCCA AATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCGCACCCCTCTAGCGGGCGCGGGC GAAGCGGTGCGGCGCGGAGGAAGAAATGGGCGGGGAGGGCCTTCTGTCGTGCGCGC GCCGCGTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTCG GGGGGACGGGGCAGGGCGGGGTTCCGGCTTCTGCGGTGTGACCGGCGGCTCTAGAGCCT CTGCTAACCATGTTATGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATT GTGCTGTCTCATCATTTTGGCAAAGAAATCCGTTTATTAATTAAGTGAACCGTCAGAGCCAC CATGTGGCCCTGGTAGCGGCGCTGTGCTGGGCTCGGCGTGCTGCGGATCAGCTCAGCT ACTATTTAATAAAACAAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCATTCCAT GCTTTGTTACTAATATGGAGGCACAAAACACTACTGAAGTATACGTAAAGTGAAATTTAA GGAAGAGATATTTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCACTGACTTTAG TAGTGCAAAATTTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGATAAG AGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGAGAAG GTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATGAACAGCTT GAGAACGGGGGCACATCTCTCTCCGAAAAGACTGTCCTGTTACTGGTGACCCCTTTCTTGG CTGCCGCATGGAGCCTGCACCCCTAA

Table 25. Exemplary Amino Acid Sequences for CD47 Protein Domains and Additional Domains

Description	SEQ ID NO:	Amino Acid Sequence
CD47 SP domain	SEQ ID NO:20	MWPLVAALLLSACCGSA
CD47 IgV domain	SEQ ID NO:21	QLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGRDIYTFDGLNKSTVPTDF SSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETII
CD47 EC domain	SEQ ID NO:22	QLLFNKTKSVEFTFCNDTVVPCFVTNMEAQNTTEVYVKWKFGRDIYTFDGLNKSTVPTDF SSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREGETIIELKYRVVSWFSPNE

CD47 TM1 domain	SEQ ID NO:23	NILIVIFPIFAILLFWGQFGI
CD47 IC1 domain	SEQ ID NO:24	KTLKYRSGGMDEKT
CD47 TM2 domain	SEQ ID NO:25	IALLVAGLVITVIVIVGAILF
CD47 EC1 domain	SEQ ID NO:26	VPGEYSLKNA
CD47 TM3 domain	SEQ ID NO:27	TGLGLIVTSTGILILLHYVVF
CD47 IC2 domain	SEQ ID NO:28	STAIGLT
CD47 TM4 domain	SEQ ID NO:29	SFVIAILVIQVIAYILAVVGL
CD47 EC2 domain	SEQ ID NO:30	SLCIAACIPMHG
CD47 TM5 domain	SEQ ID NO:31	PLGISGLSILALAQLLGLVYM
CD47(202) IC3 domain	SEQ ID NO:32	KFVASNQKTIQPPRKAVEEPLNAFKESKGMMNDE
CD47(201) IC3 Domain	SEQ ID NO:33	KFVASNQKTIQPPRNN
DAF/CD55 GPI	SEQ ID NO:34	PNKGS GTTSGTTRLLSGHTCFTLTGLLGLVTMGLLT
TRAILR3 GPI Long (with TAPE repeats)	SEQ ID NO:35	ATVETPAAEETMNTSPGTPAPAAEETMNTSPGTPAPAAEETMTTSPGTPAPAAEETMTTSPGT PAPAAEETMITSPGTPASSHYLSCTIVGIIVLIVLLIVFV
TRAILR3 GPI Short (without TAPE repeats)	SEQ ID NO:36	MITSPGTPASSHYLSCTIVGIIVLIVLLIVFV
CD59 GPI	SEQ ID NO:37	QLENGGTSLSSEKTVLLLVTPFLAAAWSLHP
CD8a TM domain	SEQ ID NO:38	IYIWAPLAGTCGVLLLSLVIT
CD8a 9AA ICD	SEQ ID NO:39	LYCNHRNRR
CD28 TM domain	SEQ ID NO:40	FWVLVVGGVLACYSLLVTVAFIIFWV
CD28 9AA ICD	SEQ ID NO:41	RSKRSRLH
PDGFR TM domain	SEQ ID NO:42	VVISAILALVVLTIISLIILI
PDGFR 9AA ICD	SEQ ID NO:43	MLWQKKPRY
CD47 3AA Hinge domain	SEQ ID NO:44	ELK

CD8a Hinge domain	SEQ ID NO: 45	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
CD28 Hinge domain	SEQ ID NO: 46	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKP
PDGFR Hinge domain	SEQ ID NO: 47	AVGQDTQEIVVPHSLPFKV
IgG4 Hinge domain	SEQ ID NO: 48	ESKYGPPCPPCP
CD47(205) IC3 Domain	SEQ ID NO: 343	KFVASNQKTIQPPRKAVEEPLNE
CD47 (205) EC1	SEQ ID NO: 344	XKNA
CD47(203) IC3 Domain	SEQ ID NO: 345	ASNQKTIQPPRKAVEEPLNAFKESKGMNDE
CD47(202) with "Velcro" insertion sequence	SEQ ID NO: 346	MWPLVAALLLSACCGSAWQPPLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGLNKTVPPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREG ETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
CD47(202) with alternate "Velcro" insertion sequence	SEQ ID NO: 347	MWPLVAALLLSACCGSAWQPQLLFNKTGSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWK FKGRDIYTFDGLNKTVPPTDFSSAKIEVSQLLKGDASLMDKSDAVSHTGNYTCEVTELTREG ETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYVVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCI AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMNDE
"Velcro" insertion sequence	SEQ ID NO: 348	WQP

Table 26. Exemplary Nucleotide Sequences for CD47 Protein Domains and Additional Domains

Description	SEQ ID NO:	Nucleotide Sequence
CAG promoter	SEQ ID NO: 49	ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGG CGTTACATAAATTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTG ACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATG GGTGGAGTATTTACGGTAAATGCCCACTTGGCAGTACATCAAGTGATCATATGCCAAGT ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGCCAGTACATGA CCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTC GAGGTGAGCCCCACGTTCTGCTTCACTCTCCCATCTCCCCCCCCCTCCCCACCCCAATTTT GTATTTATTTATTTTAAATTATTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCG CGGCCAGGCGGGGCGGGGCGGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCG GCGGCAGCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGG CGGCGGCCCTATAAAAAGCGAAGCGCGCGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGC CCCGTGCCCGCTCCGCGCGCGCTCGCGCCGCCCGCCCGGCTCTGACTGACCGCGTTAC TCCACAGGTGAGCGGGCGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGTTTTA ATGACGGCTTGTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCC TTTGTGCGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGCGTGCGGGAGCGCCG CGTGCGGCTCCGCGCTGCCCGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGT GCGCTCCGCAAGTGTGCGGAGGGGAGCGCGGCGGGGGCGGTGCCCGCGGTGCGGGG GGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGTGAGCAGGGG GTGTGGGCGGTGCGTGGGCTGCAACCCCCCTGCACCCCCCTCCCCAGTTGCTGAGCA CGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGCGCGCGGGGCTCGCGGTGCCG GCGGGGGTGCGGCAGGTGGGGTGCCGGGCGGGGCGGGGCGGCTCGGGCGGGGA GGGCTCGGGGGAGGGGCGCGGCGGCCCGGAGCGCGCGGCTGTGAGGCGCGGCGA GCCGAGCCATTGCCTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGCCCA AATCTGTGCGGAGCGGAAATCTGGGAGGGCGCGCGCACCCCTCTAGCGGGCGCGGGG

		GAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGC GCCGCCGTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGACGGCTGCCTTCG GGGGGACGGGGCAGGGCGGGGTTCCGGCTTCTGCGGTGTGACCGCGGGCTCTAGAGCCT CTGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGCTCCTGGCAACGTGCTGGTTATT GTGCTGTCTCATCATTTTGCAAGAATTTC
EF1a promoter	SEQ ID NO:50	GCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGG GAGGGGTGCGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGTAAACTGGGAAAGTG ATGTCGTGTACTGGCTCCGCCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCACT AGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTG TGTGTTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCTTGAATTACTT CCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGA GTTGAGGGCTTGCCTTAAGGAGCCCTTCGCCTCGTGCTTGAGTTGAGGCCCTGGCCTGG GCGCTGGGGCGCGCGTGCGAATCTGGTGGCACCTTCGCGCTGTCTCGTGCTTTTCGAT AAGTCTCTAGCCATTTAAATTTTTGATGACCTGTGCGACGCTTTTTTCTGGCAAGATAG TCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTGGGGCCGCGGGCGGC GACGGGGCCGTGCGTCCCGAGCGCACATGTTTCGGCGAGGGCGGGCCTTCGAGCGCGGCCA CCGAGAATCGGACGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCG CCGCGGTGTATCGCCCCGCCCTGGCGCGCAAGGCTGGCCCGGTTCGGCACCAAGTTGCGTGA GCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAATGGAGGACGCGGCGC TCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAGGGCCTTCCGTCCTCAGCC GTCGCTTCATGTGACTCCACTGAGTACCGGGCGCGTCCAGGCACCTCGATTAGTTCTCGA GCTTTTGGAGTACGTGCTTTAGGTTGGGGGAGGGGTTTATGCGATGGAGTTTCCCC ACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGA ATTTGCCCTTTTGAAGTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTT TTTTCTTCCATTTTCAGGTGTCGTGA
CD47 SP domain	SEQ ID NO:51	ATGTGGCCCTGGTAGCGGCGCTGTTGCTGGGCTCGGCGTGCTGCGGATCAGCT
CD47 IgV domain	SEQ ID NO:52	CAGCTACTATTTAATAAAACAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCAT TCCATGCTTTGTTACTAATATGGAGGCACAAACACTACTGAAGTATACGTAAAGTGAAAT TTAAAGGAAGAGATATTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGAC TTTAGTAGTGCAAAATTTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGA TAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGA GAAGGTGAAACGATCATC
CD47 EC domain	SEQ ID NO:53	CAGCTACTATTTAATAAAACAAATCTGTAGAATTCACGTTTTGTAATGACACTGTCGTCAT TCCATGCTTTGTTACTAATATGGAGGCACAAACACTACTGAAGTATACGTAAAGTGAAAT TTAAAGGAAGAGATATTACACCTTTGATGGAGCTCTAAACAAGTCCACTGTCCCCACTGAC TTTAGTAGTGCAAAATTTGAAGTCTCACAATTACTAAAAGGAGATGCCTCTTTGAAGATGGA TAAGAGTGATGCTGTCTCACACACAGGAACTACACTTGTGAAGTAACAGAATTAACCAGA GAAGGTGAAACGATCATCGAGCTAAAATATCGTGTTGTTTCATGGTTTTCTCCAAATGAA
CD47 TM1 domain	SEQ ID NO:54	AATATTCTTATTGTTATTTTCCCAATTTTGTCTATACTCCTGTTCTGGGGACAGTTTGGTAT T
CD47 IC1 domain	SEQ ID NO:55	AAAACACTTAAATATAGATCCGGTGGTATGGATGAGAAAACA
CD47 TM2 domain	SEQ ID NO:56	ATTGCTTTACTTGTGCTGGACTAGTGATCACTGTCATTGTCAATTGTTGGAGCCATTCTTTT C
CD47 EC1 domain	SEQ ID NO:57	GTCCCAGGTGAATATTCATTAAAGAATGCT
CD47 TM3 domain	SEQ ID NO:58	ACTGGCCTTGGTTTAATTGTGACTTCTACAGGGATATTAATATTACTTCACTACTATGTGTT T
CD47 IC2 domain	SEQ ID NO:59	AGTACAGCGATTGGATTAACC
CD47 TM4 domain	SEQ ID NO:60	TCCTTCGTCATTGCCATATTGGTTATTCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACT G
CD47 EC2 domain	SEQ ID NO:61	AGTCTCTGTATTGCGGCGTGATACCAATGCATGGC

CD47 TM5 domain	SEQ ID NO:62	CCTCTTCTGATTTTCAGGTTTTGAGTATCTTAGCTCTAGCACAATTACTTGGAAGTATTTATATG
CD47(202) IC3 domain	SEQ ID NO:63	AAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAAGCTGTAGAGGAACCCCTTAATGCATTCAAAGAATCAAAAGGAATGATGAATGATGAA
CD47(201) IC3 Domain	SEQ ID NO:64	AAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAATAAC
DAF/CD55 GPI	SEQ ID NO:65	CCAAATAAAGGAAGTGGAACCACTTCAGGTACTACCGTCTTCTATCTGGGCACACGTGTTTCACGTTGACAGTTTTGCTTGGGACGCTAGTAACCATGGGCTTGCTGACT
TRAILR3 GPI Long (with TAPE repeats)	SEQ ID NO:66	GCCACTGTGGAAAccccagctgctgaagagacaatgaacaccagcccggggactcctgccccagctgctgaagagaatgaacaccagcccggggactcctgccccagctgctgaagagacaatgaacaccagcccggggactcctgccccagctgctgaagagacaatgatcaccagcccggggactcctgccTCTTCTCATTTACCTCTCATGCACCATCGTAGGGATCATAGTTCTAATTGTGCTTCTGATTGTGTTTTGTT
TRAILR3 GPI Short (without TAPE repeats)	SEQ ID NO:67	atgatcaccagcccggggactcctgccTCTTCTCATTACCTCTCATGCACCATCGTAGGGATCATAGTTCTAATTGTGCTTCTGATTGTGTTTTGTT
CD59 GPI	SEQ ID NO:68	CAGCTTGAGAACGGGGGCACATCTCTCTCCGAAAAGACTGTCCTGTTACTGGTGACCCCTTCTTGGCTGCCGCATGGAGCCTGCACCCC
CD8a TM domain	SEQ ID NO:69	ATCTACATCTGGGCGCCCTTGCGCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCC
CD8a 9AA ICD	SEQ ID NO:70	CTTTACTGCAACCACAGGAACCGAAGA
CD28 TM domain	SEQ ID NO:71	TTTTGGGTGCTGGTGGTGGTTGGTGGAGTCTGGCTTGCTATAGCTTGTCTAGTAACAGTGGCCTTTATTATTTTCTGGGTG
CD28 9AA ICD	SEQ ID NO:72	AGGAGTAAGAGGAGCAGGCTCCTGCAC
PDGFR TM domain	SEQ ID NO:73	gtggtgatctcgacctcctggccctggtggtgctcaccatcatctccctTATCATCCTCATC
PDGFR 9AA ICD	SEQ ID NO:74	ATGCTTTGGCAGAAGAAGCCACGTTAC
CD47 3AA Hinge domain	SEQ ID NO:75	GAGCTAAAA
CD8a Hinge domain	SEQ ID NO:76	ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCACCATCGCGTCGACGCCCTGTCCCTGCGCCAGAGGCGTGCCGGCCAgcggcggggggcgagTGCACACGAGGGGGCTGGACTTCGCTGTGAT
CD28 Hinge domain	SEQ ID NO:77	ATTGAAGTTATGTATCCTCCTCTTACCTAGACAATGAGAAGAGCAATGGAACCATTTCCA TGTGAAAGGGAAACACCTTTGTCCAAGTCCCTATTTCCCGACCTTCTAAGCCC
PDGFR Hinge domain	SEQ ID NO:78	GCTGTGGGCCAGGACACGCAGGAGTCATCGTGGTGCCACACTCCTTGCCCTTTAAGGtg
IgG4 Hinge domain	SEQ ID NO:79	gaatctaagtacggaccgcccctgcccccttgcct
CD47(205) IC3 Domain	SEQ ID NO:349	AAATTTGTGGCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAAGCTGTAGAGGAACCCCTTAATGAATAA
CD47 (205) EC1	SEQ ID NO:350	AAGAATGCT
CD47(203) IC3 Domain	SEQ ID NO:351	GCTTCCAATCAGAAGACTATACAACCTCCTAGGAAAAGCTGTAGAGGAACCCCTTAATGCATTCAAAGAATCAAAAGGAATGATGAATGATGAATAA

Table 27. Exemplary Anti-SIRPα Antibody Amino Acid Sequences

Ab Domain	SEQ ID NO:	Amino Acid Sequence
VH or HC	SEQ ID NO:186	EVQLQQSGAELVKPGASVKLSCTASGFNIKDYIHWVQQRTEQGLEWIGRIDPEDGETKYAPK FQDKATITADTSSNTAYLHLSSLTSEDVAVYYCARWGAYWGQGTLLTVSA
VL or LC	SEQ ID NO:187	QIVLTQSPAISASPGKEVTLTCSASSSVSSSYLYWYQKPGSSPKLWIYSTNLSAGVPA RFS GSGSGTSYSLTISSMEAEDAASYFCHQWSSYPRTFGAGTKLELK
VH or HC	SEQ ID NO:188	EVQLQQSGAELVKPGASVKLSCTASGFNIKDYIHWVQQRTEQGLEWIGRIDPEDGETKYAPK FQDKATITADTSSNTAYLHLSSLTSEDVAVYYCARWGAYWGQGTLLTVSS
scFv	SEQ ID NO:189	EVQLQQSGAELVKPGASVKLSCTASGFNIKDYIHWVQQRTEQGLEWIGRIDPEDGETKYAPK FQDKATITADTSSNTAYLHLSSLTSEDVAVYYCARWGAYWGQGTLLTVSSGGGGSGGGSGG GGSQIVLTQSPAISASPGKEVTLTCSASSSVSSSYLYWYQKPGSSPKLWIYSTNLSAGVPA RFS GSGSGTSYSLTISSMEAEDAASYFCHQWSSYPRTFGAGTKLELK
scFv	SEQ ID NO:190	QIVLTQSPAISASPGKEVTLTCSASSSVSSSYLYWYQKPGSSPKLWIYSTNLSAGVPA RFS GSGSGTSYSLTISSMEAEDAASYFCHQWSSYPRTFGAGTKLELKGTAAAGSSGGSSGAEVQ LQQSGAELVKPGASVKLSCTASGFNIKDYIHWVQQRTEQGLEWIGRIDPEDGETKYAPKFQD KATITADTSSNTAYLHLSSLTSEDVAVYYCARWGAYWGQGTLLTVSS
VH or HC	SEQ ID NO:191	EVQLVQSGAEVKKPGESLRISCKASGYFTSYWVHWVRQMPGKGLEWIGNIDPSDSDTHYN QKFKDHVTLSDVKSISTAYLQLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLLTVSS
VL or LC	SEQ ID NO:192	DVVMTQSPSLPVTLGQPASISCRSSQSLVHSYGNTYLYWYQQRPGQSPRLTIYRVSNRFSGV PDRFSGSGSGTDFTLKISRVEAEDVGVYFCFQGTHTVPYTFGGGTKEIK
VL or LC	SEQ ID NO:193	DVVMTQSPSLPVTLGQPASISCRSSQSLVHSYGNTYLYWYQQRPGQSPRLTIYRVSNRFSGV PDRFSGSGSGTDFTLKISRVEAEDVGVYFCFQGTHTVPYTFGGGTKEIK
VH or HC	SEQ ID NO:194	EVQLVQSGAEVKKPGESLRISCKASGYFTSYWVHWVRQMPGKGLEWMGNIDPSDSDTHYN QKFKDHVTLSDVKSISTAYLQLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLLTVSS
VH or HC	SEQ ID NO:195	EVQLVQSGAEVKKPGESLRISCKASGYFTSYWVHWVRQMPGKGLEWMGNIDPSDSDTHYS PSFQGHVTLSDVKSISTAYLQLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLLTVSS
VH or HC	SEQ ID NO:196	EVQLVQSGAEVKKPGESLRISCKASGYFTSYWVHWVRQMPGKGLEWMGNIDPSDSDTHYS PSFQGHVTLSDVKSISTAYLQLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLLTVSS
VH or HC	SEQ ID NO:197	EVQLVQSGAEVKKPGESLRISCKASGYFTSYWVHWVRQMPGKGLEWMGNIDPSDSDTHYS PSFQGHVTLSDVKSISTAYLQLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLLTVSS
VH or HC	SEQ ID NO:198	EVQLVQSGAEVKKPGESLRISCKASGYFTSYWVHWVRQMPGKGLEWMGNIDPSDSDTHYS PSFQGHVTLSDVKSISTAYLQLSSLKASDTAMYYCVRGGTGTMAWFAYWGQGTLLTVSS

CERTAIN EMBODIMENTS**Embodiments Section I**

[0609] Embodiment 1. A genetically engineered cell comprising an engineered CD47 protein, wherein the engineered CD47 protein exhibits:

- decreased binding with TSP-1 relative to a wild-type CD47 protein; and/or
- decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein.

[0610] Embodiment 2. A genetically engineered cell comprising an engineered CD47 protein, wherein the genetically engineered cell:

- exhibits decreased CD47/TSP-1-mediated undesirable effects relative to a control cell; and/or
- exhibits decreased CD47/TSP-1-mediated exhaustion relative to a control cell; and/or
- exhibits decreased CD47-mediated undesirable effects relative to a control cell; and/or
- exhibits increased CD47-mediated desirable effects relative to a control cell; and/or

exhibits increased longevity relative to a control cell; and/or
exhibits increased persistence relative to a control cell; and/or
exhibits increased expansion relative to a control cell, and/or
exhibits increased response to its intended therapeutic target, and/or
exhibits increased hyp immunity relative to a control cell; and/or
exhibits increased immune evasion relative to a control cell; and/or
exhibits increased ability to evade a host immune response relative to a control cell; and/or
exhibits increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; and/or

expresses the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would experience one or more unwanted effects.

[0611] Embodiment 3. The genetically engineered cell of embodiment 1, wherein the control cell comprises a wild-type cell, an unmodified cell, a cell that has not been engineered to express CD47 or a cell that has been engineered to express wild-type CD47.

[0612] Embodiment 4. The genetically engineered cell of embodiment 1, wherein the first expression level comprises 1.25x, 1.5x, 1.75x, 2x, 2.25x, 2.5x, 2.75x, 3x, 3.25x, 3.5x, 3.75x, 4x, 4.5x, 5x, 5.5x, 6x, 6.5x, 7x, 7.5x, 8x, 8.5x, 9x, 9.5x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x the level of wild-type CD47 protein in an unmodified cell.

[0613] Embodiment 5. The genetically engineered cell of embodiment 1 or embodiment 2, wherein the CD47/TSP-1-mediated signaling and/or the CD47/TSP-1-mediated exhaustion comprises autocrine signaling.

[0614] Embodiment 6. The genetically engineered cell of embodiment 1 or embodiment 2, wherein the CD47/TSP-1-mediated signaling and/or the CD47/TSP-1-mediated exhaustion comprises paracrine signaling.

[0615] Embodiment 7. The genetically engineered cell of embodiment 1 or embodiment 2, wherein the CD47/TSP-1-mediated signaling and/or the CD47/TSP-1-mediated exhaustion comprises both autocrine signaling and paracrine signaling.

[0616] Embodiment 8. The genetically engineered cell of embodiment 2, wherein the increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via autocrine signaling.

[0617] Embodiment 9. The genetically engineered cell of embodiment 2, wherein the increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via paracrine signaling.

[0618] Embodiment 10. The genetically engineered cell of embodiment 2, wherein the increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via both autocrine signaling and paracrine signaling.

[0619] Embodiment 11. The genetically engineered cell of any one of embodiments 1-10, wherein exhaustion comprises increased expression of PD1, LAG3, TIM3, CD39, CD101, or a combination thereof.

[0620] Embodiment 12. The genetically engineered cell of any one of embodiments 1-10, wherein exhaustion comprises decreased or eliminated cytokine expression.

[0621] Embodiment 13. The genetically engineered cell of any one of embodiments 1-12, wherein the engineered CD47 protein comprises:

- an amino acid substitution at position S82 of SEQ ID NO: 2,
- an amino acid substitution at position S64 of SEQ ID NO: 3,
- an amino acid substitution at position S82 of SEQ ID NO: 1, or
- an amino acid substitution at position S37 of SEQ ID NO: 6.

[0622] Embodiment 14. The genetically engineered cell of any one of embodiments 1-13, wherein the engineered CD47 protein comprises an amino acid substitution at position S82 of SEQ ID NO: 2.

[0623] Embodiment 15. The genetically engineered cell of any one of embodiments 1-14, wherein the engineered CD47 protein comprises:

- an amino acid substitution at position S83 of SEQ ID NO: 2,
- an amino acid substitution at position S65 of SEQ ID NO: 3,
- an amino acid substitution at position S83 of SEQ ID NO: 1, or
- an amino acid substitution at position S38 of SEQ ID NO: 6.

[0624] Embodiment 16. The genetically engineered cell of any one of embodiments 1-15, wherein the engineered CD47 protein comprises an amino acid substitution at position S83 of SEQ ID NO: 2.

[0625] Embodiment 17. The genetically engineered cell of any one of embodiments 1-16, wherein the engineered CD47 protein comprises:

- amino acid substitutions at positions S82 and S83 of SEQ ID NO: 2,
- amino acid substitutions at positions S64 and S65 of SEQ ID NO: 3,
- amino acid substitutions at positions S82 and S83 of SEQ ID NO: 1, or
- amino acid substitutions at positions S37 and S38 of SEQ ID NO: 6.

[0626] Embodiment 18. The genetically engineered cell of any one of embodiments 1-17, wherein the engineered CD47 protein comprises amino acid substitutions at positions S82 and S83 of SEQ ID NO: 2.

[0627] Embodiment 19. The genetically engineered cell of any one of embodiments 1-18, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S82A relative to SEQ ID NO: 2,
- an amino acid substitution of S64A relative to SEQ ID NO: 3,
- an amino acid substitution of S82A relative to SEQ ID NO: 1, or
- an amino acid substitution of S37A relative to SEQ ID NO: 6.

[0628] Embodiment 20. The genetically engineered cell of any one of embodiments 1-19, wherein the engineered CD47 protein comprises an amino acid substitution of S82A relative to SEQ ID NO: 2.

[0629] Embodiment 21. The genetically engineered cell of any one of embodiments 1-18, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2,
- an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3,
- an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, or
- an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6.

[0630] Embodiment 22. The genetically engineered cell of any one of embodiments 1-21, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S83A relative to SEQ ID NO: 2,
- an amino acid substitution of S65A relative to SEQ ID NO: 3,
- an amino acid substitution of S83A relative to SEQ ID NO: 1, or
- an amino acid substitution of S38A relative to SEQ ID NO: 6.

[0631] Embodiment 23. The genetically engineered cell of any one of embodiments 1-22, wherein the engineered CD47 protein comprises an amino acid substitution of S83A relative to SEQ ID NO: 2.

[0632] Embodiment 24. The genetically engineered cell of any one of embodiments 1-21, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2,
- an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3,
- an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, or
- an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[0633] Embodiment 25. The genetically engineered cell of any one of embodiments 1-24, wherein the engineered CD47 protein comprises an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2.

[0634] Embodiment 26. The genetically engineered cell of any one of embodiments 1-25, wherein the engineered CD47 protein comprises:

- a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, and an amino acid substitution of S37A relative to SEQ ID NO: 6; and
- a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83A relative to SEQ ID NO: 2, an amino acid substitution of S65A relative to SEQ ID NO: 3, an amino acid substitution of S83A relative to SEQ ID NO: 1, and an amino acid substitution of S38A relative to SEQ ID NO: 6.

[0635] Embodiment 27. The genetically engineered cell of any one of embodiments 1-26, wherein the engineered CD47 protein comprises amino acid substitutions of S82A and S83A relative to SEQ ID NO: 2.

[0636] Embodiment 28. The genetically engineered cell of any one of embodiments 1-25, wherein the engineered CD47 protein comprises:

- a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2, an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3, an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, and an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6; and
- a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2, an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3, an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, and an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[0637] Embodiment 29. The genetically engineered cell of any one of embodiments 1-28, wherein the engineered CD47 protein comprises:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2.

[0638] Embodiment 30. A genetically engineered cell comprising an engineered CD47 protein, wherein the engineered CD47 protein exhibits improved binding with SIRPα relative to a wild-type CD47 protein.

[0639] Embodiment 31. A genetically engineered cell comprising an engineered CD47 protein, wherein contacting a cell expressing SIRPα with the genetically engineered cell increases CD47/SIRPα-mediated signaling in the cell expressing SIRPα relative to contacting the cell expressing SIRPα with a control cell.

[0640] Embodiment 32. A genetically engineered cell comprising an engineered CD47 protein, wherein contacting a cell expressing SIRPα with the genetically engineered cell increases CD47/SIRPα-mediated signaling in the cell expressing the engineered CD47 relative to contacting the cell expressing SIRPα with a control cell.

[0641] Embodiment 33. A genetically engineered cell comprising an engineered CD47 protein, wherein contacting a cell expressing SIRPα with the genetically engineered cell increases CD47/SIRPα-mediated signaling in both the cell expressing SIRPα and the cell expressing CD47 relative to contacting the cell expressing SIRPα with a control cell.

[0642] Embodiment 34. A genetically engineered cell comprising an engineered CD47 protein, wherein the genetically engineered cell:

exhibits decreased CD47-mediated undesirable effects relative to a control cell; and/or

exhibits increased CD47-mediated desirable effects relative to a control cell; and/or

exhibits increased longevity relative to a control cell; and/or

exhibits increased persistence relative to a control cell; and/or

exhibits increased expansion relative to a control cell; and/or

exhibits increased response to its intended therapeutic target, and/or

exhibits increased hyp immunity relative to a control cell; and/or

exhibits improved immune evasion relative to a control cell; and/or

exhibits increased ability to evade a host immune response relative to a control cell; and/or

exhibits increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell; and/or

expresses the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would be killed by one or more immune cells.

[0643] Embodiment 35. The genetically engineered cell of any one of embodiments 31-34, wherein the control cell comprises a wild-type cell, an unmodified cell, a cell that has not been engineered to express CD47 or a cell that has been engineered to express wild-type CD47.

[0644] Embodiment 36. The genetically engineered cell of embodiment 34-35, wherein the first expression level comprises 1.25x, 1.5x, 1.75x, 2x, 2.25x, 2.5x, 2.75x, 3x, 3.25x, 3.5x, 3.75x, 4x, 4.5x, 5x, 5.5x, 6x, 6.5x, 7x, 7.5x, 8x, 8.5x, 9x, 9.5x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x the level of wild-type CD47 protein in an unmodified cell.

[0645] Embodiment 37. The genetically engineered cell of any one of embodiments 30-36, wherein the engineered CD47 protein comprises:

an amino acid sequence having at least 80% identity to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6; and

an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0646] Embodiment 38. The genetically engineered cell of any one of embodiments 30-37, wherein the engineered CD47 protein comprises:

an amino acid sequence having at least 80% identity to SEQ ID NO: 2; and

an insertion of one or more amino acids relative to SEQ ID NO: 2.

[0647] Embodiment 39. The genetically engineered cell of embodiment 37, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0648] Embodiment 40. The genetically engineered cell of any one of embodiments 37-39, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 2.

[0649] Embodiment 41. The genetically engineered cell of embodiment 37 or 38, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0650] Embodiment 42. The genetically engineered cell of any one of embodiments 37-41, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2.

[0651] Embodiment 43. The genetically engineered cell of any one of embodiments 37-41, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[0652] Embodiment 44. The genetically engineered cell of any one of embodiments 37-43, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2.

[0653] Embodiment 45. The genetically engineered cell of any one of embodiments 37-43, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[0654] Embodiment 46. The genetically engineered cell of any one of embodiments 37-45, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2.

[0655] Embodiment 47. The genetically engineered cell of any one of embodiments 37-45, wherein the engineered CD47 protein comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[0656] Embodiment 48. The genetically engineered cell of any one of embodiments 37-47, wherein the engineered CD47 protein comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2.

[0657] Embodiment 49. The genetically engineered cell of any one of embodiments 37-47, wherein the engineered CD47 protein comprises:

an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2, or

an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 1 and an amino acid substitution of Q19P relative to SEQ ID NO: 1.

- [0658]** Embodiment 50. The genetically engineered cell of any one of embodiments 37-49, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2.
- [0659]** Embodiment 51. The genetically engineered cell of any one of embodiments 37-49, wherein the engineered CD47 protein comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.
- [0660]** Embodiment 52. The genetically engineered cell of any one of embodiments 37-51, wherein the engineered CD47 protein comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2.
- [0661]** Embodiment 53. The genetically engineered cell of any one of embodiments 37-52, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 346.
- [0662]** Embodiment 54. The genetically engineered cell of any one of embodiments 37-51, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 347.
- [0663]** Embodiment 55. The genetically engineered cell of any one of embodiments 37-54, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 20;
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid sequence comprising SEQ ID NO: 3.
- [0664]** Embodiment 56. The genetically engineered cell of any one of embodiments 37-54, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 20;
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid substitution of Q1P relative to SEQ ID NO: SEQ ID NO: 3.
- [0665]** Embodiment 57. The genetically engineered cell of any one of embodiments 37-54, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid sequence comprising SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.
- [0666]** Embodiment 58. The nucleic acid construct of any one of embodiments 1-57, wherein the engineered CD47 protein comprises an amino acid sequence comprising:
an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, or an amino acid substitution of S37A relative to SEQ ID NO: 6;
an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1; and an amino acid substitution of Q19P relative to SEQ ID NO: 2 or SEQ ID NO: 1.
- [0667]** Embodiment 59. The genetically engineered cell of any one of embodiments 1-58, wherein the engineered CD47 protein comprises an amino acid sequence comprising:
an amino acid substitution of S82A relative to SEQ ID NO: 2;
an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2; and
an amino acid substitution of Q19P relative to SEQ ID NO: 2.
- [0668]** Embodiment 60. A genetically engineered cell comprising an engineered CD47 protein, wherein the engineered CD47 protein exhibits:

decreased binding with TSP-1 relative to a wild-type CD47 protein;
 decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein; and
 improved binding with SIRPα relative to a wild-type CD47 protein; and
 wherein the genetically engineered cell:
 exhibits decreased CD47/TSP-1-mediated undesirable effects relative to a control cell;
 exhibits decreased CD47-mediated undesirable effects relative to a control cell;
 exhibits decreased CD47/TSP-1-mediated exhaustion relative to a control cell;
 exhibits decreased CD47-mediated undesirable effects relative to a control cell;
 exhibits increased CD47-mediated desirable effects relative to a control cell;
 exhibits increased longevity relative to a control cell;
 exhibits increased persistence relative to a control cell;
 exhibits increased expansion relative to a control cell;
 exhibits increased response to its intended therapeutic target;
 exhibits increased hyp immunity relative to a control cell;
 exhibits increased immune evasion relative to a control cell;
 exhibits increased ability to evade a host immune response relative to a control cell;
 exhibits increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response relative to a control cell;
 expresses the engineered CD47 protein at a first expression level, wherein a control cell expressing a wild-type CD47 protein at the first expression level would experience one or more unwanted effects and would be killed by one or more immune cells; and
 wherein contacting a cell expressing SIRPα with the genetically engineered cell increases CD47/SIRPα-mediated signaling in both the cell expressing SIRPα and the cell expressing CD47 relative to contacting the cell expressing SIRPα with a control cell.

[0669] Embodiment 61. The genetically engineered cell of embodiment 60, wherein the control cell comprises a wild-type cell, an unmodified cell, a cell that has not been engineered to express CD47 or a cell that has been engineered to express wild-type CD47.

[0670] Embodiment 62. The genetically engineered cell of embodiment 60, wherein the first expression level comprises 1.25x, 1.5x, 1.75x, 2x, 2.25x, 2.5x, 2.75x, 3x, 3.25x, 3.5x, 3.75x, 4x, 4.5x, 5x, 5.5x, 6x, 6.5x, 7x, 7.5x, 8x, 8.5x, 9x, 9.5x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x the level of wild-type CD47 protein in an unmodified cell.

[0671] Embodiment 63. The genetically engineered cell of any one of embodiments 60-62, wherein the CD47/TSP-1-mediated signaling and/or the CD47/TSP-1-mediated exhaustion comprises autocrine signaling.

[0672] Embodiment 64. The genetically engineered cell of any one of embodiments 60-62, wherein the CD47/TSP-1-mediated signaling and/or the CD47/TSP-1-mediated exhaustion comprises paracrine signaling.

[0673] Embodiment 65. The genetically engineered cell of any one of embodiments 60-64, wherein the CD47/TSP-1-mediated signaling and/or the CD47/TSP-1-mediated exhaustion comprises both autocrine signaling and paracrine signaling.

[0674] Embodiment 66. The genetically engineered cell of any one of embodiments 60-65, wherein the increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a

natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via autocrine signaling.

[0675] Embodiment 67. The genetically engineered cell of any one of embodiments 60-65, wherein the increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via paracrine signaling.

[0676] Embodiment 68. The genetically engineered cell any one of embodiments 60-65, wherein the increased longevity, increased persistence, increased expansion, increased response to its intended therapeutic target, increased immune evasion, increased ability to evade a host immune response, and/or increased ability to evade a natural killer (NK) cell, monocyte, or macrophage response comprises reduced CD47/TSP-1-mediated exhaustion via both autocrine signaling and paracrine signaling.

[0677] Embodiment 69. The genetically engineered cell of any one of embodiments 60-68, wherein exhaustion comprises increased expression of PD1, LAG3, TIM3, CD39, CD101, or a combination thereof.

[0678] Embodiment 70. The genetically engineered cell of any one of embodiments 60-69, wherein exhaustion comprises decreased or eliminated cytokine expression.

[0679] Embodiment 71. The genetically engineered cell of any one of embodiments 60-70, wherein the engineered CD47 protein comprises:

- an amino acid substitution at position S82 of SEQ ID NO: 2,
- an amino acid substitution at position S64 of SEQ ID NO: 3,
- an amino acid substitution at position S82 of SEQ ID NO: 1, or
- an amino acid substitution at position S37 of SEQ ID NO: 6.

[0680] Embodiment 72. The genetically engineered cell of any one of embodiments 60-71, wherein the engineered CD47 protein comprises an amino acid substitution at position S82 of SEQ ID NO: 2.

[0681] Embodiment 73. The genetically engineered cell of any one of embodiments 60-72, wherein the engineered CD47 protein comprises:

- an amino acid substitution at position S83 of SEQ ID NO: 2,
- an amino acid substitution at position S65 of SEQ ID NO: 3,
- an amino acid substitution at position S83 of SEQ ID NO: 1, or
- an amino acid substitution at position S38 of SEQ ID NO: 6.

[0682] Embodiment 74. The genetically engineered cell of any one of embodiments 60-73, wherein the engineered CD47 protein comprises an amino acid substitution at position S83 of SEQ ID NO: 2.

[0683] Embodiment 75. The genetically engineered cell of any one of embodiments 60-74, wherein the engineered CD47 protein comprises:

- amino acid substitutions at positions S82 and S83 of SEQ ID NO: 2,
- amino acid substitutions at positions S64 and S65 of SEQ ID NO: 3,
- amino acid substitutions at positions S82 and S83 of SEQ ID NO: 1, or
- amino acid substitutions at positions S37 and S38 of SEQ ID NO: 6.

[0684] Embodiment 76. The genetically engineered cell of any one of embodiments 60-75, wherein the engineered CD47 protein comprises amino acid substitutions at positions S82 and S83 of SEQ ID NO: 2.

[0685] Embodiment 77. The genetically engineered cell of any one of embodiments 60-76, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S82A relative to SEQ ID NO: 2,
- an amino acid substitution of S64A relative to SEQ ID NO: 3,
- an amino acid substitution of S82A relative to SEQ ID NO: 1, or
- an amino acid substitution of S37A relative to SEQ ID NO: 6.

[0686] Embodiment 78. The genetically engineered cell of any one of embodiments 60-77, wherein the engineered CD47 protein comprises an amino acid substitution of S82A relative to SEQ ID NO: 2.

[0687] Embodiment 79. The genetically engineered cell of any one of embodiments 60-78, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2,
- an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3,
- an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, or
- an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6.

[0688] Embodiment 80. The genetically engineered cell of any one of embodiments 60-79, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S83A relative to SEQ ID NO: 2,
- an amino acid substitution of S65A relative to SEQ ID NO: 3,
- an amino acid substitution of S83A relative to SEQ ID NO: 1, or
- an amino acid substitution of S38A relative to SEQ ID NO: 6.

[0689] Embodiment 81. The genetically engineered cell of any one of embodiments 60-80, wherein the engineered CD47 protein comprises an amino acid substitution of S83A relative to SEQ ID NO: 2.

[0690] Embodiment 82. The genetically engineered cell of any one of embodiments 60-81, wherein the engineered CD47 protein comprises:

- an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2,
- an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3,
- an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, or
- an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[0691] Embodiment 83. The genetically engineered cell of any one of embodiments 60-82, wherein the engineered CD47 protein comprises an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2

[0692] Embodiment 84. The genetically engineered cell of any one of embodiments 60-83, wherein the engineered CD47 protein comprises:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, and an amino acid substitution of S37A relative to SEQ ID NO: 6; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83A relative to SEQ ID NO: 2, an amino acid substitution of S65A relative to SEQ ID NO: 3, an amino acid substitution of S83A relative to SEQ ID NO: 1, and an amino acid substitution of S38A relative to SEQ ID NO: 6.

[0693] Embodiment 85. The genetically engineered cell of any one of embodiments 60-84, wherein the engineered CD47 protein comprises amino acid substitutions of S82A and S83A relative to SEQ ID NO: 2

[0694] Embodiment 86. The genetically engineered cell of any one of embodiments 60-85, wherein the engineered CD47 protein comprises:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2, an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3, an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, and an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2, an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3, an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, and an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[0695] Embodiment 87. The genetically engineered cell of any one of embodiments 60-86, wherein the engineered CD47 protein comprises:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2.

[0696] Embodiment 88. The genetically engineered cell of any one of embodiments 60-87, wherein the engineered CD47 protein comprises:

an amino acid sequence having at least 80% identity to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6; and

an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0697] Embodiment 89. The genetically engineered cell of any one of embodiments 60-88, wherein the engineered CD47 protein comprises:

an amino acid sequence having at least 80% identity to SEQ ID NO: 2; and

an insertion of one or more amino acids relative to SEQ ID NO: 2.

[0698] Embodiment 90. The genetically engineered cell of any one of embodiments 60-89, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0699] Embodiment 91. The genetically engineered cell of any one of embodiments 60-90, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 2.

[0700] Embodiment 92. The genetically engineered cell of any one of embodiments 60-91, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0701] Embodiment 93. The genetically engineered cell of any one of embodiments 60-92, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2

[0702] Embodiment 94. The genetically engineered cell of any one of embodiments 60-93, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

- [0703]** Embodiment 95. The genetically engineered cell of any one of embodiments 60-94, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2.
- [0704]** Embodiment 96. The genetically engineered cell of any one of embodiments 60-95, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.
- [0705]** Embodiment 97. The genetically engineered cell of any one of embodiments 60-96, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2.
- [0706]** Embodiment 98. The genetically engineered cell of any one of embodiments 60-97, wherein the engineered CD47 protein comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.
- [0707]** Embodiment 99. The genetically engineered cell of any one of embodiments 60-98, wherein the engineered CD47 protein comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2.
- [0708]** Embodiment 100. The genetically engineered cell of any one of embodiments 60-99, wherein the engineered CD47 protein comprises:
- an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2, or
 - an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 1 and an amino acid substitution of Q19P relative to SEQ ID NO: 1.
- [0709]** Embodiment 101. The genetically engineered cell of any one of embodiments 60-100, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2.
- [0710]** Embodiment 102. The genetically engineered cell of any one of embodiments 60-101, wherein the engineered CD47 protein comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.
- [0711]** Embodiment 103. The genetically engineered cell of any one of embodiments 60-102, wherein the engineered CD47 protein comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2.
- [0712]** Embodiment 104. The genetically engineered cell of any one of embodiments 60-103, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 346.
- [0713]** Embodiment 105. The genetically engineered cell of any one of embodiments 60-104, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 347.
- [0714]** Embodiment 106. The genetically engineered cell of any one of embodiments 60-105, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
- an amino acid sequence comprising SEQ ID NO: 20;
 - an amino acid sequence comprising SEQ ID NO: 348; and
 - an amino acid sequence comprising SEQ ID NO: 3.
- [0715]** Embodiment 107. The genetically engineered cell of any one of embodiments 60-106, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
- an amino acid sequence comprising SEQ ID NO: 20;
 - an amino acid sequence comprising SEQ ID NO: 348; and

an amino acid substitution of Q1P relative to SEQ ID NO: 3.

[0716] Embodiment 108. The genetically engineered cell of any one of embodiments 60-107, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:

an amino acid sequence comprising SEQ ID NO: 348; and

an amino acid sequence comprising SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.

[0717] Embodiment 109. The nucleic acid construct of any one of embodiments 60-108, wherein the engineered CD47 protein comprises an amino acid sequence comprising:

an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, or an amino acid substitution of S37A relative to SEQ ID NO: 6;

an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1; and an amino acid substitution of Q19P relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[0718] Embodiment 110. The genetically engineered cell of any one of embodiments 60-109, wherein the engineered CD47 protein comprises an amino acid sequence comprising:

an amino acid substitution of S82A relative to SEQ ID NO: 2;

an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2; and

an amino acid substitution of Q19P relative to SEQ ID NO: 2.

[0719] Embodiment 111. The genetically engineered cell of any one of embodiments 1-110, wherein the genetically engineered cell has cell surface expression of the engineered CD47 protein.

[0720] Embodiment 112. The genetically engineered cell of any one of embodiments 1-111, wherein the genetically engineered cell has decreased cell surface expression of a TCR as compared to a comparable cell that has not been genetically engineered.

[0721] Embodiment 113. The genetically engineered cell of any one of embodiments 1-112, wherein the genetically engineered cell has decreased cell surface expression of B2M as compared to a comparable cell that has not been genetically engineered.

[0722] Embodiment 114. The genetically engineered cell of any one of embodiments 1-113, wherein the genetically engineered cell has decreased expression of CIITA as compared to a comparable cell that has not been genetically engineered.

[0723] Embodiment 115. The genetically engineered cell of any one of embodiments 1-114, wherein the genetically engineered cell comprises a modification at a TCR locus, B2M locus, a TAP I locus, a NLRC5 locus, a CIITA locus, an HLA-A locus, an HLA-B locus, an HLA-C locus, an HLA-DP locus, an HLA-DM locus, an HLA-DOA locus, an HLA-DOB locus, an HLA-DQ locus, an HLA-DR locus, a RFX5 locus, a RFXANK locus, a RFXAP locus, an NFY-A locus, an NFY-B locus, an NFY-C locus, or a combination thereof.

[0724] Embodiment 116. The genetically engineered cell of any one of embodiments 1-115, wherein the genetically engineered cell has been genetically engineered to knock-out an HLA-A locus, an HLA-B locus, an HLA-C locus, or a combination thereof.

[0725] Embodiment 117. The genetically engineered cell of any one of embodiments 1-116, wherein the genetically engineered cell has been genetically engineered to knock-out an HLA-DM locus, an HLA-DO locus, an HLA-DP locus, an HLA-DQ locus, an HLA-DR locus, or a combination thereof.

[0726] Embodiment 118. The genetically engineered cell of any one of embodiments 1-117, wherein the genetically engineered cell has been genetically engineered to knock-out a TCR locus.

[0727] Embodiment 119. The genetically engineered cell of any one of embodiments 1-118, wherein the genetically engineered cell has been genetically engineered to knock-out a B2M locus.

[0728] Embodiment 120. The genetically engineered cell of any one of embodiments 1-119, wherein the genetically engineered cell has been genetically engineered to knock-out a CIITA locus.

[0729] Embodiment 121. The genetically engineered cell of any one of embodiments 1-120, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of:

(a) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or

(b) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[0730] Embodiment 122. The genetically engineered cell of any one of embodiments 1-121, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules.

[0731] Embodiment 123. The genetically engineered cell of any one of embodiments 1-122, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[0732] Embodiment 124. The genetically engineered cell of any one of embodiments 1-123, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of:

(a) one or more MHC class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and

(b) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[0733] Embodiment 125. The genetically engineered cell of any one of embodiments 1-124, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class I molecules.

[0734] Embodiment 126. The genetically engineered cell of any one of embodiments 1-125, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class I molecules.

[0735] Embodiment 127. The genetically engineered cell of any one of embodiments 1-126, wherein the one or more modifications reduce a function of the one or more MHC class I molecules, optionally wherein the function is antigen presentation.

[0736] Embodiment 128. The genetically engineered cell of any one of embodiments 1-127, wherein the one or more MHC class I molecules are one or more human leukocyte antigen (HLA) class I molecules.

[0737] Embodiment 129. The genetically engineered cell of embodiment 128, wherein the one or more HLA class I molecules are selected from the group consisting of HLA-A, HLA-B, and HLA-C.

[0738] Embodiment 130. The genetically engineered cell of any one of embodiments 1-127, wherein the one or more molecules that regulate expression of the one or more MHC class I molecules are selected from the group consisting of B2M, NLRC5 and TAP1.

[0739] Embodiment 131. The genetically engineered cell of any one of embodiments 1-127, wherein the one or more molecules that regulate cell surface trafficking of the one or more MHC class I molecules are selected from the group consisting of B2M and TAP1.

[0740] Embodiment 132. The genetically engineered cell of any one of embodiments 1-120, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class II molecules.

- [0741]** Embodiment 133. The genetically engineered cell of any one of embodiments 1-132, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class II molecules.
- [0742]** Embodiment 134. The genetically engineered cell of any one of embodiments 1-133, wherein the one or more modifications reduce a function of the one or more MHC class II molecules, optionally wherein the function is antigen presentation.
- [0743]** Embodiment 135. The genetically engineered cell of any one of embodiments 1-134, wherein the one or more MHC class II molecules are one or more human leukocyte antigen (HLA) class II molecules.
- [0744]** Embodiment 136. The genetically engineered cell of embodiment 135, wherein the one or more HLA class II molecules are selected from the group consisting of HLA-DP, HLA-DQ, and/or HLA-DR.
- [0745]** Embodiment 137. The genetically engineered cell of any one of embodiments 1-134, wherein the one or more molecules that regulate expression of the one or more MHC class II molecules is/are selected from the group consisting of CIITA and CD74.
- [0746]** Embodiment 138. The genetically engineered cell of any one of embodiments 1-137, wherein the engineered CD47 protein is encoded by a first transgene.
- [0747]** Embodiment 139. The genetically engineered cell of embodiment 138, comprising, in its genome, the first transgene at a first insertion site.
- [0748]** Embodiment 140. The genetically engineered cell of embodiment 139, wherein the first insertion site is a T-cell receptor (TCR) locus.
- [0749]** Embodiment 141. The genetically engineered cell of embodiment 140, wherein the TCR locus is or comprises: a TRAC locus, a TRBC1 locus, or a TRBC2 locus.
- [0750]** Embodiment 142. The genetically engineered cell of embodiment 140, wherein the first insertion site is a $\beta 2$ microglobulin (B2M) locus.
- [0751]** Embodiment 143. The genetically engineered cell of embodiment 140, wherein the first insertion site is a class II transactivator (CIITA) locus.
- [0752]** Embodiment 144. The genetically engineered cell of embodiment 140, wherein the first insertion site is a safe harbor locus.
- [0753]** Embodiment 145. The genetically engineered cell of embodiment 144, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.
- [0754]** Embodiment 146. The genetically engineered cell of any one of embodiments 140-145, wherein the first insertion site is an exon.
- [0755]** Embodiment 147. The genetically engineered cell of any one of embodiments 140-145, wherein the first insertion site is an intron.
- [0756]** Embodiment 148. The genetically engineered cell of any one of embodiments 140-145, wherein the first insertion site is between an intron and an exon.
- [0757]** Embodiment 149. The genetically engineered cell of any one of embodiments 140-145, wherein the first insertion site is in a regulatory region.
- [0758]** Embodiment 150. The genetically engineered cell of any one of embodiments 1-149, further comprising a second transgene encoding a tolerogenic factor.
- [0759]** Embodiment 151. The genetically engineered cell of embodiment 150, wherein the tolerogenic factor is or comprises A20/TNFAIP3, B2M-HLA-E, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, CD52, CD55,

CD59, CD64, CD200, CCL21, CCL22, CTLA4-Ig, C1 inhibitor, CR1, DUX4, FASL, HLA-C, HLA-E, HLA-E heavy chain, HLA-F, HLA-G, H2-M3, IDO1, IL-10, IL15-RF, IL-35, IL-39, MANF, Mfge8, PD-L1, or Serpinb9.

[0760] Embodiment 152. The genetically engineered cell of embodiment 150 or 151, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a TCR locus.

[0761] Embodiment 153. The genetically engineered cell of embodiment 150 or 151, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a B2M locus.

[0762] Embodiment 154. The genetically engineered cell of embodiment 150 or 151, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a CIITA locus.

[0763] Embodiment 155. The genetically engineered cell of embodiment 150 or 151, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a safe harbor locus.

[0764] Embodiment 156. The genetically engineered cell of embodiment 155, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

[0765] Embodiment 157. The genetically engineered cell of any one of embodiments 150-156, wherein the genetically engineered cell has cell surface expression of the tolerogenic factor.

[0766] Embodiment 158. The genetically engineered cell of any one of embodiments 1-157, further comprising a third transgene encoding a chimeric antigen receptor (CAR).

[0767] Embodiment 159. The genetically engineered cell of embodiment 158, wherein the CAR is or comprises a CD5-specific CAR, a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD23-specific CAR, a CD30-specific CAR, a CD33-specific CAR, CD38-specific CAR, a CD70-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a Kappa, Lambda, B cell maturation agent (BCMA)-specific CAR, a G-protein coupled receptor family C group 5 member D (GPRC5D)-specific CAR, a CD123-specific CAR, a LeY-specific CAR, a NKG2D ligand-specific CAR, a WT1-specific CAR, a GD2-specific CAR, a HER2-specific CAR, a EGFR-specific CAR, a EGFRvIII-specific CAR, a B7H3-specific CAR, a PSMA-specific CAR, a PSCA-specific CAR, a CAIX-specific CAR, a CD171-specific CAR, a CEA-specific CAR, a CSPG4-specific CAR, a EPHA2-specific CAR, a FAP-specific CAR, a FR α -specific CAR, a IL-13R α -specific CAR, a Mesothelin-specific CAR, a MUC1-specific CAR, a MUC16-specific CAR, a ROR1-specific CAR, a C-Met-specific CAR, a CD133-specific CAR, a Ep-CAM-specific CAR, a GPC3-specific CAR, a HPV16-E6-specific CAR, a IL13R α 2-specific CAR, a MAGEA3-specific CAR, a MAGEA4-specific CAR, a MART1-specific CAR, a NY-ESO-1-specific CAR, a VEGFR2-specific CAR, a α -Folate receptor-specific CAR, a CD24-specific CAR, a CD44v7/8-specific CAR, a EGP-2-specific CAR, a EGP-40-specific CAR, a erb-B2-specific CAR, a erb-B 2,3,4-specific CAR, a FBP-specific CAR, a Fetal acetylcholine e receptor-specific CAR, a GD2-specific CAR, a GD3-specific CAR, a HMW-MAA-specific CAR, a IL-11R α -specific CAR, a KDR-specific CAR, a Lewis Y-specific CAR, a L1-cell adhesion molecule-specific CAR, a MAGE-A1-specific CAR, a Oncofetal antigen (h5T4)-specific CAR, a TAG-72-specific CAR, or a CD19/CD22-bispecific CAR.

[0768] Embodiment 160. The genetically engineered cell of embodiment 158 or 159, wherein the CAR is or comprises a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD38-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a BCMA-specific CAR, or a CD19/CD22-bispecific CAR.

[0769] Embodiment 161. The genetically engineered cell of any one of embodiments 158-160, wherein the genetically engineered cell has cell surface expression of the CAR.

[0770] Embodiment 162. The genetically engineered cell of any one of embodiments 1-157, further comprising a third transgene encoding a chimeric auto antigen receptor (CAAR).

[0771] Embodiment 163. The genetically engineered cell of embodiment 162, wherein the CAAR comprises an antigen selected from the group consisting of a pancreatic β -cell antigen, synovial joint antigen, myelin basic protein,

proteolipid protein, myelin oligodendritic glycoprotein, MuSK, keratinocyte adhesion protein desmoglein 3 (Dsg3), Ro-RNP complex, La antigen, myeloperoxidase, proteinase 3, cardiolipin, citrullinated proteins, carbamylated proteins, and $\alpha 3$ chain of basement membrane collagen.

[0772] Embodiment 164. The genetically engineered cell of embodiment 162 or 163, wherein the genetically engineered cell has cell surface expression of the CAAR.

[0773] Embodiment 165. The genetically engineered cell of any one of embodiments 1-157, further comprising a third transgene encoding a B-cell autoantibody receptor (BAR).

[0774] Embodiment 166. The genetically engineered cell of embodiment 165, wherein the genetically engineered cell has cell surface expression of the BAR.

[0775] Embodiment 167. The genetically engineered cell of any one of embodiments 158-166, wherein the third transgene is inserted at a safe harbor locus.

[0776] Embodiment 168. The genetically engineered cell of any one of embodiments 158-167, wherein the third transgene is inserted at a TRAC locus, a TRBC1 locus, a TRBC2 locus, a B2M locus, a CIITA locus, a MICA locus, a MICB locus, or a safe harbor locus.

[0777] Embodiment 169. The genetically engineered cell of any one of embodiments 158-168, wherein the third transgene is inserted at an AAVS1 locus, an ABO locus, a CCR5 locus, a CLYBL locus, a CXCR4 locus, a F3 locus, a FUT1 locus, a HMGB1 locus, a KDM5D locus, a LRP1 locus, a RHD locus, a ROSA26 locus, or a SHS231 locus.

[0778] Embodiment 170. The genetically engineered cell of any one of embodiments 138-157, wherein the first transgene and the second transgene are inserted into the same insertion site.

[0779] Embodiment 171. The genetically engineered cell of any one of embodiments 138-157 or 158-169, wherein the first transgene and the third transgene are inserted into the same insertion site.

[0780] Embodiment 172. The genetically engineered cell of any one of embodiments 150-169, wherein the second transgene and the third transgene are inserted into the same insertion site.

[0781] Embodiment 173. The genetically engineered cell of any one of embodiments 138-169, wherein the first transgene, the second transgene, and the third transgene are inserted into the same insertion site.

[0782] Embodiment 174. The genetically engineered cell of any one of embodiments 138-173, wherein the first transgene, the second transgene, and the third transgene are encoded by three separate constructs.

[0783] Embodiment 175. The genetically engineered cell of any one of embodiments 138-173, wherein the first transgene, the second transgene, and the third transgene are encoded by two separate constructs.

[0784] Embodiment 176. The genetically engineered cell of any one of embodiments 138-173, wherein the first transgene, the second transgene, and the third transgene are encoded by a polycistronic construct.

[0785] Embodiment 177. The genetically engineered cell of any one of embodiments 138-176, wherein the first transgene and/or the second transgene and/or the third transgene encode a genome editing complex.

[0786] Embodiment 178. The genetically engineered cell of 177, wherein the genome editing complex comprises a genome targeting entity and a genome modifying entity.

[0787] Embodiment 179. The genetically engineered cell of 178, wherein the genome targeting entity is a nucleic acid-guided targeting entity.

[0788] Embodiment 180. The genetically engineered cell of embodiment 178 or 179, wherein the genome targeting entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc

finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

[0789] Embodiment 181. The genetically engineered cell of any one of embodiments 178-180, wherein the genome targeting entity is selected from the group consisting of: Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1, Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, dCas (D10A), dCas (H840A), dCas13a, dCas13b, or a functional portion thereof.

[0790] Embodiment 182. The genetically engineered cell of any one of embodiments 178-181, wherein the genome modifying entity cleaves, deaminates, nicks, polymerizes, interrogates, integrates, cuts, unwinds, breaks, alters, methylates, demethylates, or otherwise destabilizes a target locus.

[0791] Embodiment 183. The genetically engineered cell of any one of embodiments 178-182, wherein the genome modifying entity comprises a recombinase, integrase, transposase, endonuclease, exonuclease, nickase, helicase, DNA polymerase, RNA polymerase, reverse transcriptase, deaminase, flippase, methylase, demethylase, acetylase, a nucleic acid modifying protein, an RNA modifying protein, a DNA modifying protein, an Argonaute protein, an epigenetic modifying protein, a histone modifying protein, or a functional portion thereof.

[0792] Embodiment 184. The genetically engineered cell of any one of embodiments 178-183, wherein the genome modifying entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising the gRNA and the Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, a Cas nuclease, a core Cas protein, a TnpB nuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, base editing, prime editing, a Programmable Addition via Site-specific Targeting Elements (PASTE), or a functional portion thereof.

[0793] Embodiment 185. The genetically engineered cell of any one of embodiments 178-184, wherein the genome modifying entity is selected from the group consisting of Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1, Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, FokI, dCas (D10A), dCas (H840A), dCas13a, dCas13b, a base editor, a prime editor, a target-primed reverse transcription (TPRT) editor, APOBEC1, cytidine deaminase, adenosine deaminase, uracil glycosylase inhibitor (UGI), adenine base editors (ABE), cytosine base editors (CBE), reverse transcriptase, serine integrase, recombinase,

transposase, polymerase, adenine-to-thymine or "ATBE" (or thymine-to-adenine or "TABE") transversion base editor, ten-eleven translocation methylcytosine dioxygenases (TETs), TET1, TET3, TET1CD, histone acetyltransferase p300, histone methyltransferase SMYD3, histone methyltransferase PRDM9, H3K79 methyltransferase DOT1L, transcriptional repressor, or a functional portion thereof.

[0794] Embodiment 186. The genetically engineered cell of any one of embodiments 178-185, wherein the genome targeting entity and the genome modifying entity are different domains of a single polypeptide.

[0795] Embodiment 187. The genetically engineered cell of any one of embodiments 178-186, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are operably linked together.

[0796] Embodiment 188. The genetically engineered cell of any one of embodiments 178-186, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are not linked together.

[0797] Embodiment 189. The genetically engineered cell of any one of embodiments 178-188, wherein the genome editing complex comprises a guide nucleic acid having a targeting domain that is complementary to at least one target locus, optionally wherein the guide nucleic acid is a guide RNA (gRNA).

[0798] Embodiment 190. The genetically engineered cell of any one of embodiments 177-187, wherein the genome editing complex comprises an RNA-guided nuclease.

[0799] Embodiment 191. The genetically engineered cell of embodiment 190, wherein the RNA-guided nuclease comprises a Cas nuclease.

[0800] Embodiment 192. The genetically engineered cell of embodiment 191, wherein the genome editing complex comprises a ribonucleoprotein (RNP) complex comprising the Cas nuclease and the gRNA.

[0801] Embodiment 193. The genetically engineered cell of embodiment 191 or 192, wherein the Cas nuclease is a Type II or Type V Cas protein.

[0802] Embodiment 194. The genetically engineered cell of any one of embodiments 191-193, wherein the Cas nuclease is selected from the group consisting of Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr5, Cse1, Cse2, Csf1, Csm2, Csn2, Csx10, Csx11, Csy1, Csy2, Csy3, and Mad7.

[0803] Embodiment 195. The genetically engineered cell of any one of embodiments 1-194, wherein the genetically engineered cell is a human cell or a non-human animal cell.

[0804] Embodiment 196. The genetically engineered cell of embodiment 195, wherein the non-human animal cell is a porcine, bovine or ovine cell.

[0805] Embodiment 197. The genetically engineered cell of embodiment 195, wherein the genetically engineered cell is a human cell.

[0806] Embodiment 198. The genetically engineered cell of any one of embodiments 1-197, wherein the genetically engineered cell is a differentiated cell derived from a stem cell or a progenitor cell.

[0807] Embodiment 199. The genetically engineered cell of embodiment 198, wherein the stem cell is a pluripotent stem cell.

[0808] Embodiment 200. The genetically engineered cell of embodiment 199, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC).

[0809] Embodiment 201. The genetically engineered cell of embodiment 199, wherein the pluripotent stem cell is an embryonic stem cell (ESC).

- [0810]** Embodiment 202. The genetically engineered cell of any one of embodiments 1-201, wherein the cell is a primary cell isolated from a donor.
- [0811]** Embodiment 203. The genetically engineered cell of embodiment 202, wherein the donor is healthy and/or is not suspected of having a disease or condition at the time the primary cell is obtained from the donor.
- [0812]** Embodiment 204. The genetically engineered cell of any one of embodiments 1-203, wherein the genetically engineered cell is part of a population of primary cells isolated from multiple donors.
- [0813]** Embodiment 205. The genetically engineered cell of any one of embodiments 1-204, wherein the multiple donors are healthy and/or are not suspected of having a disease or condition at the time the primary cells are obtained from the donors.
- [0814]** Embodiment 206. The genetically engineered cell of any one of embodiments 1-205, wherein the cell is an islet cell, beta islet cell, pancreatic islet cell, immune cell, B cell, T cell, natural killer (NK) cell, natural killer T (NKT) cell, macrophage cell, endothelial cell, muscle cell, cardiac muscle cell, smooth muscle cell, skeletal muscle cell, dopaminergic neuron, retinal pigmented epithelium cell, optic cell, hepatocyte, thyroid cell, skin cell, glial progenitor cell, neural cell, cardiac cell, stem cell, hematopoietic stem cell, induced pluripotent stem cell (iPSC), mesenchymal stem cell (MSC), embryonic stem cell (ESC), pluripotent stem cell (PSC), blood cell, or a combination thereof.
- [0815]** Embodiment 207. The genetically engineered cell of any one of embodiments 1-206, wherein the cell is a T-cell.
- [0816]** Embodiment 208. The genetically engineered cell of embodiment 207, wherein the T-cell is a CD3+ T cell, CD4+ T cell, CD8+ T cell, naive T cell, regulatory T (Treg) cell, non-regulatory T cell, Th1 cell, Th2 cell, Th9 cell, Th17 cell, T-follicular helper (Tfh) cell, cytotoxic T lymphocyte (CTL), effector T (Teff) cell, central memory T cell, effector memory T cell, effector memory T cell expressing CD45RA (TEMRA cell), tissue-resident memory (Trm) cell, virtual memory T cell, innate memory T cell, memory stem cell (Tse), $\gamma\delta$ T cell, or a combination thereof.
- [0817]** Embodiment 209. The genetically engineered cell of embodiment 207 or 208, wherein the T cell is a cytotoxic T-cell, helper T-cell, memory T-cell, regulatory T-cell, tumor infiltrating lymphocyte, or a combination thereof.
- [0818]** Embodiment 210. The genetically engineered cell of any one of embodiments 207-209, wherein the cell is a human T-cell.
- [0819]** Embodiment 211. The genetically engineered cell of any one of embodiments 207-210, wherein the cell is an autologous T-cell.
- [0820]** Embodiment 212. The genetically engineered cell of any one of embodiments 207-210, wherein the cell is an allogeneic T-cell.
- [0821]** Embodiment 213. The genetically engineered cell of embodiment 212, wherein the allogeneic T-cell is a primary T cell.
- [0822]** Embodiment 214. The genetically engineered cell of 212 or 213, wherein the allogeneic T cell has been differentiated from an embryonic stem cell (ESC) or an induced pluripotent stem cell (iPSC).
- [0823]** Embodiment 215. The genetically engineered cell of any one of embodiments 207-209, wherein the cell is an islet cell, beta islet cell, or pancreatic islet cell.
- [0824]** Embodiment 216. The genetically engineered cell of embodiment 215, wherein the cell is an autologous islet cell, an autologous beta islet cell, or an autologous pancreatic islet cell.
- [0825]** Embodiment 217. The genetically engineered cell of embodiment 216, wherein the cell is an allogeneic islet cell, an allogeneic beta islet cell, or an allogeneic pancreatic islet cell.

[0826] Embodiment 218. The genetically engineered cell of embodiment 217, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell is a primary islet cell, a primary beta islet cell, or a primary pancreatic islet cell.

[0827] Embodiment 219. The genetically engineered cell of embodiment 217 or embodiment 218, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell has been differentiated from an embryonic stem cell (ESC) or an induced pluripotent stem cell (iPSC).

[0828] Embodiment 220. The genetically engineered cell of any one of embodiments 31-214, wherein the cell expressing SIRPa is an immune cell.

[0829] Embodiment 221. The genetically engineered cell of embodiment 220, wherein the immune cell is a natural killer (NK) cell, a monocyte, or a macrophage.

[0830] Embodiment 222. The genetically engineered cell of any one of embodiments 31-214, wherein the cell expressing SIRPa is a neural cell.

[0831] Embodiment 223. The genetically engineered cell of embodiment 222, wherein the neural cell is a microglia.

[0832] Embodiment 224. A composition comprising the genetically engineered cell of any one of embodiments 1-221.

[0833] Embodiment 225. A pharmaceutical composition comprising (i) the genetically engineered cell of any one of embodiments 1-221, and (ii) a pharmaceutically acceptable excipient.

[0834] Embodiment 226. A method comprising administering to a subject the genetically engineered cell of any one of embodiments 1-221, the composition of embodiment 224, or the pharmaceutical composition of embodiment 225.

[0835] Embodiment 227. The method of embodiment 226, wherein the method is a method of treating a disease in a subject.

[0836] Embodiment 228. A method of treating a disease in a subject, the method comprising administering to the subject the genetically engineered cell of any one of embodiments 1-221, the composition of embodiment 224, or the pharmaceutical composition of embodiment 225.

[0837] Embodiment 229. A population of cells comprising the genetically engineered cell of any one of embodiments 1-221 for use in treating a disease in a subject.

[0838] Embodiment 230. The composition of embodiment 224 for use in treating a disease in a subject.

[0839] Embodiment 231. The pharmaceutical composition of embodiment 225 for use in treating a disease in a subject.

[0840] Embodiment 232. Use of the genetically modified cell of any one of embodiments 1-221, the population of cells of embodiment 229, the composition of embodiment 224 or 230, or the pharmaceutical composition of embodiment 225 or 231 for use in treating a disease in a subject.

[0841] Embodiment 233. Use of the genetically modified cell of any one of embodiments 1-221, the population of cells of embodiment 229, the composition of embodiment 224 or 230, or the pharmaceutical composition of embodiment 225 or 231 in the manufacture of a medicament for the treatment of a disease.

[0842] Embodiment 234. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any of the preceding embodiments, wherein the disease is cancer.

[0843] Embodiment 235. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any of the preceding embodiments, wherein the cancer is

associated with CD5, CD19, CD20, CD22, CD23, CD30, CD33, CD70, Kappa, Lambda, B cell maturation agent (BCMA), G-protein coupled receptor family C group 5 member D (GPC5D), CD123, LeY, NKG2D ligand, WT1, GD2, HER2, EGFR, EGFRvIII, B7H3, PSMA, PSCA, CAIX, CD171, CEA, CSPG4, EPHA2, FAP, FRα, IL-13Rα, Mesothelin, MUC1, MUC16, ROR1, C-Met, CD133, Ep-CAM, GPC3, HPV16-E6, IL13Rα2, MAGEA3, MAGEA4, MART1, NY-ESO-1, VEGFR2, α-Folate receptor, CD24, CD44v7/8, EGP-2, EGP-40, erb-B2, erb-B 2,3,4, FBP, Fetal acetylcholine receptor, GD2, GD3, HMW-MAA, IL-11Rα, KDR, Lewis Y, L1-cell adhesion molecule, MAGE-A1, Oncofetal antigen (h5T4), and/or TAG-72 expression.

[0844] Embodiment 236. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any of the preceding embodiments, wherein the cancer is a hematologic malignancy.

[0845] Embodiment 237. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any of the preceding embodiments, wherein the hematologic malignancy is selected from the group consisting of myeloid neoplasm, myelodysplastic syndromes (MDS), myeloproliferative/myelodysplastic syndromes, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), blast crisis chronic myelogenous leukemia (bcCML), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), T-cell lymphoma, and B-cell lymphoma.

[0846] Embodiment 238. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any of the preceding embodiments, wherein the cancer is solid malignancy.

[0847] Embodiment 239. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any of the preceding embodiments, wherein the solid malignancy is selected breast cancer, ovarian cancer, colon cancer, prostate cancer, epithelial cancer, renal-cell carcinoma, pancreatic adenocarcinoma, cervical carcinoma, colorectal cancer, glioblastoma, rhabdomyosarcoma, neuroblastoma, melanoma, Ewing sarcoma, osteosarcoma, mesothelioma and adenocarcinoma.

[0848] Embodiment 240. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any one of embodiments 1-233, wherein the disease is an autoimmune disease.

[0849] Embodiment 241. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of embodiment 240, wherein the autoimmune disease is selected from the group consisting of lupus, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, and celiac disease.

[0850] Embodiment 242. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any one of embodiments 1-233, wherein the disease is diabetes mellitus.

[0851] Embodiment 243. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition or the use of embodiment 242, wherein the diabetes is selected from the group consisting Type I diabetes, Type II diabetes, prediabetes, and gestational diabetes.

[0852] Embodiment 244. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of any one of embodiments 1-233, wherein the disease is a neurological disease.

- [0853]** Embodiment 245. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the method or the use of embodiment 244, wherein the neurological disease is selected from the group consisting of catalepsy, epilepsy, encephalitis, meningitis, migraine, Huntington's, Alzheimer's, Parkinson's, Pelizaeus-Merzbacher disease, and multiple sclerosis.
- [0854]** Embodiment 246. A method of making a genetically engineered cell comprising an engineered CD47 protein and a nucleic acid construct encoding the engineered CD47 protein, the method comprising:
- a. delivering to a cell a nucleic acid construct comprising a first transgene encoding the engineered CD47 protein, thereby making a genetically engineered cell.
- [0855]** Embodiment 247. The method of embodiment 246, wherein the method comprises inserting the first transgene encoding the engineered CD47 protein into a first insertion site in the genome of the cell.
- [0856]** Embodiment 248. The method of embodiment 247, wherein the first insertion site is a T-cell receptor (TCR) locus.
- [0857]** Embodiment 249. The method of embodiment 248, wherein the TCR locus is or comprises: a TRAC locus, a TRBC1 locus, or a TRBC2 locus.
- [0858]** Embodiment 250. The method of embodiment 247, wherein the first insertion site is a $\beta 2$ microglobulin (B2M) locus.
- [0859]** Embodiment 251. The method of embodiment 247, wherein the first insertion site is a class II transactivator (CIITA) locus.
- [0860]** Embodiment 252. The method of embodiment 247, wherein the first insertion site is a safe harbor locus.
- [0861]** Embodiment 253. The method of embodiment 146, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.
- [0862]** Embodiment 254. The method of any one of embodiments 247-253, wherein the first insertion site is an exon.
- [0863]** Embodiment 255. The method of any one of embodiments 247-253, wherein the first insertion site is an intron.
- [0864]** Embodiment 256. The method of any one of embodiments 247-253, wherein the first insertion site is between an intron and an exon.
- [0865]** Embodiment 257. The method of any one of embodiments 247-253, wherein the first insertion site is in a regulatory region.
- [0866]** Embodiment 258. The method of any one of embodiments 247-257, wherein the step of inserting comprises insertion using a gene therapy vector, transposase, lentiviral vector, retrovirus, fusosome, PiggyBac transposon, Sleeping Beauty (SB11) transposon, Mos1 transposon, or Tol2 transposon.
- [0867]** Embodiment 259. The method of any one of embodiments 247-258, wherein the step of inserting comprises insertion using a lentiviral vector.
- [0868]** Embodiment 260. The method of any one of embodiments 247-257, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using a genome-modifying protein.
- [0869]** Embodiment 261. The method of any one of embodiments 246-260, further comprising introducing into the cell a genome editing complex.
- [0870]** Embodiment 262. The method of embodiment 261, wherein the genome editing complex comprises a genome targeting entity and a genome modifying entity.

[0871] Embodiment 263. The method of embodiment 262, wherein the genome targeting entity localizes the genome editing complex to a safe harbor site, optionally wherein the genome targeting entity is a nucleic acid-guided targeting entity.

[0872] Embodiment 264. The method of 262 or 263, wherein the genome targeting entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

[0873] Embodiment 265. The method of any one of embodiments 262-264, wherein the genome targeting entity is selected from the group consisting of Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1, Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, dCas (D10A), dCas (H840A), dCas13a, dCas13b, or a functional portion thereof.

[0874] Embodiment 266. The method of any one of embodiments 262-265, wherein the genome modifying entity cleaves, deaminates, nicks, polymerizes, interrogates, integrates, cuts, unwinds, breaks, alters, methylates, demethylates, or otherwise destabilizes the target locus.

[0875] Embodiment 267. The method of any one of embodiments 262-266, wherein the genome modifying entity comprises a recombinase, integrase, transposase, endonuclease, exonuclease, nickase, helicase, DNA polymerase, RNA polymerase, reverse transcriptase, deaminase, flippase, methylase, demethylase, acetylase, a nucleic acid modifying protein, an RNA modifying protein, a DNA modifying protein, an Argonaute protein, an epigenetic modifying protein, a histone modifying protein, or a functional portion thereof.

[0876] Embodiment 268. The method of any one of embodiments 262-267, wherein the genome modifying entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising the gRNA and the Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, a Cas nuclease, a core Cas protein, a TnpB nuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, base editing, prime editing, a Programmable Addition via Site-specific Targeting Elements (PASTE), or a functional portion thereof.

[0877] Embodiment 269. The method of any one of embodiments 262-268, wherein the genome modifying entity is selected from the group consisting of Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1,

Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, FokI, dCas (D10A), dCas (H840A), dCas13a, dCas13b, a base editor, a prime editor, a target-primed reverse transcription (TPRT) editor, APOBEC1, cytidine deaminase, adenosine deaminase, uracil glycosylase inhibitor (UGI), adenine base editors (ABE), cytosine base editors (CBE), reverse transcriptase, serine integrase, recombinase, transposase, polymerase, adenine-to-thymine or "ATBE" (or thymine-to-adenine or "TABE") transversion base editor, ten-eleven translocation methylcytosine dioxygenases (TETs), TET1, TET3, TET1CD, histone acetyltransferase p300, histone methyltransferase SMYD3, histone methyltransferase PRDM9, H3K79 methyltransferase DOT1L, transcriptional repressor, or a functional portion thereof.

[0878] Embodiment 270. The method of any one of embodiments 262-269, wherein the genome targeting entity and the genome modifying entity are different domains of a single polypeptide.

[0879] Embodiment 271. The method of any one of embodiments 262-270, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are operably linked together.

[0880] Embodiment 272. The method of any one of embodiments 262-271, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are not linked together.

[0881] Embodiment 273. The method of any one of embodiments 261-272, wherein the genome editing complex comprises a guide nucleic acid having a targeting domain that is complementary to at least one sequence within the genomic safe harbor site, optionally wherein the guide nucleic acid is a guide RNA (gRNA).

[0882] Embodiment 274. The method of any one of embodiments 261-273, wherein the genome editing complex comprises an RNA-guided nuclease.

[0883] Embodiment 275. The method of embodiment 274, wherein the RNA-guided nuclease comprises a Cas nuclease.

[0884] Embodiment 276. The method of embodiment 275, wherein the genome editing complex comprises a ribonucleoprotein (RNP) complex comprising the Cas nuclease and the gRNA.

[0885] Embodiment 277. The method of embodiment 275 or 276, wherein the Cas nuclease is a Type II or Type V Cas protein.

[0886] Embodiment 278. The method of any one of embodiments 275-277, wherein the Cas nuclease is selected from the group consisting of Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr5, Cse1, Cse2, Csf1, Csm2, Csn2, Csx10, Csx11, Csy1, Csy2, Csy3, and Mad7.

[0887] Embodiment 279. The method of embodiment 264, wherein the gRNA comprises a complementary region, wherein the complementary region comprises a nucleic acid sequence that is complementary to a target nucleic acid sequence within a gene locus, and wherein the target nucleic acid sequence comprises the insertion site.

[0888] Embodiment 280. The method of embodiment 279, wherein the gene locus is a TCR locus.

[0889] Embodiment 281. The method of embodiment 279, wherein the gene locus is a B2M locus.

[0890] Embodiment 282. The method of embodiment 279, wherein the gene locus is a CIITA locus.

[0891] Embodiment 283. The method of any of embodiments 247-282, wherein the first insertion site is 25 nucleotides or less from a protospacer adjacent motif (PAM) sequence, wherein the PAM sequence is ngg, nag, ngrt, ngrrn, nnnngatt, nnnnyac, nnagaaw, naaaac, ttv, ttn, attn, ttnn, gttn, or ytnn and wherein:

(i) r = a or g,

- (ii) y = c or t,
- (iii) w = a or t,
- (iv) v = a or c or g, and
- (v) n = a, c, t, or g.

[0892] Embodiment 284. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using SpCas9 and the PAM is ngg or nag, wherein n= a, c, t, or g.

[0893] Embodiment 285. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using SaCas9 and the PAM is ngrt or ngrn, wherein:

- (i) r = a or g, and
- (ii) n = a, c, t, or g.

[0894] Embodiment 286. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using NmeCas9 and the PAM is nnnngatt, wherein n = a, c, t, or g.

[0895] Embodiment 287. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using CjCas9 and the PAM is nnnnyac, wherein:

- (i) r = a or g,
- (ii) y = c or t, and
- (iii) n= a, c, t, or g.

[0896] Embodiment 288. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using StCas9 and the PAM is nnagaaw wherein:

- (i) w = a or t, and
- (ii) n= a, c, t, or g.

[0897] Embodiment 289. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TdCas9 and the PAM is naaac, wherein n= a, c, t, or g.

[0898] Embodiment 290. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using LbCas12a and the PAM is ttv, wherein v = a or c or g.

[0899] Embodiment 291. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using AsCas12a and the PAM is ttv, wherein v = a or c or g.

[0900] Embodiment 292. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using AacCas12b and the PAM is ttn, wherein n= a, c, t, or g.

[0901] Embodiment 293. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using BhCas12b and the PAM is attn., tttn, or gttn, wherein n= a, c, t, or g.

[0902] Embodiment 294. The method of any one of embodiments 247-283, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using MAD7 (ErCas12a) and the PAM is yttn, wherein:

- (i) y= c or t, and

(ii) $n = a, c, t, \text{ or } g.$

[0903] Embodiment 295. The method of any one of embodiments 247, 265, or 284-294, wherein homology-directed repair (HDR)-mediated insertion using a site-directed nuclease is performed with an HDR efficiency equal to or greater than HDR insertion using lentivirus.

[0904] Embodiment 296. The method of any one of embodiments 247-260, 262, 263, or 264, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using ZFN.

[0905] Embodiment 297. The method of any one of embodiments 247-260, 262, 263, 264, or 190, wherein the first insertion site is 25 nucleotides or less from a zinc finger binding sequence.

[0906] Embodiment 298. The method of any one of embodiments 247-260, 262, 263, or 264, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TALEN.

[0907] Embodiment 299. The method of any one of embodiments 247-260, 262, 263, 264, or 298, wherein the first insertion site is 25 nucleotides or less from a transcription activator-like effectors (TALE) binding sequence.

[0908] Embodiment 300. The method of any one of embodiments 247-262 or 264, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using a guide RNA (gRNA) and a TnpB polypeptide.

[0909] Embodiment 301. The method of embodiment 300, wherein the gRNA comprises a complementary region,

wherein the complementary region comprises a nucleic acid sequence that is complementary to a target nucleic acid sequence within a gene locus, and wherein the target nucleic acid sequence comprises the insertion site.

[0910] Embodiment 302. The method of embodiment 301, wherein the gene locus is a TCR locus.

[0911] Embodiment 303. The method of embodiment 301, wherein the gene locus is a B2M locus.

[0912] Embodiment 304. The method of embodiment 301, wherein the gene locus is a CIITA locus.

[0913] Embodiment 305. The method of embodiment 301, wherein the gene locus is a safe harbor locus.

[0914] Embodiment 306. The method of embodiment 305, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

[0915] Embodiment 307. The method of any one of embodiments 300-306, wherein the insertion site is 25 nucleotides or less from a target adjacent motif (TAM) sequence, wherein the TAM sequence is tca, tcac, tcag, tcac, tcaa, ttcan, ttaa, ttacg, or ttgat, and wherein:

(i) $n = a, c, t, \text{ or } g.$

[0916] Embodiment 308. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tca.

[0917] Embodiment 309. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcac.

[0918] Embodiment 310. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcag.

[0919] Embodiment 311. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcac.

[0920] Embodiment 312. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcaa.

[0921] Embodiment 313. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is ttcan, wherein n= a, c, t, or g.

[0922] Embodiment 314. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is ttcaa.

[0923] Embodiment 315. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is ttcat.

[0924] Embodiment 316. The method of any one of embodiments 300-307, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is ttgat.

[0925] Embodiment 317. The method of embodiment 246, wherein the step of delivering the nucleic acid construct to the cell comprises viral transduction with a retrovirus or an adeno-associated virus (AAV) vector.

[0926] Embodiment 318. The method of embodiment 317, wherein the retrovirus is or comprises Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV), Rous Sarcoma Virus (RSV), a lentivirus, a Gammaretrovirus, an Epsilonretrovirus, an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, or a Spumaretrovirus.

[0927] Embodiment 319. The method of embodiment 317, wherein the AAV vector is an AAV6 vector or an AAV9 vector.

[0928] Embodiment 320. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, wherein the first transgene encoding the engineered protein at the insertion site at a TCR locus reduces expression of a functional TCR.

[0929] Embodiment 321. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-320, wherein the first transgene encoding the engineered protein at the insertion site at a TCR locus disrupts expression of a functional TCR.

[0930] Embodiment 322. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, wherein the first transgene encoding the engineered protein at the insertion site at a B2M locus reduces expression of a functional B2M.

[0931] Embodiment 323. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, or 322, wherein the first transgene encoding the engineered protein at the insertion site at a B2M locus reduces expression of a functional MHC I molecule.

[0932] Embodiment 324. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, or 322-323, wherein the first transgene encoding the engineered protein at the insertion site at a B2M locus disrupts expression of a functional B2M.

[0933] Embodiment 325. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, or 322-324, wherein the first transgene encoding the engineered protein at the insertion site at a B2M gene locus disrupts expression of a functional MHC I molecule.

[0934] Embodiment 326. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA locus reduces expression of a functional CIITA.

[0935] Embodiment 327. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, or 326, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA locus reduces expression of a functional MHC II molecule.

[0936] Embodiment 328. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, or 326-327, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA locus disrupts expression of a functional CIITA.

[0937] Embodiment 329. The method of any one of embodiments 247, 248, 249, 260-280, 283-302, or 307-319, or 326-328, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA gene locus disrupts expression of a functional MHC II molecule.

[0938] Embodiment 330. The method of any one of embodiments 246-329, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of:

one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or

one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[0939] Embodiment 331. The method of any one of embodiments 246-330, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules.

[0940] Embodiment 332. The method of any one of embodiments 246-331, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[0941] Embodiment 333. The method of any one of embodiments 246-332, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of:

one or more MHC class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and

one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[0942] Embodiment 334. The method of any one of embodiments 330-333, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class I molecules.

[0943] Embodiment 335. The method of any one of embodiments 330-334, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class I molecules.

[0944] Embodiment 336. The method of any one of embodiments 330-335, wherein the one or more modifications reduce a function of the one or more MHC class I molecules, optionally wherein the function is antigen presentation.

[0945] Embodiment 337. The method of any one of embodiments 330-336, wherein the one or more MHC class I molecules are one or more human leukocyte antigen (HLA) class I molecules.

[0946] Embodiment 338. The method of embodiment 337, wherein the one or more HLA class I molecules are selected from the group consisting of HLA-A, HLA-B, and HLA-C.

[0947] Embodiment 339. The method of any one of embodiments 330-338, wherein the one or more molecules that regulate expression of the one or more MHC class I molecules are selected from the group consisting of B2M, NLRC5 and TAP1.

- [0948]** Embodiment 340. The method of any one of embodiments 330-338, wherein the one or more molecules that regulate cell surface trafficking of the one or more MHC class I molecules are selected from the group consisting of B2M and TAP1.
- [0949]** Embodiment 341. The method of any one of embodiments 330-339, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class II molecules.
- [0950]** Embodiment 342. The method of any one of embodiments 330-341, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class II molecules.
- [0951]** Embodiment 343. The method of any one of embodiments 330-342, wherein the one or more modifications reduce a function of the one or more MHC class II molecules, optionally wherein the function is antigen presentation.
- [0952]** Embodiment 344. The method of any one of embodiments 330-343, wherein the one or more MHC class II molecules are one or more human leukocyte antigen (HLA) class II molecules.
- [0953]** Embodiment 345. The method of embodiment 344, wherein the one or more HLA class II molecules are selected from the group consisting of HLA-DP, HLA-DQ, and/or HLA-DR.
- [0954]** Embodiment 346. The method of any one of embodiments 330-345, wherein the one or more molecules that regulate expression of the one or more MHC class II molecules is/are selected from the group consisting of CIITA and CD74.
- [0955]** Embodiment 347. The method of any one of embodiments 247-346, wherein the first transgene encoding the engineered protein at the insertion site at a safe harbor locus disrupts expression of a functional AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231.
- [0956]** Embodiment 348. The method of any one of embodiments 247-346, wherein the first transgene encoding the engineered protein at the insertion site at a safe harbor locus disrupts expression of a functional AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 molecule.
- [0957]** Embodiment 349. The method of any one of embodiments 246-348, wherein the genetically engineered cell has cell surface expression of the engineered protein.
- [0958]** Embodiment 350. The method of any one of embodiments 246-349, wherein the nucleic acid construct further comprises a second transgene encoding a tolerogenic factor.
- [0959]** Embodiment 351. The method of embodiment 350, wherein the method further comprises inserting the second transgene into a second insertion site in the genome of the cell.
- [0960]** Embodiment 352. The method of embodiment 350, wherein the tolerogenic factor is or comprises A20/TNFAIP3, B2M-HLA-E, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, CD52, CD55, CD59, CD64, CD200, CCL21, CCL22, CTLA4-Ig, C1 inhibitor, CR1, DUX4, FASL, HLA-C, HLA-E, HLA-E heavy chain, HLA-F, HLA-G, H2-M3, IDO1, IL-10, IL15-RF, IL-35, IL-39, MANF, Mfge8, PD-L1, or Serpinb9.
- [0961]** Embodiment 353. The method of any one of embodiments 350-352, wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a TCR locus.
- [0962]** Embodiment 354. The method of any one of embodiments 350-352, wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a B2M locus.
- [0963]** Embodiment 355. The method of any one of embodiments 350-352, wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a CIITA locus.
- [0964]** Embodiment 356. The method of any one of embodiments 350-352, wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a safe harbor locus.

[0965] Embodiment 357. The method of embodiment 356, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

[0966] Embodiment 358. The method of any one of embodiments 350-357, wherein the genetically engineered cell has cell surface expression of the tolerogenic factor.

[0967] Embodiment 359. The method of any one of embodiments 246-358, wherein the nucleic acid construct further comprises a third transgene encoding a chimeric antigen receptor (CAR).

[0968] Embodiment 360. The method of embodiment 359, wherein the method further comprises inserting the third transgene into a third insertion site in the genome of the cell.

[0969] Embodiment 361. The method of embodiment 359, wherein the CAR is or comprises a CD5-specific CAR, a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD23-specific CAR, a CD30-specific CAR, a CD33-specific CAR, CD38-specific CAR, a CD70-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a Kappa, Lambda, B cell maturation agent (BCMA)-specific CAR, a G-protein coupled receptor family C group 5 member D (GPRC5D)-specific CAR, a CD123-specific CAR, a LeY-specific CAR, a NKG2D ligand-specific CAR, a WT1-specific CAR, a GD2-specific CAR, a HER2-specific CAR, a EGFR-specific CAR, a EGFRvIII-specific CAR, a B7H3-specific CAR, a PSMA-specific CAR, a PSCA-specific CAR, a CAIX-specific CAR, a CD171-specific CAR, a CEA-specific CAR, a CSPG4-specific CAR, a EPHA2-specific CAR, a FAP-specific CAR, a FR α -specific CAR, a IL-13R α -specific CAR, a Mesothelin-specific CAR, a MUC1-specific CAR, a MUC16-specific CAR, a ROR1-specific CAR, a C-Met-specific CAR, a CD133-specific CAR, a Ep-CAM-specific CAR, a GPC3-specific CAR, a HPV16-E6-specific CAR, a IL13Ra2-specific CAR, a MAGEA3-specific CAR, a MAGEA4-specific CAR, a MART1-specific CAR, a NY-ESO-1-specific CAR, a VEGFR2-specific CAR, a α -Folate receptor-specific CAR, a CD24-specific CAR, a CD44v7/8-specific CAR, a EGP-2-specific CAR, a EGP-40-specific CAR, a erb-B2-specific CAR, a erb-B 2,3,4-specific CAR, a FBP-specific CAR, a Fetal acetylcholine e receptor-specific CAR, a GD2-specific CAR, a GD3-specific CAR, a HMW-MAA-specific CAR, a IL-11R α -specific CAR, a KDR-specific CAR, a Lewis Y-specific CAR, a L1-cell adhesion molecule-specific CAR, a MAGE-A1-specific CAR, a Oncofetal antigen (h5T4)-specific CAR, a TAG-72-specific CAR, or a CD19/CD22-bispecific CAR.

[0970] Embodiment 362. The method of any one of embodiments 359-361, wherein the CAR is or comprises a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD38-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a BCMA-specific CAR, or a CD19/CD22-bispecific CAR.

[0971] Embodiment 363. The method of any one of embodiments 359-362, wherein the genetically engineered cell has cell surface expression of the CAR.

[0972] Embodiment 364. The method of any one of embodiments 246-358, wherein the nucleic acid construct further comprises a third transgene encoding a chimeric auto antigen receptor (CAAR).

[0973] Embodiment 365. The method of embodiment 364, wherein the method further comprises inserting the third transgene into a third insertion site in the genome of the cell.

[0974] Embodiment 366. The method of embodiment 364, wherein the CAAR comprises an antigen selected from the group consisting of a pancreatic β -cell antigen, synovial joint antigen, myelin basic protein, proteolipid protein, myelin oligodendritic glycoprotein, MuSK, keratinocyte adhesion protien desmoglein 3 (Dsg3), Ro-RNP complex, La antigen, myeloperoxidase, proteinase 3, cardiolipin, citrullinated proteins, carbamylated proteins, and α 3 chain of basement membrane collagen.

[0975] Embodiment 367. The method of any one of embodiments 364-366, wherein the genetically engineered cell has cell surface expression of the CAAR.

- [0976] Embodiment 368. The method of any one of embodiments 246-358, wherein the nucleic acid construct further comprises a third transgene encoding a B-cell autoantibody receptor (BAR).
- [0977] Embodiment 369. The method of embodiment 368, wherein the method further comprises inserting the third transgene into a third insertion site in the genome of the cell.
- [0978] Embodiment 370. The method of embodiment 368, wherein the genetically engineered cell has cell surface expression of the BAR.
- [0979] Embodiment 371. The method of any one of embodiments 359-370, wherein the third transgene is inserted at a safe harbor locus.
- [0980] Embodiment 372. The method of any one of embodiments 359-371, wherein the third transgene is inserted at a TRAC locus, a TRBC1 locus, a TRBC2 locus, a B2M locus, a CIITA locus, a MICA locus, a MICB locus, or a safe harbor locus.
- [0981] Embodiment 373. The method of any one of embodiments 359-372, wherein the third transgene is inserted at an AAVS1 locus, an ABO locus, a CCR5 locus, a CLYBL locus, a CXCR4 locus, a F3 locus, a FUT1 locus, a HMGB1 locus, a KDM5D locus, a LRP1 locus, a RHD locus, a ROSA26 locus, or a SHS231 locus.
- [0982] Embodiment 374. The method of any one of embodiments 246-373, wherein the first transgene and the second transgene are inserted into the same insertion site.
- [0983] Embodiment 375. The method of any one of embodiments 246-356 or 368-266, wherein the first transgene and the third transgene are inserted into the same insertion site.
- [0984] Embodiment 376. The method of any one of embodiments 350-373, wherein the second transgene and the third transgene are inserted into the same insertion site.
- [0985] Embodiment 377. The method of any one of embodiments 246-373, wherein the first transgene, the second transgene, and the third transgene are inserted into the same insertion site.
- [0986] Embodiment 378. The method of any one of embodiments 246-377, wherein the first transgene, the second transgene, and the third transgene are encoded by three separate constructs.
- [0987] Embodiment 379. The method of any one of embodiments 246-377, wherein the first transgene, the second transgene, and the third transgene are encoded by two separate constructs.
- [0988] Embodiment 380. The method of any one of embodiments 246-377, wherein the first transgene, the second transgene, and the third transgene are encoded by a polycistronic construct.
- [0989] Embodiment 381. The method of any one of embodiments 246-380, wherein the genetically engineered cell is a human cell or a non-human animal cell.
- [0990] Embodiment 382. The method of embodiment 381, wherein the non-human animal cell is a porcine, bovine or ovine cell.
- [0991] Embodiment 383. The method of embodiment 381, wherein the genetically engineered cell is a human cell.
- [0992] Embodiment 384. The method of any one of embodiments 246-383, wherein the genetically engineered cell is a differentiated cell derived from a stem cell or a progenitor cell.
- [0993] Embodiment 385. The method of any one of embodiments 246-384, wherein the cell is a stem cell or a progenitor cell.
- [0994] Embodiment 386. The method of any one of embodiments 246-384, wherein the cell is a differentiated cell derived from a stem cell or a progenitor cell.
- [0995] Embodiment 387. The method of embodiment 384, wherein the stem cell is a pluripotent stem cell.

- [0996]** Embodiment 388. The method of embodiment 384, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC).
- [0997]** Embodiment 389. The method of embodiment 387, wherein the pluripotent stem cell is an embryonic stem cell (ESC).
- [0998]** Embodiment 390. The method of any one of embodiments 246-389, wherein the cell is a primary cell isolated from a donor.
- [0999]** Embodiment 391. The method of embodiment 390, wherein the donor is healthy and/or is not suspected of having a disease or condition at the time the primary cell is obtained from the donor.
- [1000]** Embodiment 392. The method of any one of embodiments 246-391, wherein the cells are islet cells, beta islet cells, pancreatic islet cells, immune cells, B cells, T cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophage cells, endothelial cells, muscle cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, dopaminergic neurons, retinal pigmented epithelium cells, optic cells, hepatocytes, thyroid cells, skin cells, glial progenitor cells, neural cells, cardiac cells, stem cells, hematopoietic stem cells, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), pluripotent stem cell (PSCs), blood cells, or a combination thereof.
- [1001]** Embodiment 393. The method of any one of embodiments 246-392, wherein the cell is a T-cell.
- [1002]** Embodiment 394. The method of embodiment 393, wherein the T-cell is a CD3+ T cell, CD4+ T cell, CDS+ T cell, naive T cell, regulatory T (Treg) cell, non-regulatory T cell, Th1 cell, Th2 cell, Th9 cell, Th17 cell, T-follicular helper (Tfh) cell, cytotoxic T lymphocyte (CTL), effector T (Teff) cell, central memory T cell, effector memory T cell, effector memory T cell expressing CD45RA (TEMRA cell), tissue-resident memory (Trm) cell, virtual memory T cell, innate memory T cell, memory stem cell (Tse), $\gamma\delta$ T cell, or a combination thereof.
- [1003]** Embodiment 395. The method of embodiment 393 or 394, wherein the T cell is a cytotoxic T-cell, helper T-cell, memory T-cell, regulatory T-cell, tumor infiltrating lymphocyte, or a combination thereof.
- [1004]** Embodiment 396. The method of any one of embodiments 393-395, wherein the cell is a human T-cell.
- [1005]** Embodiment 397. The method of any one of embodiments 393-396, wherein the cell is an autologous T-cell.
- [1006]** Embodiment 398. The method of any one of embodiments 393-396, wherein the cell is an allogeneic T-cell.
- [1007]** Embodiment 399. The method of embodiment 398, wherein the allogeneic T-cell is a primary T cell.
- [1008]** Embodiment 400. The method of 398 or 399, wherein the allogeneic T cell has been differentiated from an embryonic stem cell (ESC) or an induced pluripotent stem cell (iPSC).
- [1009]** Embodiment 401. The method of any one of embodiments 246-392, wherein the cell is an islet cell, beta islet cell, or pancreatic islet cell.
- [1010]** Embodiment 402. The method of embodiment 401, wherein the cell is an autologous islet cell, an autologous beta islet cell, or an autologous pancreatic islet cell.
- [1011]** Embodiment 403. The method of embodiment 401, wherein the cell is an allogeneic islet cell, an allogeneic beta islet cell, or an allogeneic pancreatic islet cell.
- [1012]** Embodiment 404. The method of embodiment 403, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell is a primary islet cell, a primary beta islet cell, or a primary pancreatic islet cell.

[1013] Embodiment 405. The method of embodiment 403 or 404, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell has been differentiated from an embryonic stem cell (ESC) or an induced pluripotent stem cell (iPSC).

Embodiments Section II

[1014] Embodiment 1. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered protein,

wherein the one or more nucleic acid sequences comprise a nucleic acid sequence at least 80% identical to SEQ ID NO: 17, and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.

[1015] Embodiment 2. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered protein,

wherein the one or more nucleic acid sequences comprise a nucleic acid sequence at least 80% identical to SEQ ID NO: 16, and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.

[1016] Embodiment 3. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered protein,

wherein the one or more nucleic acid sequences comprise a nucleic acid sequence at least 80% identical to SEQ ID NO: 15, and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.

[1017] Embodiment 4. The nucleic acid construct of any one of the preceding embodiments, wherein the engineered protein comprises one or more membrane tethers.

[1018] Embodiment 5. The nucleic acid construct of any one of embodiments 1-4, wherein the one or more membrane tethers are or comprise a transmembrane domain.

[1019] Embodiment 6. The nucleic acid construct of embodiment 5, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNGR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.

[1020] Embodiment 7. The nucleic acid construct of embodiment 5 or 6, wherein the transmembrane domain is or comprises a CD47 transmembrane domain.

[1021] Embodiment 8. The nucleic acid construct of any one of embodiments 5-7, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 54.

[1022] Embodiment 9. The nucleic acid construct of any one of embodiments 5-7, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 56.

[1023] Embodiment 10. The nucleic acid construct of any one of embodiments 5-7, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 58.

[1024] Embodiment 11. The nucleic acid construct of any one of embodiments 5-7, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 60.

[1025] Embodiment 12. The nucleic acid construct of any one of embodiments 5-7, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 62.

[1026] Embodiment 13. The nucleic acid construct of embodiment 5 or 6, wherein the transmembrane domain is or comprises a CD8a transmembrane domain.

- [1027] Embodiment 14. The nucleic acid construct of embodiment 5, 6, or 13, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 69.
- [1028] Embodiment 15. The nucleic acid construct of embodiment 5 or 6, wherein the transmembrane domain is or comprises a CD28 transmembrane domain.
- [1029] Embodiment 16. The nucleic acid construct of embodiment 5, 6, or 15, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 71.
- [1030] Embodiment 17. The nucleic acid construct of embodiment 5 or 6, wherein the transmembrane domain is or comprises a PDGFR transmembrane domain.
- [1031] Embodiment 18. The nucleic acid construct of embodiment 5, 6, or 17, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 73.
- [1032] Embodiment 19. The nucleic acid construct of any one of embodiments 4-18, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.
- [1033] Embodiment 20. The nucleic acid construct of embodiment 19, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
- [1034] Embodiment 21. The nucleic acid construct of embodiment 19 or 20, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor.
- [1035] Embodiment 22. The nucleic acid construct of any one of embodiments 19-21, wherein the DAF/CD55 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 65.
- [1036] Embodiment 23. The nucleic acid construct of embodiment 19 or 20, wherein the GPI anchor is or comprises a TRAILR3 GPI anchor.
- [1037] Embodiment 24. The nucleic acid construct of any one of embodiments 19, 20, or 23, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 66.
- [1038] Embodiment 25. The nucleic acid construct of any one of embodiments 19, 20, or 23, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 67.
- [1039] Embodiment 26. The nucleic acid construct of any one of embodiments 1-15, wherein the one or more extracellular domains further comprise an extracellular hinge domain.
- [1040] Embodiment 27. The nucleic acid construct of embodiment 26, wherein the extracellular hinge domain is or comprises a CD47 hinge, a CD8a hinge, a CD28 hinge, a PDGFR hinge, or an IgG4 hinge.
- [1041] Embodiment 28. The nucleic acid construct of embodiment 26 or 27, wherein the extracellular hinge domain is or comprises a CD47 hinge.
- [1042] Embodiment 29. The nucleic acid construct of any one of embodiments 26-28, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 75.
- [1043] Embodiment 30. The nucleic acid construct of embodiment 26 or 29, wherein the extracellular hinge domain is or comprises a CD8a hinge.
- [1044] Embodiment 31. The nucleic acid construct of embodiment 26, 29, or 30, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 76.
- [1045] Embodiment 32. The nucleic acid construct of embodiment 26 or 29, wherein the extracellular hinge domain is or comprises a CD28 hinge.
- [1046] Embodiment 33. The nucleic acid construct of embodiment 26, 29, or 32, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 77.

- [1047]** Embodiment 34. The nucleic acid construct of embodiment 26 or 29, wherein the extracellular hinge domain is or comprises a PDGFR hinge.
- [1048]** Embodiment 35. The nucleic acid construct of embodiment 26, 29, or 34, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 78.
- [1049]** Embodiment 36. The nucleic acid construct of embodiment 26 or 29, wherein the extracellular hinge domain is or comprises an IgG4 hinge.
- [1050]** Embodiment 37. The nucleic acid construct of embodiment 26, 29, or 36, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 79.
- [1051]** Embodiment 38. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered CD47 protein comprising:
- (a) one or more extracellular domains; and (b) one or more membrane tethers;
- wherein the one or more extracellular domains comprise: an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6; and/or
- an amino acid substitution at position S83 of SEQ ID NO: 2, an amino acid substitution at position S65 of SEQ ID NO: 3, an amino acid substitution at position S83 of SEQ ID NO: 1, or an amino acid substitution at position S38 of SEQ ID NO: 6; and/or
 - an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 1 or SEQ ID NO: 6; and
- wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.
- [1052]** Embodiment 39. The nucleic acid construct of embodiment 38, wherein the one or more extracellular domains comprise:
- an amino acid substitution of S82A relative to SEQ ID NO: 2,
 - an amino acid substitution of S64A relative to SEQ ID NO: 3,
 - an amino acid substitution of S82A relative to SEQ ID NO: 1, or
 - an amino acid substitution of S37A relative to SEQ ID NO: 6.
- [1053]** Embodiment 40. The nucleic acid construct of embodiment 38, wherein the one or more extracellular domains comprise:
- an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2,
 - an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3,
 - an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, or
 - an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6.
- [1054]** Embodiment 41. The nucleic acid construct of any one of embodiments 38-40, wherein the one or more extracellular domains comprise:
- an amino acid substitution of S83A relative to SEQ ID NO: 2,
 - an amino acid substitution of S65A relative to SEQ ID NO: 3,
 - an amino acid substitution of S83A relative to SEQ ID NO: 1, or
 - an amino acid substitution of S38A relative to SEQ ID NO: 6.
- [1055]** Embodiment 42. The nucleic acid construct of any one of embodiments 38-40, wherein the one or more extracellular domains comprise:

an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2,
 an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3,
 an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, or
 an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[1056] Embodiment 43. The nucleic acid construct of any one of embodiments 38, 39 or 41, wherein the one or more extracellular domains comprise:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, and an amino acid substitution of S37A relative to SEQ ID NO: 6; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83A relative to SEQ ID NO: 2, an amino acid substitution of S65A relative to SEQ ID NO: 3, an amino acid substitution of S83A relative to SEQ ID NO: 1, and an amino acid substitution of S38A relative to SEQ ID NO: 6.

[1057] Embodiment 44. The nucleic acid construct of any one of embodiments 38, 39 or 42, wherein the one or more extracellular domains comprise:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2, an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3, an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, and an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2, an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3, an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, and an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[1058] Embodiment 45. The nucleic acid construct of embodiment 38, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.

[1059] Embodiment 46. The nucleic acid construct of embodiment 38 or 45, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.

[1060] Embodiment 47. The nucleic acid construct of any one of embodiments 38-46, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[1061] Embodiment 48. The nucleic acid construct of any one of embodiments 38-47, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[1062] Embodiment 49. The nucleic acid construct of any one of embodiments 38-48, wherein the engineered CD47 protein comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[1063] Embodiment 50. The nucleic acid construct of any one of embodiments 38-49, wherein the engineered CD47 protein comprises: an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2, or

an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 1 and an amino acid substitution of Q19P relative to SEQ ID NO: 1.

[1064] Embodiment 51. The nucleic acid construct of any one of embodiments 38-50, wherein the engineered CD47 protein comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.

[1065] Embodiment 52. The nucleic acid construct of any one of embodiments 38-51, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 348

[1066] Embodiment 53. The nucleic acid construct of any one of embodiments 38-52, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 347.

[1067] Embodiment 54. The nucleic acid construct of any one of embodiments 38-53, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus: an amino acid sequence comprising SEQ ID NO: 20;

an amino acid sequence comprising SEQ ID NO: 348; and

an amino acid sequence comprising SEQ ID NO: 3.

[1068] Embodiment 55. The nucleic acid construct of any one of embodiments 38-53, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:

an amino acid sequence comprising SEQ ID NO: 20;

an amino acid sequence comprising SEQ ID NO: 348; and

an amino acid substitution of Q19P relative to SEQ ID NO: 3.

[1069] Embodiment 56. The nucleic acid construct of any one of embodiments 38-53, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:

an amino acid sequence comprising SEQ ID NO: 348; and

an amino acid sequence comprising SEQ ID NO: 6.

[1070] Embodiment 57. The nucleic acid construct of any one of embodiments 38-56, wherein the engineered CD47 protein comprises an amino acid sequence comprising:

an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, or an amino acid substitution of S37A relative to SEQ ID NO: 6;

an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1; and

an amino acid substitution of Q19P relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[1071] Embodiment 58. The nucleic acid construct of any one of embodiments 38-57, wherein the one or more membrane tethers are or comprise a transmembrane domain.

[1072] Embodiment 59. The nucleic acid construct of embodiment 58, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNGR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.

[1073] Embodiment 60. The nucleic acid construct of embodiment 58 or 59, wherein the transmembrane domain is or comprises a CD47 transmembrane domain.

[1074] Embodiment 61. The nucleic acid construct of any one of embodiments 58-60, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 54.

[1075] Embodiment 62. The nucleic acid construct of any one of embodiments 58-60, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 56.

- [1076] Embodiment 63. The nucleic acid construct of any one of embodiments 58-60, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 58.
- [1077] Embodiment 64. The nucleic acid construct of any one of embodiments 58-60, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 60.
- [1078] Embodiment 65. The nucleic acid construct of any one of embodiments 58-60, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 62.
- [1079] Embodiment 66. The nucleic acid construct of embodiment 58 or 59, wherein the transmembrane domain is or comprises a CD8a transmembrane domain.
- [1080] Embodiment 67. The nucleic acid construct of embodiment 58, 59, or 66, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 69.
- [1081] Embodiment 68. The nucleic acid construct of embodiment 58 or 59, wherein the transmembrane domain is or comprises a CD28 transmembrane domain.
- [1082] Embodiment 69. The nucleic acid construct of embodiment 58, 59, or 68, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 71.
- [1083] Embodiment 70. The nucleic acid construct of embodiment 58 or 59, wherein the transmembrane domain is or comprises a PDGFR transmembrane domain.
- [1084] Embodiment 71. The nucleic acid construct of embodiment 58, 59, or 70, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 73.
- [1085] Embodiment 72. The nucleic acid construct of any one of embodiments 38-71, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.
- [1086] Embodiment 73. The nucleic acid construct of embodiment 72, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
- [1087] Embodiment 74. The nucleic acid construct of embodiment 72 or 73, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor.
- [1088] Embodiment 75. The nucleic acid construct of any one of embodiments 72-74, wherein the DAF/CD55 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 65.
- [1089] Embodiment 76. The nucleic acid construct of embodiment 72 or 73, wherein the GPI anchor is or comprises a TRAILR3 GPI anchor.
- [1090] Embodiment 77. The nucleic acid construct of any one of embodiments 72, 73, or 76, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 66.
- [1091] Embodiment 78. The nucleic acid construct of any one of embodiments 72, 73, or 76, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 67.
- [1092] Embodiment 79. The nucleic acid construct of any one of embodiments 38-78, wherein the one or more extracellular domains further comprise an extracellular hinge domain.
- [1093] Embodiment 80. The nucleic acid construct of embodiment 79, wherein the extracellular hinge domain is or comprises a CD47 hinge, a CD8a hinge, a CD28 hinge, a PDGFR hinge, or an IgG4 hinge.
- [1094] Embodiment 81. The nucleic acid construct of embodiment 79 or 80, wherein the extracellular hinge domain is or comprises a CD47 hinge.
- [1095] Embodiment 82. The nucleic acid construct of any one of embodiments 79-81, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 75.

- [1096] Embodiment 83. The nucleic acid construct of embodiment 79 or 80, wherein the extracellular hinge domain is or comprises a CD8a hinge.
- [1097] Embodiment 84. The nucleic acid construct of embodiment 79, 80, or 83, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 76.
- [1098] Embodiment 85. The nucleic acid construct of embodiment 79 or 80, wherein the extracellular hinge domain is or comprises a CD28 hinge.
- [1099] Embodiment 86. The nucleic acid construct of embodiment 79, 80, or 85, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 77.
- [1100] Embodiment 87. The nucleic acid construct of embodiment 79 or 80, wherein the extracellular hinge domain is or comprises a PDGFR hinge.
- [1101] Embodiment 88. The nucleic acid construct of embodiment 79, 80, or 87, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 78.
- [1102] Embodiment 89. The nucleic acid construct of embodiment 79 or 80, wherein the extracellular hinge domain is or comprises an IgG4 hinge.
- [1103] Embodiment 90. The nucleic acid construct of embodiment 79, 80, or 89, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 79.
- [1104] Embodiment 91. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered CD47 protein, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 4 or SEQ ID NO: 5.
- [1105] Embodiment 92. The nucleic acid construct of any one of embodiments 91, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 348.
- [1106] Embodiment 93. The nucleic acid construct of 91 or 92, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 347.
- [1107] Embodiment 94. The nucleic acid construct of any one of embodiments B1-B3, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid sequence comprising SEQ ID NO: 4, or SEQ ID NO: 5.
- [1108] Embodiment 95. The nucleic acid construct of any one of embodiments 91-94, wherein the engineered protein comprises one or more membrane tethers.
- [1109] Embodiment 96. The nucleic acid construct of embodiment 95, wherein the one or more membrane tethers are or comprise a transmembrane domain.
- [1110] Embodiment 97. The nucleic acid construct of embodiment 96, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNGR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.
- [1111] Embodiment 98. The nucleic acid construct of embodiment 96 or 97, wherein the transmembrane domain is or comprises a CD47 transmembrane domain.
- [1112] Embodiment 99. The nucleic acid construct of any one of embodiments 96-98, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 54.
- [1113] Embodiment 100. The nucleic acid construct of any one of embodiments 96-98, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 56.

- [1114] Embodiment 101. The nucleic acid construct of any one of embodiments 96-98, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 58.
- [1115] Embodiment 102. The nucleic acid construct of any one of embodiments 96-98, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 60.
- [1116] Embodiment 103. The nucleic acid construct of any one of embodiments 96-98, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 62.
- [1117] Embodiment 104. The nucleic acid construct of embodiment 96 or 97, wherein the transmembrane domain is or comprises a CD8a transmembrane domain.
- [1118] Embodiment 105. The nucleic acid construct of embodiment 96, 97, or 104, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 69.
- [1119] Embodiment 106. The nucleic acid construct of embodiment 96 or 97, wherein the transmembrane domain is or comprises a CD28 transmembrane domain.
- [1120] Embodiment 107. The nucleic acid construct of embodiment 96, 97, or 106, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 71.
- [1121] Embodiment 108. The nucleic acid construct of embodiment 96 or 97, wherein the transmembrane domain is or comprises a PDGFR transmembrane domain.
- [1122] Embodiment 109. The nucleic acid construct of embodiment 96, 97, or 108, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 73.
- [1123] Embodiment 110. The nucleic acid construct of any one of embodiments 95-110, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.
- [1124] Embodiment 111. The nucleic acid construct of embodiment 110, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
- [1125] Embodiment 112. The nucleic acid construct of embodiment 110 or 111, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor.
- [1126] Embodiment 113. The nucleic acid construct of any one of embodiments 110-112, wherein the DAF/CD55 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 65.
- [1127] Embodiment 114. The nucleic acid construct of embodiment 110 or 111, wherein the GPI anchor is or comprises a TRAILR3 GPI anchor.
- [1128] Embodiment 115. The nucleic acid construct of any one of embodiments 110, 111, or 114, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 66.
- [1129] Embodiment 116. The nucleic acid construct of any one of embodiments 110, 111, or 114, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 67.
- [1130] Embodiment 117. The nucleic acid construct of any one of embodiments 38-116, wherein the one or more extracellular domains further comprise an extracellular hinge domain.
- [1131] Embodiment 118. The nucleic acid construct of embodiment 117, wherein the extracellular hinge domain is or comprises a CD47 hinge, a CD8a hinge, a CD28 hinge, a PDGFR hinge, or an IgG4 hinge.
- [1132] Embodiment 119. The nucleic acid construct of embodiment 117 or 118, wherein the extracellular hinge domain is or comprises a CD47 hinge.
- [1133] Embodiment 120. The nucleic acid construct of any one of embodiments 117-119, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 75.

- [1134] Embodiment 121. The nucleic acid construct of embodiment 117 or 118, wherein the extracellular hinge domain is or comprises a CD8a hinge.
- [1135] Embodiment 122. The nucleic acid construct of embodiment 117, 118, or 121, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 76.
- [1136] Embodiment 123. The nucleic acid construct of embodiment 117 or 118, wherein the extracellular hinge domain is or comprises a CD28 hinge.
- [1137] Embodiment 124. The nucleic acid construct of embodiment 117, 118, or 123, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 77.
- [1138] Embodiment 125. The nucleic acid construct of embodiment 117 or 118, wherein the extracellular hinge domain is or comprises a PDGFR hinge.
- [1139] Embodiment 126. The nucleic acid construct of embodiment 117, 118, or 125, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 78.
- [1140] Embodiment 127. The nucleic acid construct of embodiment 117 or 118, wherein the extracellular hinge domain is or comprises an IgG4 hinge.
- [1141] Embodiment 128. The nucleic acid construct of embodiment 117, 118, or 127, wherein the extracellular hinge domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 79.
- [1142] Embodiment 129. The nucleic acid construct of any one of the preceding embodiments, further comprising one or more control sequences.
- [1143] Embodiment 130. The nucleic acid construct of embodiment 129, wherein the one or more control sequences encode an extracellular signal peptide.
- [1144] Embodiment 131. The nucleic acid construct of embodiment 129, wherein the one or more control sequences comprise a promoter.
- [1145] Embodiment 132. The nucleic acid construct of embodiment 131, wherein the promoter is a constitutive promoter or an inducible promoter.
- [1146] Embodiment 133. The nucleic acid construct of embodiment 131 or 132, wherein the promoter is a naturally occurring promoter, a hybrid promoter, or a synthetic promoter.
- [1147] Embodiment 134. The nucleic acid construct of any one of embodiments 131-133, wherein the promoter is or comprises an EF1 α promoter, an EF1 α short promoter, a CAG promoter, a ubiquitin/S27a promoter, an SV40 early promoter, an adenovirus major late promoter, a mouse metallothionein-I promoter, an RSV promoter, an MMTV promoter, a Moloney murine leukemia virus Long Terminal repeat region, a CMV promoter, an actin promoter, an immunoglobulin promoter, a heat shock promoter, polyoma virus promoter, a fowlpox virus promoter, a bovine papilloma virus promoter, an avian sarcoma virus promoter, a retrovirus promoter, a hepatitis-B virus promoter, a PGK promoter, an adenovirus late promoter, a vaccinia virus 7.5K promoter, a SV40 promoter, a tk promoter of HSV, a mouse mammary tumor virus (MMTV) promoter, an LTR promoter of HIV, a promoter of moloney virus, an Epstein Barr virus (EBV) promoter, a Rous sarcoma virus (RSV) promoter, a U6 promoter, or an UBC promoter.
- [1148] Embodiment 135. The nucleic acid construct of any one of embodiments 131-134, wherein the promoter is or comprises a CAG promoter.
- [1149] Embodiment 136. The nucleic acid construct of any one of embodiments 131-135, wherein the promoter comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 49.
- [1150] Embodiment 137. The nucleic acid construct of any one of embodiments 131-134, wherein the promoter is or comprises an EF1 α promoter.

[1151] Embodiment 138. The nucleic acid construct of any one of embodiments 131-135, or 137 wherein the promoter comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 50.

[1152] Embodiment 139. The nucleic acid construct of embodiment 129, wherein the one or more control sequences comprise ribosomal binding sites, enhancer elements, activator elements, translational start sequences, translational termination sequences, transcription start sequences, transcription termination sequences, polyadenylation signal sequences, replication elements, RNA processing and export elements, transposon sequences, transposase sequences, insulator sequences, internal ribosome entry sites (IRES), 5'UTRs, 3'UTRs, mRNA 3' end processing sequences, boundary elements, locus control regions (LCR), matrix attachment regions (MAR), recombination or cassette exchange sequences, linker sequences, secretion signals, resistance markers, anchoring peptides, localization signals, fusion tags, affinity tags, chaperonins, proteases, or combinations thereof.

[1153] Embodiment 140. The nucleic acid construct of any one of the preceding embodiments, further comprising one or more nucleic acid sequences encoding an intracellular domain.

[1154] Embodiment 141. The nucleic acid construct of embodiment 140, wherein the intracellular domain is or comprises a CD47 intracellular domain or a portion thereof.

[1155] Embodiment 142. The nucleic acid construct of embodiment 140 or 141, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 55.

[1156] Embodiment 143. The nucleic acid construct of embodiment 140 or 141, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 59.

[1157] Embodiment 144. The nucleic acid construct of embodiment 140 or 141, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 63.

[1158] Embodiment 145. The nucleic acid construct of embodiment 140 or 141, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 64.

[1159] Embodiment 146. The nucleic acid construct of embodiment 140 or 141, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 349.

[1160] Embodiment 147. The nucleic acid construct of embodiment 140 or 141, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 351.

[1161] Embodiment 148. The nucleic acid construct of embodiment 140, wherein the intracellular domain is or comprises a CD8a intracellular domain or a portion thereof.

[1162] Embodiment 149. The nucleic acid construct of embodiment 140 or 148, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 70.

[1163] Embodiment 150. The nucleic acid construct of embodiment 140, wherein the intracellular domain is or comprises a CD28 intracellular domain or a portion thereof.

[1164] Embodiment 151. The nucleic acid construct of embodiment 140 or 150, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 72.

[1165] Embodiment 152. The nucleic acid construct of embodiment 140, wherein the intracellular domain is or comprises a PDGFR intracellular domain or a portion thereof.

[1166] Embodiment 153. The nucleic acid construct of embodiment 140 or 152, wherein the intracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 74.

[1167] Embodiment 154. The nucleic acid construct of any one of embodiments 140-153, wherein the intracellular domain comprises one or more modifications relative to a wild-type CD47 intracellular domain.

- [1168] Embodiment 155. The nucleic acid construct of any one of embodiments 140-154, wherein the intracellular domain comprises one or more deletions relative to a wild-type CD47 intracellular domain.
- [1169] Embodiment 156. The nucleic acid construct of any one of embodiments 140-155, wherein the intracellular domain comprises one or more insertions relative to a wild-type CD47 intracellular domain.
- [1170] Embodiment 157. The nucleic acid construct of any one of embodiments 140-156, wherein the intracellular domain comprises altered function relative to a wild-type CD47 intracellular domain.
- [1171] Embodiment 158. The nucleic acid construct of any one of embodiments 140-157, wherein the intracellular domain comprises reduced function relative to a wild-type CD47 intracellular domain.
- [1172] Embodiment 159. The nucleic acid construct of any one of embodiments 140-158, wherein the intracellular domain comprises reduced levels of CD47 intracellular signaling relative to a wild-type CD47 intracellular domain.
- [1173] Embodiment 160. The nucleic acid construct of any one of embodiments 140-159, wherein the intracellular domain comprises a non-functional intracellular domain.
- [1174] Embodiment 161. The nucleic acid construct of any one of the preceding embodiments, comprising a nucleic acid sequence at least 80% identical to a sequence selected from Table 24.
- [1175] Embodiment 162. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleic acid sequence at least 80% identical to SEQ ID NO: 13.
- [1176] Embodiment 163. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleic acid sequence at least 80% identical to SEQ ID NO: 14.
- [1177] Embodiment 164. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleic acid sequence at least 80% identical to SEQ ID NO: 18.
- [1178] Embodiment 165. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 133.
- [1179] Embodiment 166. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 134.
- [1180] Embodiment 167. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 135.
- [1181] Embodiment 168. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 136.
- [1182] Embodiment 169. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 137.
- [1183] Embodiment 170. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 138.
- [1184] Embodiment 171. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 139.
- [1185] Embodiment 172. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 140.
- [1186] Embodiment 173. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 141.
- [1187] Embodiment 174. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 142.

- [1188] Embodiment 175. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 143.
- [1189] Embodiment 176. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 144.
- [1190] Embodiment 177. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 145.
- [1191] Embodiment 178. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 146.
- [1192] Embodiment 179. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 147.
- [1193] Embodiment 180. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 148.
- [1194] Embodiment 181. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 149.
- [1195] Embodiment 182. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 150.
- [1196] Embodiment 183. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 151.
- [1197] Embodiment 184. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 152.
- [1198] Embodiment 185. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 153.
- [1199] Embodiment 186. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 154.
- [1200] Embodiment 187. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 155.
- [1201] Embodiment 188. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 156.
- [1202] Embodiment 189. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 157.
- [1203] Embodiment 190. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 158.
- [1204] Embodiment 191. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 159.
- [1205] Embodiment 192. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 160.
- [1206] Embodiment 193. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 161.
- [1207] Embodiment 194. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 162.

- [1208] Embodiment 195. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 163.
- [1209] Embodiment 196. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 164.
- [1210] Embodiment 197. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 165.
- [1211] Embodiment 198. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 166.
- [1212] Embodiment 199. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 167.
- [1213] Embodiment 200. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 168.
- [1214] Embodiment 201. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 169.
- [1215] Embodiment 202. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 170.
- [1216] Embodiment 203. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 171.
- [1217] Embodiment 204. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 172.
- [1218] Embodiment 205. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 173.
- [1219] Embodiment 206. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 174.
- [1220] Embodiment 207. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 175.
- [1221] Embodiment 208. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 176.
- [1222] Embodiment 209. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 177.
- [1223] Embodiment 210. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 178.
- [1224] Embodiment 211. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 179.
- [1225] Embodiment 212. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 180.
- [1226] Embodiment 213. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 181.
- [1227] Embodiment 214. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 182.

- [1228] Embodiment 215. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 183.
- [1229] Embodiment 216. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 184.
- [1230] Embodiment 217. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 185.
- [1231] Embodiment 218. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 341.
- [1232] Embodiment 219. The nucleic acid construct of any one of embodiments 1-161, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 342.
- [1233] Embodiment 220. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to one or more sequences selected from Table 26.
- [1234] Embodiment 221. The nucleic acid construct of any one of the preceding embodiments, comprising one or more nucleic acid sequences at least 80% identical to one or more sequences selected from Table 26 and/or Table 24.
- [1235] Embodiment 222. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 49 and SEQ ID NO: 14.
- [1236] Embodiment 223. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 14.
- [1237] Embodiment 224. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 49 and SEQ ID NO: 13.
- [1238] Embodiment 225. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 13.
- [1239] Embodiment 226. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 49 and SEQ ID NO: 18.
- [1240] Embodiment 227. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 18.
- [1241] Embodiment 228. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 51, 52, 54, 55, 56, and 57.
- [1242] Embodiment 229. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 51, 52, 54, 55, and 56.
- [1243] Embodiment 230. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 51, 52, 54, and 55.
- [1244] Embodiment 231. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, and 62.
- [1245] Embodiment 232. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 51, 52, 54, 55, 56, 57, 58, 59, 60, and 61.
- [1246] Embodiment 233. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 51, 52, 54, 55, 56, 57, 58, 59, and 60.
- [1247] Embodiment 234. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 51, 52, 54, 55, 56, 57, 58, and 59.

- [1248] Embodiment 235. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 53, and 65.
- [1249] Embodiment 236. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 53, and 65.
- [1250] Embodiment 237. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 53, and 66.
- [1251] Embodiment 238. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 53, and 66.
- [1252] Embodiment 239. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 53, and 67.
- [1253] Embodiment 240. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 53, and 67.
- [1254] Embodiment 241. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 53, 69, and 70.
- [1255] Embodiment 242. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 53, 69, and 70.
- [1256] Embodiment 243. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 53, 71, and 72.
- [1257] Embodiment 244. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 53, 71, and 72.
- [1258] Embodiment 245. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 53, 73, and 74.
- [1259] Embodiment 246. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 53, 73, and 74.
- [1260] Embodiment 247. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 52, 78, 73, and 74.
- [1261] Embodiment 248. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 52, 78, 73, and 74.
- [1262] Embodiment 249. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 52, 79, 71, and 72.
- [1263] Embodiment 250. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 52, 79, 71, and 72.
- [1264] Embodiment 251. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 52, 79, 69, and 70.
- [1265] Embodiment 252. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 52, 79, 69, and 70.
- [1266] Embodiment 253. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 52, 77, 71, and 72.
- [1267] Embodiment 254. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 52, 77, 71, and 72.

- [1268]** Embodiment 255. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 52, 77, 69, and 70.
- [1269]** Embodiment 256. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 52, 77, 69, and 70.
- [1270]** Embodiment 257. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 52, 76, 71, and 72.
- [1271]** Embodiment 258. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 52, 76, 71, and 72.
- [1272]** Embodiment 259. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49, 52, 76, 69, and 70.
- [1273]** Embodiment 260. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 52, 76, 69, and 70.
- [1274]** Embodiment 261. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 49 and 200.
- [1275]** Embodiment 262. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID Nos: 50 and 200.
- [1276]** Embodiment 263. The nucleic acid construct of any one of embodiments 1-153, comprising one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 200.
- [1277]** Embodiment 264. A vector comprising the nucleic acid construct of any one of the preceding embodiments.
- [1278]** Embodiment 265. The vector of embodiment 264, wherein the vector is a polycistronic vector.
- [1279]** Embodiment 266. The vector of embodiment 265, wherein the polycistronic vector is a bicistronic vector or a tricistronic vector.
- [1280]** Embodiment 267. The vector of any one of embodiments 264-266, wherein the vector is a plasmid or a viral vector.
- [1281]** Embodiment 268. The vector of embodiment 267, wherein the viral vector is a pseudotyped, self-inactivating lentiviral vector.
- [1282]** Embodiment 269. An engineered protein encoded by the nucleic acid construct of any one of embodiments 1-268
- [1283]** Embodiment 270. An engineered protein:
 wherein the engineered protein is or comprises an amino acid sequence at least 80% identical to SEQ ID NO: 5,
 and
 wherein the engineered protein does not comprise one or more full-length CD47 intracellular domains.
- [1284]** Embodiment 271. An engineered protein:
 wherein the engineered protein is or comprises an amino acid sequence at least 80% identical to SEQ ID NO: 4
 and
 wherein the engineered protein does not comprise one or more full-length CD47 intracellular domains.
- [1285]** Embodiment 272. The engineered CD47 protein of embodiment 270 or 271, wherein the engineered protein comprises one or more membrane tethers.
- [1286]** Embodiment 273. The engineered CD47 protein of embodiment 272, wherein the one or more membrane tethers are or comprise a transmembrane domain.

- [1287]** Embodiment 274. The engineered CD47 protein of embodiment 273, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNGR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.
- [1288]** Embodiment 275. The engineered CD47 protein of embodiment 270 or 271, wherein the transmembrane domain is or comprises a CD47 transmembrane domain.
- [1289]** Embodiment 276. The engineered CD47 protein of any one of embodiments 273-275, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 23.
- [1290]** Embodiment 277. The engineered CD47 protein of any one of embodiments 273-275, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 25.
- [1291]** Embodiment 278. The engineered CD47 protein of any one of embodiments 273-275, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 27.
- [1292]** Embodiment 279. The engineered CD47 protein of any one of embodiments 273-275, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 29.
- [1293]** Embodiment 280. The engineered CD47 protein of any one of embodiments 273-275, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 31.
- [1294]** Embodiment 281. The engineered CD47 protein of embodiment 273 or 274, wherein the transmembrane domain is or comprises a CD8a transmembrane domain.
- [1295]** Embodiment 282. The engineered CD47 protein of embodiment 273, 274, or 281, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 38.
- [1296]** Embodiment 283. The engineered CD47 protein of embodiment 273 or 274, wherein the transmembrane domain is or comprises a CD28 transmembrane domain.
- [1297]** Embodiment 284. The engineered CD47 protein of embodiment 273, 274, or 283, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 40.
- [1298]** Embodiment 285. The engineered CD47 protein of embodiment 273 or 274, wherein the transmembrane domain is or comprises a PDGFR transmembrane domain.
- [1299]** Embodiment 286. The engineered CD47 protein of embodiment 273, 274, or 285, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 42.
- [1300]** Embodiment 287. The engineered CD47 protein of any one of embodiments 272-286, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.
- [1301]** Embodiment 288. The engineered CD47 protein of embodiment 287, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
- [1302]** Embodiment 289. The engineered CD47 protein of embodiment 287 or 288, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor.
- [1303]** Embodiment 290. The engineered CD47 protein of any one of embodiments 287-289, wherein the DAF/CD55 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 34.
- [1304]** Embodiment 291. The engineered CD47 protein of embodiment 287 or 288, wherein the GPI anchor is or comprises a TRAILR3 GPI anchor.
- [1305]** Embodiment 292. The engineered CD47 protein of any one of embodiments 287, 288, or 291, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 35.

[1306] Embodiment 293. The engineered CD47 protein of any one of embodiments 287, 288, or 291, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 36.

[1307] Embodiment 294. The engineered protein of embodiment 287 or 288, wherein the GPI anchor is or comprises a CD59 GPI anchor.

[1308] Embodiment 295. The engineered protein of any one of embodiments 287, 288, or 294, wherein the CD59 GPI anchor comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 37.

[1309] Embodiment 296. The engineered protein of any one of embodiments 270-295, further comprising an extracellular signal peptide.

[1310] Embodiment 297. The engineered protein of embodiment 296, wherein the extracellular signal peptide is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 20.

[1311] Embodiment 298. An engineered CD47 protein comprising:

(a) one or more extracellular domains; and (b) one or more membrane tethers;

wherein the one or more extracellular domains comprise: an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6; and/or

an amino acid substitution at position S83 of SEQ ID NO: 2, an amino acid substitution at position S65 of SEQ ID NO: 3, an amino acid substitution at position S83 of SEQ ID NO: 1, or an amino acid substitution at position S38 of SEQ ID NO: 6; and/or

an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 1 or SEQ ID NO: 6; and

wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.

[1312] Embodiment 299. The engineered CD47 protein of embodiment 298, wherein the one or more extracellular domains comprise:

an amino acid substitution of S82A relative to SEQ ID NO: 2,

an amino acid substitution of S64A relative to SEQ ID NO: 3,

an amino acid substitution of S82A relative to SEQ ID NO: 1, or

an amino acid substitution of S37A relative to SEQ ID NO: 6.

[1313] Embodiment 300. The engineered CD47 protein of embodiment 298, wherein the one or more extracellular domains comprise:

an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2,

an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3,

an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, or

an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6.

[1314] Embodiment 301. The engineered CD47 protein of any one of embodiments 298-300, wherein the one or more extracellular domains comprise:

an amino acid substitution of S83A relative to SEQ ID NO: 2,

an amino acid substitution of S65A relative to SEQ ID NO: 3,

an amino acid substitution of S83A relative to SEQ ID NO: 1, or

an amino acid substitution of S38A relative to SEQ ID NO: 6.

[1315] Embodiment 302. The engineered CD47 protein of any one of embodiments 298-300, wherein the one or more extracellular domains comprise:

- an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2,
- an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3,
- an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, or
- an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[1316] Embodiment 304. The engineered CD47 protein of any one of embodiments 298, 299 or 301, wherein the one or more extracellular domains comprise:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, and an amino acid substitution of S37A relative to SEQ ID NO: 6; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83A relative to SEQ ID NO: 2, an amino acid substitution of S65A relative to SEQ ID NO: 3, an amino acid substitution of S83A relative to SEQ ID NO: 1, and an amino acid substitution of S38A relative to SEQ ID NO: 6.

[1317] Embodiment 305. The engineered CD47 protein of any one of embodiments 298, 299 or 302, wherein the one or more extracellular domains comprise:

a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2, an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3, an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, and an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6; and

a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2, an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3, an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, and an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

[1318] Embodiment 306. The engineered CD47 protein of embodiment 298, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.

[1319] Embodiment 307. The engineered CD47 protein of embodiment 298 or 306, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.

[1320] Embodiment 308. The engineered CD47 protein of any one of embodiments 298-307, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[1321] Embodiment 309. The engineered CD47 protein of any one of embodiments 298-308, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

[1322] Embodiment 310. The engineered CD47 protein of any one of embodiments 298-309, wherein the engineered CD47 protein comprises an amino acid substitution at position Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.

- [1323]** Embodiment 311. The engineered CD47 protein of any one of embodiments 298-311, wherein the engineered CD47 protein comprises: an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2, or
an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 1 and an amino acid substitution of Q19P relative to SEQ ID NO: 1.
- [1324]** Embodiment 312. The engineered CD47 protein of any one of embodiments 298-312, wherein the engineered CD47 protein comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.
- [1325]** Embodiment 313. The engineered CD47 protein of any one of embodiments 298-312, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 348.
- [1326]** Embodiment 314. The engineered CD47 protein of any one of embodiments 298-313, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 347.
- [1327]** Embodiment 315. The engineered CD47 protein of any one of embodiments 298-314, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 20;
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid sequence comprising SEQ ID NO: 3.
- [1328]** Embodiment 316. The engineered CD47 protein of any one of embodiments 298-314, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 20;
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid substitution of Q19P relative to SEQ ID NO: 3.
- [1329]** Embodiment 317. The engineered CD47 protein of any one of embodiments 298-314, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid sequence comprising SEQ ID NO: 6.
- [1330]** Embodiment 318. The engineered CD47 protein of any one of embodiments 298-317, wherein the engineered CD47 protein comprises an amino acid sequence comprising:
an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, or an amino acid substitution of S37A relative to SEQ ID NO: 6;
an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1; and
an amino acid substitution of Q19P relative to SEQ ID NO: 2 or SEQ ID NO: 1.
- [1331]** Embodiment 319. The engineered CD47 protein of any one of embodiments 298-318, wherein the one or more membrane tethers are or comprise a transmembrane domain.
- [1332]** Embodiment 320. The engineered CD47 protein of embodiment 319, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNCR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.
- [1333]** Embodiment 321. The engineered CD47 protein of embodiment 319 or 320, wherein the transmembrane domain is or comprises a CD47 transmembrane domain.

- [1334] Embodiment 322. The engineered CD47 protein of any one of embodiments 319-321, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 23.
- [1335] Embodiment 323. The engineered CD47 protein of any one of embodiments 319-321, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 25.
- [1336] Embodiment 324. The engineered CD47 protein of any one of embodiments 319-321, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 27.
- [1337] Embodiment 325. The engineered CD47 protein of any one of embodiments 319-321, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 29.
- [1338] Embodiment 326. The engineered CD47 protein of any one of embodiments 319-321, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 31.
- [1339] Embodiment 327. The engineered CD47 protein of embodiment 319 or 320, wherein the transmembrane domain is or comprises a CD8a transmembrane domain.
- [1340] Embodiment 328. The engineered CD47 protein of embodiment 319, 320, or 327, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 28.
- [1341] Embodiment 329. The engineered CD47 protein of embodiment 319 or 320, wherein the transmembrane domain is or comprises a CD28 transmembrane domain.
- [1342] Embodiment 330. The engineered CD47 protein of embodiment 319, 320, or 329, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 40.
- [1343] Embodiment 331. The engineered CD47 protein of embodiment 319 or 320, wherein the transmembrane domain is or comprises a PDGFR transmembrane domain.
- [1344] Embodiment 332. The engineered CD47 protein of embodiment 319, 320, or 331, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 42.
- [1345] Embodiment 333. The engineered CD47 protein of any one of embodiments 298-332, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.
- [1346] Embodiment 334. The engineered CD47 protein of embodiment A35, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
- [1347] Embodiment 335. The engineered CD47 protein of embodiment A35 or A36, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor.
- [1348] Embodiment 336. The engineered CD47 protein of any one of embodiments 333-335, wherein the DAF/CD55 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 34.
- [1349] Embodiment 337. The engineered CD47 protein of embodiment 333 or 334, wherein the GPI anchor is or comprises a TRAILR3 GPI anchor.
- [1350] Embodiment 338. The engineered CD47 protein of any one of embodiments 333, 335 or 337, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 35.
- [1351] Embodiment 339. The engineered CD47 protein of any one of embodiments 333, 335 or 337, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 36.
- [1352] Embodiment 340. The engineered protein of embodiment 333 or 334, wherein the GPI anchor is or comprises a CD59 GPI anchor.
- [1353] Embodiment 341. The engineered protein of any one of embodiments 333, 335 or 340, wherein the CD59 GPI anchor comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 37.

- [1354] Embodiment 342. The engineered protein of any one of embodiments 298-341, further comprising an extracellular signal peptide.
- [1355] Embodiment 343. The engineered protein of embodiment 342, wherein the extracellular signal peptide is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 20.
- [1356] Embodiment 344. An engineered CD47 protein, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 4 or SEQ ID NO: 5.
- [1357] Embodiment 345. The engineered CD47 protein of any one of embodiments 344, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 348.
- [1358] Embodiment 346. The engineered CD47 protein of 344 or 345, wherein the engineered CD47 protein comprises an amino acid sequence comprising SEQ ID NO: 347.
- [1359] Embodiment 347. The engineered CD47 protein of any one of embodiments 344-346, wherein the engineered CD47 protein comprises an amino acid sequence comprising, from N-terminus to C-terminus:
an amino acid sequence comprising SEQ ID NO: 348; and
an amino acid sequence comprising SEQ ID NO: 4 or SEQ ID NO: 5.
- [1360] Embodiment 348. The engineered CD47 protein of embodiment 347, wherein the engineered protein comprises one or more membrane tethers.
- [1361] Embodiment 349. The engineered CD47 protein of any one of embodiments 344-348, wherein the engineered protein comprises one or more membrane tethers.
- [1362] Embodiment 350. The engineered CD47 protein of embodiment 349, wherein the one or more membrane tethers are or comprise a transmembrane domain.
- [1363] Embodiment 351. The engineered CD47 protein of embodiment 350, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNCR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.
- [1364] Embodiment 352. The engineered CD47 protein of embodiment 350 or 351, wherein the transmembrane domain is or comprises a CD47 transmembrane domain.
- [1365] Embodiment 353. The engineered CD47 protein of any one of embodiments 350-352, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 23.
- [1366] Embodiment 354. The engineered CD47 protein of any one of embodiments 350-352, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 25.
- [1367] Embodiment 355. The engineered CD47 protein of any one of embodiments 350-352, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 27.
- [1368] Embodiment 356. The engineered CD47 protein of any one of embodiments 350-352, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 29.
- [1369] Embodiment 357. The engineered CD47 protein of any one of embodiments 350-352, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 31.
- [1370] Embodiment 358. The engineered CD47 protein of embodiment 350 or 351, wherein the transmembrane domain is or comprises a CD8a transmembrane domain.
- [1371] Embodiment 359. The engineered CD47 protein of embodiment 350, 351, or 358, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 38.

- [1372] Embodiment 360. The engineered CD47 protein of embodiment 350 or 351, wherein the transmembrane domain is or comprises a CD28 transmembrane domain.
- [1373] Embodiment 361. The engineered CD47 protein of embodiment 350, 351, or 360, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 40.
- [1374] Embodiment 362. The engineered CD47 protein of embodiment 350 or 351, wherein the transmembrane domain is or comprises a PDGFR transmembrane domain.
- [1375] Embodiment 363. The engineered CD47 protein of embodiment 350, 351, or 362, wherein the transmembrane domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 42.
- [1376] Embodiment 364. The engineered CD47 protein of any one of embodiments 348-363, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.
- [1377] Embodiment 365. The engineered CD47 protein of embodiment 364, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
- [1378] Embodiment 366. The engineered CD47 protein of embodiment 364 or 365, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor.
- [1379] Embodiment 367. The engineered CD47 protein of any one of embodiments 364-366, wherein the DAF/CD55 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 34.
- [1380] Embodiment 368. The engineered CD47 protein of embodiment 364 or 365, wherein the GPI anchor is or comprises a TRAILR3 GPI anchor.
- [1381] Embodiment 369. The engineered CD47 protein of any one of embodiments 364, 365, or 368, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 35.
- [1382] Embodiment 370. The engineered CD47 protein of any one of embodiments 364, 365, or 368, wherein the TRAILR3 GPI anchor is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 36.
- [1383] Embodiment 371. The engineered protein of embodiment 364 or 365, wherein the GPI anchor is or comprises a CD59 GPI anchor.
- [1384] Embodiment 372. The engineered protein of any one of embodiments 364, 365, or 371, wherein the CD59 GPI anchor comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 37.
- [1385] Embodiment 373. The engineered protein of any one of embodiments 347-372, further comprising an extracellular signal peptide.
- [1386] Embodiment 374. The engineered protein of embodiment 373, wherein the extracellular signal peptide is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 20.
- [1387] Embodiment 375. The engineered protein of any one of the preceding embodiments, further comprising an intracellular domain.
- [1388] Embodiment 376. The engineered protein of embodiment 375, wherein the intracellular domain is or comprises a CD47 intracellular domain or a portion thereof.
- [1389] Embodiment 377. The engineered protein of embodiment 375 or 376, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 24.
- [1390] Embodiment 378. The engineered protein of embodiment 375 or 376, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 28.
- [1391] Embodiment 379. The engineered protein of embodiment 375 or 376, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 32.

- [1392] Embodiment 380. The engineered protein of embodiment 375 or 376, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 33.
- [1393] Embodiment 381. The engineered protein of embodiment 375 or 376, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 343.
- [1394] Embodiment 382. The engineered protein of embodiment 375 or 376, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 345.
- [1395] Embodiment 383. The engineered protein of embodiment 375, wherein the intracellular domain is or comprises a CD8a intracellular domain or a portion thereof.
- [1396] Embodiment 384. The engineered protein of embodiment 375 or 383, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 39.
- [1397] Embodiment 385. The engineered protein of embodiment 375, wherein the intracellular domain is or comprises a CD28 intracellular domain or a portion thereof.
- [1398] Embodiment 386. The engineered protein construct of embodiment 375 or 385, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 41.
- [1399] Embodiment 387. The engineered protein of embodiment 375, wherein the intracellular domain is or comprises a PDGFR intracellular domain or a portion thereof.
- [1400] Embodiment 388. The engineered protein of embodiment 375 or 387, wherein the intracellular domain comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 43.
- [1401] Embodiment 389. The engineered protein of any one of embodiments 375-388, wherein the intracellular domain comprises one or more modifications relative to a wild-type CD47 intracellular domain.
- [1402] Embodiment 390. The engineered protein of any one of embodiments 375-389, wherein the intracellular domain comprises one or more deletions relative to a wild-type CD47 intracellular domain.
- [1403] Embodiment 391. The engineered protein of any one of embodiments 375-390, wherein the intracellular domain comprises one or more insertions relative to a wild-type CD47 intracellular domain.
- [1404] Embodiment 392. The engineered protein of any one of embodiments 375-391, wherein the intracellular domain comprises altered function relative to a wild-type CD47 intracellular domain.
- [1405] Embodiment 393. The engineered protein of any one of embodiments 375-392, wherein the intracellular domain comprises reduced function relative to a wild-type CD47 intracellular domain.
- [1406] Embodiment 394. The engineered protein of any one of embodiments 375-393, wherein the intracellular domain comprises reduced levels of CD47 intracellular signaling relative to a wild-type CD47 intracellular domain.
- [1407] Embodiment 395. The engineered protein of any one of embodiments 375-394, wherein the intracellular domain comprises a non-functional intracellular domain.
- [1408] Embodiment 396. The engineered protein of any one of embodiments 270-395, comprising an amino acid sequence at least 80% identical to a sequence selected from Table 30.
- [1409] Embodiment 397. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 1.
- [1410] Embodiment 398. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 2.
- [1411] Embodiment 399. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 6.

- [1412] Embodiment 400. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 80.
- [1413] Embodiment 401. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 81.
- [1414] Embodiment 402. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 82.
- [1415] Embodiment 403. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 83.
- [1416] Embodiment 404. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 84.
- [1417] Embodiment 405. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 85.
- [1418] Embodiment 406. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 86.
- [1419] Embodiment 407. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 87.
- [1420] Embodiment 408. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 88.
- [1421] Embodiment 409. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 89.
- [1422] Embodiment 410. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 90.
- [1423] Embodiment 411. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 91.
- [1424] Embodiment 412. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 92.
- [1425] Embodiment 413. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 93.
- [1426] Embodiment 414. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 94.
- [1427] Embodiment 415. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 95.
- [1428] Embodiment 416. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 96.
- [1429] Embodiment 417. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 97.
- [1430] Embodiment 418. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 98.
- [1431] Embodiment 419. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 99.

- [1432] Embodiment 420. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 100.
- [1433] Embodiment 421. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 101.
- [1434] Embodiment 422. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 102.
- [1435] Embodiment 423. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 103.
- [1436] Embodiment 424. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 104.
- [1437] Embodiment 425. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 105.
- [1438] Embodiment 426. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 106.
- [1439] Embodiment 427. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 107.
- [1440] Embodiment 428. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 108.
- [1441] Embodiment 429. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 109.
- [1442] Embodiment 430. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 110.
- [1443] Embodiment 431. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 111.
- [1444] Embodiment 432. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 112.
- [1445] Embodiment 433. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 113.
- [1446] Embodiment 434. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 114.
- [1447] Embodiment 435. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 115.
- [1448] Embodiment 436. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 116.
- [1449] Embodiment 437. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 117.
- [1450] Embodiment 438. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 118.
- [1451] Embodiment 439. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 119.

- [1452] Embodiment 440. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 120.
- [1453] Embodiment 441. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 121.
- [1454] Embodiment 442. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 122.
- [1455] Embodiment 443. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 123.
- [1456] Embodiment 444. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 124.
- [1457] Embodiment 445. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 125.
- [1458] Embodiment 446. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 126.
- [1459] Embodiment 447. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 127.
- [1460] Embodiment 448. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 128.
- [1461] Embodiment 449. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 129.
- [1462] Embodiment 450. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 130.
- [1463] Embodiment 451. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 131.
- [1464] Embodiment 452. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 132.
- [1465] Embodiment 453. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 340.
- [1466] Embodiment 454. The engineered protein of any one of embodiments 270-396, comprising an amino acid sequence at least 80% identical to SEQ ID NO: 199.
- [1467] Embodiment 455. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to one or more sequences selected from Table 32.
- [1468] Embodiment 456. The engineered protein of any one of the preceding embodiments, comprising one or more nucleic acid sequences at least 80% identical to one or more sequences selected from Table 32 and/or Table 30.
- [1469] Embodiment 457. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 20, 21, 23, 24, 25, and 26.
- [1470] Embodiment 458. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 20, 21, 23, 24, and 25.
- [1471] Embodiment 459. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 20, 21, 23, and 24.

- [1472] Embodiment 460. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, and 31.
- [1473] Embodiment 461. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 20, 21, 23, 24, 25, 26, 27, 28, 29, and 30.
- [1474] Embodiment 462. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 20, 21, 23, 24, 25, 26, 27, 28, and 29.
- [1475] Embodiment 463. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 20, 21, 23, 24, 25, 26, 27, and 28.
- [1476] Embodiment 464. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 22 and 34.
- [1477] Embodiment 465. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 22 and 35.
- [1478] Embodiment 466. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 22 and 36.
- [1479] Embodiment 467. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 22, 38, and 39.
- [1480] Embodiment 468. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 22, 40, and 41.
- [1481] Embodiment 469. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 22, 42, and 43.
- [1482] Embodiment 470. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 21, 47, 42, and 43.
- [1483] Embodiment 471. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 21, 48, 40, and 41.
- [1484] Embodiment 472. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 21, 48, 38, and 39.
- [1485] Embodiment 473. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 21, 46, 40, and 41.
- [1486] Embodiment 474. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 21, 46, 38, and 39.
- [1487] Embodiment 475. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 21, 45, 40, and 41.
- [1488] Embodiment 476. The engineered protein of any one of embodiments 270-388, comprising one or more amino acid sequences at least 80% identical to SEQ ID NOs: 21, 45, 38, and 39.
- [1489] Embodiment 477. The engineered protein of any one of embodiments 270-476, comprising fewer glycosylation modification sites than a wild-type human CD47 protein.
- [1490] Embodiment 478. The engineered protein of embodiment 477, wherein the engineered protein does not comprise an N206 glycosylation site.
- [1491] Embodiment 479. A genetically engineered cell comprising the engineered protein of any one of embodiments 270-478.

- [1492]** Embodiment 480. A genetically engineered cell comprising a first transgene encoding an engineered protein of any one of embodiments 270-478, and wherein the engineered protein does not comprise one or more full-length CD47 intracellular domains
- [1493]** Embodiment 481. The genetically engineered cell of embodiment 480, wherein the wherein the engineered protein comprises: (a) one or more extracellular domains and/or (b) one or more membrane tethers.
- [1494]** Embodiment 482. The genetically engineered cell of embodiment 481, wherein the one or more extracellular domains comprises a CD47 extracellular domain.
- [1495]** Embodiment 483. The genetically engineered cell of embodiment 482, wherein the CD47 extracellular domain is a CD47 immunoglobulin variable (IgV)-like domain and/or a human CD47 extracellular domain.
- [1496]** Embodiment 484. The genetically engineered cell of any one of embodiments 481-483, wherein the CD47 extracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 52.
- [1497]** Embodiment 485. The genetically engineered cell of any one of embodiments 481-483, wherein the CD47 extracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 53.
- [1498]** Embodiment 486. The genetically engineered cell of any one of embodiments 481-483, wherein the CD47 extracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 57.
- [1499]** Embodiment 487. The genetically engineered cell of any one of embodiments 481-483, wherein the CD47 extracellular domain is encoded by a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 59.
- [1500]** Embodiment 488. The genetically engineered cell of embodiment 480, wherein the wherein the engineered protein comprises a SIRPa interaction motif, optionally wherein is or comprises a SIRPa antibody or a portion thereof.
- [1501]** Embodiment 489. The genetically engineered cell of any one of embodiments 480-488, wherein the one or more membrane tethers are or comprise a transmembrane domain.
- [1502]** Embodiment 490. The genetically engineered cell of embodiment 489, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNGR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.
- [1503]** Embodiment 491. The genetically engineered cell of embodiment 489 or 490, wherein the transmembrane domain is or comprises a CD47 transmembrane domain.
- [1504]** Embodiment 492. The genetically engineered cell of any one of embodiments 489-491, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 54.
- [1505]** Embodiment 493. The genetically engineered cell of any one of embodiments 489-491, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 56.
- [1506]** Embodiment 494. The genetically engineered cell of any one of embodiments 489-491, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 58.

- [1507]** Embodiment 495. The genetically engineered cell of any one of embodiments 489-491, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 60.
- [1508]** Embodiment 496. The genetically engineered cell of any one of embodiments 489-491, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 62.
- [1509]** Embodiment 497. The genetically engineered cell of embodiment 489 or 490, wherein the transmembrane domain is or comprises a CD8a transmembrane domain.
- [1510]** Embodiment 498. The genetically engineered cell of embodiment 489, 490, or 497, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 69.
- [1511]** Embodiment 499. The genetically engineered cell of embodiment 489 or 490, wherein the transmembrane domain is or comprises a CD28 transmembrane domain.
- [1512]** Embodiment 500. The genetically engineered cell of embodiment 489, 490, or 499, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 71.
- [1513]** Embodiment 501. The genetically engineered cell of embodiment 489 or 490, wherein the transmembrane domain is or comprises a PDGFR transmembrane domain.
- [1514]** Embodiment 502. The genetically engineered cell of embodiment 489, 490, or 501, wherein the transmembrane domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 73.
- [1515]** Embodiment 503. The genetically engineered cell of any one of embodiments 480-488, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.
- [1516]** Embodiment 504. The genetically engineered cell of embodiment 503, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
- [1517]** Embodiment 505. The genetically engineered cell of embodiment 503 or 504, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor.
- [1518]** Embodiment 506. The genetically engineered cell of any one of embodiments 503-505, wherein the DAF/CD55 GPI anchor is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 65.
- [1519]** Embodiment 507. The genetically engineered cell of embodiment 503 or 504, wherein the GPI anchor is or comprises a TRAILR3 GPI anchor.
- [1520]** Embodiment 508. The genetically engineered cell of any one of embodiments 503, 504, or 507, wherein the TRAILR3 GPI anchor is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 66.
- [1521]** Embodiment 509. The genetically engineered cell of any one of embodiments 503, 504, or 507, wherein the TRAILR3 GPI anchor is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 67.
- [1522]** Embodiment 510. The genetically engineered cell of embodiment 503 or 504, wherein the GPI anchor is or comprises a CD59 GPI anchor.

- [1523]** Embodiment 511. The genetically engineered cell of any one of embodiments 503, 504, or 510, wherein the CD59 GPI anchor is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 68.
- [1524]** Embodiment 512. The genetically engineered cell of any one of embodiments 480-503, further comprising one or more control sequences.
- [1525]** Embodiment 513. The genetically engineered cell of embodiment 512, wherein the one or more control sequences encode an extracellular signal peptide.
- [1526]** Embodiment 514. The genetically engineered cell of embodiment 512 or 513, wherein the extracellular signal peptide is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 51.
- [1527]** Embodiment 515. The genetically engineered cell of embodiment 512, wherein the one or more control sequences comprise a promoter.
- [1528]** Embodiment 516. The genetically engineered cell of embodiment 515, wherein the promoter is a constitutive promoter or an inducible promoter.
- [1529]** Embodiment 517. The genetically engineered cell of embodiment 512 or 516, wherein the promoter is a naturally occurring promoter, a hybrid promoter, or a synthetic promoter.
- [1530]** Embodiment 518. The genetically engineered cell of any one of embodiments 512-517, wherein the promoter is or comprises an EF1 α promoter, an EF1 α short promoter, a CAG promoter, a ubiquitin/S27a promoter, an SV40 early promoter, an adenovirus major late promoter, a mouse metallothionein-I promoter, an RSV promoter, an MMTV promoter, a Moloney murine leukemia virus Long Terminal repeat region, a CMV promoter, an actin promoter, an immunoglobulin promoter, a heat shock promoter, polyoma virus promoter, a fowlpox virus promoter, a bovine papilloma virus promoter, an avian sarcoma virus promoter, a retrovirus promoter, a hepatitis-B virus promoter, a PGK promoter, an adenovirus late promoter, a vaccinia virus 7.5K promoter, a SV40 promoter, a tk promoter of HSV, a mouse mammary tumor virus (MMTV) promoter, an LTR promoter of HIV, a promoter of moloney virus, an Epstein Barr virus (EBV) promoter, a Rous sarcoma virus (RSV) promoter, or an UBC promoter.
- [1531]** Embodiment 519. The genetically engineered cell of any one of embodiments 512-518, wherein the promoter is or comprises a CAG promoter.
- [1532]** Embodiment 520. The genetically engineered cell of any one of embodiments 512-519, wherein the first transgene comprises the promoter, and wherein the promoter comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 49.
- [1533]** Embodiment 521. The genetically engineered cell of any one of embodiments 512-518, wherein the promoter is or comprises an EF1 α promoter.
- [1534]** Embodiment 522. The genetically engineered cell of any one of embodiments 512-519, or 521, wherein the first transgene comprises the promoter, and wherein the promoter comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 50.
- [1535]** Embodiment 523. The genetically engineered cell of embodiment 512, wherein the one or more control sequences comprise ribosomal binding sites, enhancer elements, activator elements, translational start sequences, translational termination sequences, transcription start sequences, transcription termination sequences, polyadenylation signal sequences, replication elements, RNA processing and export elements, transposon sequences, transposase sequences, insulator sequences, internal ribosome entry sites (IRES), 5'UTRs, 3'UTRs, mRNA 3' end processing sequences, boundary elements, locus control regions (LCR), matrix attachment regions (MAR), recombination or cassette

exchange sequences, linker sequences, secretion signals, resistance markers, anchoring peptides, localization signals, fusion tags, affinity tags, chaperonins, proteases, or combinations thereof.

[1536] Embodiment 524. The genetically engineered cell of any one of embodiments 480-523, wherein the one or more extracellular domains further comprise an extracellular hinge domain.

[1537] Embodiment 525. The genetically engineered cell of embodiment 524, wherein the extracellular hinge domain is or comprises a CD47 hinge, a CD8a hinge, a CD28 hinge, a PDGFR hinge, or an IgG4 hinge.

[1538] Embodiment 526. The genetically engineered cell of embodiment 524 or 525, wherein the extracellular hinge domain is or comprises a CD47 hinge.

[1539] Embodiment 527. The genetically engineered cell of any one of embodiments 524-526, wherein the extracellular hinge domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 75.

[1540] Embodiment 528. The genetically engineered cell of embodiment 524 or 525, wherein the extracellular hinge domain is or comprises a CD8a hinge.

[1541] Embodiment 529. The genetically engineered cell of embodiment 524, 525, or 528, wherein the extracellular hinge domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 76.

[1542] Embodiment 530. The genetically engineered cell of embodiment 524 or 525, wherein the extracellular hinge domain is or comprises a CD28 hinge.

[1543] Embodiment 531. The genetically engineered cell of embodiment 524, 525, or 530, wherein the extracellular hinge domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 77.

[1544] Embodiment 532. The genetically engineered cell of embodiment 524 or 525, wherein the extracellular hinge domain is or comprises a PDGFR hinge.

[1545] Embodiment 533. The genetically engineered cell of embodiment 524, 525, or 532, wherein the extracellular hinge domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 78.

[1546] Embodiment 534. The genetically engineered cell of embodiment 524 or 525, wherein the extracellular hinge domain is or comprises an IgG4 hinge.

[1547] Embodiment 535. The genetically engineered cell of embodiment 524, 525, or 534, wherein the extracellular hinge domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 79.

[1548] Embodiment 536. The genetically engineered cell of any one of embodiments 480-535, wherein the first transgene further comprises one or more nucleic acid sequences encoding an intracellular domain.

[1549] Embodiment 537. The genetically engineered cell of embodiment 56, wherein the intracellular domain is or comprises a CD47 intracellular domain or a portion thereof.

[1550] Embodiment 538. The genetically engineered cell of embodiment 536 or 537, wherein the intracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 55.

[1551] Embodiment 539. The genetically engineered cell of embodiment 536 or 537, wherein the intracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 59.

[1552] Embodiment 540. The genetically engineered cell of embodiment 536 or 537, wherein the intracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 63.

[1553] Embodiment 541. The genetically engineered cell of embodiment 536 or 537, wherein the intracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 64.

[1554] Embodiment 542. The genetically engineered cell of embodiment 536, wherein the intracellular domain is or comprises a CD8a intracellular domain or a portion thereof.

[1555] Embodiment 543. The genetically engineered cell of embodiment 536 or 542, wherein the intracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 70.

[1556] Embodiment 544. The genetically engineered cell of embodiment 536, wherein the intracellular domain is or comprises a CD28 intracellular domain or a portion thereof.

[1557] Embodiment 545. The genetically engineered cell of embodiment 536 or 537, wherein the intracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 72.

[1558] Embodiment 546. The genetically engineered cell of embodiment 536, wherein the intracellular domain is or comprises a PDGFR intracellular domain or a portion thereof.

[1559] Embodiment 547. The genetically engineered cell of embodiment 536 or 546, wherein the intracellular domain is encoded by the first transgene, wherein the first transgene comprises a nucleic acid sequence that is at least 80% identical to SEQ ID NO: 74.

[1560] Embodiment 548. The genetically engineered cell of any one of embodiments 536-547, wherein the intracellular domain comprises one or more modifications relative to a wild-type CD47 intracellular domain.

[1561] Embodiment 549. The genetically engineered cell of any one of embodiments 536-548, wherein the intracellular domain comprises one or more deletions relative to a wild-type CD47 intracellular domain.

[1562] Embodiment 550. The genetically engineered cell of any one of embodiments 536-549, wherein the intracellular domain comprises one or more insertions relative to a wild-type CD47 intracellular domain.

[1563] Embodiment 551. The genetically engineered cell of any one of embodiments 536-550, wherein the intracellular domain comprises altered function relative to a wild-type CD47 intracellular domain.

[1564] Embodiment 552. The genetically engineered cell of any one of embodiments 536-551, wherein the intracellular domain comprises reduced function relative to a wild-type CD47 intracellular domain.

[1565] Embodiment 553. The genetically engineered cell of any one of embodiments 536-552, wherein the intracellular domain comprises reduced levels of CD47 intracellular signaling relative to a wild-type CD47 intracellular domain.

[1566] Embodiment 554. The genetically engineered cell of any one of embodiments 536-553, wherein the intracellular domain comprises a non-functional intracellular domain.

[1567] Embodiment 555. The genetically engineered cell of any one of embodiments 536-554, wherein the first transgene comprises a nucleic acid sequence at least 80% identical to a sequence selected from Table 24.

[1568] Embodiment 556. The genetically engineered cell of any one of embodiments 480-555, wherein the first transgene comprises a nucleic acid sequence at least 80% identical to SEQ ID NO: 13.

- [1569] Embodiment 557. The genetically engineered cell of any one of embodiments 480-555, wherein the first transgene comprises a nucleic acid sequence at least 80% identical to SEQ ID NO: 14.
- [1570] Embodiment 558. The genetically engineered cell of any one of embodiments 480-555, wherein the first transgene comprises a nucleic acid sequence at least 80% identical to SEQ ID NO: 18.
- [1571] Embodiment 559. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 133.
- [1572] Embodiment 560. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 134.
- [1573] Embodiment 561. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 135.
- [1574] Embodiment 562. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 136.
- [1575] Embodiment 563. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 137.
- [1576] Embodiment 564. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 138.
- [1577] Embodiment 565. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 139.
- [1578] Embodiment 566. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 140.
- [1579] Embodiment 567. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 141.
- [1580] Embodiment 568. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 142.
- [1581] Embodiment 569. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 143.
- [1582] Embodiment 570. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 144.
- [1583] Embodiment 571. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 145.
- [1584] Embodiment 572. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 146.
- [1585] Embodiment 573. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 147.
- [1586] Embodiment 574. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 148.
- [1587] Embodiment 575. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 149.
- [1588] Embodiment 576. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 150.

- [1589]** Embodiment 577. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 151.
- [1590]** Embodiment 578. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 152.
- [1591]** Embodiment 579. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 153.
- [1592]** Embodiment 580. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 154.
- [1593]** Embodiment 581. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 155.
- [1594]** Embodiment 582. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 156.
- [1595]** Embodiment 583. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 157.
- [1596]** Embodiment 584. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 158.
- [1597]** Embodiment 585. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 159.
- [1598]** Embodiment 586. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 160.
- [1599]** Embodiment 587. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 161.
- [1600]** Embodiment 588. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 162.
- [1601]** Embodiment 589. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 163.
- [1602]** Embodiment 590. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 164.
- [1603]** Embodiment 591. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 165.
- [1604]** Embodiment 592. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 166.
- [1605]** Embodiment 593. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 167.
- [1606]** Embodiment 594. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 168.
- [1607]** Embodiment 595. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 169.
- [1608]** Embodiment 596. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 170.

- [1609] Embodiment 597. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 171.
- [1610] Embodiment 598. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 172.
- [1611] Embodiment 599. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 173.
- [1612] Embodiment 600. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 174.
- [1613] Embodiment 601. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 175.
- [1614] Embodiment 602. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 176.
- [1615] Embodiment 603. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 177.
- [1616] Embodiment 604. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 178.
- [1617] Embodiment 605. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 179.
- [1618] Embodiment 606. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 180.
- [1619] Embodiment 607. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 181.
- [1620] Embodiment 608. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 182.
- [1621] Embodiment 609. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 183.
- [1622] Embodiment 610. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 184.
- [1623] Embodiment 611. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 185.
- [1624] Embodiment 612. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 341.
- [1625] Embodiment 613. The genetically engineered cell of any one of embodiments 480-555, comprising a nucleotide sequence at least 80% identical to SEQ ID NO: 342.
- [1626] Embodiment 614. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to one or more sequences selected from Table 26.
- [1627] Embodiment 615. The genetically engineered cell of any one of embodiments 480, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to one or more sequences selected from Table 26 and/or Table 24.

- [1628] Embodiment 616. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 49 and SEQ ID NO: 14.
- [1629] Embodiment 617. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 14.
- [1630] Embodiment 618. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 49 and SEQ ID NO: 13.
- [1631] Embodiment 619. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 13.
- [1632] Embodiment 620. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 49 and SEQ ID NO: 18.
- [1633] Embodiment 621. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 18.
- [1634] Embodiment 622. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 51, 52, 54, 55, 56, and 57.
- [1635] Embodiment 623. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 51, 52, 54, 55, and 56.
- [1636] Embodiment 624. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 51, 52, 54, and 55.
- [1637] Embodiment 625. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, and 62.
- [1638] Embodiment 626. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 51, 52, 54, 55, 56, 57, 58, 59, 60, and 61.
- [1639] Embodiment 627. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 51, 52, 54, 55, 56, 57, 58, 59, and 60.
- [1640] Embodiment 628. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 51, 52, 54, 55, 56, 57, 58, and 59.
- [1641] Embodiment 629. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 53, and 65.
- [1642] Embodiment 630. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 53, and 65.
- [1643] Embodiment 631. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 53, and 66.

- [1644] Embodiment 632. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 53, and 66.
- [1645] Embodiment 633. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 53, and 67.
- [1646] Embodiment 634. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 53, and 67.
- [1647] Embodiment 635. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 53, 69, and 70.
- [1648] Embodiment 636. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 53, 69, and 70.
- [1649] Embodiment 637. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 53, 71, and 72.
- [1650] Embodiment 638. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 53, 71, and 72.
- [1651] Embodiment 639. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 53, 73, and 74.
- [1652] Embodiment 640. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 53, 73, and 74.
- [1653] Embodiment 641. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 52, 78, 73, and 74.
- [1654] Embodiment 642. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 52, 78, 73, and 74.
- [1655] Embodiment 643. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 52, 79, 71, and 72.
- [1656] Embodiment 644. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 52, 79, 71, and 72.
- [1657] Embodiment 645. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 52, 79, 69, and 70.
- [1658] Embodiment 646. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 52, 79, 69, and 70.
- [1659] Embodiment 647. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 52, 77, 71, and 72.
- [1660] Embodiment 648. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 52, 77, 71, and 72.
- [1661] Embodiment 649. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 52, 77, 69, and 70.

- [1662] Embodiment 650. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 52, 77, 69, and 70.
- [1663] Embodiment 651. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 52, 76, 71, and 72.
- [1664] Embodiment 652. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 52, 76, 71, and 72.
- [1665] Embodiment 653. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49, 52, 76, 69, and 70.
- [1666] Embodiment 654. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 52, 76, 69, and 70.
- [1667] Embodiment 655. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 49 and 200.
- [1668] Embodiment 656. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NOs: 50 and 200.
- [1669] Embodiment 657. The genetically engineered cell of any one of embodiments 480-547, wherein the first transgene comprises one or more nucleic acid sequences at least 80% identical to SEQ ID NO: 200.
- [1670] Embodiment 658. The genetically engineered cell of any one of claims 480-657, comprising, in its genome, the first transgene at a first insertion site.
- [1671] Embodiment 659. The genetically engineered cell of embodiment 658, wherein the first insertion site is a T-cell receptor (TCR) locus.
- [1672] Embodiment 660. The genetically engineered cell of embodiment 659, wherein the TCR locus is or comprises: a TRAC locus, a TRBC1 locus, or a TRBC2 locus.
- [1673] Embodiment 661. The genetically engineered cell of embodiment 659, wherein the first insertion site is a $\beta 2$ microglobulin (B2M) locus.
- [1674] Embodiment 662. The genetically engineered cell of embodiment 659, wherein the first insertion site is a class II transactivator (CIITA) locus.
- [1675] Embodiment 663. The genetically engineered cell of embodiment 659, wherein the first insertion site is a safe harbor locus.
- [1676] Embodiment 664. The genetically engineered cell of embodiment 663, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.
- [1677] Embodiment 665. The genetically engineered cell of any one of embodiments 659-664, wherein the first insertion site is an exon.
- [1678] Embodiment 666. The genetically engineered cell of any one of embodiments 659-664, wherein the first insertion site is an intron.
- [1679] Embodiment 667. The genetically engineered cell of any one of embodiments 659-664, wherein the first insertion site is between an intron and an exon.
- [1680] Embodiment 668. The genetically engineered cell of any one of embodiments 659-664, wherein the first insertion site is in a regulatory region.

- [1681] Embodiment 669. The genetically engineered cell of any one of embodiments 480-668, wherein the genetically engineered cell has cell surface expression of the engineered protein.
- [1682] Embodiment 670. The genetically engineered cell of any one of embodiments 480-669, wherein the genetically engineered cell has decreased cell surface expression of a TCR as compared to a comparable cell that has not been genetically engineered.
- [1683] Embodiment 671. The genetically engineered cell of any one of embodiments 480-670, wherein the genetically engineered cell has decreased cell surface expression of B2M as compared to a comparable cell that has not been genetically engineered.
- [1684] Embodiment 672. The genetically engineered cell of any one of embodiments 480-671, wherein the genetically engineered cell has decreased expression of CIITA as compared to a comparable cell that has not been genetically engineered.
- [1685] Embodiment 673. The genetically engineered cell of any one of embodiments 480-672, wherein the genetically engineered cell comprises a modification at a TCR locus, B2M locus, a TAP I locus, a NLRC5 locus, a CIITA locus, an HLA-A locus, an HLA-B locus, an HLA-C locus, an HLA-DP locus, an HLA-DM locus, an HLA-DOA locus, an HLA-DOB locus, an HLA-DQ locus, an HLA-DR locus, a RFX5 locus, a RFXANK locus, a RFXAP locus, an NFY-A locus, an NFY-B locus, an NFY-C locus, or a combination thereof.
- [1686] Embodiment 674. The genetically engineered cell of any one of embodiments 480-673, wherein the genetically engineered cell has been genetically engineered to knock-out an HLA-A locus, an HLA-B locus, an HLA-C locus, or a combination thereof.
- [1687] Embodiment 675. The genetically engineered cell of any one of embodiments 480-673, wherein the genetically engineered cell has been genetically engineered to knock-out an HLA-DM locus, an HLA-DO locus, an HLA-DP locus, an HLA-DQ locus, an HLA-DR locus, or a combination thereof.
- [1688] Embodiment 676. The genetically engineered cell of any one of embodiments 480-675, wherein the genetically engineered cell has been genetically engineered to knock-out a TCR locus.
- [1689] Embodiment 677. The genetically engineered cell of any one of embodiments 480-676, wherein the genetically engineered cell has been genetically engineered to knock-out a B2M locus.
- [1690] Embodiment 678. The genetically engineered cell of any one of embodiments 480-677, wherein the genetically engineered cell has been genetically engineered to knock-out a CIITA locus.
- [1691] Embodiment 679. The genetically engineered cell of any one of embodiments 480-678, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of: (a) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (b) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.
- [1692] Embodiment 680. The genetically engineered cell of any one of embodiments 480-679, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules.
- [1693] Embodiment 681. The genetically engineered cell of any one of embodiments 480-680, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.
- [1694] Embodiment 682. The genetically engineered cell of any one of embodiments 480-681, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of: (a) one or more MHC class I

molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and (b) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[1695] Embodiment 683. The genetically engineered cell of any one of embodiments 480-682, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class I molecules.

[1696] Embodiment 684. The genetically engineered cell of any one of embodiments 480-683, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class I molecules.

[1697] Embodiment 685. The genetically engineered cell of any one of embodiments 480-684, wherein the one or more modifications reduce a function of the one or more MHC class I molecules, optionally wherein the function is antigen presentation.

[1698] Embodiment 686. The genetically engineered cell of any one of embodiments 480-685, wherein the one or more MHC class I molecules are one or more human leukocyte antigen (HLA) class I molecules.

[1699] Embodiment 687. The genetically engineered cell of embodiment 686, wherein the one or more HLA class I molecules are selected from the group consisting of HLA-A, HLA-B, and HLA-C.

[1700] Embodiment 688. The genetically engineered cell of any one of embodiments 480-685, wherein the one or more molecules that regulate expression of the one or more MHC class I molecules are selected from the group consisting of B2M, NLRC5 and TAP1.

[1701] Embodiment 689. The genetically engineered cell of any one of embodiments 480-688, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class II molecules.

[1702] Embodiment 690. The genetically engineered cell of any one of embodiments 480-689, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class II molecules.

[1703] Embodiment 691. The genetically engineered cell of any one of embodiments 480-690, wherein the one or more modifications reduce a function of the one or more MHC class II molecules, optionally wherein the function is antigen presentation.

[1704] Embodiment 692. The genetically engineered cell of any one of embodiments 480-691, wherein the one or more MHC class II molecules are one or more human leukocyte antigen (HLA) class II molecules.

[1705] Embodiment 693. The genetically engineered cell of embodiment 692, wherein the one or more HLA class II molecules are selected from the group consisting of HLA-DP, HLA-DQ, and/or HLA-DR.

[1706] Embodiment 694. The genetically engineered cell of any one of embodiments 480-693, wherein the one or more molecules that regulate expression of the one or more MHC class II molecules is/are selected from the group consisting of CIITA and CD74.

[1707] Embodiment 695. The genetically engineered cell of any one of embodiments 480-694, further comprising a second transgene encoding a tolerogenic factor.

[1708] Embodiment 696. The genetically engineered cell of embodiment 695, wherein the tolerogenic factor is or comprises A20/TNFAIP3, B2M-HLA-E, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, CD52, CD55, CD59, CD64, CD200, CCL21, CCL22, CTLA4-Ig, C1 inhibitor, CR1, DUX4, FASL, HLA-C, HLA-E, HLA-E heavy chain, HLA-F, HLA-G, H2-M3, IDO1, IL-10, IL15-RF, IL-35, IL-39, MANF, Mfge8, PD-L1, or Serpinb9.

[1709] Embodiment 697. The genetically engineered cell of embodiment 695 or 696, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a TCR locus.

[1710] Embodiment 698. The genetically engineered cell of embodiment 695 or 696, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a B2M locus.

- [1711]** Embodiment 699. The genetically engineered cell of embodiment 695 or 696, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a CIITA locus.
- [1712]** Embodiment 700. The genetically engineered cell of embodiment 695 or 696, wherein the second transgene encoding the tolerogenic factor is inserted at an insertion site at a safe harbor locus.
- [1713]** Embodiment 701. The genetically engineered cell of embodiment 700, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.
- [1714]** Embodiment 702. The genetically engineered cell of any one of embodiments 694-701, wherein the genetically engineered cell has cell surface expression of the tolerogenic factor.
- [1715]** Embodiment 703. The genetically engineered cell of any one of embodiments 480-702, further comprising a third transgene encoding a chimeric antigen receptor (CAR).
- [1716]** Embodiment 704. The genetically engineered cell of embodiment 703, wherein the CAR is or comprises a CD5-specific CAR, a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD23-specific CAR, a CD30-specific CAR, a CD33-specific CAR, CD38-specific CAR, a CD70-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a Kappa, Lambda, B cell maturation agent (BCMA)-specific CAR, a G-protein coupled receptor family C group 5 member D (GPRC5D)-specific CAR, a CD123-specific CAR, a LeY-specific CAR, a NKG2D ligand-specific CAR, a WT1-specific CAR, a GD2-specific CAR, a HER2-specific CAR, a EGFR-specific CAR, a EGFRvIII-specific CAR, a B7H3-specific CAR, a PSMA-specific CAR, a PSCA-specific CAR, a CAIX-specific CAR, a CD171-specific CAR, a CEA-specific CAR, a CSPG4-specific CAR, a EPHA2-specific CAR, a FAP-specific CAR, a FRa-specific CAR, a IL-13R α -specific CAR, a Mesothelin-specific CAR, a MUC1-specific CAR, a MUC16-specific CAR, a ROR1-specific CAR, a C-Met-specific CAR, a CD133-specific CAR, a Ep-CAM-specific CAR, a GPC3-specific CAR, a HPV16-E6-specific CAR, a IL13R α 2-specific CAR, a MAGEA3-specific CAR, a MAGEA4-specific CAR, a MART1-specific CAR, a NY-ESO-1-specific CAR, a VEGFR2-specific CAR, a α -Folate receptor-specific CAR, a CD24-specific CAR, a CD44v7/8-specific CAR, a EGP-2-specific CAR, a EGP-40-specific CAR, a erb-B2-specific CAR, a erb-B 2,3,4-specific CAR, a FBP-specific CAR, a Fetal acetylcholine e receptor-specific CAR, a GD2-specific CAR, a GD3-specific CAR, a HMW-MAA-specific CAR, a IL-11R α -specific CAR, a KDR-specific CAR, a Lewis Y-specific CAR, a L1-cell adhesion molecule-specific CAR, a MAGE-A1-specific CAR, a Oncofetal antigen (h5T4)-specific CAR, a TAG-72-specific CAR, or a CD19/CD22-bispecific CAR.
- [1717]** Embodiment 705. The genetically engineered cell of embodiment 703 or 704, wherein the CAR is or comprises a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD38-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a BCMA-specific CAR, or a CD19/CD22-bispecific CAR.
- [1718]** Embodiment 706. The genetically engineered cell of any one of embodiments 703-705, wherein the genetically engineered cell has cell surface expression of the CAR.
- [1719]** Embodiment 707. The genetically engineered cell of any one of embodiments 480-701, further comprising a third transgene encoding a chimeric auto antigen receptor (CAAR).
- [1720]** Embodiment 708. The genetically engineered cell of embodiment 707, wherein the CAAR comprises an antigen selected from the group consisting of a pancreatic β -cell antigen, synovial joint antigen, myelin basic protein, proteolipid protein, myelin oligodendritic glycoprotein, MuSK, keratinocyte adhesion protein desmoglein 3 (Dsg3), Ro-RNP complex, La antigen, myeloperoxidase, proteinase 3, cardiolipin, citrullinated proteins, carbamylated proteins, and α 3 chain of basement membrane collagen.
- [1721]** Embodiment 709. The genetically engineered cell of embodiment 707 or 708, wherein the genetically engineered cell has cell surface expression of the CAAR.

- [1722] Embodiment 710. The genetically engineered cell of any one of embodiments 480- 701, further comprising a third transgene encoding a B-cell autoantibody receptor (BAR).
- [1723] Embodiment 711. The genetically engineered cell of embodiment 710, wherein the genetically engineered cell has cell surface expression of the BAR.
- [1724] Embodiment 712. The genetically engineered cell of any one of embodiments 703-711, wherein the third transgene is inserted at a safe harbor locus.
- [1725] Embodiment 713. The genetically engineered cell of any one of embodiments 703-712, wherein the third transgene is inserted at a TRAC locus, a TRBC1 locus, a TRBC2 locus, a B2M locus, a CIITA locus, a MICA locus, a MICB locus, or a safe harbor locus.
- [1726] Embodiment 714. The genetically engineered cell of any one of embodiments 703-713, wherein the third transgene is inserted at an AAVS1 locus, an ABO locus, a CCR5 locus, a CLYBL locus, a CXCR4 locus, a F3 locus, a FUT1 locus, a HMGB1 locus, a KDM5D locus, a LRP1 locus, a RHD locus, a ROSA26 locus, or a SHS231 locus.
- [1727] Embodiment 715. The genetically engineered cell of any one of embodiments 480-700, wherein the first transgene and the second transgene are inserted into the same insertion site.
- [1728] Embodiment 716. The genetically engineered cell of any one of embodiments 480-700 or 710-714, wherein the first transgene and the third transgene are inserted into the same insertion site.
- [1729] Embodiment 717. The genetically engineered cell of any one of embodiments 695-714, wherein the second transgene and the third transgene are inserted into the same insertion site.
- [1730] Embodiment 718. The genetically engineered cell of any one of embodiments 480-714, wherein the first transgene, the second transgene, and the third transgene are inserted into the same insertion site.
- [1731] Embodiment 719. The genetically engineered cell of any one of embodiments 480-718, wherein the first transgene, the second transgene, and the third transgene are encoded by three separate constructs.
- [1732] Embodiment 720. The genetically engineered cell of any one of embodiments 480-718, wherein the first transgene, the second transgene, and the third transgene are encoded by two separate constructs.
- [1733] Embodiment 721. The genetically engineered cell of any one of embodiments 480-718, wherein the first transgene, the second transgene, and the third transgene are encoded by a polycistronic construct.
- [1734] Embodiment 722. The genetically engineered cell of any one of embodiments 480-721, wherein the first transgene and/or the second transgene and/or the third transgene encode a genome editing complex.
- [1735] Embodiment 723. The genetically engineered cell of 722, wherein the genome editing complex comprises a genome targeting entity and a genome modifying entity.
- [1736] Embodiment 724. The genetically engineered cell of 723, wherein the genome targeting entity is a nucleic acid-guided targeting entity.
- [1737] Embodiment 725. The genetically engineered cell of embodiment 723 or 724, wherein the genome targeting entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

[1738] Embodiment 726. The genetically engineered cell of any one of embodiments 723-725, wherein the genome targeting entity is selected from the group consisting of: Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1, Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, dCas (D10A), dCas (H840A), dCas13a, dCas13b, or a functional portion thereof.

[1739] Embodiment 727. The genetically engineered cell of any one of embodiments 723-726, wherein the genome modifying entity cleaves, deaminates, nicks, polymerizes, interrogates, integrates, cuts, unwinds, breaks, alters, methylates, demethylates, or otherwise destabilizes a target locus.

[1740] Embodiment 728. The genetically engineered cell of any one of embodiments 723-727, wherein the genome modifying entity comprises a recombinase, integrase, transposase, endonuclease, exonuclease, nickase, helicase, DNA polymerase, RNA polymerase, reverse transcriptase, deaminase, flippase, methylase, demethylase, acetylase, a nucleic acid modifying protein, an RNA modifying protein, a DNA modifying protein, an Argonaute protein, an epigenetic modifying protein, a histone modifying protein, or a functional portion thereof.

[1741] Embodiment 729. The genetically engineered cell of any one of embodiments 723-728, wherein the genome modifying entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising the gRNA and the Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, a Cas nuclease, a core Cas protein, a TnpB nuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, base editing, prime editing, a Programmable Addition via Site-specific Targeting Elements (PASTE), or a functional portion thereof.

[1742] Embodiment 730. The genetically engineered cell of any one of embodiments 723-729, wherein the genome modifying entity is selected from the group consisting of Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1, Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, FokI, dCas (D10A), dCas (H840A), dCas13a, dCas13b, a base editor, a prime editor, a target-primed reverse transcription (TPRT) editor, APOBEC1, cytidine deaminase, adenosine deaminase, uracil glycosylase inhibitor (UGI), adenine base editors (ABE), cytosine base editors (CBE), reverse transcriptase, serine integrase, recombinase, transposase, polymerase, adenine-to-thymine or "ATBE" (or thymine-to-adenine or "TABE") transversion base editor, ten-eleven translocation methylcytosine dioxygenases (TETs), TET1, TET3, TET1CD, histone acetyltransferase p300, histone methyltransferase SMYD3, histone methyltransferase PRDM9, H3K79 methyltransferase DOT1L, transcriptional repressor, or a functional portion thereof.

- [1743] Embodiment 731. The genetically engineered cell of any one of embodiments 723-730, wherein the genome targeting entity and the genome modifying entity are different domains of a single polypeptide.
- [1744] Embodiment 732. The genetically engineered cell of any one of embodiments 723-731, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are operably linked together.
- [1745] Embodiment 733. The genetically engineered cell of any one of embodiments 723-731, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are not linked together.
- [1746] Embodiment 734. The genetically engineered cell of any one of embodiments 723-733, wherein the genome editing complex comprises a guide nucleic acid having a targeting domain that is complementary to at least one target locus, optionally wherein the guide nucleic acid is a guide RNA (gRNA).
- [1747] Embodiment 735. The genetically engineered cell of any one of embodiments 722-732, wherein the genome editing complex comprises an RNA-guided nuclease.
- [1748] Embodiment 736. The genetically engineered cell of embodiment 735, wherein the RNA-guided nuclease comprises a Cas nuclease.
- [1749] Embodiment 737. The genetically engineered cell of embodiment 736, wherein the genome editing complex comprises a ribonucleoprotein (RNP) complex comprising the Cas nuclease and the gRNA.
- [1750] Embodiment 738. The genetically engineered cell of embodiment 735 or 736, wherein the Cas nuclease is a Type II or Type V Cas protein.
- [1751] Embodiment 739. The genetically engineered cell of any one of embodiments 735-738, wherein the Cas nuclease is selected from the group consisting of Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr5, Cse1, Cse2, Csf1, Csm2, Csn2, Csx10, Csx11, Csy1, Csy2, Csy3, and Mad7.
- [1752] Embodiment 740. The genetically engineered cell of any one of embodiments 480-721, wherein the genetically engineered cell is a human cell or a non-human animal cell.
- [1753] Embodiment 741. The genetically engineered cell of embodiment 740, wherein the non-human animal cell is a porcine, bovine or ovine cell.
- [1754] Embodiment 742. The genetically engineered cell of embodiment 740, wherein the genetically engineered cell is a human cell.
- [1755] Embodiment 743. The genetically engineered cell of any one of embodiments 480-742, wherein the genetically engineered cell is a differentiated cell derived from a stem cell or a progenitor cell.
- [1756] Embodiment 744. The genetically engineered cell of embodiment 743, wherein the stem cell is a pluripotent stem cell.
- [1757] Embodiment 745. The genetically engineered cell of embodiment 744, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC).
- [1758] Embodiment 746. The genetically engineered cell of embodiment 744, wherein the pluripotent stem cell is an embryonic stem cell (ESC).
- [1759] Embodiment 747. The genetically engineered cell of any one of embodiments 480-746, wherein the cell is a primary cell isolated from a donor.
- [1760] Embodiment 748. The genetically engineered cell of embodiment 747, wherein the donor is healthy and/or is not suspected of having a disease or condition at the time the primary cell is obtained from the donor.

- [1761]** Embodiment 749. The genetically engineered cell of any one of embodiments 480-748, wherein the genetically engineered cell is part of a population of primary cells isolated from multiple donors.
- [1762]** Embodiment 750. The genetically engineered cell of any one of embodiments 480-749, wherein the multiple donors are healthy and/or are not suspected of having a disease or condition at the time the primary cells are obtained from the donors.
- [1763]** Embodiment 751. The genetically engineered cell of any one of embodiments 480-750, wherein the cells are islet cells, beta islet cells, pancreatic islet cells, immune cells, B cells, T cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophage cells, endothelial cells, muscle cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, dopaminergic neurons, retinal pigmented epithelium cells, optic cells, hepatocytes, thyroid cells, skin cells, glial progenitor cells, neural cells, cardiac cells, stem cells, hematopoietic stem cells, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), pluripotent stem cell (PSCs), blood cells, or a combination thereof.
- [1764]** Embodiment 752. The genetically engineered cell of any one of embodiments 480-751, wherein the cell is a T-cell.
- [1765]** Embodiment 753. The genetically engineered cell of embodiment 752, wherein the T-cell is a CD3+ T cell, CD4+ T cell, CDS+ T cell, naive T cell, regulatory T (Treg) cell, non-regulatory T cell, Th1 cell, Th2 cell, Th9 cell, Th17 cell, T-follicular helper (Tfh) cell, cytotoxic T lymphocyte (CTL), effector T (Teff) cell, central memory T cell, effector memory T cell, effector memory T cell expressing CD45RA (TEMRA cell), tissue-resident memory (Trm) cell, virtual memory T cell, innate memory T cell, memory stem cell (Tse), $\gamma\delta$ T cell, or a combination thereof.
- [1766]** Embodiment 754. The genetically engineered cell of embodiment 752 or 753, wherein the T cell is a cytotoxic T-cell, helper T-cell, memory T-cell, regulatory T-cell, tumor infiltrating lymphocyte, or a combination thereof.
- [1767]** Embodiment 755. The genetically engineered cell of any one of embodiments 752-754, wherein the cell is a human T-cell.
- [1768]** Embodiment 756. The genetically engineered cell of any one of embodiments 752-755, wherein the cell is an autologous T-cell.
- [1769]** Embodiment 757. The genetically engineered cell of any one of embodiments 752-755, wherein the cell is an allogeneic T-cell.
- [1770]** Embodiment 758. The genetically engineered cell of embodiment 757, wherein the allogeneic T-cell is a primary T cell.
- [1771]** Embodiment 759. The genetically engineered cell of 757 or 758, wherein the allogeneic T cell has been differentiated from an embryonic stem cells (ESC) or an induced pluripotent stem cells (iPSC).
- [1772]** Embodiment 760. The genetically engineered cell of any one of embodiments 752-759, wherein the cell is an islet cell, beta islet cell, or pancreatic islet cell.
- [1773]** Embodiment 761. The genetically engineered cell of embodiment 760, wherein the cell is an autologous islet cell, an autologous beta islet cell, or an autologous pancreatic islet cell.
- [1774]** Embodiment 762. The genetically engineered cell of embodiment 760, wherein the cell is an allogeneic islet cell, an allogeneic beta islet cell, or an allogeneic pancreatic islet cell.
- [1775]** Embodiment 763. The genetically engineered cell of embodiment 762, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell is a primary islet cell, a primary beta islet cell, or a primary pancreatic islet cell.

[1776] Embodiment 764. The genetically engineered cell of embodiment 762 or embodiment 763, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell has been differentiated from an embryonic stem cell (ESC) or an induced pluripotent stem cell (iPSC).

[1777] Embodiment 765. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 1x, 2x, 3x, 4x, 5x, 10x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, 100x, 150x, 200x, 300x, 400x, 500x, 600x, 700x, 800x, 900x, 1000x, 1100x, 1200x, 1300x, 1400x, 1500x, 2000x, 2500x, 3000x, 3500x, 4000x, 4500x, or 5000x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1778] Embodiment 766. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 1x to at least about 15x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1779] Embodiment 767. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 1x to at least about 10x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1780] Embodiment 768. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 1x to at least about 5x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1781] Embodiment 769. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 3x to at least about 4x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1782] Embodiment 770. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 3x to at least about 10x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1783] Embodiment 771. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 4x to at least about 10x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1784] Embodiment 772. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 500x to at least about 2000x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1785] Embodiment 773. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 500x to at least about 1500x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1786] Embodiment 774. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 900x to at least about 1000x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1787] Embodiment 775. The genetically engineered cell of any one of embodiments 480-764, wherein the engineered protein is expressed in the genetically engineered cell at a level at least about 1000x greater than an endogenous CD47 protein in a control cell of the same cell type as the genetically engineered cell.

[1788] Embodiment 776. A composition comprising genetically engineered cell of any one of embodiments 479-775.

- [1789]** Embodiment 777. A pharmaceutical composition comprising (i) a genetically engineered cell of any one of embodiments 479-775, and (ii) a pharmaceutically acceptable excipient.
- [1790]** Embodiment 778. A method comprising administering to a subject a genetically engineered cell of any one of embodiments 479-775, a composition of embodiment 777, or a pharmaceutical composition of embodiment 638.
- [1791]** Embodiment 779. The method of embodiment 778, wherein the method is a method of treating a disease in a subject.
- [1792]** Embodiment 780. A method of treating a disease in a subject, the method comprising administering to the subject the genetically engineered cell of any one of embodiments 479-775, the composition of embodiment 776, or the pharmaceutical composition of embodiment 777.
- [1793]** Embodiment 781. A population of cells comprising a genetically engineered cell of any one of embodiments 479-775 for use in treating a disease in a subject.
- [1794]** Embodiment 782. A composition of embodiment 776 for use in treating a disease in a subject.
- [1795]** Embodiment 783. A pharmaceutical composition of embodiment 777 for use in treating a disease in a subject.
- [1796]** Embodiment 784. Use of a genetically modified cell of any one of embodiments 479-775, a population of cells of embodiment 781, a composition of embodiment 776, a pharmaceutical composition of embodiment 777, or a method of embodiment 779 for use in treating a disease in a subject.
- [1797]** Embodiment 785. Use of a genetically modified cell of any one of embodiments 479-775, a population of cells of embodiment 781, a composition of embodiment 776, or a pharmaceutical composition of embodiment 777, or a method of embodiment 779 in the manufacture of a medicament for the treatment of a disease.
- [1798]** Embodiment 786. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the disease is cancer.
- [1799]** Embodiment 787. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the cancer is associated with CD5, CD19, CD20, CD22, CD23, CD30, CD33, CD70, Kappa, Lambda, B cell maturation agent (BCMA), G-protein coupled receptor family C group 5 member D (GPRC5D), CD123, LeY, NKG2D ligand, WT1, GD2, HER2, EGFR, EGFRvIII, B7H3, PSMA, PSCA, CAIX, CD171, CEA, CSPG4, EPHA2, FAP, FRα, IL-13Rα, Mesothelin, MUC1, MUC16, ROR1, C-Met, CD133, Ep-CAM, GPC3, HPV16-E6, IL13Ra2, MAGEA3, MAGEA4, MART1, NY-ESO-1, VEGFR2, α-Folate receptor, CD24, CD44v7/8, EGP-2, EGP-40, erb-B2, erb-B 2,3,4, FBP, Fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-11Rα, KDR, Lewis Y, L1-cell adhesion molecule, MAGE-A1, Oncofetal antigen (h5T4), and/or TAG-72 expression.
- [1800]** Embodiment 788. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the cancer is a hematologic malignancy.
- [1801]** Embodiment 789. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the hematologic malignancy is selected from the group consisting of myeloid neoplasm, myelodysplastic syndromes (MDS), myeloproliferative/myelodysplastic syndromes, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), blast crisis chronic myelogenous leukemia (bcCML), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), T-cell lymphoma, and B-cell lymphoma.

[1802] Embodiment 790. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the cancer is solid malignancy.

[1803] Embodiment 791. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the solid malignancy is selected breast cancer, ovarian cancer, colon cancer, prostate cancer, epithelial cancer, renal-cell carcinoma, pancreatic adenocarcinoma, cervical carcinoma, colorectal cancer, glioblastoma, rhabdomyosarcoma, neuroblastoma, melanoma, Ewing sarcoma, osteosarcoma, mesothelioma and adenocarcinoma.

[1804] Embodiment 792. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the disease is an autoimmune disease.

[1805] Embodiment 793. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of the preceding embodiments, wherein the autoimmune disease is selected from the group consisting of lupus, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjögren's syndrome, Hashimoto's thyroiditis, and celiac disease.

[1806] Embodiment 794. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, the pharmaceutical composition of any of the preceding embodiments, or the use of any of the preceding embodiments, wherein the disease is diabetes mellitus.

[1807] Embodiment 795. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of any of the preceding embodiments, wherein the diabetes is selected from the group consisting Type I diabetes, Type II diabetes, prediabetes, and gestational diabetes.

[1808] Embodiment 796. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of the preceding embodiments, wherein the disease is a neurological disease.

[1809] Embodiment 797. The genetically modified cell, the population of cells, the composition, the pharmaceutical composition, the use, or the method of the preceding embodiments, wherein the neurological disease is selected from the group consisting of catalepsy, epilepsy, encephalitis, meningitis, migraine, Huntington's, Alzheimer's, Parkinson's, Pelizaeus-Merzbacher disease, and multiple sclerosis.

[1810] Embodiment 798. A method of making a genetically engineered cell comprising a nucleic acid construct, the method comprising: delivering to a cell a nucleic acid construct of any one of embodiments 1-478, thereby making a genetically engineered cell.

[1811] Embodiment 799. The method of embodiment 798, wherein the nucleic acid construct comprises a first transgene encoding the engineered protein.

[1812] Embodiment 800. The method of embodiment 798 or 799, wherein the method comprises inserting a first transgene encoding the engineered protein into a first insertion site in the genome of the cell.

[1813] Embodiment 801. The method of embodiment 800, wherein the first insertion site is a T-cell receptor (TCR) locus.

[1814] Embodiment 802. The method of embodiment 801, wherein the TCR locus is or comprises: a TRAC locus, a TRBC1 locus, or a TRBC2 locus.

- [1815]** Embodiment 803. The method of embodiment 801, wherein the first insertion site is a $\beta 2$ microglobulin (B2M) locus.
- [1816]** Embodiment 804. The method of embodiment 801, wherein the first insertion site is a class II transactivator (CIITA) locus.
- [1817]** Embodiment 805. The method of embodiment 801, wherein the first insertion site is a safe harbor locus.
- [1818]** Embodiment 806. The method of embodiment 805, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.
- [1819]** Embodiment 807. The method of any one of embodiments 800-804, wherein the first insertion site is an exon.
- [1820]** Embodiment 808. The method of any one of embodiments 800-804, wherein the first insertion site is an intron.
- [1821]** Embodiment 809. The method of any one of embodiments 800-804, wherein the first insertion site is between an intron and an exon.
- [1822]** Embodiment 810. The method of any one of embodiments 800-804, wherein the first insertion site is in a regulatory region.
- [1823]** Embodiment 811. The method of any one of embodiments 800-810, wherein the step of inserting comprises insertion using a gene therapy vector, transposase, lentiviral vector, retrovirus, fusosome, PiggyBac transposon, Sleeping Beauty (SB11) transposon, Mos1 transposon, or Tol2 transposon.
- [1824]** Embodiment 812. The method of any one of embodiments 800-811, wherein the step of inserting comprises insertion using a lentiviral vector.
- [1825]** Embodiment 813. The method of any one of embodiments 800-810, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using a genome-modifying protein.
- [1826]** Embodiment 814. The method of any one of embodiments 798-813, further comprising introducing into the cell a genome editing complex.
- [1827]** Embodiment 815. The method of embodiment 814, wherein the genome editing complex comprises a genome targeting entity and a genome modifying entity.
- [1828]** Embodiment 816. The method of embodiment 815, wherein the genome targeting entity localizes the genome editing complex to a safe harbor site, optionally wherein the genome targeting entity is a nucleic acid-guided targeting entity.
- [1829]** Embodiment 817. The method of 815 or 816, wherein the genome targeting entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.
- [1830]** Embodiment 818. The method of any one of embodiments 815-817, wherein the genome targeting entity is selected from the group consisting of Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g,

Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1, Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, dCas (D10A), dCas (H840A), dCas13a, dCas13b, or a functional portion thereof.

[1831] Embodiment 819. The method of any one of embodiments 815-818, wherein the genome modifying entity cleaves, deaminates, nicks, polymerizes, interrogates, integrates, cuts, unwinds, breaks, alters, methylates, demethylates, or otherwise destabilizes the target locus.

[1832] Embodiment 820. The method of any one of embodiments 815-819, wherein the genome modifying entity comprises a recombinase, integrase, transposase, endonuclease, exonuclease, nickase, helicase, DNA polymerase, RNA polymerase, reverse transcriptase, deaminase, flippase, methylase, demethylase, acetylase, a nucleic acid modifying protein, an RNA modifying protein, a DNA modifying protein, an Argonaute protein, an epigenetic modifying protein, a histone modifying protein, or a functional portion thereof.

[1833] Embodiment 821. The method of any one of embodiments 815-820, wherein the genome modifying entity is selected from the group consisting of: a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising the gRNA and the Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, a Cas nuclease, a core Cas protein, a TnpB nuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, base editing, prime editing, a Programmable Addition via Site-specific Targeting Elements (PASTE), or a functional portion thereof.

[1834] Embodiment 822. The method of any one of embodiments 815-821, wherein the genome modifying entity is selected from the group consisting of Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr1, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csd1, Csd2, Cas5d, Cse1, Cse2, Cse3, Cse4, Cas5e, Csf1, Csm1, Csm2, Csm3, Csm4, Csm5, Csn1, Csn2, Cst1, Cst2, Cas5t, Csh1, Csh2, Cas5h, Csa1, Csa2, Csa3, Csa4, Csa5, Cas5a, Csx10, Csx11, Csy1, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HF1, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCas12a, AsCas12a, AacCas12b, BhCas12b v4, TnpB, FokI, dCas (D10A), dCas (H840A), dCas13a, dCas13b, a base editor, a prime editor, a target-primed reverse transcription (TPRT) editor, APOBEC1, cytidine deaminase, adenosine deaminase, uracil glycosylase inhibitor (UGI), adenine base editors (ABE), cytosine base editors (CBE), reverse transcriptase, serine integrase, recombinase, transposase, polymerase, adenine-to-thymine or "ATBE" (or thymine-to-adenine or "TABE") transversion base editor, ten-eleven translocation methylcytosine dioxygenases (TETs), TET1, TET3, TET1CD, histone acetyltransferase p300, histone methyltransferase SMYD3, histone methyltransferase PRDM9, H3K79 methyltransferase DOT1L, transcriptional repressor, or a functional portion thereof.

[1835] Embodiment 823. The method of any one of embodiments 815-822, wherein the genome targeting entity and the genome modifying entity are different domains of a single polypeptide.

[1836] Embodiment 824. The method of any one of embodiments 815-823, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are operably linked together.

- [1837]** Embodiment 825. The method of any one of embodiments 815-823, wherein the genome targeting entity and genome modifying entity are two different polypeptides that are not linked together.
- [1838]** Embodiment 826. The method of any one of embodiments 815-825, wherein the genome editing complex comprises a guide nucleic acid having a targeting domain that is complementary to at least one sequence within the genomic safe harbor site, optionally wherein the guide nucleic acid is a guide RNA (gRNA).
- [1839]** Embodiment 827. The method of any one of embodiments 815-816, wherein the genome editing complex comprises an RNA-guided nuclease.
- [1840]** Embodiment 828. The method of embodiment 827, wherein the RNA-guided nuclease comprises a Cas nuclease.
- [1841]** Embodiment 829. The method of embodiment 828, wherein the genome editing complex comprises a ribonucleoprotein (RNP) complex comprising the Cas nuclease and the gRNA.
- [1842]** Embodiment 830. The method of embodiment 828 or 829, wherein the Cas nuclease is a Type II or Type V Cas protein.
- [1843]** Embodiment 831. The method of any one of embodiments 828-830, wherein the Cas nuclease is selected from the group consisting of Cas3, Cas4, Cas5, Cas8a, Cas8b, Cas8c, Cas9, Cas10, Cas12, Cas12a (Cpf1), Cas12b (C2c1), Cas12c (C2c3), Cas12d (CasY), Cas12e (CasX), Cas12f (C2c10), Cas12g, Cas12h, Cas12i, Cas12k (C2c5), Cas13, Cas13a (C2c2), Cas13b, Cas13c, Cas13d, C2c4, C2c8, C2c9, Cmr5, Cse1, Cse2, Csf1, Csm2, Csn2, Csx10, Csx11, Csy1, Csy2, Csy3, and Mad7.
- [1844]** Embodiment 832. The method of embodiment 818, wherein the gRNA comprises a complementary region, wherein the complementary region comprises a nucleic acid sequence that is complementary to a target nucleic acid sequence within a gene locus, and wherein the target nucleic acid sequence comprises the insertion site.
- [1845]** Embodiment 833. The method of embodiment 832, wherein the gene locus is a TCR locus.
- [1846]** Embodiment 834. The method of embodiment 832, wherein the gene locus is a B2M locus.
- [1847]** Embodiment 835. The method of embodiment 832, wherein the gene locus is a CIITA locus.
- [1848]** Embodiment 836. The method of any of embodiments 799-835, wherein the first insertion site is 25 nucleotides or less from a protospacer adjacent motif (PAM) sequence, wherein the PAM sequence is ngg, nag, ngrrt, ngrrn, nnnngatt, nnnnyac, nnagaaw, naaaac, ttv, ttn, attn, ttn, gttn, or ytn and wherein: (i) r = a or g, (ii) y = c or t, (iii) w = a or t, (iv) v = a or c or g, and (v) n = a, c, t, or g.
- [1849]** Embodiment 837. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using SpCas9 and the PAM is ngg or nag, wherein n = a, c, t, or g.
- [1850]** Embodiment 838. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using SaCas9 and the PAM is ngrt or ngrn, wherein: (i) r = a or g, and (ii) n = a, c, t, or g.
- [1851]** Embodiment 839. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using NmeCas9 and the PAM is nnnngatt, wherein n = a, c, t, or g.
- [1852]** Embodiment 840. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using CjCas9 and the PAM is nnnnyac, wherein: (i) r = a or g, (ii) y = c or t, and (iii) n = a, c, t, or g.

[1853] Embodiment 841. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using StCas9 and the PAM is nnagaaw wherein: (i) w = a or t, and (ii) n= a, c, t, or g.

[1854] Embodiment 842. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TdCas9 and the PAM is naaaac, wherein n= a, c, t, or g.

[1855] Embodiment 843. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using LbCas12a and the PAM is ttv, wherein v = a or c or g.

[1856] Embodiment 844. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using AsCas12a and the PAM is ttv, wherein v = a or c or g.

[1857] Embodiment 845. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using AacCas12b and the PAM is ttn, wherein n= a, c, t, or g.

[1858] Embodiment 846. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using BhCas12b and the PAM is attn., ttnn, or gttn, wherein n= a, c, t, or g.

[1859] Embodiment 847. The method of any one of embodiments 799-836, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using MAD7 (ErCas12a) and the PAM is yttn, wherein: (i) y= c or t, and (ii) n= a, c, t, or g.

[1860] Embodiment 848. The method of any one of embodiments 800, 818, or 836-847, wherein homology-directed repair (HDR)-mediated insertion using a site-directed nuclease is performed with an HDR efficiency equal to or greater than HDR insertion using lentivirus.

[1861] Embodiment 849. The method of any one of embodiments 800-813, 815, 816, or 817, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using ZFN.

[1862] Embodiment 850. The method of any one of embodiments 800-813, 815, 816, 817, or 849, wherein the first insertion site is 25 nucleotides or less from a zinc finger binding sequence.

[1863] Embodiment 851. The method of any one of embodiments 800-813, 815, 816, or 817, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TALEN.

[1864] Embodiment 852. The method of any one of embodiments 800-813, 815, 816, 817, or 851, wherein the first insertion site is 25 nucleotides or less from a transcription activator-like effectors (TALE) binding sequence.

[1865] Embodiment 853. The method of any one of embodiments 800-815 or 817, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using a guide RNA (gRNA) and a TnpB polypeptide.

[1866] Embodiment 854. The method of embodiment 853, wherein the gRNA comprises a complementary region, wherein the complementary region comprises a nucleic acid sequence that is complementary to a target nucleic acid sequence within a gene locus, and wherein the target nucleic acid sequence comprises the insertion site.

[1867] Embodiment 855. The method of embodiment 854, wherein the gene locus is a TCR locus.

[1868] Embodiment 856. The method of embodiment 854, wherein the gene locus is a B2M locus.

[1869] Embodiment 857. The method of embodiment 854, wherein the gene locus is a CIITA locus.

- [1870]** Embodiment 858. The method of embodiment 854, wherein the gene locus is a safe harbor locus.
- [1871]** Embodiment 859. The method of embodiment 858, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.
- [1872]** Embodiment 860. The method of any one of embodiments 853-859, wherein the insertion site is 25 nucleotides or less from a target adjacent motif (TAM) sequence, wherein the TAM sequence is tca, tcac, tcag, tcat, tcaa, ttcan, tcaa, ttcat, or ttgat, and wherein: (i) n= a, c, t, or g.
- [1873]** Embodiment 861. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tca.
- [1874]** Embodiment 862. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcac.
- [1875]** Embodiment 863. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcag.
- [1876]** Embodiment 864. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcat.
- [1877]** Embodiment 865. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcaa.
- [1878]** Embodiment 866. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is ttcan, wherein n= a, c, t, or g.
- [1879]** Embodiment 867. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is tcaa.
- [1880]** Embodiment 868. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is ttcat.
- [1881]** Embodiment 869. The method of any one of embodiments 853-860, wherein the step of inserting comprises homology-directed repair (HDR)-mediated insertion using TnpB polypeptide and the TAM is ttgat.
- [1882]** Embodiment 870. The method of embodiment 798 or 799, wherein the step of delivering the nucleic acid construct to the cell comprises viral transduction with a retrovirus or an adeno-associated virus (AAV) vector.
- [1883]** Embodiment 871. The method of embodiment 870, wherein the retrovirus is or comprises Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV), Rous Sarcoma Virus (RSV), a lentivirus, a Gammaretrovirus, an Epsilonretrovirus, an Alpharetrovirus, a Betaretrovirus, a Deltaretrovirus, or a Spumaretrovirus.
- [1884]** Embodiment 872. The method of embodiment 870, wherein the AAV vector is an AAV6 vector or an AAV9 vector.
- [1885]** Embodiment 873. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 720-732, wherein the first transgene encoding the engineered protein at the insertion site at a TCR locus reduces expression of a functional TCR.
- [1886]** Embodiment 874. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-873, wherein the first transgene encoding the engineered protein at the insertion site at a TCR locus disrupts expression of a functional TCR.

[1887] Embodiment 875. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, wherein the first transgene encoding the engineered protein at the insertion site at a B2M locus reduces expression of a functional B2M.

[1888] Embodiment 876. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, or 875, wherein the first transgene encoding the engineered protein at the insertion site at a B2M locus reduces expression of a functional MHC I molecule.

[1889] Embodiment 877. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, or 875-876, wherein the first transgene encoding the engineered protein at the insertion site at a B2M locus disrupts expression of a functional B2M.

[1890] Embodiment 878. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, or 875-877, wherein the first transgene encoding the engineered protein at the insertion site at a B2M gene locus disrupts expression of a functional MHC I molecule.

[1891] Embodiment 879. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA locus reduces expression of a functional CIITA.

[1892] Embodiment 880. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, or 879, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA locus reduces expression of a functional MHC II molecule.

[1893] Embodiment 881. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, or 879-880, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA locus disrupts expression of a functional CIITA.

[1894] Embodiment 882. The method of any one of embodiments 800, 801, 802, 813-832, 836-854, or 860-872, or 879-880, wherein the first transgene encoding the engineered protein at the insertion site at a CIITA gene locus disrupts expression of a functional MHC II molecule.

[1895] Embodiment 883. The method of any one of embodiments 798-882, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of: (a) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or (b) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[1896] Embodiment 884. The method of any one of embodiments 798-883, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules.

[1897] Embodiment 885. The method of any one of embodiments 798-884, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[1898] Embodiment 886. The method of any one of embodiments 798-885, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of: (a) one or more MHC class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and (b) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

[1899] Embodiment 887. The method of any one of embodiments 883-886, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class I molecules.

- [1900]** Embodiment 888. The method of any one of embodiments 883-887, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class I molecules.
- [1901]** Embodiment 889. The method of any one of embodiments 883-888, wherein the one or more modifications reduce a function of the one or more MHC class I molecules, optionally wherein the function is antigen presentation.
- [1902]** Embodiment 890. The v of any one of embodiments 883-889, wherein the one or more MHC class I molecules are one or more human leukocyte antigen (HLA) class I molecules.
- [1903]** Embodiment 891. The method of embodiment 890, wherein the one or more HLA class I molecules are selected from the group consisting of HLA-A, HLA-B, and HLA-C.
- [1904]** Embodiment 892. The method of any one of embodiments 883-891, wherein the one or more molecules that regulate expression of the one or more MHC class I molecules are selected from the group consisting of B2M, NLRC5 and TAP1.
- [1905]** Embodiment 893. The method of any one of embodiments 883-892, wherein the one or more modifications reduce cell surface protein expression of the one or more MHC class II molecules.
- [1906]** Embodiment 894. The method of any one of embodiments 883-893, wherein the one or more modifications reduce cell surface trafficking of the one or more MHC class II molecules.
- [1907]** Embodiment 895. The method of any one of embodiments 883-894, wherein the one or more modifications reduce a function of the one or more MHC class II molecules, optionally wherein the function is antigen presentation.
- [1908]** Embodiment 896. The method of any one of embodiments 883-895, wherein the one or more MHC class II molecules are one or more human leukocyte antigen (HLA) class II molecules.
- [1909]** Embodiment 897. The method of embodiment 896, wherein the one or more HLA class II molecules are selected from the group consisting of HLA-DP, HLA-DQ, and/or HLA-DR.
- [1910]** Embodiment 898. The method of any one of embodiments 883-897, wherein the one or more molecules that regulate expression of the one or more MHC class II molecules is/are selected from the group consisting of CIITA and CD74.
- [1911]** Embodiment 899. The method of any one of embodiments 800-898, wherein the first transgene encoding the engineered protein at the insertion site at a safe harbor locus disrupts expression of a functional AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231.
- [1912]** Embodiment 900. The method of any one of embodiments 800-898, wherein the first transgene encoding the engineered protein at the insertion site at a safe harbor locus disrupts expression of a functional AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 molecule.
- [1913]** Embodiment 901. The method of any one of embodiments 798-900, wherein the genetically engineered cell has cell surface expression of the engineered protein.
- [1914]** Embodiment 902. The method of any one of embodiments 798-901, wherein the nucleic acid construct further comprises a second transgene encoding a tolerogenic factor.
- [1915]** Embodiment 903. The method of embodiment 902, wherein the method further comprises inserting the second transgene into a second insertion site in the genome of the cell.
- [1916]** Embodiment 904. The method of embodiment 902, wherein the tolerogenic factor is or comprises A20/TNFAIP3, B2M-HLA-E, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, CD52, CD55, CD59, CD64,

CD200, CCL21, CCL22, CTLA4-Ig, C1 inhibitor, CR1, DUX4, FASL, HLA-C, HLA-E, HLA-E heavy chain, HLA-F, HLA-G, H2-M3, IDO1, IL-10, IL15-RF, IL-35, IL-39, MANF, Mfge8, PD-L1, or Serpinb9.

[1917] Embodiment 905. The method of any one of embodiments 902-904, wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a TCR locus.

[1918] Embodiment 906. The method of any one of embodiments 902-904, wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a B2M locus.

[1919] Embodiment 907. The method of any one of embodiments 902-904 wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a CIITA locus.

[1920] Embodiment 908. The method of any one of embodiments 902-904, wherein the second transgene encoding the tolerogenic factor is inserted at the second insertion site at a safe harbor locus.

[1921] Embodiment 909. The method of embodiment 908, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

[1922] Embodiment 910. The method of any one of embodiments 902-909, wherein the genetically engineered cell has cell surface expression of the tolerogenic factor.

[1923] Embodiment 911. The method of any one of embodiments 798-910, wherein the nucleic acid construct further comprises a third transgene encoding a chimeric antigen receptor (CAR).

[1924] Embodiment 912. The method of embodiment 911, wherein the method further comprises inserting the third transgene into a third insertion site in the genome of the cell.

[1925] Embodiment 913. The method of embodiment 911, wherein the CAR is or comprises a CD5-specific CAR, a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD23-specific CAR, a CD30-specific CAR, a CD33-specific CAR, CD38-specific CAR, a CD70-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a Kappa, Lambda, B cell maturation agent (BCMA)-specific CAR, a G-protein coupled receptor family C group 5 member D (GPRC5D)-specific CAR, a CD123-specific CAR, a LeY-specific CAR, a NKG2D ligand-specific CAR, a WT1-specific CAR, a GD2-specific CAR, a HER2-specific CAR, a EGFR-specific CAR, a EGFRvIII-specific CAR, a B7H3-specific CAR, a PSMA-specific CAR, a PSCA-specific CAR, a CAIX-specific CAR, a CD171-specific CAR, a CEA-specific CAR, a CSPG4-specific CAR, a EPHA2-specific CAR, a FAP-specific CAR, a FR α -specific CAR, a IL-13R α -specific CAR, a Mesothelin-specific CAR, a MUC1-specific CAR, a MUC16-specific CAR, a ROR1-specific CAR, a C-Met-specific CAR, a CD133-specific CAR, a Ep-CAM-specific CAR, a GPC3-specific CAR, a HPV16-E6-specific CAR, a IL13R α 2-specific CAR, a MAGEA3-specific CAR, a MAGEA4-specific CAR, a MART1-specific CAR, a NY-ESO-1-specific CAR, a VEGFR2-specific CAR, a α -Folate receptor-specific CAR, a CD24-specific CAR, a CD44v7/8-specific CAR, a EGP-2-specific CAR, a EGP-40-specific CAR, a erb-B2-specific CAR, a erb-B 2,3,4-specific CAR, a FBP-specific CAR, a Fetal acetylcholine e receptor-specific CAR, a GD2-specific CAR, a GD3-specific CAR, a HMW-MAA-specific CAR, a IL-11R α -specific CAR, a KDR-specific CAR, a Lewis Y-specific CAR, a L1-cell adhesion molecule-specific CAR, a MAGE-A1-specific CAR, a Oncofetal antigen (h5T4)-specific CAR, a TAG-72-specific CAR, or a CD19/CD22-bispecific CAR.

[1926] Embodiment 914. The method of any one of embodiments 911-913, wherein the CAR is or comprises a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD38-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a BCMA-specific CAR, or a CD19/CD22-bispecific CAR.

[1927] Embodiment 915. The method of any one of embodiments 911-914, wherein the genetically engineered cell has cell surface expression of the CAR.

[1928] Embodiment 916. The method of any one of embodiments 798-910, wherein the nucleic acid construct further comprises a third transgene encoding a chimeric auto antigen receptor (CAAR).

- [1929] Embodiment 917. The method of embodiment 916, wherein the method further comprises inserting the third transgene into a third insertion site in the genome of the cell.
- [1930] Embodiment 918. The method of embodiment 916, wherein the CAAR comprises an antigen selected from the group consisting of a pancreatic β -cell antigen, synovial joint antigen, myelin basic protein, proteolipid protein, myelin oligodendritic glycoprotein, MuSK, keratinocyte adhesion protein desmoglein 3 (Dsg3), Ro-RNP complex, La antigen, myeloperoxidase, proteinase 3, cardiolipin, citrullinated proteins, carbamylated proteins, and α 3 chain of basement membrane collagen.
- [1931] Embodiment 919. The method of any one of embodiments 916-918, wherein the genetically engineered cell has cell surface expression of the CAAR.
- [1932] Embodiment 920. The method of any one of embodiments 798-910, wherein the nucleic acid construct further comprises a third transgene encoding a B-cell autoantibody receptor (BAR).
- [1933] Embodiment 921. The method of embodiment 920, wherein the method further comprises inserting the third transgene into a third insertion site in the genome of the cell.
- [1934] Embodiment 922. The method of embodiment 920, wherein the genetically engineered cell has cell surface expression of the BAR.
- [1935] Embodiment 923. The method of any one of embodiments 911-922, wherein the third transgene is inserted at a safe harbor locus.
- [1936] Embodiment 924. The method of any one of embodiments 911-923, wherein the third transgene is inserted at a TRAC locus, a TRBC1 locus, a TRBC2 locus, a B2M locus, a CIITA locus, a MICA locus, a MICB locus, or a safe harbor locus.
- [1937] Embodiment 925. The method of any one of embodiments 911-924, wherein the third transgene is inserted at an AAVS1 locus, an ABO locus, a CCR5 locus, a CLYBL locus, a CXCR4 locus, a F3 locus, a FUT1 locus, a HMGB1 locus, a KDM5D locus, a LRP1 locus, a RHD locus, a ROSA26 locus, or a SHS231 locus.
- [1938] Embodiment 926. The method of any one of embodiments 798-925, wherein the first transgene and the second transgene are inserted into the same insertion site.
- [1939] Embodiment 927. The method of any one of embodiments 798-908 or 920-925, wherein the first transgene and the third transgene are inserted into the same insertion site.
- [1940] Embodiment 928. The method of any one of embodiments 902-925, wherein the second transgene and the third transgene are inserted into the same insertion site.
- [1941] Embodiment 929. The method of any one of embodiments 798-925, wherein the first transgene, the second transgene, and the third transgene are inserted into the same insertion site.
- [1942] Embodiment 930. The method of any one of embodiments 798-929, wherein the first transgene, the second transgene, and the third transgene are encoded by three separate constructs.
- [1943] Embodiment 931. The method of any one of embodiments 798-929, wherein the first transgene, the second transgene, and the third transgene are encoded by two separate constructs.
- [1944] Embodiment 932. The method of any one of embodiments 798-929, wherein the first transgene, the second transgene, and the third transgene are encoded by a polycistronic construct.
- [1945] Embodiment 933. The method of any one of embodiments 798-932, wherein the genetically engineered cell is a human cell or a non-human animal cell.
- [1946] Embodiment 934. The method of embodiment 933, wherein the non-human animal cell is a porcine, bovine or ovine cell.

- [1947]** Embodiment 935. The method of embodiment 933, wherein the genetically engineered cell is a human cell.
- [1948]** Embodiment 936. The method of any one of embodiments 798-935, wherein the genetically engineered cell is a differentiated cell derived from a stem cell or a progenitor cell.
- [1949]** Embodiment 937. The method of any one of embodiments 798-936, wherein the cell is a stem cell or a progenitor cell.
- [1950]** Embodiment 938. The method of any one of embodiments 798-936, wherein the cell is a differentiated cell derived from a stem cell or a progenitor cell.
- [1951]** Embodiment 939. The method of embodiment 936, wherein the stem cell is a pluripotent stem cell.
- [1952]** Embodiment 940. The method of embodiment 739, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC).
- [1953]** Embodiment 941. The method of embodiment 739, wherein the pluripotent stem cell is an embryonic stem cell (ESC).
- [1954]** Embodiment 942. The method of any one of embodiments 798-991, wherein the cell is a primary cell isolated from a donor.
- [1955]** Embodiment 943. The method of embodiment 942, wherein the donor is healthy and/or is not suspected of having a disease or condition at the time the primary cell is obtained from the donor.
- [1956]** Embodiment 944. The method of any one of embodiments 798-943, wherein the cells are islet cells, beta islet cells, pancreatic islet cells, immune cells, B cells, T cells, natural killer (NK) cells, natural killer T (NKT) cells, macrophage cells, endothelial cells, muscle cells, cardiac muscle cells, smooth muscle cells, skeletal muscle cells, dopaminergic neurons, retinal pigmented epithelium cells, optic cells, hepatocytes, thyroid cells, skin cells, glial progenitor cells, neural cells, cardiac cells, stem cells, hematopoietic stem cells, induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), pluripotent stem cell (PSCs), blood cells, or a combination thereof.
- [1957]** Embodiment 945. The method of any one of embodiments 798-944, wherein the cell is a T-cell.
- [1958]** Embodiment 946. The method of embodiment 945, wherein the T-cell is a CD3+ T cell, CD4+ T cell, CDS+ T cell, naive T cell, regulatory T (Treg) cell, non-regulatory T cell, Th1 cell, Th2 cell, Th9 cell, Th17 cell, T-follicular helper (Tfh) cell, cytotoxic T lymphocyte (CTL), effector T (Teff) cell, central memory T cell, effector memory T cell, effector memory T cell expressing CD45RA (TEMRA cell), tissue-resident memory (Trm) cell, virtual memory T cell, innate memory T cell, memory stem cell (Tse), $\gamma\delta$ T cell, or a combination thereof.
- [1959]** Embodiment 947. The method of embodiment 945 or 946, wherein the T cell is a cytotoxic T-cell, helper T-cell, memory T-cell, regulatory T-cell, tumor infiltrating lymphocyte, or a combination thereof.
- [1960]** Embodiment 948. The method of any one of embodiments 945-947, wherein the cell is a human T-cell.
- [1961]** Embodiment 949. The method of any one of embodiments 945-948, wherein the cell is an autologous T-cell.
- [1962]** Embodiment 950. The method of any one of embodiments 945-948, wherein the cell is an allogeneic T-cell.
- [1963]** Embodiment 951. The method of embodiment 950, wherein the allogeneic T-cell is a primary T cell.
- [1964]** Embodiment 952. The method of 950 or 951, wherein the allogeneic T cell has been differentiated from an embryonic stem cells (ESC) or an induced pluripotent stem cells (iPSC).

[1965] Embodiment 953. The method of any one of embodiments 798-952, wherein the cell is an islet cell, beta islet cell, or pancreatic islet cell.

[1966] Embodiment 954. The method of embodiment 953, wherein the cell is an autologous islet cell, an autologous beta islet cell, or an autologous pancreatic islet cell.

[1967] Embodiment 955. The method of embodiment 953, wherein the cell is an allogeneic islet cell, an allogeneic beta islet cell, or an allogeneic pancreatic islet cell.

[1968] Embodiment 956. The method of embodiment 955, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell is a primary islet cell, a primary beta islet cell, or a primary pancreatic islet cell.

[1969] Embodiment 957. The method of 955 or 956, wherein the allogeneic islet cell, allogeneic beta islet cell, or allogeneic pancreatic islet cell has been differentiated from an embryonic stem cell (ESC) or an induced pluripotent stem cell (iPSC).

EQUIVALENTS

[1970] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various embodiments from different headings and sections as appropriate according to the spirit and scope of the technology described herein.

[1971] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[1972] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

[1973] It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS

We claim:

1. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered protein, wherein the one or more nucleic acid sequences comprise a nucleic acid sequence at least 80% identical to SEQ ID NO: 17, and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.
2. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered protein, wherein the one or more nucleic acid sequences comprise a nucleic acid sequence at least 80% identical to SEQ ID NO: 16, and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.
3. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered protein, wherein the one or more nucleic acid sequences comprise a nucleic acid sequence at least 80% identical to SEQ ID NO: 15, and wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.
4. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered CD47 protein comprising:
 - (a) one or more extracellular domains; and (b) one or more membrane tethers;

wherein the one or more extracellular domains comprise: an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6; and/or

an amino acid substitution at position S83 of SEQ ID NO: 2, an amino acid substitution at position S65 of SEQ ID NO: 3, an amino acid substitution at position S83 of SEQ ID NO: 1, or an amino acid substitution at position S38 of SEQ ID NO: 6; and/or

an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 1 or SEQ ID NO: 6; and

wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.
5. The nucleic acid construct of claim 4, wherein the one or more extracellular domains comprise:

an amino acid substitution of S82A relative to SEQ ID NO: 2,

an amino acid substitution of S64A relative to SEQ ID NO: 3,

an amino acid substitution of S82A relative to SEQ ID NO: 1, or

an amino acid substitution of S37A relative to SEQ ID NO: 6.
6. The nucleic acid construct of claim 4, wherein the one or more extracellular domains comprise:

an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2,

an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3,

an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, or

- an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6.
7. The nucleic acid construct of any one of claims 4-6, wherein the one or more extracellular domains comprise:
an amino acid substitution of S83A relative to SEQ ID NO: 2,
an amino acid substitution of S65A relative to SEQ ID NO: 3,
an amino acid substitution of S83A relative to SEQ ID NO: 1, or
an amino acid substitution of S38A relative to SEQ ID NO: 6.
8. The nucleic acid construct of any one of claims 4-6, wherein the one or more extracellular domains comprise:
an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2,
an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3,
an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, or
an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.
9. The nucleic acid construct of any one of claims 4-8, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.
10. The nucleic acid construct of any one of claims 4-9, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP relative to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.
11. The nucleic acid construct of any one of claims 4-10, wherein the engineered CD47 protein comprises an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 or SEQ ID NO: 1.
12. A nucleic acid construct comprising one or more nucleic acid sequences encoding an engineered CD47 protein, wherein the engineered CD47 protein comprises an insertion of three amino acids relative to SEQ ID NO: 4 or SEQ ID NO: 5.
13. The nucleic acid construct of any one of claims 1-3 and 12, wherein the engineered protein comprises one or more membrane tethers.
14. The nucleic acid construct of any one of claims 4-11 and 13, wherein the one or more membrane tethers are or comprise a transmembrane domain.
15. The nucleic acid construct of claim 14, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNGR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.
16. The nucleic acid construct of any one of claims 4-11 and 13, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.

17. The nucleic acid construct of claim 16, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.
18. The nucleic acid construct of any one of claims 4-11, wherein the one or more extracellular domains further comprise an extracellular hinge domain.
19. The nucleic acid construct of claim 18, wherein the extracellular hinge domain is or comprises a CD47 hinge, a CD8a hinge, a CD28 hinge, a PDGFR hinge, or an IgG4 hinge.
20. A vector comprising the nucleic acid construct of any one of the preceding claims.
21. An engineered protein encoded by the nucleic acid construct of any one of claims 1-19.
22. An engineered protein:
wherein the engineered protein is or comprises an amino acid sequence at least 80% identical to SEQ ID NO: 5,
and
wherein the engineered protein does not comprise one or more full-length CD47 intracellular domains.
23. An engineered protein:
wherein the engineered protein is or comprises an amino acid sequence at least 80% identical to SEQ ID NO: 4
and
wherein the engineered protein does not comprise one or more full-length CD47 intracellular domains.
24. An engineered CD47 protein comprising:
(a) one or more extracellular domains; and (b) one or more membrane tethers;
wherein the one or more extracellular domains comprise:
an amino acid substitution at position S82 of SEQ ID NO: 2, an amino acid substitution at position S64 of SEQ ID NO: 3, an amino acid substitution at position S82 of SEQ ID NO: 1, or an amino acid substitution at position S37 of SEQ ID NO: 6; and/or
an amino acid substitution at position S83 of SEQ ID NO: 2, an amino acid substitution at position S65 of SEQ ID NO: 3, an amino acid substitution at position S83 of SEQ ID NO: 1, or an amino acid substitution at position S38 of SEQ ID NO: 6; and/or
an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 3 SEQ ID NO: 1 or SEQ ID NO: 6; and
wherein the nucleic acid construct does not comprise a nucleic acid sequence encoding one or more full-length CD47 intracellular domains.
25. The engineered CD47 protein of claim 24, wherein the one or more extracellular domains comprise:
an amino acid substitution of S82A relative to SEQ ID NO: 2,
an amino acid substitution of S64A relative to SEQ ID NO: 3,
an amino acid substitution of S82A relative to SEQ ID NO: 1, or

an amino acid substitution of S37A relative to SEQ ID NO: 6.

26. The engineered CD47 protein of claim 24, wherein the one or more extracellular domains comprise:
an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2,
an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3,
an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, or
an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6.
27. The engineered CD47 protein of any one of claims 24-26, wherein the one or more extracellular domains comprise:
an amino acid substitution of S83A relative to SEQ ID NO: 2,
an amino acid substitution of S65A relative to SEQ ID NO: 3,
an amino acid substitution of S83A relative to SEQ ID NO: 1, or
an amino acid substitution of S38A relative to SEQ ID NO: 6.
28. The engineered CD47 protein of any one of claims 24-26, wherein the one or more extracellular domains comprise:
an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2,
an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3,
an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 1, or
an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.
29. The engineered CD47 protein of any one of claims 24, 25, or 27, wherein the one or more extracellular domains comprise:
a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, and an amino acid substitution of S37A relative to SEQ ID NO: 6; and
a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83A relative to SEQ ID NO: 2, an amino acid substitution of S65A relative to SEQ ID NO: 3, an amino acid substitution of S83A relative to SEQ ID NO: 1, and an amino acid substitution of S38A relative to SEQ ID NO: 6.
30. The engineered CD47 protein of any one of claims 24, 26, or 28, wherein the one or more extracellular domains comprise:
a first amino acid substitution selected from the group consisting of: an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 2, an amino acid substitution of S64V, S64I, S64L, S64F, S64W, S64G, or S64P relative to SEQ ID NO: 3, an amino acid substitution of S82V, S82I, S82L, S82F, S82W, S82G, or S82P relative to SEQ ID NO: 1, and an amino acid substitution of S37V, S37I, S37L, S37F, S37W, S37G, or S37P relative to SEQ ID NO: 6; and
a second amino acid substitution selected from the group consisting of: an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or S83P relative to SEQ ID NO: 2, an amino acid substitution of S65V, S65I, S65L, S65F, S65W, S65G, or S65P relative to SEQ ID NO: 3, an amino acid substitution of S83V, S83I, S83L, S83F, S83W, S83G, or

S83P relative to SEQ ID NO: 1, and an amino acid substitution of S38V, S38I, S38L, S38F, S38W, S38G, or S38P relative to SEQ ID NO: 6.

31. The engineered CD47 protein of any one of claims 24-30, wherein the engineered CD47 protein comprises: an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 2 and an amino acid substitution of Q19P relative to SEQ ID NO: 2, or

an insertion of the amino acids WQP after A18 relative to SEQ ID NO: 1 and an amino acid substitution of Q19P relative to SEQ ID NO: 1.

32. The engineered CD47 protein of any one of claims 24-31, wherein the engineered CD47 protein comprises an amino acid sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2, SEQ ID NO: 1 or SEQ ID NO: 6.

33. The engineered CD47 protein of any one of claims 22-23, wherein the engineered protein comprises one or more membrane tethers.

34. The engineered CD47 protein of any one of claims 24-33, wherein the one or more membrane tethers are or comprise a transmembrane domain.

35. The engineered CD47 protein of claim 34, wherein the transmembrane domain is or comprises a CD3zeta, CD8a, CD16a, CD28, CD32a, CD32c, CD40, CD47, CD64, ICOS, Dectin-1, DNGR1, EGFR, GPCR, MyD88, PDGFR, SLAMF7, TRL1, TLR2, TLR3, TRL4, TLR5, TLR6, TLR7, TLR8, TLR9, or VEGFR transmembrane domain.

36. The engineered CD47 protein of any one of claims 24-33, wherein the one or more membrane tethers are or comprise a glycosylphosphatidylinositol (GPI) anchor.

37. The engineered CD47 protein of claim 36, wherein the GPI anchor is or comprises a DAF/CD55 GPI anchor, a TRAILR3 GPI anchor, or a CD59 GPI anchor.

38. A genetically engineered cell comprising the engineered protein of any one of claims 21-37.

39. A genetically engineered cell comprising an engineered CD47 protein, wherein the engineered CD47 protein exhibits:

decreased binding with TSP-1 relative to a wild-type CD47 protein; and/or
decreased CD47/TSP-1-mediated signaling relative to a wild-type CD47 protein.

40. The genetically engineered cell of claim 39, wherein the control cell comprises a wild-type cell, an unmodified cell, a cell that has not been engineered to express CD47 or a cell that has been engineered to express wild-type CD47.

41. The genetically engineered cell of claim 39 or 40, wherein the engineered CD47 protein is expressed at a level 1.25x, 1.5x, 1.75x, 2x, 2.25x, 2.5x, 2.75x, 3x, 3.25x, 3.5x, 3.75x, 4x, 4.5x, 5x, 5.5x, 6x, 6.5x, 7x, 7.5x, 8x, 8.5x, 9x,

9.5x, 10x, 15x, 20x, 25x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x the level of wild-type CD47 protein in an unmodified cell.

42. The genetically engineered cell of any one of claims 39-41, wherein the engineered CD47 protein comprises:
an amino acid substitution at position S82 of SEQ ID NO: 2,
an amino acid substitution at position S64 of SEQ ID NO: 3,
an amino acid substitution at position S82 of SEQ ID NO: 1, or
an amino acid substitution at position S37 of SEQ ID NO: 6.
43. The genetically engineered cell of any one of claims 39-42, wherein the engineered CD47 protein comprises:
an amino acid substitution at position S83 of SEQ ID NO: 2,
an amino acid substitution at position S65 of SEQ ID NO: 3,
an amino acid substitution at position S83 of SEQ ID NO: 1, or
an amino acid substitution at position S38 of SEQ ID NO: 6.
44. The genetically engineered cell of any one of claims 39-43, wherein the engineered CD47 protein comprises:
amino acid substitutions at positions S82 and S83 of SEQ ID NO: 2,
amino acid substitutions at positions S64 and S65 of SEQ ID NO: 3,
amino acid substitutions at positions S82 and S83 of SEQ ID NO: 1, or
amino acid substitutions at positions S37 and S38 of SEQ ID NO: 6.
45. The genetically engineered cell of any one of claims 39-44, wherein the engineered CD47 protein comprises:
an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or
99% identity to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6; and
an insertion of one or more amino acids relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5
or SEQ ID NO: 6.
46. The genetically engineered cell of claim 45, wherein the engineered CD47 protein comprises an insertion of the
amino acids WQP relative to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5 or SEQ ID NO: 6.
47. The genetically engineered cell of claim 45 or 46, wherein the engineered CD47 protein comprises an insertion
of the amino acids WQP after A18 relative to SEQ ID NO: 2 or SEQ ID NO: 1.
48. The genetically engineered cell of any one of claims 45-47, wherein the engineered CD47 protein comprises an
amino acid substitution at position Q19 relative to SEQ ID NO: 2 or SEQ ID NO: 1.
49. The genetically engineered cell of any one of claims 39-48, wherein the engineered CD47 protein comprises an
amino acid sequence comprising:
an amino acid substitution of S82A relative to SEQ ID NO: 2, an amino acid substitution of S64A relative to SEQ
ID NO: 3, an amino acid substitution of S82A relative to SEQ ID NO: 1, or an amino acid substitution of S37A relative to
SEQ ID NO: 6;

an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2 or SEQ ID: 1; and
an amino acid substitution of Q19P relative to SEQ ID NO: 2 or SEQ ID NO: 1.

50. The genetically engineered cell of any one of claims 39-49, wherein the engineered CD47 protein comprises an amino acid sequence comprising:

an amino acid substitution of S82A relative to SEQ ID NO: 2;
an insertion of the amino acids WQP between A18 and Q19 relative to SEQ ID NO: 2; and
an amino acid substitution of Q19P relative to SEQ ID NO: 2.

51. The genetically engineered cell of any one of claims 38-50, wherein the genetically engineered cell comprises a modification at a TCR locus, B2M locus, a TAP I locus, a NLRC5 locus, a CIITA locus, an HLA-A locus, an HLA-B locus, an HLA-C locus, an HLA-DP locus, an HLA-DM locus, an HLA-DOA locus, an HLA-DOB locus, an HLA-DQ locus, an HLA-DR locus, a RFX5 locus, a RFXANK locus, a RFXAP locus, an NFY-A locus, an NFY-B locus, an NFY-C locus, or a combination thereof.

52. The genetically engineered cell of any one of claims 38-51, wherein the cell comprises one or more modifications that inactivate or disrupt one or more alleles of:

(a) one or more major histocompatibility complex (MHC) class I molecules or one or more molecules that regulate expression of the one or more MHC class I molecules, and/or
(b) one or more MHC class II molecules or one or more molecules that regulate expression of the one or more MHC class II molecules.

53. The genetically engineered cell of any one of claims 38-52, wherein the genetically engineered cell has been genetically engineered to knock-out a B2M locus.

54. The genetically engineered cell of any one of claims 38-53, wherein the genetically engineered cell has been genetically engineered to knock-out a CIITA locus.

55. The genetically engineered cell of any one of claims 38-54, wherein the cell is an islet cell, beta islet cell, pancreatic islet cell, immune cell, B cell, T cell, natural killer (NK) cell, natural killer T (NKT) cell, macrophage cell, endothelial cell, muscle cell, cardiac muscle cell, smooth muscle cell, skeletal muscle cell, dopaminergic neuron, retinal pigmented epithelium cell, optic cell, hepatocyte, thyroid cell, skin cell, glial progenitor cell, neural cell, cardiac cell, stem cell, hematopoietic stem cell, induced pluripotent stem cell (iPSC), mesenchymal stem cell (MSC), embryonic stem cell (ESC), pluripotent stem cell (PSC), blood cell, or a combination thereof.

56. The genetically engineered cell of any one of claims 38-55, wherein the genetically engineered cell is a differentiated cell derived from a pluripotent stem cell.

57. The genetically engineered cell of claim 56, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC) or an embryonic stem cell (ESC).

58. The genetically engineered cell of any one of claims 38-57, wherein the cell is a primary cell isolated from a donor.
59. The genetically engineered cell of any one of claims 38-58, wherein the cell is an islet cell, beta islet cell, or pancreatic islet cell.
60. The genetically engineered cell of any one of claims 38-58, wherein the cell is an allogeneic T-cell.
61. The genetically engineered cell of claim 60, wherein the allogeneic T-cell is a primary T cell.
62. The genetically engineered cell of claim 60 or 61, wherein the genetically engineered cell has decreased cell surface expression of a TCR as compared to a comparable cell that has not been genetically engineered.
63. The genetically engineered cell of any one of claims 38-62, further comprising a transgene encoding a chimeric antigen receptor (CAR).
64. The genetically engineered cell of claim 63, wherein the CAR is or comprises a CD5-specific CAR, a CD19-specific CAR, a CD20-specific CAR, a CD22-specific CAR, a CD23-specific CAR, a CD30-specific CAR, a CD33-specific CAR, a CD38-specific CAR, a CD70-specific CAR, a CD123-specific CAR, a CD138-specific CAR, a Kappa, Lambda, B cell maturation agent (BCMA)-specific CAR, a G-protein coupled receptor family C group 5 member D (GPRC5D)-specific CAR, a CD123-specific CAR, a LeY-specific CAR, a NKG2D ligand-specific CAR, a WT1-specific CAR, a GD2-specific CAR, a HER2-specific CAR, a EGFR-specific CAR, a EGFRvIII-specific CAR, a B7H3-specific CAR, a PSMA-specific CAR, a PSCA-specific CAR, a CAIX-specific CAR, a CD171-specific CAR, a CEA-specific CAR, a CSPG4-specific CAR, a EPHA2-specific CAR, a FAP-specific CAR, a FRa-specific CAR, a IL-13Ra-specific CAR, a Mesothelin-specific CAR, a MUC1-specific CAR, a MUC16-specific CAR, a ROR1-specific CAR, a C-Met-specific CAR, a CD133-specific CAR, a Ep-CAM-specific CAR, a GPC3-specific CAR, a HPV16-E6-specific CAR, a IL13Ra2-specific CAR, a MAGEA3-specific CAR, a MAGEA4-specific CAR, a MART1-specific CAR, a NY-ESO-1-specific CAR, a VEGFR2-specific CAR, a α -Folate receptor-specific CAR, a CD24-specific CAR, a CD44v7/8-specific CAR, a EGP-2-specific CAR, a EGP-40-specific CAR, a erb-B2-specific CAR, a erb-B 2,3,4-specific CAR, a FBP-specific CAR, a Fetal acetylcholine e receptor-specific CAR, a G_{D2}-specific CAR, a G_{D3}-specific CAR, a HMW-MAA-specific CAR, a IL-11Ra-specific CAR, a KDR-specific CAR, a Lewis Y-specific CAR, a L1-cell adhesion molecule-specific CAR, a MAGE-A1-specific CAR, a Oncofetal antigen (h5T4)-specific CAR, a TAG-72-specific CAR, or a CD19/CD22-bispecific CAR.
65. A composition comprising the genetically engineered cell of any one of claims 38-64.
66. A pharmaceutical composition comprising (i) the genetically engineered cell of any one of claims 38-64, and (ii) a pharmaceutically acceptable excipient.
67. A method comprising administering to a subject the genetically engineered cell of any one of claims 38-64, the composition of claim 65, or the pharmaceutical composition of claim 66.

68. A method of treating a disease in a subject, the method comprising administering to the subject the genetically engineered cell of any one of claims 38-64, the composition of claim 65, or the pharmaceutical composition of claim 66.

69. A population of cells comprising the genetically engineered cell of any one of claims 38-64 for use in treating a disease in a subject.

70. Use of the genetically modified cell of any one of claims 38-64, the population of cells of claim 69, the composition of claim 65, or the pharmaceutical composition of claim 66 for use in treating a disease in a subject.

71. Use of the genetically modified cell of any one of claims 38-64, the population of cells of claim 69, the composition of claim 65, or the pharmaceutical composition of claim 66 in the manufacture of a medicament for the treatment of a disease.