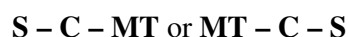


CLAIMS

What is claimed is:

1. A membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula:



wherein

S comprises a secretable effector molecule,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

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

2. The membrane-cleavable chimeric protein of claim 1, wherein the secretable effector molecule comprises a signal peptide or a signal-anchor sequence, optionally wherein the signal peptide comprises a native signal peptide native to the secretable effector molecule or the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF.
3. The membrane-cleavable chimeric protein of claim 1 or claim 2, wherein the secretable effector molecule is selected from a therapeutic class, wherein the therapeutic class is selected from the group consisting of: a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a peptide, and an enzyme, optionally wherein the cytokine is selected from the group consisting of: IL-1-beta, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, an IL-12p70 fusion protein, IL-15, IL-17A, IL-18, IL-21, IL-22, Type I interferons, Interferon-gamma, and TNF-alpha, optionally wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10,

CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1, optionally wherein the homing molecule is selected from the group consisting of: anti-integrin $\alpha 4, \beta 7$; anti-MAdCAM; SDF1; and MMP-2, optionally wherein the growth factor is selected from the group consisting of: FLT3L and GM-CSF, optionally wherein the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L, optionally wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, a TGF β inhibitor, an immune checkpoint inhibitor, a VEGF inhibitor, and HPGE2, optionally wherein the TGF β inhibitor is selected from the group consisting of: an anti-TGF β peptide, an anti-TGF β antibody, a TGF β -TRAP, and a combination thereof, optionally wherein the immune checkpoint inhibitor is selected from the group consisting of: an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-LAG-3 antibody, an anti-TIM-3 antibody, an anti-TIGIT antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-GAL9 antibody, an anti-A2AR antibody, an anti-phosphatidylserine antibody, an anti-CD27 antibody, an anti-TNF α antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody, or optionally wherein the VEGF inhibitor comprises an anti-VEGF antibody, an anti-VEGF peptide, or a combination thereof.

4. The membrane-cleavable chimeric protein of any one of claims 1-3, wherein the secretable effector molecule comprises IL-15, IL-12, or an IL-12p70 fusion protein.
5. The membrane-cleavable chimeric protein of any one of claims 1-4, wherein the protease cleavage site is cleavable by a protease selected from the group consisting of: a Type 1 transmembrane protease, a Type II transmembrane protease, a GPI anchored protease, an ADAM8 protease, an ADAM9 protease, an ADAM10 protease, an ADAM12 protease, an ADAM15 protease, an ADAM17 protease, an ADAM19 protease, an ADAM20 protease, an ADAM21 protease, an ADAM28 protease, an ADAM30 protease, an ADAM33 protease, a BACE1 protease, a BACE2 protease, a SIP protease, an MT1-MMP protease, an MT3-MMP protease, an MT5-MMP protease, a furin protease, a PCSK7 protease, a matriptase protease, a matriptase-2 protease, an MMP9 protease, and an NS3 protease.

6. The membrane-cleavable chimeric protein of any one of claims 1-5, wherein the protease cleavage site is cleavable by an ADAM17 protease, optionally wherein the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176) and/or a second region having the amino acid sequence of KGG (SEQ ID NO: 177), optionally wherein the first region is located N-terminal to the second region.
7. The membrane-cleavable chimeric protein of any one of claims 1-6, wherein the protease cleavage site comprises the amino acid sequence of PRAEX₁X₂KGG (SEQ ID NO: 178),
wherein X₁ is A, Y, P, S, or F, and
wherein X₂ is V, L, S, I, Y, or T.
8. The membrane-cleavable chimeric protein of any one of claims 1-7, wherein:
 - a. the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180); or
 - b. the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180); or
 - c. the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181); or
 - d. the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182); or
 - e. the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183); or
 - f. the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184); or
 - g. the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185); or
 - h. the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187); or
 - i. the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188); or
 - j. the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189); or

- k. the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190); or
 - l. the protease cleavage site comprises the amino acid sequence of VTPEPIFSLI (SEQ ID NO: 191); or
 - m. the protease cleavage site comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198).
- 9. The membrane-cleavable chimeric protein of any one of claims 1-8, wherein the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain, optionally wherein the transmembrane-intracellular domain and/or transmembrane domain is derived from PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, or BTLA, optionally wherein the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof.
- 10. The membrane-cleavable chimeric protein of any one of claims 1-9, wherein the cell membrane tethering domain comprises a post-translational modification tag, or motif capable of post-translational modification to modify the chimeric protein to include a post-translational modification tag, where the post-translational modification tag is capable of association with a cell membrane, optionally wherein the post-translational modification tag comprises a lipid-anchor domain, optionally wherein the lipid-anchor domain is selected from the group consisting of: a GPI lipid-anchor, a myristoylation tag, and a palmitoylation tag.
- 11. The membrane-cleavable chimeric protein of any one of claims 1-10, wherein:
 - a. when expressed in a cell, the secretable effector molecule is tethered to a cell membrane of the cell; and/or
 - b. when expressed in a cell expressing a protease capable of cleaving the protease cleavage site, the secretable effector molecule is released from the cell membrane.; and/or
 - c. wherein the protease expressed on the cell membrane is endogenous to the cell; and/or

- d. the protease is selected from the group consisting of: a Type 1 transmembrane protease, a Type II transmembrane protease, a GPI anchored protease, an ADAM8 protease, an ADAM9 protease, an ADAM10 protease, an ADAM12 protease, an ADAM15 protease, an ADAM17 protease, an ADAM19 protease, an ADAM20 protease, an ADAM21 protease, an ADAM28 protease, an ADAM30 protease, an ADAM33 protease, a BACE1 protease, a BACE2 protease, a SIP protease, an MT1-MMP protease, an MT3-MMP protease, an MT5-MMP protease, a furin protease, a PCSK7 protease, a matriptase protease, a matriptase-2 protease, and an MMP9 protease
12. An engineered nucleic acid comprising an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding the membrane-cleavable chimeric protein of any one of claims 1-11, optionally wherein the promoter is selected from the group consisting of a constitutive promoter, an inducible promoter, a tissue-specific promoter, and a synthetic promoter.
 13. An expression vector comprising the engineered nucleic acid of claim 12.
 14. An isolated cell comprising the membrane-cleavable chimeric protein of any one of claims 1-11, the engineered nucleic acid of claim 12, or the expression vector of claim 13.
 15. The isolated cell of claim 14, wherein the cell is selected from the group consisting of: a T cell, a CD8⁺ T cell, a CD4⁺ T cell, a gamma-delta T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, a dendritic cell, an erythrocyte, a platelet cell, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, a mesenchymal stromal cell (MSC), an induced pluripotent stem cell (iPSC), and an iPSC-derived cell.
 16. The isolated cell of claim 14 or claim 15, wherein the cell further comprises a protease capable of cleaving the protease cleavage site, optionally wherein the protease is an endogenous protease, and/or optionally wherein the endogenous protease is selected from the group consisting of: a Type 1 transmembrane protease, a

Type II transmembrane protease, a GPI anchored protease, an ADAM8 protease, an ADAM9 protease, an ADAM10 protease, an ADAM12 protease, an ADAM15 protease, an ADAM17 protease, an ADAM19 protease, an ADAM20 protease, an ADAM21 protease, an ADAM28 protease, an ADAM30 protease, an ADAM33 protease, a BACE1 protease, a BACE2 protease, a SIP protease, an MT1-MMP protease, an MT3-MMP protease, an MT5-MMP protease, a furin protease, a PCSK7 protease, a matriptase protease, a matriptase-2 protease, and an MMP9 protease.

17. The isolated cell of any one of claims 14-16, wherein the cell further comprises an antigen recognizing receptor, optionally wherein the antigen recognizing receptor is a CAR.
18. A composition comprising the membrane-cleavable chimeric protein of any one of claims 1-11, the engineered nucleic acid of claim 12, the expression vector of claim 13, or the isolated cell of any one of claims 14-17 and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.
19. ~~A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the~~ The isolated cells of any one of claims 14-17 or the composition of claim 18 for use in therapy.
20. A method of inducing release of a membrane-tethered effector molecule, comprising:
 - a) providing a cell of any one of claims 14-17; and
 - b) culturing the cell under conditions suitable for expression of the membrane-bound protease and the membrane-cleavable chimeric protein, wherein upon expression, the membrane-cleavable chimeric protein is tethered to the cell membrane of the cell, and wherein, upon expression, the membrane-bound protease cleaves the cognate membrane-bound protease cleavage site of the membrane-cleavable chimeric protein, thereby releasing the secretable effector molecule from the cell membrane.