

PROTEIN PAYLOAD RELEASE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Nos. 63/193,004
5 filed May 25, 2021 and 63/109,812 filed November 4, 2020, each of which is hereby
incorporated in their entirety by reference for all purposes.

SEQUENCE LISTING

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

BACKGROUND

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

SUMMARY

Provided herein, in some embodiments, is a cell-based therapy platform involving
regulated armoring of the cell-based therapy, such as regulated secretion of payload effector
30 molecules. Also provided herein, in some embodiments, is a combinatorial cell-based
immunotherapy involving regulated armoring for the targeted treatment of cancer, such as
ovarian cancer, breast cancer, colon cancer, lung cancer, and pancreatic cancer.

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

Non-limiting examples of effector molecules encompassed by the present disclosure include cytokines, antibodies, chemokines, nucleotides, peptides, enzymes, and oncolytic viruses. For example, cells may be engineered to express and secrete in a regulated manner at least one, two, three or more of the following effector molecules: IL-12, IL-16, IFN- β , IFN- γ , IL-2, IL-15, IL-7, IL-36 γ , IL-18, IL-1 β , IL-21, OX40-ligand, CD40L, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-CTLA-4 antibodies, anti-TGF β antibodies, anti-TNFR2, MIP1 α (CCL3), MIP1 β (CCL5), CCL21, CpG oligodeoxynucleotides, and anti-tumor peptides (*e.g.*, anti-microbial peptides having anti-tumor activity, see, *e.g.*, Gaspar, D. *et al. Front Microbiol.* 2013; 4: 294; Chu, H. *et al. PLoS One.* 2015; 10(5): e0126390, and website:aps.unmc.edu/AP/main.php).

Provided for herein is an engineered nucleic acid comprising an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula: S – C – MT or MT – C – S, wherein S comprises a secretable effector molecule, C comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein the promoter is operably linked to the exogenous polynucleotide sequence, and wherein S – C – MT or MT – C – S is configured to be expressed as a single polypeptide.

Also provided for herein is a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula: S – C – MT or MT – C – S, wherein S comprises a secretable effector molecule, 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.

Also provided for herein is an isolated cell comprising an engineered nucleic acid, wherein the engineered nucleic acid comprises an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding a membrane-cleavable chimeric protein,

oriented from N-terminal to C-terminal, having the formula: S – C – MT or MT – C – S, wherein S comprises a secretable effector molecule, C comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein the promoter is operably linked to the exogenous polynucleotide sequence, and wherein S – C – MT or MT – C – S is
 5 configured to be expressed as a single polypeptide.

Also provided for herein is an isolated cell comprising a membrane-cleavable chimeric protein, wherein the membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula: S – C – MT or MT – C – S, wherein S comprises a secretable effector molecule, C comprises a protease cleavage site, and MT comprises a cell
 10 membrane tethering domain, and wherein S – C – MT or MT – C – S is configured to be expressed as a single polypeptide.

Also provided for herein is a method of inducing release of a membrane-tethered effector molecule, comprising: a) providing a cell, wherein the cell comprises a membrane-bound protease and a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula: S – C – MT or MT – C – S, wherein S comprises a secretable
 15 effector molecule, C comprises a cognate protease cleavage site of the membrane-bound protease, wherein S – C – MT or MT – C – S is configured to be expressed as a single polypeptide; and b) culturing the cell under conditions suitable for expression of the membrane-bound protease and the membrane-cleavable chimeric protein, wherein upon
 20 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.

In some aspects, the promoter is a constitutive promoter. In some aspects, the
 25 constitutive promoter is selected from the group consisting of: CAG, HLP, CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, hEF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb. In some aspects, the promoter is an inducible promoter. In some aspects, the inducible promoter comprises a minimal promoter and a responsive element selected from the group consisting of: NFkB response
 30 element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, inducer molecule responsive promoters, and tandem repeats thereof.

In some aspects, the promoter is a synthetic promoter. In some aspects, the synthetic promoter comprises an activation-conditional control polypeptide- (ACP-) binding domain sequence and a promoter sequence. In some aspects, the promoter sequence is derived from a promoter selected from the group consisting of: minP, NFkB response element, CREB
5 response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, minCMV, YB_TATA, minTK, inducer molecule responsive promoters, and tandem repeats thereof. In some aspects, the ACP-binding domain comprises one or more zinc finger binding sites. In some aspects, the
10 synthetic promoter is regulatable by an activation-conditional control polypeptide (ACP) that binds to the ACP-binding domain of the synthetic promoter.

In some aspects, the ACP is a transcriptional modulator. In some aspects, the ACP is a transcriptional repressor. In some aspects, the ACP is a transcriptional activator. In some aspects, the ACP further comprises a repressible protease and one or more cognate cleavage
15 sites of the repressible protease. In some aspects, the ACP further comprises a hormone-binding domain of estrogen receptor (ERT2 domain).

In some aspects, the ACP is a transcription factor. In some aspects, the transcription factor is a zinc-finger-containing transcription factor. In some aspects, the ACP comprises a DNA-binding zinc finger protein domain (ZF protein domain) and a transcriptional effector
20 domain. In some aspects, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). In some aspects, the ZF protein domain comprises one to ten ZFA.

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

In some aspects, the one or more cognate cleavage sites of the repressible protease are localized between the ZF protein domain and the effector domain. In some aspects, the

repressible protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3). In some aspects, the cognate cleavage site comprises an NS3 protease cleavage site. In some aspects, the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site. In some aspects, the NS3 protease can be repressed by a protease inhibitor. In some aspects, the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.

In some aspects, the ACP is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof. In some aspects, the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

In some aspects, the ACP further comprises a degron domain, and wherein the degron domain is operably linked to the ACP. In some aspects, the degron domain is derived from a degron selected from the group consisting of HCV NS4 degron, PEST (two copies of residues 277-307 of human I κ B α), GRR (residues 352-408 of human p105), DRR (residues 210-295 of yeast Cdc34), SNS (tandem repeat of SP2 and NB (SP2-NB-SP2 of influenza A or influenza B), RPB (four copies of residues 1688-1702 of yeast RPB), SPmix (tandem repeat of SP1 and SP2 (SP2-SP1-SP2-SP1-SP2 of influenza A virus M2 protein), NS2 (three copies of residues 79-93 of influenza A virus NS protein), ODC (residues 106-142 of ornithine decarboxylase), Nek2A, mouse ODC (residues 422-461), mouse ODC_DA (residues 422-461 of mODC including D433A and D434A point mutations), an APC/C degron, a COP1 E3 ligase binding degron motif, a CRL4-Cdt2 binding PIP degron, an actinfilin-binding degron, a KEAP1 binding degron, a KLHL2 and KLHL3 binding degron, an MDM2 binding motif, an N-degron, a hydroxyproline modification in hypoxia signaling, a phytohormone-dependent SCF-LRR-binding degron, an SCF ubiquitin ligase binding phosphodegron, a phytohormone-dependent SCF-LRR-binding degron, a DSGxxS phospho-dependent degron, an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box. In some aspects, the degron domain comprises a cereblon (CRBN) polypeptide substrate domain capable of binding CRBN in response to an immunomodulatory drug (IMiD) thereby promoting ubiquitin pathway-mediated degradation of the ACP. In some aspects, the CRBN polypeptide substrate domain is selected from the group consisting of: IKZF1, IKZF3, CK1a, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN. In some aspects, the CRBN polypeptide substrate domain is a chimeric

fusion product of native CRBN polypeptide sequences. In some aspects, the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of

FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRR

- 5 DAL (SEQ ID NO: 175). In some aspects, the IMiD is an FDA-approved drug. In some aspects, the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide. In some aspects, the degron domain is N-terminal of the repressible protease, C-terminal of the repressible protease, N-terminal of the ZF protein domain, C-terminal of the ZF protein domain, N-terminal of the effector domain, or C-terminal of the effector
- 10 domain.

In some aspects, the promoter is a tissue-specific promoter.

- In some aspects, the secretable effector molecule comprises a signal peptide or a signal-anchor sequence. In some aspects, the signal peptide comprises a native signal peptide native to the secretable effector molecule. In some aspects, the signal peptide comprises a
- 15 non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule. In some aspects, the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein,
- 20 osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL21, CD8, NKG2D, TNFR2, and GMCSF.

- In some aspects, 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
- 25 microenvironment modifier, a ligand, an antibody, a peptide, and an enzyme. In some aspects, 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. In some aspects, the secretable effector molecule comprises IL-15, IL-12, or an IL-12p70 fusion protein. In some aspects, the
- 30 secretable effector molecule comprises IL-15. In some aspects, the secretable effector molecule comprises IL-15 having the amino acid sequence of SEQ ID NO: 199. In some aspects, the secretable effector molecule comprises IL-15 and an IL-15R α sushi domain. In some aspects, the secretable effector molecule comprises and IL-15/IL-15R α sushi domain fusion protein having the amino acid sequence of SEQ ID NO: 202. In some aspects, the

secretable effector molecule consists of IL-15 and IL-15R α sushi domain. In some aspects, the secretable effector molecule comprises IL-12. In some aspects, the secretable effector molecule comprises an IL-12p70 fusion protein. In some aspects, the secretable effector molecule comprises an IL-12p70 fusion protein having the amino acid sequence of SEQ ID

5 NO: 203. In some aspects, the secretable effector molecule consists of IL-12. In some aspects, the secretable effector molecule consists of an IL-12p70 fusion protein. In some aspects, the secretable effector molecule is IL-15. In some aspects, the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1. In some aspects, the homing molecule is selected

10 from the group consisting of: anti-integrin α 4, β 7; anti-MAdCAM; SDF1; and MMP-2. In some aspects, the growth factor is selected from the group consisting of: FLT3L and GM-CSF. In some aspects, the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L. In some aspects, the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, a TGF β inhibitor, an immune checkpoint

15 inhibitor, a VEGF inhibitor, and HPGE2. In some aspects, 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. In some aspects, 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

20 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. In some aspects, the VEGF inhibitor comprises an anti-VEGF antibody, an anti-

25 VEGF peptide, or a combination thereof. In some aspects, the secretable effector molecule is a human-derived effector molecule.

In some aspects, the protease cleavage site is selected from the group consisting of: a Type 1 transmembrane protease cleavage site, a Type II transmembrane protease cleavage site, a GPI anchored protease cleavage site, an ADAM8 protease cleavage site, an ADAM9

30 protease cleavage site, an ADAM10 protease cleavage site, an ADAM12 protease cleavage site, an ADAM15 protease cleavage site, an ADAM17 protease cleavage site, an ADAM19 protease cleavage site, an ADAM20 protease cleavage site, an ADAM21 protease cleavage site, an ADAM28 protease cleavage site, an ADAM30 protease cleavage site, an ADAM33 protease cleavage site, a BACE1 protease cleavage site, a BACE2 protease cleavage site, a

SIP protease cleavage site, an MT1-MMP protease cleavage site, an MT3-MMP protease cleavage site, an MT5-MMP protease cleavage site, a furin protease cleavage site, a PCSK7 protease cleavage site, a matriptase protease cleavage site, a matriptase-2 protease cleavage site, an MMP9 protease cleavage site, and an NS3 protease cleavage site. In some aspects, 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.

In some aspects, the protease cleavage site is cleavable by an ADAM17 protease. In some aspects, the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176). In some aspects, the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177). In some aspects, the first region is located N-terminal to the second region. In some aspects, 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, T, or A. In some aspects, 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. In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186). In some aspects, the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187). In some aspects, the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188). In

some aspects, the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189). In some aspects, the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190). In some aspects, the protease cleavage site comprises the amino acid sequence of VTPEPIFSLI (SEQ ID NO: 191). In some aspects, the protease cleavage site comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198)

In some aspects, the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain. In some aspects, 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. In some aspects, the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof.

In some aspects, 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. In some aspects, 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.

In some aspects, when expressed in a cell, the secretable effector molecule is tethered to a cell membrane of the cell. In some aspects, 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. In some aspects, the protease expressed on the cell membrane is endogenous to the cell. In some aspects, the protease is selected from the group consisting of: a Type I 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. In some aspects, the protease is an ADAM17 protease.

In some aspects, the protease expressed on the cell membrane is heterologous to the cell. In some aspects, the protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).

In some aspects, the protease cleavage site comprises an NS3 protease cleavage site. In some aspects, the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site. In some aspects, the protease can be repressed by a protease inhibitor. In some aspects, the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir. In some aspects, expression and/or localization of the protease is capable of regulation. In some aspects, the expression and/or localization is regulated by a cell state of the cell.

In some aspects, the engineered nucleic acid is a single-stranded or double-stranded nucleic acid selected from the group consisting of: a DNA, cDNA, an RNA, an mRNA, and a naked plasmid.

In some aspects, the isolated 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. In some aspects, the isolated cell is a Natural Killer (NK) cell.

In some aspects, the isolated cell is autologous. In some aspects, the isolated cell is allogeneic. In some aspects, the isolated cell is a tumor cell selected from the group consisting of: a bladder tumor cell, a brain tumor cell, a breast tumor cell, a cervical tumor cell, a colorectal tumor cell, an esophageal tumor cell, a glioma cell, a kidney tumor cell, a liver tumor cell, a lung tumor cell, a melanoma cell, an ovarian tumor cell, a pancreatic tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

In some aspects, the isolated cell was engineered via transduction with an oncolytic virus.

In some aspects, the isolated cell further comprises a protease capable of cleaving the protease cleavage site. In some aspects, the protease is an endogenous protease. In some aspects, the endogenous protease is selected from the group consisting of: a Type I 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. In some aspects, the endogenous protease is an ADAM17 protease. In some aspects, the protease is a heterologous protease. In some aspects, the heterologous protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3). In some aspects, the protease is expressed on the cell membrane of the isolated cell. In some aspects, the protease is capable of cleaving the protease cleavage site. In some aspects, cleavage of the protease cleavage site releases the secretable effector molecule from the cell membrane of the isolated cell.

In some aspects, the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176). In some aspects, the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177). In some aspects, the first region is located N-terminal to the second region. In some aspects, 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, T, or A. In some aspects, 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. In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185). In some aspects, the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186). In some aspects, the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187). In some aspects, the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188). In some aspects, the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189). In some aspects, the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190). In some aspects, the protease cleavage site

comprises the amino acid sequence of VTPEPIFSLI (SEQ ID NO: 191). In some aspects, the protease cleavage site comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198).

In some aspects, the isolated cell further comprises an antigen recognizing receptor. In some aspects, the antigen recognizing receptor recognizes an antigen selected from the group consisting of: 5T4, ADAM9, AFP, AXL, B7-H3, B7-H4, B7-H6, C4.4, CA6, Cadherin 3, Cadherin 6, CCR4, CD123, CD133, CD138, CD142, CD166, CD25, CD30, CD352, CD37, CD38, CD44, CD56, CD66e, CD70, CD71, CD74, CD79b, CD80, CEA, CEACAM5, Claudin18.2, cMet, CSPG4, CTLA, DLK1, DLL3, DR5, EGFR, ENPP3, EpCAM, EphA2, Ephrin A4, ETBR, FGFR2, FGFR3, FRalpha, FRb, GCC, GD2, GFRa4, gpA33, GPC3, gpNBM, GPRC5, HER2, IL-13R, IL-13Ra, IL-13Ra2, IL-8, IL-15, IL1RAP, Integrin aV, KIT, L1CAM, LAMP1, Lewis Y, LeY, LIV-1, LRRC, LY6E, MCSP, Mesothelin, MUC1, MUC16, MUC1C, NaPi2B, Nectin 4, NKG2D, NOTCH3, NY ESO 1, Ovarin, P-cadherin, pan-Erb2, PSCA, PSMA, PTK7, ROR1, S Aures, SCT, SLAMF7, SLITRK6, SSTR2, STEAP1, Survivin, TDGF1, TIM1, TROP2, and WT1. In some aspects, the antigen recognizing receptor comprises an antigen-binding domain. In some aspects, the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some aspects, the antigen-binding domain comprises a single chain variable fragment (scFv). In some aspects, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some aspects, the VH and VL are separated by a peptide linker. In some aspects, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain. In some aspects, the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR).

In some aspects, the antigen recognizing receptor is a CAR. In some aspects, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling

domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain. In some aspects, the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28

5 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain. In some aspects, the CAR comprises a spacer region between the
10 antigen-binding domain and the transmembrane domain.

Also provided for herein is a composition comprising any of the isolated cells described herein, and a pharmaceutically acceptable carrier.

Also provided for herein is a method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells
15 described herein or any of the compositions described herein. In some aspects, the isolated cell is derived from the subject. In some aspects, the isolated cell is allogeneic with reference to the subject. In some aspects, the method further comprises administering a checkpoint inhibitor. In some aspects, the 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
20 antibody, an anti-LAG-3 antibody, an anti-TIM-3 antibody, an anti-TIGIT antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-GAL9 antibody, an anti-A2AR antibody, an anti-phosphatidylserine antibody, an anti-CD27 antibody, an anti-TNFa antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody. In some aspects, the
25 method further comprises administering an anti-CD40 antibody.

Also provided for herein is a lipid-based structure comprising any of the engineered nucleic acids described herein, or any of the expression vectors described herein, or any of the membrane-cleavable chimeric proteins described herein. In some aspects, the lipid-based structure comprises an extracellular vesicle, a lipid nanoparticle, a micelle, or a liposome. In
30 some aspects, the extracellular vesicle is selected from the group consisting of: a nanovesicle and an exosome. In some aspects, the lipid-based structure comprises a lipid nanoparticle or a micelle. In some aspects, the lipid-based structure comprises a liposome.

Also provided for herein is a composition comprising any of the lipid-based structures described herein, and a pharmaceutically acceptable carrier.

Also provided for herein is a method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the lipid-based structures described herein or any of the compositions described herein. In some aspects, the administering comprises systemic administration. In some aspects, the lipid-based structure is capable of engineering a cell in the subject. In some aspects, the method further comprises administering a checkpoint inhibitor. In some aspects, the 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. In some aspects, the method further comprises administering an anti-CD40 antibody.

Also provided for herein is a nanoparticle comprising any of the engineered nucleic acids described herein or any of the membrane-cleavable chimeric proteins described herein. In some aspects, the nanoparticle comprises an inorganic material.

Also provided for herein is a composition comprising any of the nanoparticles described herein.

Also provided for herein is a method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the nanoparticles described herein, or any of the compositions described herein. In some aspects, the administering comprises systemic administration. In some aspects, the nanoparticle is capable of engineering a cell in the subject. In some aspects, the method further comprises administering a checkpoint inhibitor. In some aspects, the 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. In some aspects, the method further comprises administering an anti-CD40 antibody.

Also provided for herein is a virus engineered to comprise any of the engineered nucleic acids described herein or any of the expression vectors described herein. In some aspects, the virus is selected from the group consisting of: a lentivirus, a retrovirus, an

oncolytic virus, an adenovirus, an adeno-associated virus (AAV), and a virus-like particle (VLP).

Also provided for herein is a composition comprising any of the engineered viruses described herein, and a pharmaceutically acceptable carrier.

5 Also provided for herein is a method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the engineered viruses described herein, or any of the compositions described herein. In some aspects, the administering comprises systemic administration. In some aspects, the engineered virus infects a cell in the subject and expresses the expression cassette. In some aspects, the method
10 further comprises administering a checkpoint inhibitor. In some aspects, the 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
15 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. In some aspects, the method further comprises administering an anti-CD40 antibody.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A illustrates a schematic of the Membrane-Cleavable system described herein in which a desired payload is expressed as a chimeric protein in which a protease cleavage site is inserted between the payload and membrane-tethering domain. The left panel
25 illustrates a schematic of the Membrane-Cleavable system using a transmembrane spanning architecture (*e.g.*, the chimeric protein contains a transmembrane domain). The right panel illustrates a schematic of the Membrane-Cleavable system using a membrane-associated architecture (*e.g.*, the chimeric protein contains a post-translational modification tag allowing association with a cell membrane).

FIG. 1B illustrates a representative Membrane-Cleavable system for the regulated
30 secretion of IL-15 in which IL-15 is expressed as a chimeric protein having a cleavage site capable of cleavage by TACE inserted between the IL-15 payload and membrane-tethering domain. The left panel illustrates a Type I transmembrane architecture (*e.g.*, S – C – MT) through use of Type I transmembrane domains, such as PDGFR-beta and CD8. The right

panel a Type II transmembrane architecture (*e.g.*, MT – C – S) through use of Type II transmembrane domains, such as NKG2D and TNFR2.

FIG. 2 provides a plot of membrane-bound expression as assayed by flow cytometry (calculated as geometric mean fluorescent intensity, “gMFI,” y axis) and IL-15 secretion as determined by ELISA (measured as pg/ml, x-axis) for each Membrane-Cleavable IL-15 construct.

FIGs. 3A-3C provide membrane-bound expression and secretion of IL-12 Membrane-Cleavable systems. **FIG. 3A** indicates membrane-bound expression of each Membrane-Cleavable IL-12 system in NK cells, as measured by flow cytometry. **FIG. 3B** indicates membrane-bound expression of each Membrane-Cleavable IL-12 system in T cells, as measured by flow cytometry. **FIG. 3C** shows IL-12 secretion for each Membrane-Cleavable IL-12 system expressed in NK cells and T cells.

FIGs. 4A-4B provide membrane-bound NK cell expression and secretion of various IL-15 Membrane-Cleavable systems. **FIG. 4A** indicates membrane-bound expression of each Membrane-Cleavable IL-15 system in NK cells, as measured by flow cytometry. **FIG. 4B** shows IL-15 secretion for each Membrane-Cleavable IL-15 system expressed in NK cells.

DETAILED DESCRIPTION

Chimeric proteins (or engineered nucleic acids encoding the chimeric proteins) are provided for herein having the formula **S – C – MT** or **MT – C – S**, oriented from N-terminal to C-terminal and expressed as a single polypeptide. S refers to a secretable effector molecule. C refers to a protease cleavage site. MT refers to a cell membrane tethering domain. The membrane-cleavable chimeric protein is engineered such that secretion of the effector molecule can be regulated in a protease-dependent manner. Specifically, the membrane-cleavable chimeric protein is engineered such that secretion of the effector molecule can be regulated as part of a “Membrane-Cleavable” system, where incorporation of a protease cleavage site (“C”) and a cell membrane tethering domain (“MT”) allow for regulated secretion of an effector molecule in a protease-dependent manner. Without wishing to be bound by theory, the components of the Membrane-Cleavable system present in the membrane-cleavable chimeric protein generally regulate secretion through the below cellular processes:

- MT: The cell membrane tethering domain contains a transmembrane domain (or a transmembrane-intracellular domain) that directs cellular-trafficking of the

chimeric protein such that the protein is inserted into, or otherwise associated with, a cell membrane (“tethered”)

- C: Following expression and localization of the chimeric protein into the cell membrane, the protease cleavage site directs cleavage of the chimeric protein such that the effector molecule is released (“secreted”) into the extracellular space.

Generally, the protease cleavage site is protease-specific, including sites engineered to be protease-specific. The protease cleavage site can be selected or engineered to achieve optimal protein expression, cell-type specific cleavage, cell-state specific cleavage, and/or cleavage and release of the payload at desired kinetics (*e.g.*, ratio of membrane-bound to secreted chimeric protein levels)

In some aspects, membrane-cleavable chimeric proteins (or engineered nucleic acids encoding the membrane-cleavable chimeric proteins) are provided for herein having a protein of interest (*e.g.*, any of the effector molecules described herein), a protease cleavage site, and a cell membrane tethering domain.

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

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

The term “modulate” encompasses maintenance of a biological activity, inhibition (partial or complete) of a biological activity, and stimulation/activation (partial or complete) of a biological activity. The term also encompasses decreasing or increasing (*e.g.*, enhancing) a biological activity. Two different effector molecules are considered to “modulate different tumor-mediated immunosuppressive mechanisms” when one effector molecule modulates a

tumor-mediated immunosuppressive mechanism (*e.g.*, stimulates T cell signaling) that is different from the tumor-mediated immunosuppressive mechanism modulated by the other effector molecule (*e.g.*, stimulates antigen presentation and/or processing).

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

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

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

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

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

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

mechanism results in a decrease in an immunosuppressive response by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold.

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

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

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

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

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

In some embodiments, effector molecules may be selected from the following non-limiting classes of molecules: cytokines, antibodies, chemokines, nucleotides, peptides, and enzymes. Non-limiting examples of the foregoing classes of effector molecules are listed in Table 1 and specific sequences encoding exemplary effector molecules are listed in Table 2. Effector molecules can be human, such as those listed in Table 1 or Table 2 or human equivalents of murine effector molecules listed in Table 1 or Table 2. Effector molecules can be human-derived, such as the endogenous human effector molecule or an effector molecule modified and/or optimized for function, *e.g.*, codon optimized to improve expression, modified to improve stability, or modified at its signal sequence (*see below*). Various programs and algorithms for optimizing function are known to those skilled in the art and can be selected based on the improvement desired, such as codon optimization for a specific species (*e.g.*, human, mouse, bacteria, etc.).

In some embodiments, the effector molecule comprises interleukin 12 (IL-12), for example, p35 and p40 as a dimer that is generally referred to in the art as IL12p70. In some embodiments, the first effector molecule comprises an IL12p70 fusion protein. In some embodiments, the IL12p70 fusion protein is a human IL12p70 fusion protein. In some

embodiments, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 203.

- In some embodiments, the effector molecule comprises interleukin 15 (IL-15). In some embodiments, the effector molecule consists of IL-15 (see, e.g., SEQ ID NO: 199). In some
- 5 embodiments, the effector molecule comprises a fusion protein including IL-15 and an extracellular portion of IL-15 Receptor α (IL-15R α), such as the sushi domain as shown in SEQ ID NO: 201. An exemplary IL-15/IL-15R α sushi domain fusion is provided as SEQ ID NO: 202.

10 **Table 1. Exemplary Effector Molecules**

Effector name	Category	Function
anti-CD40 or CD40 Ligand	Agonist antibody	Stimulates B-cells and antigen presenting cells.
Flt3L	Ligand agonist	Stimulates myeloid cells and antigen presenting cells
CXCL10-11 fusion	Chemokine	Attracts T-cells
TGFb blocking peptides	Antagonist peptides	Inhibit TGFb pathway, TME modifier
Adenosine deaminase (ADA)	TME modifier	Degradation of suppressive adenosine in the TME
Kynurinease	TME modifier	Degradation of kynurine
HPGE2	TME modifier	Degradation of PGE2
CXCL13	Chemokine	Attracts B-cells
anti PD-1/PD-L1	Agonist antibody	Remove checkpoint
anti-CTLA-4	Agonist antibody	Remove checkpoint
anti-VEGF	Antagonist antibody	Neutralizes an immunosuppressive/angiogenesis factor
anti-TNF α	Antagonist antibody	Neutralizes cytokine/pro-tumor factor
anti-IL-10	Antagonist antibody	Neutralizes immunosuppressive cytokine
anti-SDF1/CXCL12	Antagonist antibody	Neutralizes pro-tumor chemokine
(T β RII)2 trap	Capture trap	Neutralizes an immunosuppressive cytokine
CCL21	Chemokine	Attracts leukocytes/NK
CCL1	Chemokine	Attracts leukocytes/NK
CCL17	Chemokine	Attracts leukocytes/NK
CCL19	Chemokine	Attracts leukocytes/NK
CCL21	Chemokine	Attracts leukocytes/NK
CCL20	Chemokine	Attracts leukocytes/NK
CCL21a	Chemokine	Attracts leukocytes/NK
MIP1b (CCL5)	Chemokine	Attracts leukocytes/NK

Effector name	Category	Function
CXCL10	Chemokine	Attracts leukocytes/NK
CXCL11	Chemokine	Attracts leukocytes/NK
CCL2	Chemokine	Attracts monocytes
MIP-1alpha (CCL3)	Chemokine	Attracts leukocytes/NK
XCL1	Chemokine	Attracts leukocytes/NK
IFNbeta	Cytokine	T cell response, tumor cell killing
IFNgamma	Cytokine	T cell response, tumor cell killing
IL-12	Cytokine	T cells, NK cells
IL-1beta	Cytokine	T cells, NK cells
IL-15	Cytokine	Stimulates T-cells and NK
IL-2	Cytokine	Stimulates T-cells and NK
IL-21	Cytokine	Stimulates T-cells
IL-24	Cytokine	Stimulates T-cells
IL36-gamma	Cytokine	Stimulates T-cells
IL-7	Cytokine	Stimulates T-cells
IL-22	Cytokine	Stimulates T-cells
IL-18	Cytokine	Stimulates T-cells
Granzymes/Perforin	Enzyme	Direct tumor cell killing
OX86 (anti-OX40)	ligand	Stimulates T-cells
anti-TGFbeta	Neutralizing antibody	Neutralizes an Immunosuppressive cytokine
TRAIL	Receptor/ligand	Direct tumor cell killing
FASL (CD49L)	Receptor/ligand	Direct tumor cell killing
OX40-L	Receptor/Ligand	Stimulates T-cells
cGAS	secreted molecule	Stimulates antigen-presenting cells
41BBL	secreted molecule	Co-activation of T-cells
CD40L	secreted molecule	Stimulates T-cells
GM-CSF	secreted molecule	Growth factor for monocytes
STING	secreted molecule	Stimulates antigen-presenting cells
HAC-V 'microbody' PD1	Antagonist antibody	inhibits checkpoint
yCD	Pro-drug	Converts to cytotoxic molecule upon activation
CpG/Nucleotides	Nucleotides	STING agonist

Table 2: Exemplary effector molecule sequences

IL-12 (Human) (SEQ ID NO: 56)
ATGTGCCATCAGCAGCTTGTCATATCTTGGTTTTCACTTGATTCTCGGCCAGCCCTTTGGTTGC GATCTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGT GAGATGGTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACCAGT CTCCGAAAGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGG ACAATACACATGCCACAAGGGAGGCGAGGTGTTGTCTCATAGTTTGCTGCTTCTCCACAAGAAA GAGGATGGAATCTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAAATAAGACATTT CTGCGATGTGAGGCTAAGAACTATAGTGGCCGCTTCACTTGTTGGTGGCTGACTACCATCAGCA CAGATCTCACGTTTTAGTAAAAAGTAGAGGTTCAAGTGATCCTCAAGGGGTAACGTGCGG TGCTGCAACACTGTCTGCTGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCGTAGA

ATGCCAAGAGGACAGTGCTTGTCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGA
 CGCGGTGCATAAACTGAAATATGAGAACTACACAAGCAGTTTTTTTATAAGAGATATCATCAAG
 CCCGATCCGCCGAAGAATTTGCAACTTAAACCGCTTAAAACTCACGCCAGGTGAAGTATCCT
 GGGAGTATCCGGATACATGGTCAACACCACACAGCTATTTTTCCCTTACCTTCTGTGTGCAGGT
 CAAGGGAAGAGCAAAAGGGAGAAGAAGGACAGGGTATTCACTGATAAACTTCCGCGACGGT
 CATCTGCCGAAAAACGCTAGTATATCTGTACGGGCGCAGGATAGGTACTATAGTTCTTCTTGG
 TCTGAGTGGGCCTCAGTTCCGTGCTCTGGGGGAGGAAGTGGAGGAGGGTCCGCGCGTGGAAGC
 GGGGGAGGGAGTCGCAACTTGCCAGTGGCTACACCAGATCCAGGCATGTTTCCATGTCTGCATC
 ATTCCAGAATCTCCTGAGAGCGGTGTCAAATATGCTCCAAAAAGCGAGACAAACACTGGAAT
 TTTACCCGTGTACCAAGTGAAGAGATTGATCACAGGACATAACCAAGGACAAGACCTCAACTG
 TAGAAGCGTGTGTGCGCTGGAGTTGACTAAGAATGAGTCCTGCCTCAATTCCAGAGAACTTC
 ATTCACTACTAACGGCAGTTGTCTTGCATCCCGGAAAAACGTCCTTTATGATGGCCCTTTGCCTTA
 GTTCAATTTACGAGGATCTTAAAAATGTATCAAGTGGAGTTTAAAACCATGAATGCTAAACTTCT
 TATGGACCCCAAACGACAAATTTTCTGGATCAGAATATGCTTGCCGTGATAGACGAACTCATG
 CAGGCGCTTAATTTTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAGAACCTGATTTTT
 ATAAAACGAAGATTAAACTGTGTATCCTGCTGCATGCCTTCGCATCCGAGCTGTCACAATCGA
 TAGGGTTATGTCCTACCTTAACGCGAGCtaG

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

ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCTCGCTTCCCCTCTGG
TCGCCATTTGGGAAGTGAAGAAGGACGCTACGTGGTTCGAGCTGGATTGGTACCCGGACGCC
 CTGGAGAAATGGTCGTGCTGACTTGCATACGCCAGAAGAGGACGGCATAACCTGGACCCTGG
 ATCAGAGCTCCGAGGTGCTCGGAAGCGGAAACCTGACCATTCAAGTCAAGGAGTTCCGGC
 ACGCGGGCCAGTACACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCA
 CAAGAAAGAGGATGGAATCTGGTCCACTGACATCTCAAGGACCAAAAAAGAACCGAAGAACA
 AGACCTTCTCCGTGCGAAGCCAAGAACTACAGCGGTTCGGTTACCTGTTGGTGGCTGACGAC
 AATCTCCACCGACCTGACTTTCTCCGTGAAGTCGTACGCGGATCAAGCGATCCTCAGGGCGTG
 ACCTGTGGAGCCGCACTCTGTCCGCCGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTAC
 TCCGTGGAATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCCGAGGT
 ATGGTCCGTGCCGTGATAAGCTGAAATACAGAACTACACTTCTCCTTCTTATCCGCGACA
 TCATCAAGCCTGACCCCCCAAGAACTTGCAGCTGAAGCCACTCAAGAACTCCCGCCAAGTGA
 AGTGTCTTGGGAATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTG
 TGCAAGTGCAGGGAAGTCCAAACGGGAGAAGAAAGACCGGGTTCACCGACAAAACCTCCG
 CCACTGTGATTTGTGCGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTC
 CTCCTGGAGCGAATGGGCCAGCGTGCCTTGTTCGGTGGCGGATCAGGCGGAGGTTACAGGAG
 AGGCTCCGAGGAGGTTCCCGGAACCTCCCTGTGGCAACCCCGACCTGGAATGTTCCCGTGC
 CTACACCACTCCCAAAACCTCTGAGGGCTGTGTGCAACATGTTGCAGAAGGCCCGCCAGACCC
 TTGAGTTTCTACCCCTGCACCTCGGAAGAAATTGATCACGAGGACATACCAAGGACAAGACCTC
 GACCGTGAAGCCTGCCTGCCGCTGGAAGTACCAAGAACGAATCGTGTCTGAACTCCCGCGA
 GACAAGCTTTATCACTAACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTATGATGGCGCTC
 TGCTTTTCTCGATCTACGAAGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGAACGCCA
 AGCTGCTCATGGACCCGAAGCGGCAGATCTTCTGGACCAGAATATGCTCGCCGTGATTGATGA
 ACTGATGCAAGGCCCTGAATTTCAACTCCGAGCTGTGCCTCAAAAGTCCAGCTGGAAGAACC
 GACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGTTGCACGCTTTCCGCATTTCGAGCCGTGA
 CCATTGACCGCGTGATGTCCTACCTGAACGCCAGT

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

ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCTCCTGGTGAGCCCACTCATGG
 CAATGTGGGAAGTCAAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAG
 GGGAGACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACC
 AACGCCATGGCGTAATAGGGAGTGGGAAAAACACTCACGATCACAGTTAAAGAGTTCTTGATG
 CTGGTCAATATACTTGCCATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCATAA
 AAAAGAGAATGGCATTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAAGACATTTCTCAA
 GTGTGAGGCCCTAATTACAGCGGCAGGTTACGTGCTCATGGCTGGTCCAGCGCAACATGGAC
 CTCAAGTTTAACATAAAATCTTCTCTCTTACCTGACTCCAGAGCTGTTACTTGCGGCATGGC
 TTCTCTGAGCGCAGAAAAAGTAACGTTGGATCAAAGAGACTACGAAAAAGTACTCTGTTTCTTGT
 CAAGAGGATGTTACGTGCCCCGACGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAAGCT
 CGCCAACAAAACAAGTATGAAAACTACAGTACAAGCTTCTTTATACGGGATATAATTAACCCG
 ATCCCCCAAGAAGTGAATGAAACCACTTAAGAACAGCCAGGTGGAAGTTTCTGGGAGT
 ATCCAGACTCATGGAGTACTCCTCACAGCTATTTTCTCTGAAATCTTTGTAAGGATACAACGG
 AAGAAAGAGAAGATGAAAGAGACCGAGGAGGGTGTAAATCAGAAGGGAGCGTTTCTCGTGGA
 GAAAACGTCTACCGAAGTCCAATGTAAAGGTGGCAATGTGTGCGTCCAAGCTCAGGATAGATA

CTATAATTCAAGTTGCTCCAAGTGGGCTGTGTTCCATGCCGCGTTCGGAGCGGGGAGGTAGC GGAGGAGGTAGTGGGGGTGGGTGTCAGGAGGAGGGAGTCGAGTTATCCCGGTGTGAGGCCCCGCA CGTGTCTGAGCCAGAGTCGCAACCTCCTTAAGACAACAGATGACATGGTGAAAACAGCACGC GAAAAGCTTAAACACTACTCTTGTACGGCGGAGGATATTGATCACGAGGATATTACCCGAGACC AACTAGCACTTTGAAAACCTGTCTGCCCTTGAACCTCATAAAAATGAGAGCTGTCTGGCTAC ACGAGAGACGTCAAGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAAGACAAGCCTCATGAT GACGCTCTGTTTGGGTTCATTTACGAGGACTTGAAAATGTATCAAACGGAGTTCAGGCTATA AATGCGGCGTTGCAGAACCATAACCATCAACAAATTATACTTGATAAAGGCATGTTGGTGGCGA TTGATGAACTCATGCAGAGTCTCAATCACAAACGGGGAAACGTTGAGACAGAAACCCCACTCG GTGAAGCGGACCCATATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACGCATTACGACTAG AGTTGTTACCATCAACCGGGTAATGGGATATCTCTCCAGTGCCGtaG
IL21 (Human; codon optimized; bold denotes signal sequence) (SEQ ID NO: 59)
ATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGTCG AGCAGCC AGGGACAGGACAGGCACATGATTAGAATGCGCCAGCTCATCGATATCGTGGACCA TTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTCCTGCCGGCCCCCGAAGATGTGGAAACC AATTGCGAATGGTCGGCATTTCCTGCTTTCAAAAGGCACAGCTCAAGTCCGCTAACACCGGGA ACAACGAACGGATCATCAACGTGTCCATCAAAAAGCTGAAGCGGAAGCCTCCCTCCACCAACG CCGGACGGAGGCAGAAGCATAGGCTGACTTGCCCGTCATGCGACTCCTACGAGAAGAAGCCGC CGAAGGAGTTCCTGGAGCGGTTCAAGTCGCTCCTGCAAAAGATGATTCATCAGCACCTGTCTC CCGACTCATGGGTCTGAGGATTCA
IL-12p70 T2A IL21 (Human; codon optimized; bold denotes signal sequences) (SEQ ID NO: 60)
ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTTGTTCTCGCTTCCCTCTGG TCGCC ATTTGGGAAGTGAAGAAGGACGTCTACGTGGTTCGAGCTGGATTGGTACCCGAGCGCC CTGGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCCTGG ATCAGAGCTCCGAGGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCGGCG ACGCGGGCCAGTACACTTGCCACAAGGGTGGCGAAGTGCTGTCCCACTCCCTGCTGCTGCTGCA CAAGAAAGAGGATGGAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACA AGACCTTCCTCCGTGCGAAGCCAAGAAGTACAGCGGTGGTTCACCTGTTGGTGGCTGACGAC AATCTCCACCGACCTGACTTTCTCCGTGAAGTCGTACGGGGATCAAGCGATCTCAGGCGGTG ACCTGTGGAGCCGCACTCTGTCCGCGGAGAGATCAAGGGAGACAACAAGCAATATGAGTAC TCCGTGGAATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTC ATGGTCGATGCCGTGCATAAGCTGAAATACGAGAAGTACACTTCCTCCTTCTTTATCCGCGACA TCATCAAGCCTGACCCCCCAAGAACTTGCAAGTGAAGCCACTCAAGAACTCCCGCCAAGTGGA AGTGTCTTGGAATATCCAGACACTTGAGACACCCCGCACTCATACTTCTCGCTCACTTTCTGTG TGCAAGTGCAGGGAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCG CCACTGTGATTTGTGCGGAAGAAGCGGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTC CTCCTGGAGCGAATGGGCCAGCGTGCCTTGTTCGGTGGCGGATCAGGCGGAGGTTTCAGGAGG AGGCTCCGGAGGAGGTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGGAATGTTCCCGTGC CTACACCACTCCCAAAACCTCCTGAGGGCTGTGTGCAACATGTTGCAGAAGGCCCGCCAGACCC TTGAGTTCTACCCCTGCACCTCGGAAGAAATTGATCACGAGGACATACCAAGGACAAGACCTC GACCGTGGAAGCCTGCCTGCCGTGGAAGTACCAAGAACGAATCGTGTCTGAACTCCCGCGA GACAAGCTTTTACTACTAACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTTCATGATGGCGCTC TGCTTTTCTCGATCTACGAAGATCTGAAGATGTATCAGGTTCGAGTTCAAGACCATGAACGCCA AGCTGCTCATGGACCCGAAGCGGCAGATCTTCCTGGACCAGAATATGCTCGCCGTGATTGATGA ACTGATGCAGGCCCTGAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCG GACTTCTACAAGACCAAGATCAAGCTGTGCATCCTGTTGCACGCTTTCCGCATTTCGAGCCGTGA CCATTGACCGGTGATGTCTACCTGAACGCCAGTAGACGGAACGCGGAAGCGGAGAGGGCA GAGGCTCGTGTCTACATGCGGGGACGTGGAAGAGAACCCCGGTCCGAT TGGAACGCATTGTG ATCTGCCTGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGTCGAGCAGCCAGGGACAG GACAGGCACATGATTAGAATGCGCCAGCTCATCGATATCGTGGACCAGTTGAAGAAGTACGTG AACGACCTGGTGCCCGAGTTCTGCGCGCCCCCGAAGATGTGGAAACCAATTGCGAATGGTCG GCATTTTCTGCTTTCAAAAGGCACAGCTCAAGTCCGCTAACACCGGGAACAACGAACGGATCA TCAACGTGTCCATCAAAAAGCTGAAGCGGAAGCCTCCCTCCACCAACGCCGGACGGAGGCAGA AGCATAGGCTGACTTGCCCGTCATGCGACTCTACGAGAAGAAGCCCGCCGAAGGAGTTCTCTGG AGCGGTTCAAGTCGCTCCTGCAAAAGATGATTATCAGCACCTGTCTCCCGGACTCATGGGTC TGAGGATTCA
IL-12 2A CCL21a (Human) (SEQ ID NO: 61)
ATGTGCCATCAGCAGCTTGTATATCTTGGTTTCACTTGTATTCTGGCCAGCCCTTGGTTGC GATCTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGT GAGATGGTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACCAGT

CCTCCGAAGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGG
 ACAATACACATGCCACAAGGGAGGCGAGGTGTGTCTCATAGTTTGCTGCTTCTCCACAAGAAA
 GAGGATGGAATCTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAATAAGACATTT
 CTGCGATGTGAGGCTAAGAAGTATAGTGGCCGCTTCACTTGTGGTGGCTGACTACCATCAGCA
 CAGATCTCACGTTTTTCAGTAAAAAGTAGTAGAGGTTCAAGTGATCCTCAAGGGGTAACGTGCGG
 TGCTGCAACACTGTCTGCTGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCGTAGA
 ATGCCAAGAGGACAGTGCTTGTCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGA
 CGCGGTGCATAAACTGAAATATGAGAACTACACAAGCAGTTTTTTTATAAGAGATATCATCAAG
 CCCGATCCGCCGAAGAATTTGCAACTTAAACCGCTTAAAACTCACGCCAGGTTGAAGTATCCT
 GGGAGTATCCGGATACATGGTCAACACCACACAGCTATTTTTCCCTTACCTTCTGTGTGCAGGTC
 CAAGGGAAGAGCAAAAAGGGAGAAGAAGGACAGGGTATTCACTGATAAACTTCCGCGACGGT
 CATCTGCCGAAAAACGCTAGTATATCTGTACGGGCGCAGGATAGGTACTATAGTTCTTCTTGG
 TCTGAGTGGGCCTCAGTTCCGTGCTCTGGGGGAGGAAGTGAGGAGGGTCCGCGCGTGGAAGC
 GGGGGAGGGAGTCGCAACTTGCCAGTGCTACACCAGATCCAGGCATGTTTCCATGTCTGCATC
 ATTCCCAGAATCTCCTGAGAGCGGTGTCAAATATGCTCCAAAAGCGAGACAAACACTGGAAT
 TTTACCCGTGTACCAAGTGAGGAGATTGATCAGGAGACATAACCAAGGACAAGACCTCAACTG
 TAGAAGCGTGTTTGCCGCTGGAGTTGACTAAGAATGAGTCCTGCCTCAATTCCAGAGAACTTC
 ATTCACTACTAACGGCAGTTGTCTTGCATCCCGGAAAAACGTCCTTTATGATGGCCCTTTGCCTTA
 GTTCAATTTACGAGGATCTTAAAATGTATCAAGTGAGTTTAAACCATGAATGCTAAACTTCT
 TATGGACCCCAAACGACAAATTTTTCTGGATCAGAATATGCTTGCCGTGATAGACGAACTCATG
 CAGGCGCTTAATTTTAACTCCGAAACAGTTCACAAAAATCTAGCCTTGAAGAACCTGATTTTT
 ATAAAACGAAGATTAAGTGTGTATCCTGCTGCATGCCTTTTCGCATCCGAGCTGTCAATCGA
 TAGGGTTATGTCTACCTTAACGCGAGCCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAG
 TCTGCTCACCTGCGGCGATGTTGAAGAAAAATCCTGGTCCCATGGCGCAAAGTCTGGCTCTTCA
 CTCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCAAAGGAAGTGATGGTGGCGCCCAAG
 ATTGTTGCCTTAAATACAGCCAGCGGAAAAATACCCGCGAAAGTGCTCAGGAGTTATAGAAAAC
 AGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCTCCCGAGAAAAACGGAGCCAGGC
 CGAAGTGTGCGTGACCCTAAGGAACCTTGGGTGCAACAACCTTATGCAACACCTGGATAAGACA
 CTTTCTCTCAAAAAGCCAGCTCAGGGCTGCCGAAAAAGATAGAGGCGCTCAAAAACCGGAAAA
 AAGGGCAAAGGTTCTAAAGGATGTAAGCGGACTGAACGCTCTCAAACGCCTAAAGGGCCGtaG

IL-12 2A CCL21a (Mouse) (SEQ ID NO: 62)

ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCTCTCTGGTGAGCCCACTCATGG
 CAATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAG
 GGGAGACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACC
 AACGCCATGGCGTAATAGGGAGTGGGAAAACTACGATCAGATCAGTTAAAGAGTTCTTGGATG
 CTGGTCAATATACTTGCCATAAAGGCGGCGAGACTCAGCCACTCACATTTGCTTTTGCCATAA
 AAAAGAGAATGGCATTGGAGCACTGAAATACTTAAGAACTTTAAGAACAGACATTTCTCAA
 GTGTGAGGCCCCTAATTACAGCGGCAGGTTACGTGCTCATGGCTGGTCCAGCGCAACATGGAC
 CTCAAGTTTAACATAAAATCTTCTTCTCTTACCTGACTCCAGAGCTGTTACTTGCGGCATGGC
 TTCTCTGAGCGCAGAAAAAGTAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGT
 CAAGAGGATGTTACGTGCCCCGACGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCT
 CGCCAACAAAACAAGTATGAAAACTACAGTACAAGCTTCTTTATACGGGATATAATTAACCCCG
 ATCCCCCAAGAAGTTGCAAATGAAACCACTTAAGAACAGCCAGGTGGAAGTTTCTGGGAGT
 ATCCAGACTCATGGAGTACTCCTCACAGCTATTTTTCTCTGAAATTCTTTGTAAGGATACAACGG
 AAGAAAGAGAAGATGAAAGAGACCGAGGAGGGTTGTAATCAGAAGGGAGCGTTTCTCGTGGA
 GAAAACGTCTACCGAAGTCCAATGTAAAGGTGGCAATGTGTGCGTCCAAGCTCAGGATAGATA
 CTATAATTCAAGTTGCTCCAAGTGGGCTGTGTTCCATGCCGCGTTCGGAGCGGGGGAGGTAGC
 GGAGGAGGTAGTGGGGGTGGGTCAAGAGGAGGGAGTCGAGTTATCCCGGTGTAGGCCCCGCA
 CGCTGCTTGAGCCAGAGTCGCAACCTCCTTAAGACAACAGATGACATGGTGAAAAACGCACGC
 GAAAAGCTTAAACACTACTCTTGTACGGCGGAGGATATTGATCACGAGGATATTACCCGAGACC
 AAAGTAGCACTTTGAAAACCTGTCTGCCCCCTTGAACCTCATAAAAATGAGAGCTGTCTGGCTAC
 ACGAGAGACGTCAAGTACGACTAGGGGCGAGCTGTCTCCCGCCGCAAAAAGACAAGCCTCATGAT
 GACGCTCTGTTTGGGTTCCATTTACGAGGACTTGAATAATGTATCAAACGGAGTTCCAGGCTATA
 AATGCGGCGTGTGAGAACCATCAACCAAAATTATACTTGATAAAGGCATGTTGGTGGCGA
 TTGATGAACTCATGCAGAGTCTCAATCACAACGGGGAAACGTTGAGACAGAAACCCCAAGTCG
 GTGAAGCGGACCCATATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACGCATTACGACTAG
 AGTTGTTACCATCAACCGGGTAATGGGATATCTCTCCAGTGCGCGGCGCAAGAGGGGTTCCGGA
 GAGGGAAGGGGTAGTCTGCTCACCTGCGGCGATGTTGAAGAAAAATCCTGGTCCCATGGCGCAA
 ATGATGACCCCTTCCCTGCTGAGTCTTGTCTCGCGCTCTGCATCCCGTGGACGCAGGGGTCTGA
 TGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAGATACCGTACTCTATTGTCAGA

GGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTCTCCCCACGCA AGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTGATGCGAA GGCTTGACCAGCCCCAGCCCCGGCAAGCAGTCACCTGGGTGCAGAAAAACAGAGGTACTT CAAAGAGCGGCAAGAAAGGCAAGGGAGTAAAGGATGTAAAGAACGGAGCAGACCCAGCCT TCACGAGGCTaG
CCL21a 2A IL-12 (Mouse) (SEQ ID NO: 63)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGTCCTCGCGCTCTGCATCCCGTGGACGCA GGGGTCTGATGGGGGGGGCCAAAGACTGTTGCCTGAAGTATTCACAAAAAAGATACCGTACTC TATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTC TCCCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAAC TTGATGCGAAGGCTTGACCAGCCCCAGCCCCGGCAAGCAGTCACCTGGGTGCAGAAAAAC AGAGGTACTTCAAAGAGCGGCAAGAAAGGCAAGGGAGTAAAGGATGTAAAGAACGGAGCA GACCCAGCCTTCACGAGGCCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCAC CTGCGGCGATGTTGAAGAAAATCCTGGTCCCATGTGTCCACAGAAGCTGACAATAAGTTGGTTT GCCATTGTCTCCTGGTGAGCCCACTCATGGCAATGTGGGAACTCGAAAAGGATGTCTACGTGG TAGAAGTAGATTGGACTCCAGACGCGCCAGGGGAGACAGTGAATTTGACATGTGACACACCAG AAGAAGATGACATTACATGGACATCTGACCAACGCCATGGCGTAATAGGGAGTGGGAAAAACAC TCACGATCACAGTTAAAGAGTTCTTGGATGCTGGTCAATATACTTGCCATAAAGGCGGCGAGAC ACTCAGCCACTCACATTTGCTTTTGCATAAAAAAGAGAATGGCATTGAGCACTGAAATACTT AAGAAGTTTAAAGAACAGACATTTCTCAAGTGTGAGGCCCTAATTACAGCGGCAGGTTACAGT GCTCATGGCTGGTCCAGCGCAACATGGACCTCAAGTTAACATAAAATCTTCTCTCTTACCT GACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTGAGCGCAGAAAAAGTAACGTTGGATCAAA GAGACTACGAAAAGTACTCTGTTTCTGTCAAGAGGATGTTACGTGCCCCGACGGCCGAAGAAAC GCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACAAAACAAGTATGAAAACTACAGTACAAG CTTCTTTATACGGGATATAATTAACCCGATCCCCCAAGAACTTGCAAATGAAACCACTTAAG AACAGCCAGGTGGAAGTTTCTGGGAGTATCCAGACTCATGGAGTACTCCTCACAGCTATTTTT CTCTGAAATTTCTTTGTAAGGATACAACGGAAGAAAGAGAAGATGAAAGAGACCGAGGAGGGTT GTAATCAGAAGGGAGCGTTTCTCGTGGAGAAAACGCTTACCGAAGTCCAATGTAAAGGTGGCA ATGTGTGCGTCCAAAGCTCAGGATAGATACTATAATTCAAGTTGCTCCAAGTGGGCTGTGTTCC ATGCCGCGTTCCGAGCGGGGAGGTAGCGGAGGAGGTAGTGGGGGTGGGTGAGGAGGAGGGA GTCGAGTTATCCCGGTGTCAGGCCCCGACGCTGCTTGAGCCAGAGTCGCAACCTCCTTAAGAC AACAGATGACATGGTGAAAACAGCACGCGAAAAGCTTAAACACTACTCTTGTACGGCGGAGGA TATTGATCACGAGGATATTACCGAGACCAAACTAGCACTTTGAAAACCTGTCTGCCCTTGAA CTTCATAAAAAATGAGAGCTGTCTGGCTACACGAGAGACGTCAAGTACGACTAGGGGCAGCTGT CTCCCGCCGCAAAAGACAAGCCTCATGATGACGCTCTGTTTGGGTTCCATTACGAGGACTTGA AAATGTATCAAACGGAGTTCCAGGCTATAAATGCGGCGTTGCAGAACCATAACCATCAACAAA TTATACTTGATAAAGGCATGTTGGTGGCGATTGATGAACTCATGCAGAGTCTCAATCACAACGG GGAAACGTTGAGACAGAAACCCCCAGTCGGTGAAAGCGGACCCATATCGAGTAAAAATGAAGCT CTGCATTCTGCTTCACGCATTACGACTAGAGTTGTTACCATCAACCGGGTAATGGGATATCTCT CCAGTGCGTtaG
IL7 (Mouse) (SEQ ID NO: 64)
ATGTTTCATGTGTCCTTCAGGTACATATTTGGTATCCCACCACCTTATATTGGTGCTCTTGCTGTGA ACCAGCTCTGAATGTCATATAAAAGACAAGGAGGGCAAAGCATACGAGTCCGTATTGATGATC TCAATCGATGAACTTGACAAGATGACAGGGACCGATTCTAATTGTCCAAATAACGAGCCAACT TCTTTCGGAAACACGTGTGTGATGATACAAAAGAAGCTGCTTTTCTTAACAGAGCTGCCAGAAA ACTCAAGCAGTTCCCAAGATGAATATATCCGAGGAATTTAACGTGCATCTCCTCACAGTATCT CAGGGAACCTCAAACCTTGTAACCTGCACTTCTAAGGAGGAGAAAGATGTCAAAGAGCAGAAG AAAAATGATGCATGTTTTTTGAAACGGCTGTTGAGGGAGATCAAAACATGCTGGAATAAAATCC TCAAGGGCTCAATTtaG
IL-15 (Human) (SEQ ID NO: 65)
ATGGAAACAGACACATTGCTGCTTTGGGTATTGTTGCTCTGGGTGCCTGGATCAACAGGAACT GGGTAAACGTAATTTAGATCTGAAGAAGATCGAGGACCTTATTCAATCCATGCACATCGATGC CACTCTCTACACCGAAAGCGACGTTACCCATCTTGCAAGGTGACCGCTATGAAATGTGAATTG TTGGAACCTCAGGTAATTTCTCTGGAGAGCGGCGATGCCTCAATACATGACACCGTTGAAAATC TTATCATCCTTGCTAATGATTCACCTCTAGTAATGGGAACGTAACAGAGAGCGGGTGAAGGA GTGTGAAGAACTGGAGGAGAAAAACATTAAGGAATTTTGCAGTCATTTCGTCCATATAGTGCAA ATGTTTCATAAACACTTCCAGAAGAAAGCGAGGCTCTGGGGAGGGGCGAGGCTCTCTGCTGACC TGTGGGGATGTAGAAGAGAATCCAGGTCCCATGGACCGGCTGACCAGCTCATCTCTGCTTCTGA TTGTGCCAGCCTACGTGCTCTCCATCACATGCTCTCCCCAATGAGCGTCGAGCATGCTGACATC TGGGTGAAGTCATACTCCTTGATACAGCAGAGAGAGATACATTTGTAATTCGGGATTCAAGCGCA

AGGCCGGCACCTCCTCTCTGACAGAGTGCCTCTTAACAAAGCAACCAACGTAGCACATTGGAC CACACCATCCTTGAAGTGCATACGAGAACCTAAATCTTGCAGATAAGACTCATACTTGTCCACCT TGTCAGCCCCAGAACTGCTTGGCGGACCCTCAGTATTTTTGTTCCCAACCAAGCCAAAGACACA CACTCATGATATCCAGAACTCCTGAGGTGACCTGTGTCTGTTGTAGACGTTTCCCAAGATCC TGAAGTAAAATTCAACTGGTACGTGGATGGGGTGAAGTCCATAACGCCAAGACTAAACCAAG GGAGGAACAGTATAACTCTACTTACCGAGTAGTTTCTGTGTTGACCGTGTGACCAGGACTGG TTGAACGGGAAGGAGTACAAATGCAAGGTGAGCAATAAAGCTCTGCCCCACCAATCGAAAAG ACAATATCTAAGGCCAAGGGGCAGCCACGAGAGCCCCAGGTATACACACTGCCACCCTCACGC GATGAATTGACTAAGAACCAGGTTTCCCTGACCTGTCTGTAAAAGGTTTCTACCCTTCCGACAT AGCTGTTGAGTGGGAAAAGTAACGGGCAGCGAACAATTACAAGACAACCTCCACCCGTTCT TGATAGCGATGGATCATTTTTTCTGTATTCCAACTCACTGTGATAAAAGTCGCTGGCAGCAA GGCAATGTTTTAGCTGCTCAGTCATGCACGAAGCACTGCATAATCACTACACACAAAAAAGTT TGTCCTTAGCCCTGGTAAGtaG
IL-15 (Human) (SEQ ID NO: 66)
ATGTAATCAATGCAGTTGGCCTCCTGTGTAACATTGACCTTGGTCTCTTGGTCAACAGCAATTG ATCGATGTACGCTACGACTTGGAGAAGATTGAGTCCCTTATACAGAGTATACATAGATACACA ACCTGTATGATGACAGTGACTTCCATCCCAGCTGTAAAGTGACTGCAATGAAGTGTGTGTGTT GGAGTTGCAAGTAATTCTGCATGAATACAGCAACATGACCCTCAATGAAACCGTTAGGAATGTC CTTTATCTCGAAATTCTACTCTGAGTAGCAATAAGAATGTTGCCGAAAGCGGCTGCAAGGAGT GCCAAGAACTGGAGGAAAAAACTTTCACCGAGTTTCTCCAGAGTTTCATCAGAATTGTCCAAAT GTTTCATTAATACAAGTAGTGGTGGTGGGAGCGGGGGTGGAGGCAGTGGGGGAGGTGGGAGCGG AGGTGGAGGGTCCGGAGGGGGGAGCCTTCAAGGCACTACTTGTCTCCACCCGTATCCATCGAG CAGCCGATATTTCGAGTTAAAAATTATAGTGTAAATAGCAGAGAAACGATACGCTCTGCAACTCAG GGTTTAAGAGAAAGGCCGGAACCTTCAACTCTCATAGAATGCGTGATTAATAAGAATACTAACGT CGCACATTGGACTACTCCAGTCTCAAGTGCATACGCGATCCATCTCTCGCTCATTACTCACCAG TACCTACAGTGGTTACTCCTAAGGTGACCTCTCAGCCCGAATCACCATCTCCAGCGCAAAAAGA GCCTGAGGCCTTTTCTCCTAAATCAGACACTGCTATGACTACAGAAACAGCCATAATGCCAGGA AGCCGGCTGACACCATCTCAAACCTACCAGCGCAGGCACAACCTGGGACTGGCTCCCAAAAAGC TCACGATACCAAGTCTCGCCGCAACAATGACATTGGAGCCTACAGCCAGCACATCTCTTAGAA TCACAGAAATTTCTCCCCACAGTAGCAAGATGACCAAGGTGGCAATTAGTACCAGCGTCTTCT TGTAGGAGCTGGAGTTGTGATGGCATTTTTGGCATGGTATATCAAAAGCAGGtaG
IL-15 (Mouse) (SEQ ID NO: 67)
ATGAAGATCCTCAAGCCATACATGCGAAACACTAGTATTAGCTGTTACTTGTGTTTTCTGCTGAA TAGTCATTTTTTGAAGCAGGAATCCATGTATTTATACTCGGTTGTGTGTCTGTAGGTCTGC CAAAGACTGAGGCTAATTGGATTGACGTGCGCTATGATCTTGAAAAAATAGAGTCCTTGATTCA ATCAATACACATCGATACCACTCTTACACCGACAGTGATTTCATCCTTCTGCAAGGTAAGA GCTATGAATTGCTTCTCCTGGAGCTCCAAGTCATTCTCCATGAGTACTCCAACATGACTTTGAA CGAACTGTAAGAAACGTATTGTATCTGGCTAATAGCACCTTGTCTAGTAACAAAAATGTGGCA GAGAGCGGCTGCAAGAATGTGAAGAATTGGAAGAGAAAACATTTACAGAGTTCTGCAATCC TTTATTCGCATCGTCCAAATGTTTATCAATACCTCTtaG
IL-15 (Mouse) (SEQ ID NO: 68)
ATGTATTCCATGCAACTTGCCAGTTGTGTAACCTTACTCTCGTCCTGCTCGTTAATTCCGCTGG TGCTAACTGGATAGATGTTTCGATACGATCTGGAAAAGATTGAGTCCCTTATCCAATCCATTCT ATAGATACCACCTTTATACTGACAGCGACTTCCATCCTTCTTGCAAGGTGACCGCTATGAATTG TTTCTGCTGGAACCTCAAGTTATTCTGCATGAATACTCTAATATGACACTTAACGAGACCGTAA GAAATGTTCTCTATCTCGCTAATAGTACTTTGAGCTCAAATAAGAACGTGGCCGAGTCTGGGTG TAAGGAATGCGAAGAGCTGGAAGAAAAGACATTCACCGAGTTTCTCCAGTCTTTCATACGGATT GTGCAGATGTTTATCAACACATCAGATTACAAAGACGACGATGATAAGtaG
IL-18 (Mouse) (SEQ ID NO: 69)
ATGGCAGCCATGTCTGAGGACTCTTGTGTGAACCTTAAAGAAATGATGTTTCATAGACAATACAC TCTACTTTATACTGAGGAGAATGGAGATTGGAATCTGACAACTTTGGCAGGCTGCATTGCAC TACCGCAGTTATCCGAAACATCAACGATCAGGTACTGTTTGTGATAAAAGACAACCTGTATTCT GAGGACATGACCGACATAGATCAGTCTGCCTCAGAGCCCCAGACTAGGCTTATCATCTATATGT ACAAGGACAGCGAAGTACGAGGCCTGGCTGTTACACTCTCAGTCAAAGACTCTAAGATGAGCA CCCTGTCTATGCAAGAACAAAATTATCAGTTTGGAGGAGATGGACCCACCTGAAAACATAGATG ACATTCAGTCAGACCTCATTTTTTTTCAAAGCGGGTACCAGGACACAACAAAATGGAATTTGA ATCATCACTCTACGAAGGACATTTCTTGCATGCCAGAAAGAGGATGACGCATTCAAATTGATC CTGAAAAAAAAGGACGAAAATGGTGATAAATCAGTCATGTTTACATTGACCAATCTTCAACAA AGTtaG
IL-18 (Mouse) (SEQ ID NO: 70)

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ATGAAGTACACTAGCTATATATTGGCCCTCCAGCTTTGCATCGTATTGGGTAGCCTCGGATGCTA TTGCCAAGACCCGTATGTCAAAGAAGCCGAAAATCTCAAAAAGTATTTCAATGCCGGACACTCA GACGTCGCGGATAACGGTACACTGTTTCTTGGCATCCTGAAAAATTGGAAGGAAGAGAGTGAC AGAAAAATAATGCAGTCACAAATAGTGTCTTTTACTTTAAGCTGTTCAAAAATTTCAAGGATG ACCAAAGTATCCAGAAGAGTGTTGAAACTATCAAAGAGGACATGAATGTGAAATCTTTAACA GTAATAAGAAGAAGCGCGATGACTTCGAGAACTCACTAATTACAGCGTAACGGATCTTAACG TCCAACGCAAGGCAATCCACGAGCTTATACAGGTAATGGCTGAGCTTAGTCCCGCAGCCAAGA CAGGGAAGAGAAAAAGGTCTCAAATGCTTTTTTCGGGGCCGGCGAGCTTCACAAaG
IFN-gamma (Mouse) (SEQ ID NO: 77)
ATGAACGCTACGCATTGCATCCTCGCACTCCAATTGTTCTCATGGCTGTGTGAGGGTGTACTG TCACGGTACTGTCATAGAAAGCCTCGAATCCCTGAATAACTATTTTAACAGTAGCGGTATAGAT GTAGAAGAAAAGTCTCTCTTTCTTGACATCTGGAGGAATTGGCAAAAGGATGGAGACATGAAG ATTCTCCAATCTCAGATTATATCATTCTTACTTGAGGCTTTTGGAGGTTCTGAAGGATAACCAGGC GATCAGCAATAATATCAGCGTAATTGAATCTCACCTTATTACAACATTTTCTCAAATTTCAAGG CAAAGAAAGATGCTTTCATGTCTATCGCGAAATTTGAGGTGAACAATCCTCAGGTACAAAGGCA AGCCTTTAACGAGCTGATTAGAGTTGTACATCAGTTGTTGCCCGAAAGTAGTCTTAGAAAACGC AAACGGAGCCGATGCTaG
IFN-alpha (Mouse) (SEQ ID NO: 78)
ATGGCAAGGTTGTGCGCTTTTCTCATGGTACTGGCTGTGCTCTCCTATTGGCCTACTTGTCTCTG GGATGCGACTTGCCACAGACCCACAATCTGCGGAATAAGAGGGCTCTGACTCTGCTGGTGCAA ATGAGACGGCTCTCTCCACTTAGCTGTTTGAAGATAGAAAGGATTTCCGGTTCCTCCAGGAGA AGGTGGATGCCAGCAGATCAAGAAGGCACAGGCTATCCCCGTCCTTTCCGAGCTTGACCCAGC AAATTTGAACATCTTTACAAGTAAGGATAGTTAGCTGCATGGAATACCACACTTTTGGATTCT TTTTGTAACAGCTCTGCATCAGCAGCTGAACGATCTCCAGGGATGCCTGATGCAGCAAGTCGGCG TGCAAGAATTTCCACTCACCCAGGAGGACGCTCTGCTCGCAGTGCGAAAGTATTTTACCAGAT TACCGTGACCTCCGGGAGAAAAAGCATTACCCCTGCGCTTGGGAAGTAGTCAGGGCCGAAAGT ATGGAGAGCCCTTAGTAGCTCCGCTAATGTACTGGGCCGGTTGCGGGAAGAGAAAaG
CCL21 (Human) (SEQ ID NO: 79)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCA AGGAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAAATACCCGCGAA AGTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTC CTCCCGAGAAAACGGAGCCAGGCCGAACCTGTGCGCTGACCCTAAGGAACTTTGGGTGCAACAA CTTATGCAACACCTGGATAAGACACCTTCTCCTCAAAAGCCAGCTCAGGGCTGCCGAAAAGATA GAGGCGCCTCAAAAACGGGAAAAAAGGGCAAAGGTTCTAAAGGATGTAAGCGGACTGAACGC TCTCAAACGCCTAAAGGGCCGtaG
CCL21a (Mouse) (SEQ ID NO: 80)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGCTCTCGCGCTCTGCATCCCGTGGACGCA GGGGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAGATACCGTACTC TATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTC TCCCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAAC TTGATGCGAAGGCTTGACCAGCCCCAGCCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAAC AGAGGTACTTCAAAGAGCGGCAAGAAAGGCCAAAGGGAGTAAAGGATGTAAAGAACGGAGCA GACCCAGCCTTCACGAGGCTaG
Tail-less CCL21 (Human) (SEQ ID NO: 81)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCA AGGAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAAATACCCGCGAA AGTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTC CTCCCGAGAAAACGGAGCCAGGCCGAACCTGTGCGCTGACCCTAAGGAACTTTGGGTGCAACAA CTTATGCAACACCTGGATAAGACACCTTCTCCTCAAAAGCCAGCTCAGGGCTaG
Tail-less CCL21 (Mouse) (SEQ ID NO: 82)
ATGGCGCAAATGATGACCCTTTCCCTGCTGAGTCTTGCTCTCGCGCTCTGCATCCCGTGGACGCA GGGGTCTGATGGGGGGGGCCAAGACTGTTGCCTGAAGTATTCACAAAAAAGATACCGTACTC TATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTTC TCCCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAAC TTGATGCGAAGGCTTGACCAGCCCCAGCCCCCTGGCAAGCAGTCACCTGGGTaG
CCL19 (Mouse) (SEQ ID NO: 83)
ATGGCACCCCGCGTCACACCCTTGCTTGCTTTTTCTCTGCTTGCTCTGACCTTCCCCGCTCCT ACCCTTGAGGAGCCAATGATGCCGAGGATTGCTGCTGAGTGTTACACAAAGGCCAATACCA GGGAATATAGTGAAGGCATTCCGGTATCTGCTCAATGAAGATGGGTGCAGAGTCCCCGAGTTG TCTTTACAACATTGCGAGGTTACCAGCTTTGTGCTCCCCAGACCAGCCTTGGGTAGATCGCATT

ATTCGCCGGTTGAAGAAGAGCTCAGCAAAGAATAAGGGCAATTCCACACGGAGAAGCCCCGTC TCCtaG
CCL19 (Mouse) (SEQ ID NO: 84)
ATGAAATCAGCAGTCCTTTTCTTGCTCGGGATTATTTTTCTGGAACAATGTGGAGTGAGGGGAA CACTCGTAATAAGAAACGCTCGGTGCTCATGCATATCAACATCACGGGGCACTATCCACTACAA ATCCCTGAAGGATCTGAAGCAGTTCGCCCCAAGCCCTAACTGTAACAAGACCGAAATTATCGCA ACTCTCAAAAATGGAGATCAGACTTGTCTTGACCCAGATTACAGCAAATGTCAAGAAGCTGATGA AAGAGTGGGAAAAGAAGATTTACAAAAAAGCAAAAAACGCGGCAAGAAACATCAAAAG AACATGAAAAACAGGAAACCTAAGACTCCCCAGTCAAGGAGAAGATCCCGCAAGACAACCTaG
CXCL11 (Mouse) (SEQ ID NO: 85)
ATGAACAGAAAAGTTACCGCTATAGCACTTGCTGCCATAATATGGGCCACCGCAGCTCAAGGGT TCCTGATGTTCAAGCAGGGCCGATGCCTCTGCATTGGCCCTGGAATGAAGGCCGTGAAAATGGC CGAAATAGAAAAAGCTAGTGTATATACCCCTCTAACGGTTGCGATAAAGTCGAGGTTATAGTC ACAATGAAAGCTCATAAACGCCAACGCTGCCTCGACCCCCGGTCTAAGCAGGCTAGGCTCATA ATGCAAGCAATCGAGAAGAAAACTTTCTTAGACGGCAAAACATGtaG
CXCL10 (Mouse) (SEQ ID NO: 86)
ATGAACCCATCTGCCCGCTTATTTCTGTCTGATACTCCTTGGGCTGAGTGGCACACAAGGCAT ACCCCTCGCCCGCACAGTCCGGTGTAATTGTATACATATTGACGACGGCCCTGTTAGAATGCGG GCCATCGGTAAGCTGGAGATTATACCAGCAAGCCTTAGTTGTCCAGGGTTGAAATCATAGCAA CTATGAAAAAAGACGACGAACAAAGATGTTTGAATCCCGAGAGCAAGACAATCAAAAACCTTA TGAAAGCATTAGTCAAAAACGCTCTAAACGCGCTCCaG
CXCL10 (Human) (SEQ ID NO: 87)
ATGAATCAGACGGCAATCCTTATATGCTGCCTTATATTCCTTACTCTCTCAGGGATACAAGGGGT ACCACTTCTCGGACTGTTCTGCTGCACTTGCAATTTCAATATCTAACCAACCTGTAAATCCGCGGA GCCTGGAAAAATTGGAGATTATACCTGCTTCTCAATTCTGCCCTCGGGTGGAATCATCGCCAC TATGAAGAAGAAGGGCGAGAAAAGGTGTCTGAATCCAGAGTCAAAGGCAATCAAAAACCTGCT GAAAGCGGTGTCAAAGGAACGGTCCAAGAGATCACCCtaG
CXCL11-CXCL10 (Mouse) (SEQ ID NO: 88)
ATGAACAGGAAAGTAACAGCCATTGCATTGGCTGCCATCATCTGGGCCACCGCAGCACAGGGT TTTCTGATGTTTAAGCAAGGGCGCTGTCTCTGTATAGGCCAGGCATGAAGGCCGTGAAGATGG CAGAGATTGAGAAGGCATCTGTGATTTATCCTTCTAACGGGTGCGATAAAGTCGAAGTTATTGT GACAATGAAGGCACACAAACGCCAACGGTGTGTTGGACCCACGATCTAAACAGGCAAGATTGAT TATGCAAGCCATCGAGAAAAAGAACTTTCTCCGAAGGCAAAATATGATCCCTTTGGCTCGGACA GTGCGGTGTAAGTGTATTACATCGACGATGGGCCAGTACGGATGAGAGCAATAGGAAAGCTC GAAATCATACCCGCCTCATTGTCTTGTCCAGGGTGGAATAATCGCCACTATGAAAAAGAACG ATGAACAGAGGTGTCTCAACCCAGAGAGTAAGACTATCAAGAACCCTATGAAGGCATTCAAGT AGAAGAGGTCAAAGCGAGCACCaG
XCL1 (Human) (SEQ ID NO: 89)
ATGAGACTTCTCATATTGGCGCTTCTCGGGATATGTTCTCTTACGGCATAACATAGTTGAGGGGT GGGATCTGAGGTTAGCGATAAACGAACCTGTGTTAGTCTTACAACACAGAGGCTTCCAGTCTCC AGGATAAAAACATATACGATAACTGAGGGATCTCTCAGAGCGGTCACTTTCATAACGAAGAGG GGCTGAAGGTCTGTGCTGACCCACAAGCGACTTGGGTAAAGGGACGTTGTGCGGAGCATGGAC AGGAAGAGCAATACTCGCAACAACATGATCCAAACCAACCTACGGGCACCCAACAGTCAACC AATACTGCGGTAACATTGACGGGGtaG
XCL1 (Mouse) (SEQ ID NO: 90)
ATGCGCCTCCTTCTGCTGACTTTTCTGGGTGTATGTTGCCTGACACCCTGGGTTCGTAGAAGGAGT AGGAACCGAGGTTCTGGAAGAGTCCTCATGTGTAACTTGCAGACACAACGACTCCCCGTCCAA AAAATCAAGACCTATATAATCTGGGAGGGGGCAATGCGGGCCGTCAATTTCTGTGACTAAACGA GGTCTCAAAATCTGCGCCGACCCGAGGCTAAGTGGGTGAAGGCAGCCATTAAGACCGTGGAT GGGAGAGCCAGCACAGAAAGAACATGGCCGAAACAGTACCTACTGGCGCACAGCGGTCAACC TCAACTGCTATAACCTTGACAGGAtaG
m_sCD40L #1 (SEQ ID NO: 91)
ATGGAGACTGACACTCTGCTTCTGTGGGTGTTGCTGCTGTGGGTGCCTGGCAGTACAGGGCGATA TGCAACGAGGTGACGAGGACCCTCAAATCGCCGCCCATGTAGTCTCTGAAGCTAATAGCAACG CTGCATCCGTCTTGCAAGTGGGCAAAGAAAGGCTACTATACTATGAAGTCCAACCTTGGTAATGCT TGAAAACGGCAAGCAGTTGACTGTCAAGAGAGAGGGGACTTTATTACGTCTATACCCAAGTCAC ATTCTGTAGCAATCGAGAACCCTCCTCACAGAGGCTTTTATAGTGGGACTCTGGCTTAAACCA AGTAGCGGCTGAGCGCATACTGTTGAAAGCCGAAACACACACAGCTCTTCCCACTCTGCG AGCAGCAATCCGTGCATCTCGGTGGAGTATTTGAGCTTCAAGCCGGTGCCTCAGTGTGTTGTGAA

CGTCACTGAGGCCTCCCAGGTCATACATCGAGTTGGGTTCAGCTCCTTCGGCTTGCTCAAGCTCta G
m_sCD40L #2 (SEQ ID NO: 92)
ATGGAAACTGATACATTGCTGCTCTGGGTTTTGCTGCTCTGGGTGCCTGGGAGTACAGGCGACA TGAGGAGGCAGTTCGAGGATCTCGTTAAGGATATTACCCTTAATAAGGAGGAGAAGAAAGAAA ACTCTTTTGAGATGCAACGAGGGGACGAAGATCCTCAGATCGCTGCTCACGTGGTCTCTGAAGC TAACAGCAACGCCGCTTCTGTCTCCAGTGGGCCAAGAAAGGTTATTACACCATGAAATCAAAC CTTGTAATGCTTGAAAAACGGGAAACAGCTTACAGTGAAGAGGGAAAGGTCTTTACTACGTCTATA CCCAGGTAACCTTCTGCTCAAAACAGAGAACCATCAAGCCAGAGGCCATTATAGTGGGGCTCTG GCTCAAACCTTCAGATGGCAGCGAGAGAATCTTGTGAAAGCTGCTAATACACATAGTAGTAGC CAGCTTTGCGAGCAACAGTCAGTCCACCTCGGGGGGGTGTGTTGAGTTGCAAGCAGGGGCCTCAG TATTCGTGAATGTCAGTGAAGGCTTCCAGGTAATTCACAGGGTAGGCTTTAGTTCATTTCGGTTTG CTGAAGCTTtaG
m_sCD40L #3 (SEQ ID NO: 93)
ATGCGAAGAATGCAGCTTCTGCTCCTTATTGCTCTGAGTCTCGCCCTTGTCACCAACTCCGGGGA CAGAATGAAACAAATCGAGGACAAAATTGAAGAAATACTGAGTAAAATATATCACATCGAAAA CGAAATTGCACGCATTAAGAAATTGATTGGCGAACGCACCCAGTGGCGGCTCTGGTGGCACCGG AGGTTTCAGGCGGGACCGGGGGCTCTGACAAAAGTCGAAGAGGAGGTTAACCTTCATGAGGACTT TGTGTTTCATCAAGAAGCTGAAACGGTGAATAAAGGAGAAGGTTCTTTGAGCCTCCTTAATTGC GAAGAGATGCGACGACAGTTCGAGGATCTGGTTAAGGACATTACACTTAATAAGGAAGAGAAA AAGGAGAACTCTTTCGAAATGCAGCGCGGCGATGAAGATCCCCAGATAGCCGCCCATGTCTGCT CTGAGGCCAATCTAACGCAGCATCCGTCCTCCAGTGGGCTAAGAAAGGATATTATACTATGAA AAGCAATTTGGTCATGCTCGAAAACGGTAAACAGCTCACTGTTAAGAGAGAGAGGCCCTATTAC GTATATACTCAAGTAACTTTCTGTTCTAATAGGGAACCTCCTCTCAAAGACCTTTTATCGTAGG ACTCTGGTTGAAACCAAGTAGCGGTAGTGAAGGATTCTGCTCAAAGCAGCTAATACTCACTCC AGCAGTCAACTGTGCGAACAACAAAGCGTTTACCTCGGGGGCGTCTTTGAACCTTCAGGCAGGTG CCAGTGTTTTCTGTCACGTAACAGAAGCATCCCAGGTAATTCATCGAGTAGGGTTTTCTAGCTTT GGTTTGCTGAAGCTGtaG
anti-CD40_FGK4.5 (SEQ ID NO: 94)
ATGGAAACTGATCGCCTGTTGCTCTGGGTACTTCTTCTGTGGGTGCCTGGGTCCACTGGTGACAC TGTACTIONACACAATCACCCGCTTTGGCCGTTTCTCCTGGTGAACGGGTACAATTAGTTGCCGAG CTTCCGATTCTGTATCTACTCTTATGCATTGGTATCAACAAAAACCTGGTCAGCAGCCAAAATTG CTCATTTATCTTGCTAGTCACTTGGAGTCCGGCGTACCTGCTCGATTACGCGGTAGTGGGTCTGG CACAGATTTCACTTTGACCATAGATCCCGTGGAGGCCGATGACACTGCAACCTACTATTGCCAG CAATCCTGGAACGACCCTTGGACTTTTCGGCGGGCGGCACCAAGCTGGAACCTCAAGCGAGCAGAT GCTGCCCAACCGTTAGTATATTTCCACCCCTCAACCGAACAACCTCGCCACAGGAGGCGTAGTG TCGTGTGCTTATGAACAATTTCTATCCACGAGACATTAGCGTCAAGTGGAATAATTGATGGGAC AGAAAGGCGAGATGGAGTTTTGGATTACGTAACAGACCAGGATTCAAAGGATTCTACCTATAG CATGAGCTCCACCTTGAGCCTGACCAAAGCTGATTATGAATCTCATAACCTGTATACTTGTGAA GTGGTGCATAAGACTTCTAGCTCACCAAGTGGTTAAATCTTTTAACCGCAACGAATGTCGGCGCA AGAGGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTG GTCCCATGGACATTTCGGCTCTCTTTGGTATTCTGGTACTTTTTATAAGGGGGTGCAATGTGAA GTCCAGCTCGTGGAAGCGGTGGGGGCTGGTTTTCAGCCCGGTGCGAGCCTTAAACTTAGTTGCG CAGCATCCGGATTTACATTTTCTGACTATAACATGGCCTGGGTTCGACAGGCACCCAAAAAAGG GCTGGAGTGGGTGCGAACTATCATATACGATGGTTCCCGGACATACTATAGAGATTCACTGAAG GGGCGCTTTACAATAAGCAGGGACAATGCTAAGTCTACCTTGTATCTTCAGATGGACTCCCTGA GGAGCGAAGATACAGCAACATATTATTGTGCTACAAACCGCTGGTTGCTGCTTCATTATTTCGA CTACTGGGGTCAGGGCGTCATGGTAACTGTATCAAGCGCCGAGACCACAGCCCCTTCTGTATAT CCATTGGCACCAAGTACTGCTCTGAAATCCAACCTCAATGGTAACCCCTGGATGTCTGGTTAAGG GTTATTTTCCCGAGCCCGTCACAGTTACTTGGAACTCTGGGGCCCTTTCTAGCGGAGTCCATACC TTTCCCGCCGTTTTGTCAGAGTGGTCTGTACACCTTACCTCAAGCGTCACAGTTCCATCTAGCAC ATGGAGCTCCCAGGCAGTAACTTGTAAATGTGGCCCATCCAGCCTCCTCAACTAAGGTAGATAAA AAGATCGTTCCAGAGAATGCAATCCATGTGGATGCACCGGGTCTGAGGTACAGAGTGTGTTCA TTTTCCACCCAAAGACTAAAGATGTATTGACTATTACTTTACACCCAAAGTAACCTGCGTGGTG GTTGATATTAGTCAAAAATGATCCCGAGGTACGGTTCTCTTGGTTTATCGACGACGTCGAAGTAC ATACAGCTCAGACACACGCTCCCGAGAAACAAAGCAATTCCACTCTTAGGAGCGTGTCCGAGTT GCCAATCGTACATAGGGATTGGCTTAATGGCAAGACCTTTAAGTGTAAGGTCAATTCAGGGGCA TTCCCCGCACCAATAGAGAAGAGTATAAGCAAACCCGAGGGGACACCCAGAGGTCCACAGGTC TATACAATGGCTCCCCCAAGGAAGAGATGACCCAAAGTCAAGTCTCAATTACATGTATGGTGA AGGGCTTTTATCCACCCGACATATACACTGAGTGGAAGATGAATGGACAGCCCCAAGAGAATT

ATAAAAACACTCCCCCTACCATGGACACCGACGGGTCCTATTTTCTTTATAGTAAATTGAACGT GAAAAAGGAGACCTGGCAACAAGGCAACACTTTACCTGCTCCGTTCTTCACGAGGGCCTGCAT AATCATCATACCGAAAAGTCTCTCAGTCATTCTCCAGGTAAAGtaG
CD40L 2 (Human) (SEQ ID NO: 95)
ATGGAAACAGATACGTTGCTGTTGTGGGTACTTCTCCTTTGGGTCCCTGGCAGCACAGGGGACG AGAATAGTTTCGAAATGCAGAAGGGCGACCAGAACCCACAGATCGCGGCTCACGTTATATCAG AAGCAAGTAGTAAGACCACTTCCGTACTTCAGTGGGCTGAAAAAGGATATTACACCATGTCCAA CAATCTCGTGACACTGGAGAACGGTAAACAACCTTACGGTGAAACGACAGGGCCTCTATTACATC TACGCTCAGGTGACATTCTGCTCAAAATAGGAGGGCTTCTAGTCAAGCGCCCTTCATCGCCAGCC TGTGCCTCAAACTCTCCCGCCGGTTCGAACGAATTCCTGTTGCGAGCGGCCAATACCCATAGCTC AGCTAAACCTTGGCGGCCAGCAGAGTATTATCTTGGTGGTGTGTTTGAACCTCAGCCGGGAGCA TCTGTGTTCTGTAACGTAACGGACCTAGCCAAGTGTCTCATGGGACAGGTTTTACATCCTTCGG ACTCCTCAAGTTGtaG
Flt3L (Human) (SEQ ID NO: 96)
ATGACAGTTCTCGCGCCAGCTTGGAGTCCCACCACATACTTGCTTTTGCTTCTGCTTCTGTCTCT GGCCTGAGTGGGACCCAAGATTGTTCTTTCAACATTCCCAATTAGTTCTGATTTTGCAGTGAA GATTAGAGAGCTCTCAGACTATCTGCTGCAAGATTATCCTGTACAGTCGTTCTCAACCTGCAA GACGAAGAGCTCTGCGGTGCCTTGTGGCGGTTGGTCTTGGCTCAAAGATGGATGGAGAGACTG AAAACCGTAGCAGGCAGCAAGATGCAGGGTCTCCTGGAAAGGGTGAACACGGAAATCCATTTT GTGACCAAGTGCGGTTCCAGCCCCACCGAGTTGTCTCCGTTTGTTCAAACGAATATATCCC GGTGTCTCCAGGAAACCTCAGAACAACTGGTGGCTTTGAAACCTGGATCACAAGACAAAACCT TAGTCGGTGCCTCGAACTCCAGTGCCAACAGATTCTTCTACACTTCCCCCCCCGTGGTCCCCGC GCCCCTTGGAAGCAACGGCCCCAaG
TGFb TRAP (Human) (SEQ ID NO: 97)
ATGGCCTGGAGTCTCTGTTTCTGACTCTTATAACTCACTGTGCCGGCAGTTGGGCTATACCCCC TCATGTACAGAAGTCTGTAAACAACGACATGATTGTAACCGACAATAATGGCGCAGTGAAATTC CCACAACCTGTGTAAGTTCTGTGATGTACGGTTAGTACATGCGACAATCAAAAAAGCTGTATGT CTAACTGCTCTATTACATCCATATGTGAAAAACCTCAGGAGGTGTGTGTTGCCGTTTGGCGAAA AAATGATGAGAATATCACACTGGAGACAGTATGTACACCTAACTGCCATAACCATGATTTTC ATACTGGAGGACGCCCGCCAGTCTTAAGTGCATTATGAAAGAGAAAAAGAAACCCGGTGAAACA TTCTTTATGTGCTCTTGTAGCTCTGACGAGTGTAAACGACAACATTATATTACGCGAGGAGTACA ATACAAGCAACCCCGATATACCACCTCACGTACAAAAAGTGTCAACAACGATATGATTGTTAC CGACAATAACGGAGCTGTAAAGTTTCTCAGTTGTGCAAGTTCTGCGATGTACGATTCTTACCT GCGACAACCAAAAGTCATGTATGTCTAACTGTTCCATAACCTCCATCTGCGAGAAGCCCCAGGA AGTCTGCGTCGCCGTGTGGCGGAAAAACGACGAGAATATCACTCTTGAAACCGTTTGTCTATGAT CCTAAACTGCCCTATCAGACTTTATTCTGGAAGATGCTGCTTCCCCTAAGTGTATCATGAAAGA AAAGAAGAAACCTGGGGAGACATTCTTTATGTGTTTATGCTCCTCCGATGAGTGTAACGACAAT ATCATCTTCTCTGAGGAATACAACACTTCTAACCTGATtaG
Fresolimumab (Human) (SEQ ID NO: 98)
ATGGCCTGGTCCCCCTTTTTCTGACCCTCATCACACACTGTGCAGGCTCATGGGCTGAGACCGT CTTGACCCAGTCCCCAGGAACCTTTGTCTCTGTCTCCTGGTGAAAGAGCTACCCCTTAGTTGTGAG CCTCTCAGTCCCTTGGTTCTAGCTATCTCGCTTGGTACCAGCAAAAGCCAGGCCAGGCCCCACG ACTGCTGATCTACGGAGCATCTTCACGGGCTCCCGGCATTCCCGATCGATTTTCCGGATCTGGTA GTGGTACAGATTTCACTGACCATATCTCGCCTGGAGCCCGAGGACTTTGCTGTTTATTATTGT CAGCAGTACGCCGATTCTCCTATCACTTTTGGACAGGGAACCCGCCTGGAGATTAAGCGCACAG TAGCAGCTCCATCCGTTTATCTTTCCACCATCAGATGAACAGCTCAAGAGTGGGACCGCAAG TGAGTATGCCTGCTGAACAATTTTTACCCTAGAGAGGGCCAAAGTGCAGTGGAAGGTGGATAAC GCCCTCCAGAGTGGCAATAGTCAAGAAAGTGTTACTGAGCAAGATAGTAAGGACTCTACATAC TCTTTGAGTTCTACTTTGACCCTGTCAAAAGCAGATTATGAAAAACATAAGGTGTATGCTATGTG AAGTTACACACCAAGGGTTGTCTCTCCAGTTACAAAAATCTTTAATAGAGGAGATGCCGCCG CAAACGCGGTAGTGGAGAAGGTGAGGCTCACTCTTGACCTGTGGCGACGTGGAAGAAAATCC CGGTCCTATGGATTGGACTTGGAGGGTATTTTGTCTTTGGCAGTAACACCTGGAGCTCACCCCC AAGTACAGCTCGTCCAATCTGGTGCCGAGGTTAAAAAGCCTGGAAGTTCAAGTGAAGGTCTCTTG CAAGGCATCTGGATACACCTTTTCATCTAACGTCATATCTGGGTACGGCAAGCCCCAGGACAG GGACTTGAGTGGATGGGAGGGGTCATCCCCATCGTGGACATTGCTAATTACGCTCAGCGATTCA AAGGGCGGGTTACTATAACTGCCGACGAGTCTACCTCAACTACCTACATGGAGTTGTCTCTCT CCGCTCCGAGGACACTGCTGTATATTACTGTGCCAGCACTCTCGGGTTGGTGTGGATGCCATG GACTATTGGGGACAAGGAACCTGGTGACAGTTAGCTCCGCAAGCACTAAAGGCCCTTCTGTTT TCCCTTGGCACCTTGTAGTAGGTCTACCTCTGAGTCTACAGCAGCACTTGGATGCTTGGTTAAG GACTATTTTCCCGAGCCAGTTACAGTCTCTTGGAAACAGTGGTGCCCTCACAAAGTGGGGTTCATA

CCTTCCCCGAGTCCTCCAGAGTAGTGCCCTTTACAGCCTCTCATCAGTTGTGACTGTTCTAGT TCATCACTCGGTACTAAGACATATACATGTAACGTAGACCACAAGCCAAGCAACACAAAAGTA GACAAACGAGTCGAATCTAAGTATGGACCCCTTGTCCCTCTGCTGCTCCCGAGTCTCTTGG GGGCCCTTCCGTGTTCTTGTTCCTCCCAAGCCCAAGGATACCCTCATGATCTACGAACCCAG AGGTAACATGTGTGGTTGTTGACGTAAGTCAGGAAGATCCCGAAGTGCAATTTAATTGGTACGT GGATGGCGTCGAAGTCCATAACGCTAAAACAAAACCCCGAGAGGAACAATTCAATTCCACATA TCGGGTGGTGAGTGTATTGACCGTCTTCACCAAGATTGGCTGAACGGCAAGGAGTATAAGTGT AAAGTAAGCAACAAAGGTCTGCCAAGTAGCATAGAAAAACAATATCTAAAGCTAAGGGCCAA CCAAGGGAACCACAAGTATATACATTGCCCCCTCTCAGGAAGAGATGACAAAGAATCAAGTT AGCCTGACCTGTTTGGTAAAGGGGTTCTATCCCTCAGATATAGCAGTCGAGTGGGAATCTAACG GCCAGCCCGAGAATAATTATAAAACAACCCCCCTGTGTTGGACTCAGACGGCAGCTTCTTTCT CTATTCACGGCTCACTGTTGATAAGTCCCGATGGCAGGAGGGGAATGTTTTCAGCTGTAGCGTG ATGCACGAAGCTCTCCACAACCACTATACACAGAAAAGTTTGTCTTTGTCCCTTGAAAAtaG
TGfB neutralizing peptide (Human) (SEQ ID NO: 99) ATGAGTACATCCTTTCCAGAGCTGGATCTGGAGAATTTTGAGTATGACGACAGTGCCGAAGCCT GCTACCTCGGGACATAGTCGCATTTCGGGACAATCTTTTGTCTGTATTTACGCCCTGGTGT ACATTTGGCTGGTTGGAAATCTGTTGGTCGTACTCGCTCTACCAATTTCCGAAAAACCAAAA GTATAACAGACATATACCTGTTGAATCTGGCACTGAGTGACCTTTTGTTCGTCGCCACCTTCT TTTTGGACACACTACCTTATCAGTCACGAGGGGCTTCATAATGCTATGTGCAAGCTCACTACTGC CTTCTTCTTTATCGGATTCTTCGGGGTATCTTTTATCACAGTTATTAGCATTGACCGATACCT TGCCATAGTGCTCGCAGCCAACCTCAATGAACAACCGCACCGTGCAGCATGGAGTGACTATTTCC TTGGGTGTGTGGCCGCTGCTATACTTGTGCCAGCCCTCAATTCATGTTTACCAAAAGGAAAG ACAATGAGTGCCTCGGAGATTACCCTGAGGTGTTGCAAGAAATGTGGCCTGTTCGAATAAG CGAAGTGAATATACTCGGCTTTGCTCTTCTCTGCTCATCATGTCTATTCTGTTATTTTGAATAAT CCAAACATTGTTTCAGCTGTAAGAACCGAAAGAAAGCCCGCGCCGTACGCCTGATTCTGCTCGTT GTGTTGCGCTTTTTTCTGTTTGGACTCCTTACAACATAATGATATTCCTGGAGACTCTCAAAATC TATAACTTTTTTCCCTCCTGTGATATGAAAAGGGACCTTAGATTGGCTCTCAGTGTCACTGAAAC AGTAGCCTTTAGCCATTGTTGTCTCAACCCTTTCATATATGCATTTGCAGGGGAAAAGTTCCGGC GGTATCTCGGACATTGTATCGGAAGTGTGTTGCGCGTGTGTGTGGTCACTCTCCATCCGGA TTCTCTCTGAGAGTCAACGGAGCCGCCAAGATTCAATCCTGTCCAGTTTCACTCACTATACTTC AGAGGGGGATGGCAGCCTTCTGCTC
Kynurine #1 (SEQ ID NO: 100) ATGGAGACCGACACTTTGTTGCTGTGGGTACTTTGTTGTGGGTCCCAGGATCTACCGGGGATA TGGAACCCCTCTCTTGAAGTCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATTG CGATCCAACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAATTGTCACATTTTCAG GAATGCTTTTACATTTCCAAAGATGAGGGATCTCCATCCATAGATCTTAGCCTCGTGTCGAG GATGACGATGCCATATATTTTCTTGGGAACAGTCTTGGGTTGCAGCCAAAAATGGTACGGACAT ATCTCGAAGAGGAGCTGGACAAATGGGCTAAAAATGGGTGCTTACGGCCACGACGTGGGAAAAC GCCCCGATAGTTGGCGACGAATCTATCGTGAGTCTTATGAAAGATATAGTTGGAGCACATGA GAAAGAAATTGCACTGATGAATGCCCTTACTATCAATCTGCATCTCCTCTTGTCTTCTATTCTTTA AGCCCACTCTAAACGCCACAAAATACTTTTGAAGCAAAAGCCTTTCCAAGCGACCACTACGC TATTGAGTCACAAATACAATCCATGGACTGTGTTGGAAAAGTCTATGCGGATGTTGAAATACCA CGCAAGGCGAGGAGACCTTTCGAATGGAGGACATACTTGAGGTCTATCGAAGAAGAAGGAGAT AGTATAGCAGTTATCCTTTTTCAGCGGGCTGCACTTCTACACAGGTCAACTCTTTAATCATTCCAGC TATTACTAAGGCAGGCCACGCTAAAGGATGCTTCGTGGGCTTTGACCTTGCACACGCAGTAGGA AACGTAGAGCTCCGCTTGCACGATTGGGGCGTTGATTTGCGCTGCTGGTGTTCATATAAGTATCT TAACTCAGGAGCTGGTGGGTTGGCAGGCGCATTCGTACACGAGAAACACGCTCATACCGTAAA GCCTGCACTGGTAGGGTGGTTCGGACACGATCTCTACCCGCTTCAATATGGATAATAAACTC CAGCTTATACCTGGCGCCAATGGATTACAGGATCTCAAATCCTCCTATTTTGTCTGTTTGCAGTTT GCACGCATCTCTTGAAGGTGTTCCAGCAGGCTACCATGACTGCACTCCGCCGGAAGTCAATCCTT TTGACCGGATACTTGGAGTATATGCTGAAACATTATCACTCAAAAGATAAAGTGAAGATAAAG GCCCCATAGTAAACATTATCACTCCATCTCGGGCTGAAGAGCGCGGCTGCCAATCATTGAC TTTTTCCATTCCCAAGAAGTCAGTGTCAAAGAGTTGGAGAAACGGGGGGTGTATGTGATAAG CGGGAGCCAGATGGAATCCGCGTTGCCCAAGTCCCCCTCTATAATCTTTTACGATGTATACA AGTTTATTAGACTGCTGACAAGTATCTTGACTCATCTGAGCGATCTtaG
Kynurine #2 (SEQ ID NO: 101) ATGGAACCCCTCTCTTGAAGTCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATT GCGATCCAACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAATTGTCACATTTCA GGAAGTGTCTTTACATTTCCAAAGATGAGGGATCTCCATCCATAGATCTTAGCCTCGTGTCGGA GGATGACGATGCCATATATTTTCTTGGGAACAGTCTTGGGTTGCAGCCAAAAATGGTACGGACA

TATCTCGAAGAGGAGCTGGACAAATGGGCTAAAAATGGGTGCTTACGGCCACGACGTGGGAAAA CGCCCCGGATAGTTGGCGACGAATCTATCGTGAGTCTTATGAAAGATATAGTTGGAGCACATG AGAAAGAAATGCACTGATGAATGCCCTTACTATCAATCTGCATCTCCTCTTCATTCTTT AAGCCCACTCCTAAACGCCACAAAATACTTTTGGGAAGCAAAAGCCTTTCCAAGCGACCACTACG CTATTGAGTCACAAATACAACCTCCATGGACTTGATGTGGAAAAGTCTATGCGGATGGTAAAACC ACGCGAAGGCGAGGAGACCCCTTCGAATGGAGGACATACTTGAGGTCATCGAAGAAGAAGGAG ATAGTATAGCAGTTATCCTTTTCAGCGGGCTGCACTTCTACACAGGTCAACTCTTTAACATTCCA GCTATTACTAAGGCAGGCCACGCTAAAGGATGCTTCGTGGGCTTTGACCTTGACACACGCAGTAG GAAACGTAGAGCTCCGCTTGCACGATTGGGGCGTTGATTTGCCTGCTGGTGTTTCATATAAGTA TCTTAACTCAGGAGCTGGTGGGTGGCAGGCGCATTTCGTACACGAGAAAACACGCTCATACCGTA AAGCCTGCACTGGTAGGGTGGTTCGGACACGATCTCTTACCCGCTTCAATATGGATAATAAAC TCCAGCTTATACCTGGCGCCAATGGATTCAAGGATCTCAAATCCTCTATTTTGCTCGTTTGCAGT TTGCACGCATCTCTTGAGGTGTTCCAGCAGGCTACCATGACTGCACTCCGCCGGAAGTCAATCC TTTTGACCGGATACTTGAGGTATATGCTGAAAACATTATCACTCAAAAGATAACACTGAGAATAA GGGCCCCATAGTAAACATTATCACTCCATCTCGGGCTGAAGAGCGCGGCTGCCAACTCACATTG ACTTTTCCATTCCCAAGAAGTCAGTGTTCAAAGAGTTGGAGAAAACGGGGGGTTGTATGTGATA AGCGGGAGCCAGATGGAATCCGCGTTGCCCCAGTCCCCCTCTATAATTCTTTTCACGATGTATA CAAGTTTATTAGACTGCTGACAAGTATCTTGACTCATCTGAGCGATCTtaG
VEGF (SEQ ID NO: 102)
ATGAATTTCTTGCTGAGCTGGGTGCATTGGACACTCGCATTGTTGCTGTACTTGACCATGCCAA GTGGTCCCAGGCTGCACCCACTACTGAGGGCGAGCAAAAGTCTCATGAGGTGATTAATTTATG GACGTTTACCAACGATCATACTGTCGGCCAATCGAAACCCTCGTAGATATATCCAGGAGTACC CAGACGAGATCGAATACATTTTCAAGCCCTCATGTGTGCCATTGATGCGATGTGCTGGGTGCTG TAACGACGAAGCACTTGAATGTGTCCCCACCTCCGAGAGTAACATCACAATGCAAAATAATGAG AATCAAGCCCCACCAATCCCAACATATCGGTGAAATGTCATTCTTCAGCATTCCCGCTGCGAG TGCCGGCCTAAGAAGGACCGCACCAAAACCAGAGAACCATTGTGAACCCTGTTCTGAGAGACGG AAGCACTTGTTCGTACAGGACCCCTCAAACATGCAAGTGCAGCTGTAAGAATACCGACTCACGGT GTAAAGCTAGGCAACTGGAGCTTAATGAAAGGACCTGCCGATGCGATAAACCCAGGAGGtaa
GM-CSF (SEQ ID NO: 103)
ATGTGGTTGCAGAATTTGCTCTTCTGGGGATTGTGGTCTACAGCCTCTCCGCACCTACCCGCTC TCCTATCACAGTTACAAGACCCTGGAACATGTGGAGGCCATTAAAGAAGCATTGAATTTGTTG GACGATATGCCCGTCACCCTGAATGAAGAAGTAGAAGTTGTTTCTAATGAGTTCAGCTTTAAAA AATTGACCTGTGTGCAGACACGGCTTAAATTTTGAACAGGGACTTAGAGGAAACTTTACTAA GCTGAAGGGGGCACTTAACATGACAGCTTCTTATTATCAGACCTATTGTCCTCCAACACCTGAA ACCGACTGTGAAACACAGGTAACCACTTACGCCGATTTTATTGATTCTTTGAAAACATTCTCTAC CGATATACCATTGTAGTGTAAAGAAGCCAGGCCAAAAGtaG
Anti-PD1 (SEQ ID NO: 104)
ATGGAAACTGACACACTTCTTCTGTGGGTCTTGCTCCTGTGGGTCCAGGCTCTACTGGTGACAG TCCTGATAGGCCATGGAACCCACCTACCTTTAGTCCAGCCTTGCTCGTCGTAACCGAAGGGGAC AACGCTACATTACCTGCTCTTTTAGCAATACTTCTGAGAGTTTTTCATGTAGTCTGGCATCGGGA GAGTCCATCCGGACAAACAGATACTTTGGCCGCTTTTCCAGAGGATAGGTCTCAACCTGGGCAA GACGCAAGGTTTCGAGTCACACAGCTTCTTAACGGGAGAGATTTTCACATGTCTGTAGTTCGGG CACGCCGAAATGATTCTGGCACATATGTTTGCAGGTGTGATCTCACTTGCTCCAAGATTCAAAT AAAGGAGAGCCTTCGCGCCGAGTTGCGGGTGACTGAGCGGGAGCCCAAGTCCTGCGACAAAAC CCATACTTGTCCACCCTGTGGCGGCGGGTCATCCGGTGGCGGGTCTGGGGGGCAACCAAGAGA GCCACAGGTATATACTCTCCCCCAGCAGAGAAGAAATGACAAAAAACCAAGTGTCCCTGAC ATGTCTGGTTAAAGGATTTTATCCAGTGACATTGCTGTAGAATGGGAATCCAATGGTCAACCC GAGAATAACTACAAAACCACTCCTCCAGTATTGGACAGTGACGGTTCCTTCTTCTCTATTCCAA ACTTACAGTGGATAAATCCCGCTGGCAGCAAGGGAATGTATTACGCTGTAGTGTATGCACGAA GCTCTTCATAACCAATTATACACAGAAATCTCTTTCCCTGAGCCCAGGTAAAtaG
Adenosine Deaminase (ADA) #1 (Mouse) (SEQ ID NO: 105)
ATGGAGACTGATACACTTTTGCTCTGGGTTTGTCTTGTGGGTACCAGGGTCTACTGGAGATGC ACAAACTCCTGCATTCAACAAGCCTAAGGTAGAGCTTCATGTCCATTTGGACGGAGCCATAAAA CCTGAAACCATACTCTATTTGCGCAAGAAACGGGGTATAGCACTTCCCGCTGATACCGTGGAAG AGTTGAGAAATATCATTGGCATGGACAAACCTCTTAGCCTGCCTGGCTTTCTTGCAAGTTTCA CTACTATATGCCAGTTATAGCAGGGTGTAGAGAAGCAATAAAGCGAATCGCCTATGAGTTCGTT GAGATGAAGGCTAAAGAAGGAGTTGTTTACGTGGAAGTCCGGTACTCACCTCATCTGCTTGCTA ATAGCAAGGTGGACCCAATGCCATGGAATCAAACCTGAAGGTGATGTAACCCCTGACGATGTGG TCGATTTGGTCAATCAAGGTCTCCAAGAAGGCGAGCAGGCTTTCGGCATTAAAGGTAAGAAGTAT ATTGTGCTGTATGCGACATCAACCTTCATGGTCCCTGGAGGTCTCGAATTGTGCAAAAAGTAC

AATCAAAAAACAGTGGTCGCAATGGATCTCGCTGGAGATGAGACCATAGAAGGTTCTCTCTTT TCCCCGGTCATGTGCAAGCATATGAAGGGGCTGTCAAAAATGGTATCCACCGCACCGTCCACGC AGGGGAAGTAGGGTCCCCAGAAAGTAGTCAGGGAAGCCGTTGACATTTTGAAAACAGAAAGAGT CGGGCATGGCTACCATAACAATAGAGGACGAAGCCTTGTACAATCGACTTTTGAAAAGAAAATAT GCACTTCGAGGTCTGTCCCTGGAGTTCATATCTCACCGGAGCATGGGACCCCAAAACAACCCAC GCCGTCGTACGCTTCAAGAATGATAAGGCAAACCTACAGTTTGAATACAGATGATCCACTGATAT TCAAGTCAACACTTGACACTGACTACCAGATGACAAAAAAGATATGGGTTTCACCGAAGAAG AGTTCAAGAGATTGAACATTAACGCAGCAAAAAGCTCCTTCCTGCCAGAGGAAGAGAAAAAAG AATTGCTTGAAAGGTTGTATCGAGAATACCAA
Adenosine Deaminase (ADA) #2 (Mouse) (SEQ ID NO: 106)
ATGGCACAAACTCCAGCTTTTAATAAGCCCAAGTGGAACTTCATGTTTCATCTGGATGGGGCAA TTAAGCCCGAACTATATTGTACTTTGGCAAAAAGAGGGGTATTGCCCTGCCAGCAGATACCGT TGAGGAGCTTCGCAACATCATTGGGATGGACAAGCCCTCTCTCTGCCAGGTTTCTCGCTAAA TTCGATTATTATATGCCTGTTATTGCTGGTTGCCGGGAGGCCATCAAGAGGATAGCCCTACGAGT TTGTTGAGATGAAGGCCAAAGAGGGCGTGGTGTACGTAGAGGTCAGATACAGCCCTCACCTGC TTGCCAACAGCAAGGTGGACCCAATGCCCTGGAACCAACCGAGGGGGATGTCACCTCCGACG ACGTTGTAGACCTCGTAAATCAGGGCCTTCAAGAGGGCGAGCAGGCATTTGGCATAAAAAGTCC GGTCTATACTCTGCTGTATGAGGCACCAACCCCTCCTGGTCTTTGGAGGTACTTGAGTTGTGTAAG AAATACAATCAAAAGACTGTAGTCGCCATGGATCTTGCAGGCGATGAAACCATCGAGGGTAGC TCCTTGTTCCCTGGACATGTTGAAGCCTACGAGGGGGCCGTAAAAAATGGGATACACAGGACTG TCCACGCTGGTGAAGTCGGAAGCCCAGAGGTGGTAAGGGAGGCAGTTGACATACTCAAGACAG AGCGGGTTGGACACGGATACCAACAATTGAGGACGAGGCCCTGTATAACCGCCTCCTCAAAG AGAACATGCATTTTGAGGTGTGTCTTGGTCCAGCTACCTGACTGGTGTGCTTGGGACCCCTAAAC AACTCACGCCGTGGTCCGGTTCAAGAACGATAAAGCCAATTACTCTTTGAATACCGACGACCCC CTCATATTCAAATCAACATTGGATACCGACTACCAAATGACCAAAAAGGATATGGGGTTTACTG AAGAGGAGTTCAAGAGGCTCAACATAAATGCCGCTAAATCCTCCTTTCTCCCCGAGGAAGAAA AAAAAGAACTCCTTGAGCGGCTGTATAGGGAGTATCAA
4-1BBL #1 (Mouse) (SEQ ID NO: 107)
ATGGAAACAGATACACTCTTGCTGTTGGTACTGCTTCTGTGGGTCCCCGGCTCTACTGGGGATG AAGATGATGTAACACTACTACAGAAGCTCGTCCCCGCTCTTGTCACCCCAACCAAGGGTACCTG CGCCGGTTGGATGGCTGGCATCCCAGGACATCCAGGTCAACCGTACCCCGGAAGAGATGG TCGGGATGGAACCTCCCGGCGAGAAGGGCGAAAAAGGGGATGCAGGGCTTCTGGGACCTAAAG GTGAAACAGGGGACGTTGGAATGACTGGTGCAGAAAGGGCCTCGCGGCTTCTCTGGCACCCCTG GGAGGAAAGGAGAGCCCCGAGAGCTCCAGAGAACTGAACCTCGGCCTGCACTCACTATAACTA CTTCCCCTAATCTTGGGACCCGCGAGAACAACGCCGATCAGGTTACACCTGTAAGCCATATCGG GTGCCCCAATACTACCCAGCAAGGGAGTCCCGTGTTCGCAAAAGCTTTTGGCTAAAAACCAAGCA TCCCTGTGTAACTACTCTTAATTGGCATTCAACAGACGGTGTGGTAGCTCTTATCTTTCTCA GGGGCTGCGGTACGAAGAAGATAAGAAGGAATTGGTTGTGGATTCTCCAGGACTCTATTATGTC TTTCTCGAATTGAAGCTCAGTCCCACCTTCACAAACACTGGACACAAAGTCCAGGGCTGGGTAA GTCTGGTACTCCAAGCAAAGCCCCAGGTTGACGATTTGACAATTTGGCACTACCCGTAGAGCT TTTCCCATGCTCCATGGAAAAATAAATTGTTGATCGGTCATGGTCACAGCTCTTGCTGCTTAAGG CAGGCATCGCCTCTCAGTGGGTCTGAGAGCTTATTGTCATGGTGCACAAGATGCTTACAGGGA TTGGGAATTGTCTACCCAAACACTACAAGTTTCGGGTGTTCCTTGTCAAACCTGATAACCCAT GGGAGtaG
4-1BBL #2 (Mouse) (SEQ ID NO: 108)
ATGGAAACTGATACACTCCTCCTGTGGGTCTTCTTTGTGGGTGCCCGGATCAACCGGCGATG GCTGGATGGCAGGCATCCCAGGACACCCAGGACACAACGGTACTCCAGGTCGAGACGGTCCGG ATGGGACTCCTGGGGAGAAAGGCGAGAAAGGGGACGCTGGTTTGCTCGGTCCTAAGGGGGAAA CCGGGGATGTAGGAATGACAGGGGCTGAAGGGCCTCGGGGATTTCTGGGACACCAGGCAGGA AGGGTGAACCAGGGGAGGCCCTCCAGCGCACCGAGCCACGGCCAGCTCTGACCATAACAACAA GTCCAAACCTGGGCACACGCGAAAACAATGCTGACCAGGTGACTCCTGTAAGTCACATCGGAT GCCCTAACACTACACAACAGGGCTCTCCTGTATTGCAAAGCTTCTCGCAAAAAATCAAGCATC ACTTTGTAATACAACCTGAACTGGCATTCTCAGGACGGAGCAGGGTCTCTTATTGTCTCAA GGGCTCCGCTACGAAGAAGATAAAAAGGAATTGGTTGTTGACAGTCCAGGTTTGATTATGTGT TTTTGGAACCTTAAGCTGTCAACCAACCTTCACTAACACCGGCCACAAGGTCCAAGGCTGGGTTAG TCTTGTTTTGCAAGCCAAACCTCAAGTGATGATTTTGACAATCTGGCTTTGACTGTTGAGCTTT TTCCATGCAGTATGGAGAATAAACTGGTTGATCGGTCATGGTCACAGCTCCTTCTGCTCAAGGC CGGACATAGGCTGAGTGTGGGACTTCGGGCCTACTTGCACGGCGCCCAGGACGCATACCGAGA CTGGGAACCTCAGCTACCCTAACACAACCTCTTTTGGGTGTTCCTTGTCAAACCCGATAATCCTT GGGAAtaG

HPGE2 #1 (Mouse) (SEQ ID NO: 109)
ATGGAGACTGATACTTTGCTCCTGTGGGTTCTTCTCCTGTGGGTTCTGGTTCACAGGGGATAT GCATGTCAATGGCAAGGTAGCACTCGTACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTGCC GAGGCCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACCTGGAGGCTGGAGTTA AATGCAAAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCTCTTTGTGCAGTGTGACGT TGCTGACCAAAAAGCAACTCAGGGACACATTGAGGAAGGTCGTAGACCATTTCGGACGCCTCGA TATACTCGTTAATAATGCCGGGGTAAACAACGAAAAGAACTGGGAACAAACATTGCAAATCAA CCTGGTAAGTGTCAATTAGCGGAACCTTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGGGC GAGGGCGGGATCATTATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCCTG TTTACTGTGCCAGCAAGCACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAAATCT TATGAAGAGTGGGGTAAGACTGAATGTTATCTGCCCAGGTTTCGTAGATACCCCAATCCTGGAG AGCATCGAGAAGGAGGAAAAATATGGGACAATACATTGAATATAAAGATCAAATCAAGGCTATG ATGAAGTTCTACGGGGTTCTGCATCCATCCACAATTGCCAACGGGCTCATTAAATCTGATTGAGG ACGACGCCTTGAACGGAGCTATAATGAAAATCACAGCTTCCAAAGGCATTCACTTCCAAGATTA TGATATATCACCTTGCTTGTCAAGGCTCCTCTGACAAGT
HPGE2 #2 (Mouse) (SEQ ID NO: 110)
ATGCATGTCAAATGGCAAGGTAGCACTCGTACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTG CCGAGGCCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACCTGGAGGCTGGAGT TAAATGCAAAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCTCTTTGTGCAGTGTGAC GTTGCTGACCAAAAAGCAACTCAGGGACACATTGAGGAAGGTCGTAGACCATTTCGGACGCCTC GATATACTCGTTAATAATGCCGGGGTAAACAACGAAAAGAACTGGGAACAAACATTGCAAATC AACCTGGTAAGTGTCAATTAGCGGAACCTTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGG GCGAGGGCGGGATCATTATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCC TGTTTACTGTGCCAGCAAGCACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAAAT CTTATGAAGAGTGGGGTAAGACTGAATGTTATCTGCCCAGGTTTCGTAGATACCCCAATCCTGG AGAGCATCGAGAAGGAGGAAAAATATGGGACAATACATTGAATATAAAGATCAAATCAAGGCTA TGATGAAGTTCTACGGGGTTCTGCATCCATCCACAATTGCCAACGGGCTCATTAAATCTGATTGA GGACGACGCCTTGAACGGAGCTATAATGAAAATCACAGCTTCCAAAGGCATTCACTTCCAAGAT TATGATATATCACCTTGCTTGTCAAGGCTCCTCTGACAAGT
Human IL-15 Polypeptide Sequence (SEQ ID NO: 199)
MVLGTIDLCSFCSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCVKVTAMKCFLL LQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECELEEKNKEFLQSFVHIVQMFINTS
Human IL-15R α Polypeptide Sequence (SEQ ID NO: 200)
MAPRRARGCRTLGLPALLLLLLRPPATRGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKA GTSSLTECVLNKATNVAHWTTPLSKCIRDPALVHQRAPPSTVTTAGVTPQPESLSPSGKEPAASSPS SNNTAATTAIVPGSQLMPSKSPSTGTTEISSHESHGTPSQTAKNWELTASASHQPPGVYPQGHSD TTVAISTSTVLLCGLSAVSLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDEDLNCSHHL
Human IL-15R α sushi domain Polypeptide Sequence (SEQ ID NO: 201)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPLSKCIR
Human IL-15/IL-15R α sushi domain Polypeptide Sequence (SEQ ID NO: 202)
MDWTWILFLVAAATRVHSNWNVISDLKKIEDLIQSMHIDATLYTESDVHPSCVKVTAMKCFLLELQ VISLESGDASIHTVENLILANNSLSSNGNVTESGCKECELEEKNKEFLQSFVHIVQMFINTSSGGS GGGSGGGSGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPLSKCIR
IL-12 (IL-12p70) Polypeptide Sequence (SEQ ID NO: 203)
MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSS EVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEA KNYSGRFTCWWLTTISTDLTFSVSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVEQEDSAC PAAEESLPIEVMVDVAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHS YFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASVPCSGGGSG GGSGGGSGGSRNLPVATPDGPMFCLHHSQNLRAVSNNMLQKARQTLEFYPCTEEIDHEDITKD KTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAK LLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS

Secretion Signals and Signal-Anchors

The one or more effector molecules of the chimeric proteins provided for herein can be secretable effector molecules having a secretion signal peptide (also referred to as a signal peptide or signal sequence) at the chimeric protein's N-terminus (*e.g.*, an effector molecule's N-terminus for **S – C – MT**) that direct newly synthesized proteins destined for secretion or

5 membrane localization (also referred to as membrane insertion) to the proper protein processing pathways. For chimeric proteins having the formula **MT – C – S**, a membrane tethering domain generally has a signal-anchor sequence (*e.g.*, signal-anchor sequences of a Type II transmembrane protein) that direct newly synthesized proteins destined for

10 having the formula **S – C – MT**, a membrane tethering domain having a reverse signal-anchor sequence (*e.g.*, signal-anchor sequences of certain Type III transmembrane proteins) can be used, generally without a separate secretion signal peptide, that direct newly synthesized proteins destined for membrane localization to the proper protein processing pathways.

15 In general, for all membrane-cleavable chimeric proteins described herein, the one or more effector molecules are secretable effector molecules (referred to as “S” in the formula **S – C – MT** or **MT – C – S**). In embodiments with two or more chimeric proteins, each chimeric protein can comprise a secretion signal. In embodiments with two or more chimeric proteins, each chimeric protein can comprise a secretion signal such that each effector

20 molecule is capable of secretion from an engineered cell following cleavage of the protease cleavage site.

The secretion signal peptide operably associated with an effector molecule can be a native secretion signal peptide (*e.g.*, the secretion signal peptide generally endogenously associated with the given effector molecule). The secretion signal peptide operably associated

25 with an effector molecule can be a non-native secretion signal peptide native secretion signal peptide. Non-native secretion signal peptides can promote improved expression and function, such as maintained secretion, in particular environments, such as tumor microenvironments. Non-limiting examples of non-native secretion signal peptide are shown in Table 3.

30 **Table 3. Exemplary Signal Secretion Peptides**

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
<i>IL-12</i>	MCHQQLVISWFSL VFLASPLVA (SEQ ID NO: 112)	P29460	ATGTGTCACCAGCAGCTCGTTATA TCCTGGTTTAGTTTGGTGTTCCTCG CTTCACCCCTGGTGGCA (SEQ ID NO: 31)

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
<i>IL-12 (Codon Optimized)</i>	MCHQQLVISWFSL VFLASPLVA (SEQ ID NO: 112)	-	ATGTGCCATCAGCAACTCGTCATC TCCTGGTTCTCCCTTGTTCTCCTCG CTTCCCCTCTGGTCGCC (SEQ ID NO: 32)
<i>IL-2 (Optimized)</i>	MQLLSIALILALV (SEQ ID NO: 113)	-	ATGCAACTGCTGTCATGTATCGCA CTCATCCTGGCGCTGGTA (SEQ ID NO: 33)
<i>IL-2 (Native)</i>	MYRMQLLSIALS LALVTNS (SEQ ID NO: 114)	P60568	ATGTATCGGATGCAACTTTTGAGC TGCATCGCATTGTCTCTGGCGCTG GTGACAAATTCC (SEQ ID NO: 34)
<i>Trypsinogen-2</i>	MNLLLILTFVAAA VA (SEQ ID NO: 115)	P07478	ATGAATCTCTTGCTCATACTTACGT TTGTCGCTGCTGCCGTTGCG (SEQ ID NO: 35)
<i>Gaussia Luciferase</i>	MGVKVLFALICIA VAEA (SEQ ID NO: 116)	-	ATGGGCGTGAAGGTCTTGTGTTGCC CTTATCTGCATAGCTGTTGCCGAG GCG (SEQ ID NO: 36)
<i>CD5</i>	MPMGSLLQPLATLY LLGMLVASCLG (SEQ ID NO: 117)	P06127	ATGCCGATGGGGAGCCTTCAACCT TTGGCAACGCTTTATCTTCTGGGG ATGTTGGTTGCTAGTTGCCTTGGG (SEQ ID NO: 37)
<i>IgKVII (mouse)</i>	METDTLLLWVLLL WVPGSTGD (SEQ ID NO: 118)		ATGGAACTGACACGTTGTTGCTG TGGGTATTGCTCTTGTTGGGTCCCA GGATCTACGGGCGAC (SEQ ID NO: 38)
<i>IgKVII (human)</i>	MDMRVPAQLLGL LLLWLRGARC (SEQ ID NO: 119)	P01597	ATGGATATGAGGGTTCCCGCCCAG CTTTTGGGGCTGCTTTTGTGTTGGC TTCGAGGGGCTCGGTGT (SEQ ID NO: 39)
<i>VSV-G</i>	MKCLLYLAFLFIG VNC (SEQ ID NO: 120)	-	ATGAAGTGTCTGTTGTACCTGGCG TTTCTGTTTCATTGGTGTAAGTGT (SEQ ID NO: 40)
<i>Prolactin</i>	MNIKGSPWKGSLL LLLVSNLLLCQSV AP (SEQ ID NO: 121)	P01236	ATGAATATCAAAGGAAGTCCGTGG AAGGGTAGTCTCCTGCTGCTCCTC GTATCTAACCTTCTCCTTTGTCAAT CCGTGGCACCC (SEQ ID NO: 41)
<i>Serum albumin preproprotein</i>	MKWVTFISLLFLFS SAYS (SEQ ID NO: 122)	P02768	ATGAAATGGGTAACATTCATATCA CTTCTCTTTCTGTTTCAGCTCTGCGT ATTCT (SEQ ID NO: 42)
<i>Azurocidin preproprotein</i>	MTRLTVLALLAGL LASSRA (SEQ ID NO: 123)	20160	ATGACAAGGCTTACTGTTTTGGCT CTCCTCGCTGGACTCTTGGCTTCCT CCCGAGCA (SEQ ID NO: 43)
<i>Osteonectin (BM40)</i>	MRAWIFFLLCLAG RALA (SEQ ID NO: 124)	P09486	ATGAGGGCTTGGATTTTTTTCTGC TCTGCCTTGCCGGTCGAGCCCTGG CG (SEQ ID NO: 44)
<i>CD33</i>	MPLLLLLPLLWAG ALA (SEQ ID NO: 125)	P20138	ATGCCTCTTCTGCTTTTGTCTCCTC TTTTGTGGGCAGGTGCCCTCGCA (SEQ ID NO: 45)
<i>IL-6</i>	MNSFSTSAFGPVA FSLGLLLVLPAAFP AP (SEQ ID NO: 126)	P05231	ATGAACTCTTTCTCAACCTCTGCGT TTGGTCCGGTCGCTTTCTCCCTTGG GCTCCTGCTTGTCTTGCCAGCAGC GTTTCCTGCGCCA (SEQ ID NO: 46)
<i>IL-8</i>	MTSKLAVALLAAF LISAALC (SEQ ID NO: 127)	P10145	ATGACAAGTAACTGGCGGTAGCC TTGCTCGCGGCCTTTTGTATTCCG CAGCCCTTTGT (SEQ ID NO: 47)
<i>CCL2</i>	MKVSAALLCLLI AATFIPQGLA (SEQ ID NO: 128)	P13500	ATGAAGGTAAGTGCAGCGTTGCTT TGCCTTCTCCTCATTGCAGCGACCT TTATTCTCAAGGGCTGGCC (SEQ ID NO: 48)

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
<i>TIMP2</i>	MGAAARTLRLAL GLLLATLLRPAD A (SEQ ID NO: 129)	P16035	ATGGGAGCGGCAGCTAGAACACTT CGACTTGCCCTTGGGCTCTTGCTCC TTGCAACCCTCCTTAGACCTGCCG ACGCA (SEQ ID NO: 49)
<i>VEGFB</i>	MSPLLRLLLAAL LQLAPAQA (SEQ ID NO: 130)	P49765	ATGTCACCGTTGTTGCGGAGATTG CTGTTGGCCGCACTTTTGCAACTG GCTCCTGCTCAAGCC (SEQ ID NO: 50)
<i>Osteoprotegerin</i>	MNLLCCALVFLD ISIKWTTQ (SEQ ID NO: 131)	O00300	ATGAATAACCTGCTCTGTTGTGCG CTCGTGTTCCTGGACATTTCTATAA AATGGACAACGCAA (SEQ ID NO: 51)
<i>Serpin E1</i>	MQMSPALTCLVLG LALVFEGESA (SEQ ID NO: 132)	P05121	ATGCAAATGTCTCCTGCCCTTACCT GTCTCGTACTTGGTCTTGCGCTCGT ATTTGGAGAGGGATCAGCC (SEQ ID NO: 52)
<i>GROalpha</i>	MARAALSAAPSNP RLLRVALLLLLV AAGRRAAG (SEQ ID NO: 133)	P09341	ATGGCAAAGGGCTGCACTCAGTGCT GCCCCGTCTAATCCCAGATTGCTT CGAGTTGCATTGCTTCTTCTGTTGC TGGTTGCAGCTGGTAGGAGAGCAG CGGGT (SEQ ID NO: 53)
<i>CXCL12</i>	MNAKVVVVVLVLV LTALCLSDG (SEQ ID NO: 134)	P48061	ATGAATGCAAAAGTCGTGGTCTGTG CTGGTTTTGGTTCTGACGGCGTTGT GTCTTAGTGATGGG (SEQ ID NO: 54)
<i>IL-21 (Codon Optimized)</i>	MERIVICLMVIFLG TLVHKSSS (SEQ ID NO: 135)	Q9HBE4	ATGGAACGCATTGTGATCTGCCTG ATGGTCATCTTCCTGGGCACCTTA GTGCACAAGTCGAGCAGC (SEQ ID NO: 55)
<i>CD8</i>	MALPVTALLPLA LLLHAARP (SEQ ID NO: 136)	-	ATGGCCTTACCAGTGACCGCCTTG CTCCTGCCGCTGGCCTTGCTGCTCC ACGCCGCCAGGCCG (SEQ ID NO: 139)
<i>CD8 (Codon Optimized)</i>	MALPVTALLPLA LLLHAARP (SEQ ID NO: 137)	-	ATGGCGCTCCCGGTGACAGCACTT CTCTTGCTCTTGCCCTGCTGTTGC ATGCCGCGCGCCCA (SEQ ID NO: 140)
<i>GMCSF</i>	MLLVTSLLCEL HPAFLIP (SEQ ID NO: 138)	-	ATGTTGCTCGTGACATCCCTCTTGC TTTGTGAGTTGCCTCATCCCGCATT CCTGCTCATCCCA (SEQ ID NO: 141)
<i>NKG2D</i>	PFFFCCFIAMGI RFIIMVA (SEQ ID NO: 192)		CCCTTCTTCTTCTGTTGCTTTATCG CCGTGGCCATGGGCATCCGCTTCA TCATTATGGTGGCC (SEQ ID NO: 193)

Protease Cleavage Site

In certain embodiments, the chimeric proteins provided for herein (*e.g.*, in general, for all membrane-cleavable chimeric proteins described herein) contain a protease cleavage site (*e.g.*, referred to as “C” in the formula **S – C – MT** or **MT – C – S** for membrane-cleavable chimeric proteins described herein). In general, the protease cleavage site can be any amino acid sequence motif capable of being cleaved by a protease. Examples of protease cleavage sites include, but are not limited to, a Type 1 transmembrane protease cleavage site, a Type II transmembrane protease cleavage site, a GPI anchored protease cleavage site, an ADAM8

protease cleavage site, an ADAM9 protease cleavage site, an ADAM10 protease cleavage site, an ADAM12 protease cleavage site, an ADAM15 protease cleavage site, an ADAM17 protease cleavage site, an ADAM19 protease cleavage site, an ADAM20 protease cleavage site, an ADAM21 protease cleavage site, an ADAM28 protease cleavage site, an ADAM30 protease cleavage site, an ADAM33 protease cleavage site, a BACE1 protease cleavage site, a BACE2 protease cleavage site, a SIP protease cleavage site, an MT1-MMP protease cleavage site, an MT3-MMP protease cleavage site, an MT5-MMP protease cleavage site, a furin protease cleavage site, a PCSK7 protease cleavage site, a matriptase protease cleavage site, a matriptase-2 protease cleavage site, an MMP9 protease cleavage site, or an NS3 protease cleavage site.

One example of a protease cleavage site is a hepatitis C virus (HCV) nonstructural protein 3 (NS3) protease cleavage site, including, but not limited to, a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B cleavage site. For a description of NS3 protease and representative sequences of its cleavage sites for various strains of HCV, see, e.g., Hepatitis C Viruses: Genomes and Molecular Biology (S.L. Tan ed., Taylor & Francis, 2006), Chapter 6, pp. 163-206; herein incorporated by reference in its entirety. For example, the sequences of HCV NS4A/4B protease cleavage site; HCV NS5A/5B protease cleavage site; C-terminal degron with NS4A/4B protease cleavage site; N-terminal degron with HCV NS5A/5B protease cleavage site are provided. Representative NS3 sequences are listed in the National Center for Biotechnology Information (NCBI) database. See, for example, NCBI entries: Accession Nos. YP_001491553, YP_001469631, YP_001469632, NP_803144, NP_671491, YP_001469634, YP_001469630, YP_001469633, ADA68311, ADA68307, AFP99000, AFP98987, ADA68322, AFP99033, ADA68330, AFP99056, AFP99041, CBF60982, CBF60817, AHH29575, AIZ00747, AIZ00744, ABI36969, ABN05226, KF516075, KF516074, KF516056, AB826684, AB826683, JX171009, JX171008, JX171000, EU847455, EF154714, GU085487, JX171065, JX171063; all of which sequences (as entered by the date of filing of this application) are herein incorporated by reference.

Another example of a protease cleavage site is an ADAM17-specific protease (also referred to as Tumor Necrosis Factor- α Converting Enzyme [TACE]) cleavage site. An ADAM17-specific protease cleavage site can be an endogenous sequence of a substrate naturally cleaved by ADAM17. An ADAM17-specific protease cleavage site can be an engineered sequence capable of being cleaved by ADAM17. An engineered ADAM17-specific protease cleavage site can be an engineered for specific desired properties including, but not limited to, optimal expression of the chimeric proteins, specificity for ADAM17, rate-

of-cleavage by ADAM17, ratio of secreted and membrane-bound chimeric protein levels, and cleavage in different cell states. A protease cleavage site can be selected for specific cleavage by ADAM17. For example, certain protease cleavage sites capable of being cleaved by ADAM17 are also capable of cleavage by additional ADAM family proteases, such as

5 ADAM10. Accordingly, an ADAM17-specific protease cleavage site can be selected and/or engineered such that cleavage by other proteases, such as ADAM10, is reduced or eliminated. A protease cleavage site can be selected for rate-of-cleavage by ADAM17. For example, it can be desirable to select a protease cleavage site demonstrating a specific rate-of-cleavage by ADAM17, such as reduced cleavage kinetics relative to an endogenous sequence of a

10 substrate naturally cleaved by ADAM17. In such cases, in general, a specific rate-of-cleavage can be selected to regulate the rate of processing of the chimeric protein, which in turn regulates the rate of release/secretion of the payload effector molecule. Accordingly, an ADAM17-specific protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage by ADAM17. A protease cleavage site can

15 be selected for both specific cleavage by ADAM17 and rate-of-cleavage by ADAM17. Exemplary ADAM17-specific protease cleavage sites, including those demonstrating particular specificity and rate-of-cleavage kinetics, are shown in **Table 4A** below with reference to the site of cleavage (P5-P1: N-terminal; P1'-P5': C-terminal). Further details of ADAM17 and ADAM10, including expression and protease cleavage sites, are described in

20 Sharma, *et al.* (J Immunol October 15, 2017, 199 (8) 2865-2872), Pham *et al.* (Anticancer Res. 2017 Oct;37(10):5507-5513), Caescu *et al.* (Biochem J. 2009 Oct 23; 424(1): 79-88), and Tucher *et al.* (J. Proteome Res. 2014, 13, 4, 2205-2214), each herein incorporated by reference for purposes.

Table 4A – Various ADAM17 Protease Cleavage Site Sequences

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

In some embodiments, the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176). In some embodiments, the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177). In some embodiments, the first region is located N-terminal to the second region.

- 5 In some embodiments, 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, T, or A. In some embodiments, 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. In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182). In some
- 15 embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185). In some embodiments, the protease cleavage site
- 20 comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186).

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

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

The protease cleavage site can be C-terminal of the secretable effector molecule. The protease cleavage site can be N-terminal of the secretable effector molecule. In general, for all membrane-cleavable chimeric proteins described herein, the protease cleavage site is

either: (1) C-terminal of the secretable effector molecule and N-terminal of the cell membrane tethering domain (in other words, the protease cleavage site is in between the secretable effector molecule and the cell membrane tethering domain); or (2) N-terminal of the secretable effector molecule and C-terminal of the cell membrane tethering domain (also
5 between the secretable effector molecule and the cell membrane tethering domain with domain orientation inverted). The protease cleavage site can be connected to the secretable effector molecule by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the effector molecule or protease cleavage site. The protease cleavage site can be connected to the cell membrane tethering domain by a polypeptide
10 linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or protease cleavage site. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, [GS]₄GG [SEQ ID NO:
15 182]), A(EAAAK)₃A (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a “KEGS” linker such as the amino acid sequence KESGVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional exemplary polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ
20 ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art.

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

In general, a protease that cleaves the protease cleavage site is a protease specific for that specific protease cleavage site. For example, in the case of a disintegrin and metalloproteinase (“ADAM”) family protease, the protease that cleaves a specific ADAM
30 protease cleavage site is generally limited to the ADAM protease(s) that specifically recognize the specific ADAM protease cleavage site motif. A protease cleavage site can be selected and/or engineered such that cleavage by undesired proteases is reduced or eliminated. Proteases can be membrane-bound or membrane-associated. Proteases can be

secreted, *e.g.*, secreted in a specific cellular environment, such as a tumor microenvironment (“TME”).

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

A protease that cleaves the protease cleavage site of the chimeric protein can be heterologous to a cell expressing the chimeric protein. For example, a cell engineered to express the chimeric protein can also be engineered to express a protease not generally expressed by the cell that is specific for the protease cleavage site present in the chimeric protein. A cell engineered to express both the chimeric protein and the protease can be engineered to express each from separate engineered nucleic acids or from a multicistronic systems (multicistronic and multi-promoter systems are described in greater detail in the

Section herein titled “Multicistronic and Multiple Promoter Systems”). Heterologous proteases and their corresponding protease cleavage site can be selected as described above with reference to endogenous proteases.

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

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

Proteases can be tumor associated proteases, such as, a cathepsin, a cysteine protease, an aspartyl protease, a serine protease, or a metalloprotease. Specific examples of tumor associated proteases include Cathepsin B, Cathepsin L, Cathepsin S, Cathepsin D, Cathepsin E, Cathepsin A, Cathepsin G, Thrombin, Plasmin, Urokinase, Tissue Plasminogen Activator, Metalloproteinase 1 (MMP1), MMP2, MMP3, MMP4, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP20, MMP21, MMP23, MMP24, MMP25, MMP26, MMP28, ADAM, ADAMTS, CD10 (CALLA), or prostate specific antigen. Proteases can also include, but are not limited to, proteases listed in

Table 4B below. Exemplary cognate protease cleavage sites for certain proteases are also listed in Table 4B.

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

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
HCV NS4A/4B	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS5A/5B	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS3	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS2-3	DEMEECSQHL (SEQ ID NO: 142) EDVVPCSMG (SEQ ID NO: 143)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HIV-1 protease (SEQ ID NO: 144)		Amprenavir, Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir
Signal peptidase (P67812, P15367, P00804, P0803)	preference of eukaryotic signal peptidase for cleavage after residue 20 (Xaa ^{20↓}) of pre(Δ pro)apoA-II: Ala, Cys > Gly > Ser, Thr > Pro > Asn, Val, Ile, Leu, Tyr, His, Arg, Asp.	
proprotein convertases cleaving at hydrophobic residues (e.g., Leu, Phe, Val, or Met) (Q16549, Q8NBP7, Q92824, P29120, Q6UW60, P29122, Q9QXV0)	(R/K)-X-(hydrophobic)-X↓, where X is any amino acid	
proprotein convertases cleaving at small amino acid residues such as Ala	(K/R)-(X) _n -(K/R)↓, where n is 0, 2, 4 or 6 and X is any amino acid	

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

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

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	hydrophobic amino-acid residue at P2 and a hydrophilic amino-acid residue at P3, although Val or Ala are also accepted at this position.	
caspase 4 (P70343, P49662)	Strict requirement for Asp at the P1 position. It has a preferred cleavage sequence of Tyr-Val-Ala-Asp- - (YVAD; SEQ ID NO: 146) but also cleaves at Asp-Glu-Val-Asp- -(DEVD; SEQ ID NO: 148).	
caspase 5 (P51878)	Strict requirement for Asp at the P1 position. It has a preferred cleavage sequence of Tyr-Val-Ala-Asp- -(YVAD; SEQ ID NO: 146) but also cleaves at Asp-Glu-Val-Asp- - -(DEVD; SEQ ID NO: 148).	
caspase 6 (P55212)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of Val-Glu-His-Asp- -(VEHD; SEQ ID NO: 149).	
caspase 7 (P97864, P55210)	Strict requirement for an Asp residue at position P1 and has a preferred cleavage sequence of Asp-Glu-Val-Asp- - (DEVD; SEQ ID NO: 148).	
caspase 8 (Q8IRY7, O89110, Q14790)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of (Leu/Asp/Val)-Glu-Thr-Asp- - (Gly/Ser/Ala).	
caspase 9 (P55211, Q8C3Q9, Q5IS54)	Strict requirement for an Asp residue at position P1 and with a marked preference for His at position P2. It has a preferred cleavage sequence of Leu-Gly-His-Asp- -Xaa (LGHD; SEQ ID NO: 150).	
caspase 10 (Q92851)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of Leu-Gln-Thr-Asp- -Gly (LQTDG; SEQ ID NO: 151).	
puromycin sensitive aminopeptidase (P55786, Q11011)	Release of an N-terminal amino acid, preferentially alanine, from a wide range of peptides, amides and arylamides.	

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
angiotensin converting enzyme (ACE) (P12821, P09470, Q9BYF1) SEQ ID NO: 156	Release of a C-terminal dipeptide, oligopeptide- -Xaa-Yaa, when Xaa is not Pro, and Yaa is neither Asp nor Glu.	Benazepril (Lotensin), Captopril, Enalapril (Vasotec), Fosinopril, Lisinopril (Prinivil, Zestril), Moexipril, Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), Trandolapril (Mavik), Zofenopril
pyroglutamyl peptidase II (Q9NXJ5)	Release of the N-terminal pyroglutamyl group from pGlu--His-Xaa tripeptides and pGlu--His-Xaa-Gly tetrapeptides	
dipeptidyl peptidase IV (P27487, P14740, P28843)	Release of an N-terminal dipeptide, Xaa-Yaa- -Zaa-, from a polypeptide, preferentially when Yaa is Pro, provided Zaa is neither Pro nor hydroxyproline	
N-arginine dibasic convertase (O43847, Q8BHG1)	Hydrolysis of polypeptides, preferably at -Xaa- -Arg-Lys-, and less commonly at -Arg- -Arg-Xaa-, in which Xaa is not Arg or Lys	
endopeptidase 24.15 (thimet oligopeptidase) (P52888, P24155)	Preferential cleavage of bonds with hydrophobic residues at P1, P2 and P3' and a small residue at P1' in substrates of 5 to 15 residues	
endopeptidase 24.16 (neurolysin) (Q9BYT8, Q91YP2)	Preferential cleavage in neurotensin: 10-Pro- -Tyr-11	
amyloid precursor protein secretase alpha (P05067, P12023, Q9Y5Z0, P56817)	Endopeptidase of broad specificity.	
amyloid precursor protein secretase beta (P05067, P12023, Q9Y5Z0, P56817)	Broad endopeptidase specificity. Cleaves Glu-Val-Asn-Leu- -Asp-Ala-Glu-Phe (EVNLDAAEF; SEQ ID NO: 152) in the Swedish variant of Alzheimer's amyloid precursor protein	
amyloid precursor protein secretase gamma (P05067, P12023, Q9Y5Z0, P56817)	intramembrane cleavage of integral membrane proteins	
MMP 1 (P03956, Q9EPL5uy)	Cleavage of the triple helix of collagen at about three-quarters of the length of the molecule from the N-terminus, at 775-Gly- -Ile-	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	776 in the alpha-1(I) chain. Cleaves synthetic substrates and alpha-macroglobulins at bonds where P1' is a hydrophobic residue.	
MMP 2 (P08253, P33434)	Cleavage of gelatin type I and collagen types IV, V, VII, X. Cleaves the collagen-like sequence Pro-Gln-Gly- -Ile-Ala-Gly-Gln (PQGIAGQ; SEQ ID NO: 153).	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 3 (P08254, P28862)	Preferential cleavage where P1', P2' and P3' are hydrophobic residues.	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 7 (P09237, Q10738)	Cleavage of 14-Ala- -Leu-15 and 16-Tyr- -Leu-17 in B chain of insulin. No action on collagen types I, II, IV, V. Cleaves gelatin chain alpha-2(I) > alpha-1(I).	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 8 (P22894, O70138)	Can degrade fibrillar type I, II, and III collagens. Cleavage of interstitial collagens in the triple helical domain. Unlike EC 3.4.24.7, this enzyme cleaves type III collagen more slowly than type I.	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 9 (P14780, P41245)	Cleavage of gelatin types I and V and collagen types IV and V. Cleaves KiSS1 at a Gly- -Leu bond. Cleaves type IV and type V collagen into large C-terminal three quarter fragments and shorter N-terminal one quarter fragments. Degrades fibronectin but not laminin or Pz-peptide.	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 10 (P09238, O55123)	Can degrade fibronectin, gelatins of type I, III, IV, and V; weakly collagens III, IV, and V.	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 11 (P24347, Q02853)	A(A/Q)(N/A)↓(L/Y)(T/V/M/R)(R/K) G(G/A)E↓LR	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	↓ denotes the cleavage site	
MMP 12 (P39900, P34960)	Hydrolysis of soluble and insoluble elastin. Specific cleavages are also produced at 14-Ala- -Leu-15 and 16-Tyr- -Leu-17 in the B chain of insulin Has significant elastolytic activity. Can accept large and small amino acids at the P1' site, but has a preference for leucine. Aromatic or hydrophobic residues are preferred at the P1 site, with small hydrophobic residues (preferably alanine) occupying P3	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 13 (P45452, P33435)	Cleaves triple helical collagens, including type I, type II and type III collagen, but has the highest activity with soluble type II collagen. Can also degrade collagen type IV, type XIV and type X	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
MMP 14 (P50281, P53690)	Activates progelatinase A by cleavage of the propeptide at 37-Asn- -Leu-38. Other bonds hydrolyzed include 35-Gly- -Ile-36 in the propeptide of collagenase 3, and 341-Asn- -Phe-342, 441-Asp- -Leu-442 and 354-Gln- -Thr-355 in the aggrecan interglobular domain.	SB-3CT <i>p</i> -OH SB-3CT <i>O</i> -phosphate SB-3CT RXP470.1
urokinase plasminogen activator (uPA) (P00749, P06869)	Specific cleavage of Arg- -Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
tissue plasminogen activator (tPA) (P00750, P11214)	Specific cleavage of Arg- -Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
tissue plasminogen activator (tPA) (P00750, P11214)	Specific cleavage of Arg- -Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
Plasmin (P00747, P20918)	Preferential cleavage: Lys- -Xaa > Arg- -Xaa, higher selectivity than trypsin. Converts fibrin into soluble products.	α -2-antiplasmin (AP)
Thrombin (P00734, P19221)	Cleaves bonds after Arg and Lys Converts fibrinogen to fibrin and activates factors V, VII, VIII, XIII,	

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
	and, in complex with thrombomodulin, protein C.	
BMP-1 (procollagen C-peptidase) (P13497, P98063)	Cleavage of the C-terminal propeptide at Ala- -Asp in type I and II procollagens and at Arg- -Asp in type III.	
ADAM (Q9P0K1, Q9UKQ2, Q9JLN6, O14672, Q13444, P78536, Q13443, O43184, P78325, Q9UKF5, Q9BZ11, Q9H2U9, Q99965, O75077, Q9H013, O43506)		SB-3CT p-OH SB-3CT O-phosphate SB-3CT RXP470.1
granzyme A (P12544, P11032)	Preferential cleavage: -Arg- -Xaa-, -Lys- -Xaa- >> -Phe- -Xaa- in small molecule substrates.	
granzyme B (P10144, P04187)	Preference for bulky and aromatic residues at the P1 position and acidic residues at the P3' and P4' sites.	
granzyme M (P51124, Q03238)	Cleaves peptide substrates after methionine, leucine, and norleucine.	
tobacco Etch virus (TEV) protease (P04517, P0CK09)	E-Xaa-Xaa-Y -Xaa-Q-(G/S), with cleavage occurring between Q and G/S. The most common sequence is ENLYFQS (SEQ ID NO: 154)	
chymotrypsin-like serine protease (P08217, Q9UNI1, Q91X79, P08861, P09093, P08218)		- <i>Thermobifida fusca</i> Thermopin - <i>Pyrobaculum aerophilum</i> Aeropin - <i>Thermococcus kodakaraensis</i> Tk-serpin - <i>Alteromonas sp.</i> Marinostatin - <i>Streptomyces misionensis</i> SMTI - <i>Streptomyces sp.</i> chymostatin
alphavirus proteases (P08411, P03317, P13886, Q8JUX6, Q86924, Q4QXJ8, Q8QL53, P27282, Q5XXP4)		

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
chymotrypsin-like cysteine proteases (Q86TL0, Q14790, Q99538, O15553)		- <i>Thermobifida fusca</i> Thermopin - <i>Pyrobaculum</i> <i>aerophilum</i> Aeropin - <i>Thermococcus</i> <i>kodakaraensis</i> Tk-serpin - <i>Alteromonas</i> sp. Marinostatin - <i>Streptomyces</i> <i>misionensis</i> SMTI - <i>Streptomyces</i> sp. chymostatin
papain-like cysteine proteases (P25774, P53634, Q96K76)		
picornavirus leader proteases (P03305, P03311, P13899)		
HIV proteases (P04585, P03367, P04584, P03369, P12497, P03366, P04587)		
Herpesvirus proteases (P10220, Q2HRB6, O40922, Q69527)		
adenovirus proteases (P03252, P24937, Q83906, P68985, P09569, P11825, P10381)		
<i>Streptomyces griseus</i> protease A (SGPA) (P00776)		
<i>Streptomyces griseus</i> protease B (SGPB) (P00777)		
alpha-lytic protease (P85142, P00778)		
serine proteases (P48740, P98064, Q9UL52, P05981, O60235)		
cysteine proteases (Q86TL0, Q14790, Q8WYN0, Q96DT6, P55211)		
aspartic proteases (Q9Y5Z0, P56817, Q00663, Q53RT3, P0CY27)		

Protease (UniProt Accession No.)	Cognate cleavage site	Protease inhibitors
threonine proteases (Q9UI38, Q16512, Q9H6P5, Q8IWU2)		
Mast cell (MC) chymase (CMA1) (NM_001836)	Abz-HPFHL(SEQ ID NO: 155)- Lys(Dnp)-NH ₂	BAY 1142524 SUN13834
Rat mast cell protease-1, - 2, -3, -4, -5 (NM_017145, NM_172044, NM_001170466, NM_019321, NM_013092)	Abz-HPFHL(SEQ ID NO: 155)- Lys(Dnp)-NH ₂	TY-51469
Rat vascular chymase (RVCH) (O70500)	Abz-HPFHL(SEQ ID NO: 155)- Lys(Dnp)-NH ₂	
DENV NS3pro (NS2B/NS3) SEQ ID NOs: 157, 158, 159, 160	A strong preference for basic amino acid residues (Arg/Lys) at the P1 positions was observed, whereas the preferences for the P2–4 sites were in the order of Arg > Thr > Gln/Asn/Lys for P2, Lys > Arg > Asn for P3, and Nle > Leu > Lys > Xaa for P4. The prime site substrate specificity was for small and polar amino acids in P1 and P3.	Anthraquinone BP13944 ZINC04321905 MB21 Policresulen SK-12 NSC135618 Biliverdin

- A protease can be any of the following human proteases (MEROPS peptidase database number provided in parentheses; Rawlings N. D., Morton F. R., Kok, C. Y., Kong, J. & Barrett A. J. (2008) MEROPS: the peptidase database. Nucleic Acids Res. 36 Database issue, D320-325; herein incorporated by reference for all purposes): pepsin A (MER000885),
- 5 gastricsin (MER000894), memapsin-2 (MER005870), renin (MER000917), cathepsin D (MER000911), cathepsin E (MER000944), memapsin-1 (MER005534), napsin A (MER004981), Mernase-AA034 peptidase (MER014038), pepsin A4 (MER037290), pepsin A5 (Homo sapiens) (MER037291), hCG1733572 (Homo sapiens)-type putative peptidase
- 10 (MER107386), napsin B pseudogene (MER004982), CYMP g.p. (Homo sapiens) (MER002929), subfamily A1A unassigned peptidases (MER181559), mouse mammary tumor virus retropepsin (MER048030), rabbit endogenous retrovirus endopeptidase (MER043650), S71-related human endogenous retropepsin (MER001812), RTVL-H-type putative peptidase (MER047117), RTVL-H-type putative peptidase (MER047133), RTVL-
- 15 H-type putative peptidase (MER047160), RTVL-H-type putative peptidase (MER047206), RTVL-H-type putative peptidase (MER047253), RTVL-H-type putative peptidase

- (MER047260), RTVL-H-type putative peptidase (MER047291), RTVL-H-type putative peptidase (MER047418), RTVL-H-type putative peptidase (MER047440), RTVL-H-type putative peptidase (MER047479), RTVL-H-type putative peptidase (MER047559), RTVL-H-type putative peptidase (MER047583), RTVL-H-type putative peptidase (MER015446),
- 5 human endogenous retrovirus retropepsin homologue 1 (MER015479), human endogenous retrovirus retropepsin homologue 2 (MER015481), endogenous retrovirus retropepsin pseudogene 1 (Homo sapiens chromosome 14) (MER029977), endogenous retrovirus retropepsin pseudogene 2 (Homo sapiens chromosome 8) (MER029665), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER002660),
- 10 endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER030286), endogenous retrovirus retropepsin pseudogene 3 (Homo sapiens chromosome 17) (MER047144), endogenous retrovirus retropepsin pseudogene 5 (Homo sapiens chromosome 12) (MER029664), endogenous retrovirus retropepsin pseudogene 6 (Homo sapiens chromosome 7) (MER002094), endogenous retrovirus retropepsin pseudogene 7
- 15 (Homo sapiens chromosome 6) (MER029776), endogenous retrovirus retropepsin pseudogene 8 (Homo sapiens chromosome Y) (MER030291), endogenous retrovirus retropepsin pseudogene 9 (Homo sapiens chromosome 19) (MER029680), endogenous retrovirus retropepsin pseudogene 10 (Homo sapiens chromosome 12) (MER002848), endogenous retrovirus retropepsin pseudogene 11 (Homo sapiens chromosome 17)
- 20 (MER004378), endogenous retrovirus retropepsin pseudogene 12 (Homo sapiens chromosome 11) (MER003344), endogenous retrovirus retropepsin pseudogene 13 (Homo sapiens chromosome 2 and similar) (MER029779), endogenous retrovirus retropepsin pseudogene 14 (Homo sapiens chromosome 2) (MER029778), endogenous retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER047158), endogenous
- 25 retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER047332), endogenous retrovirus retropepsin pseudogene 15 (Homo sapiens chromosome 4) (MER003182), endogenous retrovirus retropepsin pseudogene 16 (MER047165), endogenous retrovirus retropepsin pseudogene 16 (MER047178), endogenous retrovirus retropepsin pseudogene 16 (MER047200), endogenous retrovirus retropepsin pseudogene 16
- 30 (MER047315), endogenous retrovirus retropepsin pseudogene 16 (MER047405), endogenous retrovirus retropepsin pseudogene 16 (MER030292), endogenous retrovirus retropepsin pseudogene 17 (Homo sapiens chromosome 8) (MER005305), endogenous retrovirus retropepsin pseudogene 18 (Homo sapiens chromosome 4) (MER030288), endogenous retrovirus retropepsin pseudogene 19 (Homo sapiens chromosome 16) (MER001740),

- endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047222),
endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047454),
endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER047477),
endogenous retrovirus retropepsin pseudogene 21 (Homo sapiens) (MER004403),
5 endogenous retrovirus retropepsin pseudogene 22 (Homo sapiens chromosome X)
(MER030287), subfamily A2A non-peptidase homologues (MER047046), subfamily A2A
non-peptidase homologues (MER047052), subfamily A2A non-peptidase homologues
(MER047076), subfamily A2A non-peptidase homologues (MER047080), subfamily A2A
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10 (MER047089), subfamily A2A non-peptidase homologues (MER047091), subfamily A2A
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(MER047093), subfamily A2A non-peptidase homologues (MER047094), subfamily A2A
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(MER047099), subfamily A2A non-peptidase homologues MER047101), subfamily A2A
15 non-peptidase homologues (MER047102), subfamily A2A non-peptidase homologues
(MER047107), subfamily A2A non-peptidase homologues (MER047108), subfamily A2A
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(MER047110), subfamily A2A non-peptidase homologues MER047111), subfamily A2A
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20 (MER047118), subfamily A2A non-peptidase homologues (MER047121), subfamily A2A
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(MER047126), subfamily A2A non-peptidase homologues (MER047129), subfamily A2A
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(MER047134), subfamily A2A non-peptidase homologues (MER047135), subfamily A2A
25 non-peptidase homologues (MER047137), subfamily A2A non-peptidase homologues
(MER047140), subfamily A2A non-peptidase homologues (MER047141), subfamily A2A
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(MER047148), subfamily A2A non-peptidase homologues (MER047149), subfamily A2A
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30 (MER047154), subfamily A2A non-peptidase homologues (MER047155), subfamily A2A
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(MER047157), subfamily A2A non-peptidase homologues (MER047159), subfamily A2A
non-peptidase homologues (MER047161), subfamily A2A non-peptidase homologues
(MER047163), subfamily A2A non-peptidase homologues (MER047166), subfamily A2A

[illegible]

[illegible]

[illegible]

non-peptidase homologues (MER047533), subfamily A2A non-peptidase homologues (MER047537), subfamily A2A non-peptidase homologues (MER047569), subfamily A2A non-peptidase homologues (MER047570), subfamily A2A non-peptidase homologues (MER047584), subfamily A2A non-peptidase homologues (MER047603), subfamily A2A non-peptidase homologues (MER047604), subfamily A2A non-peptidase homologues (MER047606), subfamily A2A non-peptidase homologues (MER047609), subfamily A2A non-peptidase homologues (MER047616), subfamily A2A non-peptidase homologues (MER047619), subfamily A2A non-peptidase homologues (MER047648), subfamily A2A non-peptidase homologues (MER047649), subfamily A2A non-peptidase homologues (MER047662), subfamily A2A non-peptidase homologues (MER048004), subfamily A2A non-peptidase homologues (MER048018), subfamily A2A non-peptidase homologues (MER048019), subfamily A2A non-peptidase homologues (MER048023), subfamily A2A non-peptidase homologues (MER048037), subfamily A2A unassigned peptidases (MER047164), subfamily A2A unassigned peptidases (MER047231), subfamily A2A unassigned peptidases (MER047386), skin aspartic protease (MER057097), presenilin 1 (MER005221), presenilin 2 (MER005223), impas 1 peptidase (MER019701), impas 1 peptidase (MER184722), impas 4 peptidase (MER019715), impas 2 peptidase (MER019708), impas 5 peptidase (MER019712), impas 3 peptidase (MER019711), possible family A22 pseudogene (Homo sapiens chromosome 18) (MER029974), possible family A22 pseudogene (Homo sapiens chromosome 11) (MER023159), cathepsin V (MER004437), cathepsin X (MER004508), cathepsin F (MER004980), cathepsin L (MER000622), cathepsin S (MER000633), cathepsin O (MER001690), cathepsin K (MER000644), cathepsin W (MER003756), cathepsin H (MER000629), cathepsin B (MER000686), dipeptidyl-peptidase I (MER001937), bleomycin hydrolase (animal) (MER002481), tubulointerstitial nephritis antigen (MER016137), tubulointerstitial nephritis antigen-related protein (MER021799), cathepsin L-like pseudogene 1 (Homo sapiens) (MER002789), cathepsin B-like pseudogene (chromosome 4, Homo sapiens) (MER029469), cathepsin B-like pseudogene (chromosome 1, Homo sapiens) (MER029457), CTSLL2 g.p. (Homo sapiens) (MER005210), CTSLL3 g.p. (Homo sapiens) (MER005209), calpain-1 (MER000770), calpain-2 (MER000964), calpain-3 (MER001446), calpain-9 (MER004042), calpain-8 (MER021474), calpain-15 (MER004745), calpain-5 (MER002939), calpain-11 (MER005844), calpain-12 (MER029889), calpain-10 (MER013510), calpain-13 (MER020139), calpain-14 (MER029744), Mername-AA253 peptidase (MER005537), calpamodulin (MER000718), hypothetical protein 940251 (MER003201), ubiquitinyl hydrolase-L1 (MER000832), ubiquitinyl hydrolase-L3

(MER000836), ubiquitinyl hydrolase-BAP1 (MER003989), ubiquitinyl hydrolase-UCH37 (MER005539), ubiquitin-specific peptidase 5 (MER002066), ubiquitin-specific peptidase 6 (MER000863), ubiquitin-specific peptidase 4 (MER001795), ubiquitin-specific peptidase 8 (MER001884), ubiquitin-specific peptidase 13 (MER002627), ubiquitin-specific peptidase 2
5 (MER004834), ubiquitin-specific peptidase 11 (MER002693), ubiquitin-specific peptidase 14 (MER002667), ubiquitin-specific peptidase 7 (MER002896), ubiquitin-specific peptidase 9X (MER005877), ubiquitin-specific peptidase 10 (MER004439), ubiquitin-specific peptidase 1 (MER004978), ubiquitin-specific peptidase 12 (MER005454), ubiquitin-specific peptidase 16 (MER005493), ubiquitin-specific peptidase 15 (MER005427), ubiquitin-specific peptidase 17
10 (MER002900), ubiquitin-specific peptidase 19 (MER005428), ubiquitin-specific peptidase 20 (MER005494), ubiquitin-specific peptidase 3 (MER005513), ubiquitin-specific peptidase 9Y (MER004314), ubiquitin-specific peptidase 18 (MER005641), ubiquitin-specific peptidase 21 (MER006258), ubiquitin-specific peptidase 22 (MER012130), ubiquitin-specific peptidase 33 (MER014335), ubiquitin-specific peptidase 29 (MER012093), ubiquitin-specific peptidase 25
15 (MER011115), ubiquitin-specific peptidase 36 (MER014033), ubiquitin-specific peptidase 32 (MER014290), ubiquitin-specific peptidase 26 (Homo sapiens-type) (MER014292), ubiquitin-specific peptidase 24 (MER005706), ubiquitin-specific peptidase 42 (MER011852), ubiquitin-specific peptidase 46 (MER014629), ubiquitin-specific peptidase 37 (MER014633), ubiquitin-specific peptidase 28 (MER014634), ubiquitin-specific peptidase 47 (MER014636),
20 ubiquitin-specific peptidase 38 (MER014637), ubiquitin-specific peptidase 44 (MER014638), ubiquitin-specific peptidase 50 (MER030315), ubiquitin-specific peptidase 35 (MER014646), ubiquitin-specific peptidase 30 (MER014649), Mername-AA091 peptidase (MER014743), ubiquitin-specific peptidase 45 (MER030314), ubiquitin-specific peptidase 51 (MER014769), ubiquitin-specific peptidase 34 (MER014780), ubiquitin-specific peptidase 48 (MER064620),
25 ubiquitin-specific peptidase 40 (MER015483), ubiquitin-specific peptidase 41 (MER045268), ubiquitin-specific peptidase 31 (MER015493), Mername-AA129 peptidase (MER016485), ubiquitin-specific peptidase 49 (MER016486), Mername-AA187 peptidase (MER052579), USP17-like peptidase (MER030192), ubiquitin-specific peptidase 54 (MER028714), ubiquitin-specific peptidase 53 (MER027329), ubiquitin-specific endopeptidase 39
30 [misleading] (MER064621), Mername-AA090 non-peptidase homologue (MER014739), ubiquitin-specific peptidase 43 [misleading] (MER030140), ubiquitin-specific peptidase 52 [misleading] (MER030317), NEK2 pseudogene (MER014736), C19 pseudogene (Homo sapiens: chromosome 5) (MER029972), Mername-AA088 peptidase (MER014750), autophagin-2 (MER013564), autophagin-1 (MER013561), autophagin-3 (MER014316),

autophagin-4 (MER064622), Cezanne deubiquitinating peptidase (MER029042), Cezanne-2 peptidase (MER029044), tumor necrosis factor alpha-induced protein 3 (MER029050), trabsid peptidase (MER029052), VCIP135 deubiquitinating peptidase (MER152304), otubain-1 (MER029056), otubain-2 (MER029061), CylD protein (MER030104), UfSP1 peptidase

5 (MER042724), UfSP2 peptidase (MER060306), DUBA deubiquitinating enzyme (MER086098), KIAA0459 (Homo sapiens)-like protein (MER122467), Otud1 protein (MER125457), glycosyltransferase 28 domain containing 1, isoform CRA_c (Homo sapiens)-like (MER123606), hin1L g.p. (Homo sapiens) (MER139816), ataxin-3 (MER099998), ATXN3L putative peptidase (MER115261), Josephin domain containing 1 (Homo sapiens)

10 (MER125334), Josephin domain containing 2 (Homo sapiens) (MER124068), YOD1 peptidase (MER116559), legumain (plant alpha form) (MER044591), legumain (MER001800), glycosylphosphatidylinositol:protein transamidase (MER002479), legumain pseudogene (Homo sapiens) (MER029741), family C13 unassigned peptidases (MER175813), caspase-1 (MER000850), caspase-3 (MER000853), caspase-7 (MER002705),

15 caspase-6 (MER002708), caspase-2 (MER001644), caspase-4 (MER001938), caspase-5 (MER002240), caspase-8 (MER002849), caspase-9 (MER002707), caspase-10 (MER002579), caspase-14 (MER012083), paracaspase (MER019325), Mername-AA143 peptidase (MER021304), Mername-AA186 peptidase (MER020516), putative caspase (Homo sapiens) (MER021463), FLIP protein (MER003026), Mername-AA142 protein

20 (MER021316), caspase-12 pseudogene (Homo sapiens) (MER019698), Mername-AA093 caspase pseudogene (MER014766), subfamily C14A non-peptidase homologues (MER185329), subfamily C14A non-peptidase homologues (MER179956), separase (Homo sapiens-type) (MER011775), separase-like pseudogene (MER014797), SENP1 peptidase (MER011012), SENP3 peptidase (MER011019), SENP6 peptidase (MER011109), SENP2

25 peptidase (MER012183), SENP5 peptidase (MER014032), SENP7 peptidase (MER014095), SENP8 peptidase (MER016161), SENP4 peptidase (MER005557), pyroglutamyl-peptidase I (chordate) (MER011032), Mername-AA073 peptidase (MER029978), Sonic hedgehog protein (MER002539), Indian hedgehog protein (MER002538), Desert hedgehog protein (MER012170), dipeptidyl-peptidase III (MER004252), Mername-AA164 protein

30 (MER020410), LOC138971 g.p. (Homo sapiens) (MER020074), Atp23 peptidase (MER060642), prenyl peptidase 1 (MER004246), aminopeptidase N (MER000997), aminopeptidase A (MER001012), leukotriene A4 hydrolase (MER001013), pyroglutamyl-peptidase II (MER012221), cytosol alanyl aminopeptidase (MER002746), cystinyl aminopeptidase (MER002060), aminopeptidase B (MER001494), aminopeptidase PILS

(MER005331), arginyl aminopeptidase-like 1 (MER012271), leukocyte-derived arginine aminopeptidase (MER002968), aminopeptidase Q (MER052595), aminopeptidase O (MER019730), Tata binding protein associated factor (MER026493), angiotensin-converting enzyme peptidase unit 1 (MER004967), angiotensin-converting enzyme peptidase unit 2 (MER001019), angiotensin-converting enzyme-2 (MER011061), Mername-AA153 protein (MER020514), thimet oligopeptidase (MER001737), neurolysin (MER010991), mitochondrial intermediate peptidase (MER003665), Mername-AA154 protein (MER021317), leishmanolysin-2 (MER014492), leishmanolysin-3 (MER180031), matrix metallopeptidase-1 (MER001063), matrix metallopeptidase-8 (MER001084), matrix metallopeptidase-2 (MER001080), matrix metallopeptidase-9 (MER001085), matrix metallopeptidase-3 (MER001068), matrix metallopeptidase-10 (Homo sapiens-type) (MER001072), matrix metallopeptidase-11 (MER001075), matrix metallopeptidase-7 (MER001092), matrix metallopeptidase-12 (MER001089), matrix metallopeptidase-13 (MER001411), membrane-type matrix metallopeptidase-1 (MER001077), membrane-type matrix metallopeptidase-2 (MER002383), membrane-type matrix metallopeptidase-3 (MER002384), membrane-type matrix metallopeptidase-4 (MER002595), matrix metallopeptidase-20 (MER003021), matrix metallopeptidase-19 (MER002076), matrix metallopeptidase-23B (MER004766), membrane-type matrix metallopeptidase-5 (MER005638), membrane-type matrix metallopeptidase-6 (MER012071), matrix metallopeptidase-21 (MER006101), matrix metallopeptidase-22 (MER014098), matrix metallopeptidase-26 (MER012072), matrix metallopeptidase-28 (MER013587), matrix metallopeptidase-23A (MER037217), macrophage elastase homologue (chromosome 8, Homo sapiens) (MER030035), Mername-AA156 protein (MER021309), matrix metallopeptidase-like 1 (MER045280), subfamily M10A non-peptidase homologues (MER175912), subfamily M10A non-peptidase homologues (MER187997), subfamily M10A non-peptidase homologues (MER187998), subfamily M10A non-peptidase homologues (MER180000), meprin alpha subunit (MER001111), meprin beta subunit (MER005213), procollagen C-peptidase (MER001113), mammalian tolloid-like 1 protein (MER005124), mammalian-type tolloid-like 2 protein (MER005866), ADAMTS9 peptidase (MER012092), ADAMTS14 peptidase (MER016700), ADAMTS15 peptidase (MER017029), ADAMTS16 peptidase (MER015689), ADAMTS17 peptidase (MER016302), ADAMTS18 peptidase (MER016090), ADAMTS19 peptidase (MER015663), ADAM8 peptidase (MER003902), ADAM9 peptidase (MER001140), ADAM10 peptidase (MER002382), ADAM12 peptidase (MER005107), ADAM19 peptidase

(MER012241), ADAM15 peptidase (MER002386), ADAM17 peptidase (MER003094), ADAM20 peptidase (MER004725), ADAMDEC1 peptidase (MER000743), ADAMTS3 peptidase (MER005100), ADAMTS4 peptidase (MER005101), ADAMTS1 peptidase (MER005546), ADAM28 peptidase (Homo sapiens-type) (MER005495), ADAMTS5

5 peptidase (MER005548), ADAMTS8 peptidase (MER005545), ADAMTS6 peptidase (MER005893), ADAMTS7 peptidase (MER005894), ADAM30 peptidase (MER006268), ADAM21 peptidase (Homo sapiens-type) (MER004726), ADAMTS10 peptidase (MER014331), ADAMTS12 peptidase (MER014337), ADAMTS13 peptidase (MER015450), ADAM33 peptidase (MER015143), ovastacin (MER029996), ADAMTS20

10 peptidase (Homo sapiens-type) (MER026906), procollagen I N-peptidase (MER004985), ADAM2 protein (MER003090), ADAM6 protein (MER047044), ADAM7 protein (MER005109), ADAM18 protein (MER012230), ADAM32 protein (MER026938), non-peptidase homologue (Homo sapiens chromosome 4) (MER029973), family M12 non-peptidase homologue (Homo sapiens chromosome 16) (MER047654), family M12 non-

15 peptidase homologue (Homo sapiens chromosome 15) (MER047250), ADAM3B protein (Homo sapiens-type) (MER005199), ADAM11 protein (MER001146), ADAM22 protein (MER005102), ADAM23 protein (MER005103), ADAM29 protein (MER006267), protein similar to ADAM21 peptidase preproprotein (Homo sapiens) (MER026944), Mername-AA225 peptidase homologue (Homo sapiens) (MER047474), putative ADAM pseudogene

20 (chromosome 4, Homo sapiens) (MER029975), ADAM3A g.p. (Homo sapiens) (MER005200), ADAM1 g.p. (Homo sapiens) (MER003912), subfamily M12B non-peptidase homologues (MER188210), subfamily M12B non-peptidase homologues (MER188211), subfamily M12B non-peptidase homologues (MER188212), subfamily M12B non-peptidase homologues (MER188220), neprilysin (MER001050), endothelin-converting enzyme 1

25 (MER001057), endothelin-converting enzyme 2 (MER004776), DINE peptidase (MER005197), neprilysin-2 (MER013406), Kell blood-group protein (MER001054), PHEX peptidase (MER002062), i-AAA peptidase (MER001246), i-AAA peptidase (MER005755), paraplegin (MER004454), Afg3-like protein 2 (MER005496), Afg3-like protein 1A (MER014306), pappalysin-1 (MER002217), pappalysin-2 (MER014521), farnesylated-

30 protein converting enzyme 1 (MER002646), metalloprotease-related protein-1 (MER030873), aminopeptidase AMZ2 (MER011907), aminopeptidase AMZ1 (MER058242), carboxypeptidase A1 (MER001190), carboxypeptidase A2 (MER001608), carboxypeptidase B (MER001194), carboxypeptidase N (MER001198), carboxypeptidase E (MER001199), carboxypeptidase M (MER001205), carboxypeptidase U (MER001193),

carboxypeptidase A3 (MER001187), metallocarboxypeptidase D peptidase unit 1 (MER003781), metallocarboxypeptidase Z (MER003428), metallocarboxypeptidase D peptidase unit 2 (MER004963), carboxypeptidase A4 (MER013421), carboxypeptidase A6 (MER013456), carboxypeptidase A5 (MER017121), metallocarboxypeptidase O (MER016044), cytosolic carboxypeptidase-like protein 5 (MER033174), cytosolic carboxypeptidase 3 (MER033176), cytosolic carboxypeptidase 6 (MER033178), cytosolic carboxypeptidase 1 (MER033179), cytosolic carboxypeptidase 2 (MER037713), metallocarboxypeptidase D non-peptidase unit (MER004964), adipocyte-enhancer binding protein 1 (MER003889), carboxypeptidase-like protein X1 (MER013404), carboxypeptidase-like protein X2 (MER078764), cytosolic carboxypeptidase (MER026952), family M14 non-peptidase homologues (MER199530), insulysin (MER001214), mitochondrial processing peptidase beta-subunit (MER004497), nardilysin (MER003883), eupitrylsin (MER004877), mitochondrial processing peptidase non-peptidase alpha subunit (MER001413), ubiquinol-cytochrome c reductase core protein I (MER003543), ubiquinol-cytochrome c reductase core protein II (MER003544), ubiquinol-cytochrome c reductase core protein domain 2 (MER043998), insulysin unit 2 (MER046821), nardilysin unit 2 (MER046874), insulysin unit 3 (MER078753), mitochondrial processing peptidase subunit alpha unit 2 (MER124489), nardilysin unit 3 (MER142856), LOC133083 g.p. (Homo sapiens) (MER021876), subfamily M16B non-peptidase homologues (MER188757), leucyl aminopeptidase (animal) (MER003100), Mername-AA040 peptidase (MER003919), leucyl aminopeptidase-1 (Caenorhabditis-type) (MER013416), methionyl aminopeptidase 1 (MER001342), methionyl aminopeptidase 2 (MER001728), aminopeptidase P2 (MER004498), Xaa-Pro dipeptidase (eukaryote) (MER001248), aminopeptidase P1 (MER004321), mitochondrial intermediate cleaving peptidase 55 kDa (MER013463), mitochondrial methionyl aminopeptidase (MER014055), Mername-AA020 peptidase homologue (MER010972), proliferation-association protein 1 (MER005497), chromatin-specific transcription elongation factor 140 kDa subunit (MER026495), proliferation-associated protein 1-like (Homo sapiens chromosome X) (MER029983), Mername-AA226 peptidase homologue (Homo sapiens) (MER056262), Mername-AA227 peptidase homologue (Homo sapiens) (MER047299), subfamily M24A non-peptidase homologues (MER179893), aspartyl aminopeptidase (MER003373), Gly-Xaa carboxypeptidase (MER033182), carnosine dipeptidase II (MER014551), carnosine dipeptidase I (MER015142), Mername-AA161 protein (MER021873), aminoacylase (MER001271), glutamate carboxypeptidase II (MER002104), NAALADASE L peptidase (MER005239), glutamate carboxypeptidase III (MER005238),

plasma glutamate carboxypeptidase (MER005244), Mername-AA103 peptidase (MER015091), Fxna peptidase (MER029965), transferrin receptor protein (MER002105), transferrin receptor 2 protein (MER005152), glutaminy cyclise (MER015095), glutamate carboxypeptidase II (Homo sapiens)-type non-peptidase homologue (MER026971), nicalin (MER044627), membrane dipeptidase (MER001260), membrane-bound dipeptidase-2 (MER013499), membrane-bound dipeptidase-3 (MER013496), dihydro-orotase (MER005767), dihydropyrimidinase (MER033266), dihydropyrimidinase related protein-1 (MER030143), dihydropyrimidinase related protein-2 (MER030155), dihydropyrimidinase related protein-3 (MER030151), dihydropyrimidinase related protein-4 (MER030149), dihydropyrimidinase related protein-5 (MER030136), hypothetical protein like 5730457F11RIK (MER033184), 1300019j08rik protein (MER033186)), guanine aminohydrolase (MER037714), Kae1 putative peptidase (MER001577), OSGEPL1-like protein (MER013498), S2P peptidase (MER004458), subfamily M23B non-peptidase homologues (MER199845), subfamily M23B non-peptidase homologues (MER199846), subfamily M23B non-peptidase homologues (MER199847), subfamily M23B non-peptidase homologues (MER137320), subfamily M23B non-peptidase homologues (MER201557), subfamily M23B non-peptidase homologues (MER199417), subfamily M23B non-peptidase homologues (MER199418), subfamily M23B non-peptidase homologues (MER199419), subfamily M23B non-peptidase homologues (MER199420), subfamily M23B non-peptidase homologues (MER175932), subfamily M23B non-peptidase homologues (MER199665), Poh1 peptidase (MER020382), Jab1/MPN domain metalloenzyme (MER022057), Mername-AA165 peptidase (MER021865), Brcc36 isopeptidase (MER021890), histone H2A deubiquitinase MYSM1 (MER021887), AMSH deubiquitinating peptidase (MER030146), putative peptidase (Homo sapiens chromosome 2) (MER029970), Mername-AA168 protein (MER021886), COP9 signalosome subunit 6 (MER030137), 26S proteasome non-ATPase regulatory subunit 7 (MER030134), eukaryotic translation initiation factor 3 subunit 5 (MER030133), IFP38 peptidase homologue (MER030132), subfamily M67A non-peptidase homologues (MER191181), subfamily M67A unassigned peptidases (MER191144), granzyme B (Homo sapiens-type) (MER000168), testisin (MER005212), tryptase beta (MER000136), kallikrein-related peptidase 5 (MER005544), corin (MER005881), kallikrein-related peptidase 12 (MER006038), DESC1 peptidase (MER006298), tryptase gamma 1 (MER011036), kallikrein-related peptidase 14 (MER011038), hyaluronan-binding peptidase (MER003612), transmembrane peptidase, serine 4 (MER011104), intestinal serine peptidase (rodent) (MER016130), adrenal secretory serine peptidase (MER003734), tryptase delta 1

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

(MER002001), matriptase (MER003735), kallikrein-related peptidase 13 (MER005269), kallikrein-related peptidase 9 (MER005270), matriptase-2 (MER005278), umbilical vein peptidase (MER005421), LCLP peptidase (MER001900), spinesin (MER014385), marapsin-2 (MER021929), complement factor D-like putative peptidase (MER056164), ovochymase-2 (MER022410), HAT-like 4 peptidase (MER044589), ovochymase 1 domain 1 (MER022412), epidermis-specific SP-like putative peptidase (MER029900), testis serine peptidase 5 (MER029901), Mername-AA258 peptidase (MER000285), polyserase-IA unit 1 (MER030879), polyserase-IA unit 2 (MER030880), testis serine peptidase 2 (human-type) (MER033187), hypothetical acrosin-like peptidase (Homo sapiens) (MER033253), HAT-like 5 peptidase (MER028215), polyserase-3 unit 1 (MER061763), polyserase-3 unit 2 (MER061748), peptidase similar to tryptophan/serine protease (MER056263), polyserase-2 unit 1 (MER061777), Mername-AA123 peptidase (MER021930), HAT-like 2 peptidase (MER099184), hCG2041452-like protein (MER099172), hCG22067 (Homo sapiens) (MER099169), brain-rescue-factor-1 (Homo sapiens) (MER098873), hCG2041108 (Homo sapiens) (MER099173), polyserase-2 unit 2 (MER061760), polyserase-2 unit 3 (MER065694), Mername-AA201 (peptidase homologue) MER099175, secreted trypsin-like serine peptidase homologue (MER030000), polyserase-1A unit 3 (MER029880), azurocidin (MER000119), haptoglobin-1 (MER000233), haptoglobin-related protein (MER000235), macrophage-stimulating protein (MER001546), hepatocyte growth factor (MER000185), protein Z (MER000227), TESP1 protein (MER047214), LOC136242 protein (MER016132), plasma kallikrein-like protein 4 (MER016346), PRSS35 protein (MER016350), DKFZp586H2123-like protein (MER066474), apolipoprotein (MER000183), psi-KLK1 pseudogene (Homo sapiens) (MER033287), tryptase pseudogene I (MER015077), tryptase pseudogene II (MER015078), tryptase pseudogene III (MER015079), subfamily S1A unassigned peptidases (MER216982), subfamily S1A unassigned peptidases (MER216148), amidophosphoribosyltransferase precursor (MER003314), glutamine-fructose-6-phosphate transaminase 1 (MER003322), glutamine:fructose-6-phosphate amidotransferase (MER012158), Mername-AA144 protein (MER021319), asparagine synthetase (MER033254), family C44 non-peptidase homologues (MER159286), family C44 unassigned peptidases (MER185625) family C44 unassigned peptidases (MER185626), secernin 1 (MER045376), secernin 2 (MER064573), secernin 3 (MER064582), acid ceramidase precursor (MER100794), N-acyl ethanolamine acid amidase precursor (MER141667), proteasome catalytic subunit 1 (MER000556), proteasome catalytic subunit 2 (MER002625), proteasome catalytic subunit 3 (MER002149), proteasome catalytic subunit 11

- (MER000552), proteasome catalytic subunit 2i (MER001515), proteasome catalytic subunit 3i (MER000555), proteasome catalytic subunit 5t (MER026203), protein serine kinase c17 (MER026497), proteasome subunit alpha 6 (MER000557), proteasome subunit alpha 2 (MER000550), proteasome subunit alpha 4 (MER000554), proteasome subunit alpha 7 (MER033250), proteasome subunit alpha 5 (MER000558), proteasome subunit alpha 1 (MER000549), proteasome subunit alpha 3 (MER000553), proteasome subunit XAPC7 (MER004372), proteasome subunit beta 3 (MER001710), proteasome subunit beta 2 (MER002676), proteasome subunit beta 1 (MER000551), proteasome subunit beta 4 (MER001711), Mername-AA230 peptidase homologue (Homo sapiens) (MER047329),
- 10 Mername-AA231 pseudogene (Homo sapiens) (MER047172), Mername-AA232 pseudogene (Homo sapiens) (MER047316), glycosylasparaginase precursor (MER003299), isoaspartyl dipeptidase (threonine type) (MER031622), tarpase-1 (MER016969), gamma-glutamyltransferase 5 (mammalian-type) (MER001977), gamma-glutamyltransferase 1 (mammalian-type) (MER001629), gamma-glutamyltransferase 2 (Homo sapiens) (MER001976), gamma-glutamyltransferase-like protein 4 (MER002721), gamma-glutamyltransferase-like protein 3 (MER016970), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026204), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026205), Mername-AA211 putative peptidase (MER026207), gamma-glutamyltransferase 6 (MER159283), gamma-glutamyl transpeptidase homologue
- 20 (chromosome 2, Homo sapiens) (MER037241), polycystin-1 (MER126824), KIAA1879 protein (MER159329), polycystic kidney disease 1-like 3 (MER172554), gamma-glutamyl hydrolase (MER002963), guanine 5"-monophosphate synthetase (MER043387), carbamoyl-phosphate synthase (Homo sapiens-type) (MER078640), dihydro-orotase (N-terminal unit) (Homo sapiens-type) (MER060647), DJ-1 putative peptidase (MER003390), Mername-AA100 putative peptidase (MER014802), Mername-AA101 non-peptidase homologue (MER014803), KIAA0361 protein (Homo sapiens-type) (MER042827), F1134283 protein (Homo sapiens) (MER044553), non-peptidase homologue chromosome 21 open reading frame 33 (Homo sapiens) (MER160094), family C56 non-peptidase homologues (MER177016), family C56 non-peptidase homologues (MER176613), family C56 non-peptidase homologues (MER176918), EGF-like module containing mucin-like hormone
- 30 receptor-like 2 (MER037230), CD97 antigen (human type) (MER037286), EGF-like module containing mucin-like hormone receptor-like 3 (MER037288), EGF-like module containing mucin-like hormone receptor-like 1 (MER037278), EGF-like module containing mucin-like hormone receptor-like 4 (MER037294), cadherin EGF LAG seven-pass G-type receptor 2

precursor (Homo sapiens) (MER045397), Gpr64 (Mus musculus)-type protein (MER123205), GPR56 (Homo sapiens)-type protein (MER122057), latrophilin 2 (MER122199), latrophilin-1 (MER126380), latrophilin 3 (MER124612), protocadherin Flamingo 2 (MER124239), ETL protein (MER126267), G protein-coupled receptor 112 (MER126114), seven transmembrane helix receptor (MER125448), Gpr114 protein (MER159320), GPR126 vascular inducible G protein-coupled receptor (MER140015), GPR125 (Homo sapiens)-type protein (MER159279), GPR116 (Homo sapiens)-type G-protein coupled receptor (MER159280), GPR128 (Homo sapiens)-type G-protein coupled receptor (MER162015), GPR133 (Homo sapiens)-type protein (MER159334), GPR110 G-protein coupled receptor (MER159277), GPR97 protein (MER159322), KPG_006 protein (MER161773), KPG_008 protein (MER161835), KPG_009 protein (MER159335), unassigned homologue (MER166269), GPR113 protein (MER159352), brain-specific angiogenesis inhibitor 2 (MER159746), PIDD auto-processing protein unit 1 (MER020001), PIDD auto-processing protein unit 2 (MER063690), MUC1 self-cleaving mucin (MER074260), dystroglycan (MER054741), proprotein convertase 9 (MER022416), site-1 peptidase (MER001948), furin (MER000375), proprotein convertase 1 (MER000376), proprotein convertase 2 (MER000377), proprotein convertase 4 (MER028255), PACE4 proprotein convertase (MER000383), proprotein convertase 5 (MER002578), proprotein convertase 7 (MER002984), tripeptidyl-peptidase II (MER000355), subfamily S8A non-peptidase homologues (MER201339), subfamily S8A non-peptidase homologues (MER191613), subfamily S8A unassigned peptidases (MER191611), subfamily S8A unassigned peptidases (MER191612), subfamily S8A unassigned peptidases (MER191614), tripeptidyl-peptidase I (MER000357), prolyl oligopeptidase (MER000393), dipeptidyl-peptidase IV (eukaryote) (MER000401), acylaminoacyl-peptidase (MER000408), fibroblast activation protein alpha subunit (MER000399), PREPL A protein (MER004227), dipeptidyl-peptidase 8 (MER013484), dipeptidyl-peptidase 9 (MER004923), FLJ1 putative peptidase (MER017240), Mername-AA194 putative peptidase (MER017353), Mername-AA195 putative peptidase (MER017367), Mername-AA196 putative peptidase (MER017368), Mername-AA197 putative peptidase (MER017371), C14orf29 protein (MER033244), hypothetical protein (MER033245), hypothetical esterase/lipase/thioesterase (MER047309), protein bat5 (MER037840), hypothetical protein flj40219 (MER033212), hypothetical protein flj37464 (MER033240), hypothetical protein flj33678 (MER033241), dipeptidylpeptidase homologue DPP6 (MER000403), dipeptidylpeptidase homologue DPP10 (MER005988), protein similar to Mus musculus chromosome 20 open reading frame 135

- (MER037845), kynurenine formamidase (MER046020), thyroglobulin precursor (MER011604), acetylcholinesterase (MER033188), cholinesterase (MER033198), carboxylesterase D1 (MER033213), liver carboxylesterase (MER033220), carboxylesterase 3 (MER033224), carboxylesterase 2 (MER033226), bile salt-dependent lipase (MER033227),
- 5 carboxylesterase-related protein (MER033231), neuroligin 3 (MER033232), neuroligin 4, X-linked (MER033235), neuroligin 4, Y-linked (MER033236), esterase D (MER043126), arylacetamide deacetylase (MER033237), KIAA1363-like protein (MER033242), hormone-sensitive lipase (MER033274), neuroligin 1 (MER033280), neuroligin 2 (MER033283), family S9 non-peptidase homologues (MER212939), family S9 non-peptidase homologues
- 10 (MER211490), subfamily S9C unassigned peptidases (MER192341), family S9 unassigned peptidases (MER209181), family S9 unassigned peptidases (MER200434), family S9 unassigned peptidases (MER209507), family S9 unassigned peptidases (MER209142), serine carboxypeptidase A (MER000430), vitellogenic carboxypeptidase-like protein (MER005492), RISC peptidase (MER010960), family S15 unassigned peptidases
- 15 (MER199442), family S15 unassigned peptidases (MER200437), family S15 unassigned peptidases (MER212825), lysosomal Pro-Xaa carboxypeptidase (MER000446), dipeptidyl-peptidase II (MER004952), thymus-specific serine peptidase (MER005538), epoxide hydrolase-like putative peptidase (MER031614), Loc328574-like protein (MER033246), abhydrolase domain-containing protein 4 (MER031616), epoxide hydrolase (MER000432),
- 20 mesoderm specific transcript protein (MER199890), mesoderm specific transcript protein (MER017123), cytosolic epoxide hydrolase (MER029997), cytosolic epoxide hydrolase (MER213866), similar to hypothetical protein FLJ22408 (MER031608), CGI-58 putative peptidase (MER030163), Williams-Beuren syndrome critical region protein 21 epoxide hydrolase (MER031610), epoxide hydrolase (MER031612), hypothetical protein 922408
- 25 (epoxide hydrolase) (MER031617), monoglyceride lipase (MER033247), hypothetical protein (MER033249), valacyclovir hydrolase (MER033259), Ccg1-interacting factor b (MER210738), glycosylasparaginase precursor (MER003299), isoaspartyl dipeptidase (threonine type) (MER031622), taspase-1 (MER016969), gamma-glutamyltransferase 5 (mammalian-type) (MER001977), gamma-glutamyltransferase 1 (mammalian-type)
- 30 (MER001629), gamma-glutamyltransferase 2 (Homo sapiens) (MER001976), gamma-glutamyltransferase-like protein 4 (MER002721), gamma-glutamyltransferase-like protein 3 (MER016970), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026204), similar to gamma-glutamyltransferase 1 precursor (Homo sapiens) (MER026205), Mername-AA211 putative peptidase (MER026207), gamma-

glutamyltransferase 6 (MER159283). gamma-glutamyl transpeptidase homologue (chromosome 2, Homo sapiens) (MER037241). polycystin-1 (MER126824), KIAA1879 protein (MER159329). polycystic kidney disease 1-like 3 (MER172554). gamma-glutamyl hydrolase (MER002963). guanine 5''-monophosphate synthetase (MER043387). carbamoyl-phosphate synthase (Homo sapiens-type) (MER078640). dihydro-orotase (N-terminal unit) (Homo sapiens-type) (MER060647). DJ-1 putative peptidase (MER003390). Mername-AA100 putative peptidase (MER014802). Mername-AA101 non-peptidase homologue (MER014803). KIAA0361 protein (Homo sapiens-type) (MER042827). F1134283 protein (Homo sapiens) (MER044553). non-peptidase homologue chromosome 21 open reading frame 33 (Homo sapiens) (MER160094). family C56 non-peptidase homologues (MER177016), family C56 non-peptidase homologues (MER176613). family C56 non-peptidase homologues (MER176918). EGF-like module containing mucin-like hormone receptor-like 2 (MER037230). CD97 antigen (human type) (MER037286). EGF-like module containing mucin-like hormone receptor-like 3 (MER037288). EGF-like module containing mucin-like hormone receptor-like 1 (MER037278). EGF-like module containing mucin-like hormone receptor-like 4 (MER037294). cadherin EGF LAG seven-pass G-type receptor 2 precursor (Homo sapiens) (MER045397), Gpr64 (Mus musculus)-type protein (MER123205). GPR56 (Homo sapiens)-type protein (MER122057). latrophilin 2 (MER122199). latrophilin-1 (MER126380). latrophilin 3 (MER124612). protocadherin Flamingo 2 (MER124239). ETL protein (MER126267). G protein-coupled receptor 112 (MER126114). seven transmembrane helix receptor (MER125448). Gpr114 protein (MER159320). GPR126 vascular inducible G protein-coupled receptor (MER140015). GPR125 (Homo sapiens)-type protein (MER159279). GPR116 (Homo sapiens)-type G-protein coupled receptor (MER159280). GPR128 (Homo sapiens)-type G-protein coupled receptor (MER162015). GPR133 (Homo sapiens)-type protein (MER159334) GPR110 G-protein coupled receptor (MER159277), GPR97 protein (MER159322), KPG_006 protein (MER161773) KPG_008 protein (MER161835), KPG_009 protein (MER159335), unassigned homologue (MER166269), GPR113 protein (MER159352), brain-specific angiogenesis inhibitor 2 (MER159746), PIDD auto-processing protein unit 1 (MER020001), PIDD auto-processing protein unit 2 (MER063690), MUC1 self-cleaving mucin (MER074260), dystroglycan (MER054741), proprotein convertase 9 (MER022416), site-1 peptidase (MER001948), furin (MER000375), proprotein convertase 1 (MER000376), proprotein convertase 2 (MER000377), proprotein convertase 4 (MER028255), PACE4 proprotein convertase (MER000383), proprotein convertase 5 (MER002578), proprotein

convertase 7 (MER002984), tripeptidyl-peptidase II (MER000355), subfamily S8A non-peptidase homologues (MER201339), subfamily S8A non-peptidase homologues (MER191613), subfamily S8A unassigned peptidases (MER191611), subfamily S8A unassigned peptidases (MER191612), subfamily S8A unassigned peptidases (MER191614),
 5 tripeptidyl-peptidase I (MER003575), prolyl oligopeptidase (MER000393), dipeptidyl-peptidase IV (eukaryote) (MER000401), acylaminoacyl-peptidase (MER000408), fibroblast activation protein alpha subunit (MER000399), PREPL A protein (MER004227), dipeptidyl-peptidase 8 (MER013484), dipeptidyl-peptidase 9 (MER004923), FLJ1 putative peptidase (MER017240), Mername-AA194 putative peptidase (MER017353), Mername-AA195
 10 putative peptidase (MER017367), Mername-AA196 putative peptidase (MER017368), Mername-AA197 putative peptidase (MER017371), C14orf29 protein (MER033244), hypothetical protein (MER033245), hypothetical esterase/lipase/thioesterase (MER047309), protein bat5 (MER037840), hypothetical protein flj40219 (MER033212), hypothetical protein flj37464 (MER033240), hypothetical protein flj33678 (MER033241),
 15 dipeptidylpeptidase homologue DPP6 (MER000403), dipeptidylpeptidase homologue DPP10 (MER005988), protein similar to Mus musculus chromosome 20 open reading frame 135 (MER037845), kynurenine formamidase (MER046020), thyroglobulin precursor (MER011604), acetylcholinesterase (MER033188), cholinesterase (MER033198), carboxylesterase D1 (MER033213), liver carboxylesterase (MER033220), carboxylesterase 3
 20 (MER033224), carboxylesterase 2 (MER033226), bile salt-dependent lipase (MER033227), carboxylesterase-related protein (MER033231), neuroligin 3 (MER033232), neuroligin 4, X-linked (MER033235), neuroligin 4, Y-linked (MER033236), esterase D (MER043126), arylacetamide deacetylase (MER033237), KIAA1363-like protein (MER033242), hormone-sensitive lipase (MER033274), neuroligin 1 (MER033280), neuroligin 2 (MER033283),
 25 family S9 non-peptidase homologues (MER212939), family S9 non-peptidase homologues (MER211490), subfamily S9C unassigned peptidases (MER192341), family S9 unassigned peptidases (MER209181), family S9 unassigned peptidases (MER200434), family S9 unassigned peptidases (MER209507), family S9 unassigned peptidases (MER209142), serine carboxypeptidase A (MER000430), vitellogenic carboxypeptidase-like protein
 30 (MER005492), RISC peptidase (MER010960), family S15 unassigned peptidases (MER199442), family S15 unassigned peptidases (MER200437), family S15 unassigned peptidases (MER212825), lysosomal Pro-Xaa carboxypeptidase (MER000446), dipeptidyl-peptidase II (MER004952), thymus-specific serine peptidase (MER005538), epoxide hydrolase-like putative peptidase (MER031614), Loc328574-like protein (MER033246),

abhydrolase domain-containing protein 4 (MER031616), epoxide hydrolase (MER000432), mesoderm specific transcript protein (MER199890), mesoderm specific transcript protein (MER017123), cytosolic epoxide hydrolase (MER029997), cytosolic epoxide hydrolase (MER213866), similar to hypothetical protein FLJ22408 (MER031608), CGI-58 putative
 5 peptidase (MER030163), Williams-Beuren syndrome critical region protein 21 epoxide hydrolase (MER031610), epoxide hydrolase (MER031612), hypothetical protein flj22408 (epoxide hydrolase) (MER031617), monoglyceride lipase (MER033247), hypothetical protein (MER033249), valacyclovir hydrolase (MER033259), Ccg1-interacting factor b (MER210738).

10 Protease enzymatic activity can be regulated. For example, certain proteases can be inactivated by the presence or absence of a specific agent (e.g., that binds to the protease, such as specific small molecule inhibitors). Such proteases can be referred to as a “repressible protease.” Exemplary inhibitors for certain proteases are listed in Table 4B. For example, an NS3 protease can be repressed by a protease inhibitor including, but not limited to,
 15 simprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir. In another example, protease activity can be regulated through regulating expression of the protease itself, such as engineering a cell to express a protease using an inducible promoter system (e.g., Tet On/Off systems) or cell-specific promoters (promoters that can be used to express a heterologous protease are
 20 described in more detail in the Section herein titled “Promoters”). A protease can also contain a degron, such as any of the degrons described herein, and can be regulated using any of the degron systems described herein.

Protease enzymatic activity can also be regulated through selection of a specific protease cleavage site. For example, a protease cleavage site can be selected and/or
 25 engineered such that the sequence demonstrates a desired rate-of-cleavage by a desired protease, such as reduced cleavage kinetics relative to an endogenous sequence of a substrate naturally cleaved by the desired protease. As another example, a protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage in a cell-state specific manner. For example, various cell states (e.g., following cellular
 30 signaling, such as immune cell activation) can influence the expression and/or localization of certain proteases. As an illustrative example, ADAM17 protein levels and localization is known to be influenced by signaling, such as through Protein kinase C (PKC) signaling pathways (e.g., activation by the PKC activator Phorbol-12-myristat-13-acetate [PMA]). Accordingly, a protease cleavage site can be selected and/or engineered such that cleavage of

the protease cleavage site and subsequent release of an effector molecule is increased or decreased, as desired, depending on the protease properties (*e.g.*, expression and/or localization) in a specific cell state. As another example, a protease cleavage site (particularly in combination with a specific membrane tethering domain) can be selected and/or engineered for optimal protein expression of the chimeric protein.

Cell Membrane Tethering Domain

The membrane-cleavable chimeric proteins provided for herein contain a cell-membrane tethering domain (referred to as “MT” in the formula **S – C – MT** or **MT – C – S**). In general, the cell-membrane tethering domain can be any amino acid sequence motif capable of directing the chimeric protein to be localized to (*e.g.*, inserted into), or otherwise associated with, the cell membrane of the cell expressing the chimeric protein. The cell-membrane tethering domain can be a transmembrane-intracellular domain. The cell-membrane tethering domain can be a transmembrane domain. The cell-membrane tethering domain can be an integral membrane protein domain (*e.g.*, a transmembrane domain). The cell-membrane tethering domain can be derived from a Type I, Type II, or Type III transmembrane protein. The cell-membrane tethering domain can include post-translational modification tag, or motif capable of post-translational modification to modify the chimeric protein to include a post-translational modification tag, where the post-translational modification tag allows association with a cell membrane. Examples of post-translational modification tags include, but are not limited to, lipid-anchor domains (*e.g.*, a GPI lipid-anchor, a myristoylation tag, or palmitoylation tag). Examples of cell-membrane tethering domains include, but are not limited to, a transmembrane-intracellular domain and/or transmembrane domain derived from PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, or BTLA. The cell membrane tethering domain can be a cell surface receptor or a cell membrane-bound portion thereof.

In some embodiments, the cell-membrane tethering domain comprises a transmembrane domain derived from a B-71 polypeptide. In some embodiments, the transmembrane domain comprises the sequence
LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204)

In some embodiments, the cell-membrane tethering domain comprises a transmembrane domain derived from a CD8 polypeptide. Any suitable CD8 polypeptide may

be used. Exemplary CD8 polypeptides include, without limitation, NCBI Reference Nos. NP_001139345 and AAA92533.1. Examples of CD8 transmembrane domains include IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO:205),

IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO:206), and

- 5 IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO:207). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO:205). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO:206). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 207). In some embodiments, the cell-membrane tethering domain comprises a hinge and transmembrane domain derived from CD8. In some embodiments, the CD8 hinge comprises the sequence
- TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 208). In some embodiments, the CD8 hinge comprises the sequence
- 15 AAAFVPVFLPAKPTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACDI YIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 209).

In general, for all membrane-cleavable chimeric proteins described herein, the cell membrane tethering domain is either: (1) C-terminal of the protease cleavage site and N-terminal of any intracellular domain, if present (in other words, the cell membrane tethering domain is in between the protease cleavage site and, if present, an intracellular domain); or

20 (2) N-terminal of the protease cleavage site and C-terminal of any intracellular domain, if present (also between the protease cleavage site and, if present, an intracellular domain with domain orientation inverted). In embodiments featuring a degron associated with the chimeric protein, the degron domain is the terminal cytoplasmic-oriented domain, specifically relative

25 to the cell membrane tethering (in other words, the cell membrane tethering domain is in between the protease cleavage site and the degron). The cell membrane tethering domain can be connected to the protease cleavage site by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of cell membrane tethering domain or protease cleavage site. The cell membrane tethering domain can be connected to an intracellular

30 domain, if present, by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or the intracellular domain. The cell membrane tethering domain can be connected to the degron, if present, by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or degron. A polypeptide linker can be any amino acid sequence that

connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, [GS]₄GG [SEQ ID NO: 182]), A(EAAAK)₃A (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a “KEGS” linker such as the amino acid sequence KESGVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art.

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

Degron Systems and Domains

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

The degron domain can be a cereblon (CRBN) polypeptide substrate domain capable of binding CRBN in response to an immunomodulatory drug (IMiD) including, but not limited to, IKZF1, IKZF3, CK1a, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN. The CRBN polypeptide substrate domain can be a chimeric fusion product of native CRBN polypeptide sequences, such as a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRR DAL (SEQ ID NO: 175). Degron domains, and in particular CRBN degron systems, are

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

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

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

protein such that the effector molecule is released (“secreted”) into the extracellular space. In the presence of an immunomodulatory drug (IMiD), the degron domain directs ubiquitin-mediated degradation of the chimeric protein such that secretion of the effector molecule is reduced or eliminated. In general, for membrane-cleavable chimeric proteins fused to a

5 degron domain, the degron domain is the terminal cytoplasmic-oriented domain, specifically relative to the cell membrane tethering domain, *e.g.*, the most C-terminal domain in the formula **S – C – MT – D** or the most N-terminal domain in the formula **D – MT – C – S**. The degron domain can be connected to the cell membrane tethering domain by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane

10 tethering domain or the degron domain. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, [GS]₄GG [SEQ ID NO: 182]), A(EAAAK)₃A (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a “KEGS” linker such as the

15 amino acid sequence KESGVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*,

20 length, flexibility, amino acid composition etc.) and are known to those skilled in the art. In general, the degron is oriented in relation to the cell membrane tethering domain such that the degron is exposed to the cytosol following localization to the cell membrane such that the degron domain is capable of mediating degradation (*e.g.*, exposure to the cytosol and cytosol) and is capable of mediating ubiquitin-mediated degradation.

25 For degron-fusion proteins, the degron domain can be N-terminal or C-terminal of the protein of interest, *e.g.*, the effector molecule. The degron domain can be connected to the protein of interest by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the protein of interest or the degron domain. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second

30 polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, [GS]₄GG [SEQ ID NO: 182]), A(EAAAK)₃A (SEQ ID NO: 183), and Whitlow linkers (*e.g.*, a “KEGS” linker such as the amino acid sequence KESGVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO:

185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are
5 known to those skilled in the art. A polypeptide linker can be cleavable, *e.g.*, any of the protease cleavage sites described herein.

Homing Molecules

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

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

In some embodiments, a homing molecule is a chemokine receptor (cell surface
25 molecule that binds to a chemokine). Chemokines are small cytokines or signaling proteins secreted by cells that can induce directed chemotaxis in cells. Chemokines can be classified into four main subfamilies: CXC, CC, CX3C and XC, all of which exert biological effects by binding selectively to chemokine receptors located on the surface of target cells. In some embodiments, engineered nucleic acids are configured to produce CXCR4, a chemokine
30 receptor which allows engineered cells to home along a chemokine gradient towards a stromal cell-derived factor 1 (also known as SDF1, C-X-C motif chemokine 12, and CXCL12)-expressing cell, tissue, or tumor. Non-limiting examples of chemokine receptors that may be encoded by the engineered nucleic acids of the present disclosure include: CXC

chemokine receptors (*e.g.*, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7), CC chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, and CCR11), CX3C chemokine receptors (*e.g.*, CX3CR1, which binds to CX3CL1), and XC chemokine receptors (*e.g.*, XCR1). In some embodiments, a

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

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

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

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

In some embodiments, a homing molecule is an integrin. Integrins are transmembrane

20 receptors that facilitate cell-extracellular matrix (ECM) adhesion. Integrins are obligate heterodimers having two subunits: α (alpha) and β (beta). The α subunit of an integrin may be, without limitation: ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, ITGA7, ITGA8, ITGA9, ITGA10, ITGA11, ITGAD, ITGAE, ITGAL, ITGAM, ITGAV, ITGA2B, ITGAX. The β subunit of an integrin may be, without limitation: ITGB1, ITGB2, ITGB3, ITGB4,

25 ITGB5, ITGB6, ITGB7, and ITGB8. Engineered nucleic acids can be configured to produce any combination of the integrin α and β subunits.

In some embodiments, a homing molecule is a matrix metalloproteinase (MMP). MMPs are enzymes that cleave components of the basement membrane underlying the endothelial cell wall. Non-limiting examples of MMPs include MMP-2, MMP-9, and MMP.

30 In some embodiments, engineered nucleic acids are configured to produce an inhibitor of a molecule (*e.g.*, protein) that inhibits MMPs. For example, engineered nucleic acids can be configured to express an inhibitor (*e.g.*, an RNAi molecule) of membrane type 1 MMP (MT1-MMP) or TIMP metalloproteinase inhibitor 1 (TIMP-1).

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

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

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

Engineered Nucleic Acids

Provided herein are engineered nucleic acids encoding at least one chimeric protein of the present disclosure, such as such as the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein. Provided herein are engineered nucleic acids encoding two or more chimeric proteins.

In certain embodiments described herein, the engineered nucleic acids encode an expression cassette containing a promoter and an exogenous polynucleotide sequence encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S**. S refers to a secretable effector molecule. C refers to a protease cleavage site. MT refers to a cell membrane tethering domain. The promoter is operably linked to the exogenous polynucleotide sequence and **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide.

In certain embodiments described herein, the engineered nucleic acids encode an expression cassette containing a promoter and an exogenous polynucleotide sequence encoding a membrane-cleavable chimeric protein having a protein of interest (*e.g.*, any of the effector molecules described herein). The promoter is operably linked to the exogenous polynucleotide sequence and the membrane-cleavable chimeric protein is configured to be expressed as a single polypeptide.

An “engineered nucleic acid” is a nucleic acid that does not occur in nature. It should be understood, however, that while an engineered nucleic acid as a whole is not naturally-occurring, it may include nucleotide sequences that occur in nature. In some embodiments, an engineered nucleic acid comprises nucleotide sequences from different organisms (*e.g.*, from different species). For example, in some embodiments, an engineered nucleic acid includes a murine nucleotide sequence, a bacterial nucleotide sequence, a human nucleotide sequence, and/or a viral nucleotide sequence. The term “engineered nucleic acids” includes recombinant nucleic acids and synthetic nucleic acids. A “recombinant nucleic acid” refers to a molecule

that is constructed by joining nucleic acid molecules and, in some embodiments, can replicate in a live cell. A “synthetic nucleic acid” refers to a molecule that is amplified or chemically, or by other means, synthesized. Synthetic nucleic acids include those that are chemically modified, or otherwise modified, but can base pair with naturally- occurring nucleic acid

5 molecules. Modifications include, but are not limited to, one or more modified internucleotide linkages and non-natural nucleic acids. Modifications are described in further detail in U.S. Pat. No. 6,673,611 and U.S. Application Publication 2004/0019001 and, each of which is incorporated by reference in their entirety. Modified internucleotide linkages can be a phosphorodithioate or phosphorothioate linkage. Non-natural nucleic acids can be a

10 locked nucleic acid (LNA), a peptide nucleic acid (PNA), glycol nucleic acid (GNA), a phosphorodiamidate morpholino oligomer (PMO or “morpholino”), and threose nucleic acid (TNA). Non-natural nucleic acids are described in further detail in International Application WO 1998/039352, U.S. Application Pub. No. 2013/0156849, and U.S. Pat. Nos. 6,670,461; 5,539,082; 5,185,444, each herein incorporated by reference in their entirety. Recombinant

15 nucleic acids and synthetic nucleic acids also include those molecules that result from the replication of either of the foregoing. Engineered nucleic acid of the present disclosure may be encoded by a single molecule (*e.g.*, included in the same plasmid or other vector) or by multiple different molecules (*e.g.*, multiple different independently-replicating molecules). Engineered nucleic acids can be an isolated nucleic acid. Isolated nucleic acids include, but

20 are not limited to a cDNA polynucleotide, an RNA polynucleotide, an RNAi oligonucleotide (*e.g.*, siRNAs, miRNAs, antisense oligonucleotides, shRNAs, etc.), an mRNA polynucleotide, a circular plasmid, a linear DNA fragment, a vector, a minicircle, a ssDNA, a bacterial artificial chromosome (BAC), and yeast artificial chromosome (YAC), and an oligonucleotide.

25 Engineered nucleic acid of the present disclosure may be produced using standard molecular biology methods (*see, e.g.*, Green and Sambrook, Molecular Cloning, A Laboratory Manual, 2012, Cold Spring Harbor Press). In some embodiments, engineered nucleic acid constructs are produced using GIBSON ASSEMBLY® Cloning (*see, e.g.*, Gibson, D.G. *et al.* Nature Methods, 343-345, 2009; and Gibson, D.G. *et al.* Nature Methods,

30 901-903, 2010, each of which is incorporated by reference herein). GIBSON ASSEMBLY® typically uses three enzymatic activities in a single-tube reaction: 5' exonuclease, the 5' extension activity of a DNA polymerase and DNA ligase activity. The 5' exonuclease activity chews back the 5' end sequences and exposes the complementary sequence for annealing. The polymerase activity then fills in the gaps on the annealed regions. A DNA

ligase then seals the nick and covalently links the DNA fragments together. The overlapping sequence of adjoining fragments is much longer than those used in Golden Gate Assembly, and therefore results in a higher percentage of correct assemblies. In some embodiments, engineered nucleic acid constructs are produced using IN-FUSION® cloning (Clontech).

5

Promoters

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

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

orientation in relation to a nucleic acid sequence it regulates to control (“drive”) transcriptional initiation and/or expression of that sequence.

A promoter may be one naturally associated with a gene or sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment of a given gene or sequence. Such a promoter can be referred to as “endogenous.” In some embodiments, a coding nucleic acid sequence may be positioned under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with the encoded sequence in its natural environment. Such promoters may include promoters of other genes; promoters isolated from any other cell; and synthetic promoters or enhancers that are not “naturally occurring” such as, for example, those that contain different elements of different transcriptional regulatory regions and/or mutations that alter expression through methods of genetic engineering that are known in the art. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. No. 4,683,202 and U.S. Pat. No. 5,928,906).

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

A promoter is “responsive to” or “modulated by” a local tumor state (*e.g.*, inflammation or hypoxia) or signal if in the presence of that state or signal, transcription from the promoter is activated, deactivated, increased, or decreased. In some embodiments, the promoter comprises a response element. A “response element” is a short sequence of DNA within a promoter region that binds specific molecules (*e.g.*, transcription factors) that modulate (regulate) gene expression from the promoter. Response elements that may be used in accordance with the present disclosure include, without limitation, a phloretin-adjustable

control element (PEACE), a zinc-finger DNA-binding domain (DBD), an interferon-gamma-activated sequence (GAS) (Decker, T. *et al. J Interferon Cytokine Res.* 1997 Mar;17(3):121-34, incorporated herein by reference), an interferon-stimulated response element (ISRE) (Han, K. J. *et al. J Biol Chem.* 2004 Apr 9;279(15):15652-61, incorporated herein by reference), a NF-kappaB response element (Wang, V. *et al. Cell Reports.* 2012; 2(4): 824-839, incorporated herein by reference), and a STAT3 response element (Zhang, D. *et al. J of Biol Chem.* 1996; 271: 9503-9509, incorporated herein by reference). Other response elements are encompassed herein. Response elements can also contain tandem repeats (*e.g.*, consecutive repeats of the same nucleotide sequence encoding the response element) to generally increase sensitivity of the response element to its cognate binding molecule. Tandem repeats can be labeled 2X, 3X, 4X, 5X, *etc.* to denote the number of repeats present.

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

20

Table 5A. Examples of Responsive Promoters

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
				TF	T
Transcriptional activator-responsive promoters					
AIR	PAIR (OalcA-PhCMVmin)	AlcR	Acetaldehyde	n.d.	A
ART	PART (OARG-PhCMVmin)	ArgR-VP16	l-Arginine	B	A
BIT	PBIT3 (OBirA3-PhCMVmin)	BIT (BirA-VP16)	Biotin	B	A
Cumate – activator	PCR5 (OCuO6-PhCMVmin)	cTA (CymR-VP16)	Cumate	D	DA
Cumate – reverse activator	PCR5 (OCuO6-PhCMVmin)	rcTA (rCymR-VP16)	Cumate	B	A
E-OFF	PETR (OETR-PhCMVmin)	ET (E-VP16)	Erythromycin	D	DA
NICE-OFF	PNIC (ONIC-PhCMVmin)	NT (HdnoR-VP16)	6-Hydroxy-nicotine	D	DA
PEACE	PTtgR1 (OTtgR-PhCMVmin)	TtgA1 (TtgR-VP16)	Phloretin	D	DA

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
PIP-OFF	PIR (OPIR-Phsp70min)	PIT (PIP-VP16)	Pristinamycin I	D	DA
QuoRex	PSCA (OscbR-PhCMVmin)PSP A (OpapRI-PhCMVmin)	SCA (ScbR-VP16)	SCB1	D	DA
Redox	PROP (OROP-PhCMVmin)	REDOX (REX-VP16)	NADH	D	DA
TET-OFF	PhCMV*-1 (OtetO7-PhCMVmin)	tTA (TetR-VP16)	Tetracycline	D	DA
TET-ON	PhCMV*-1 (OtetO7-PhCMVmin)	rtTA (rtTetR-VP16)	Doxycycline	B	A
TIGR	PCTA (OrheO-PhCMVmin)	CTA (RheA-VP16)	Heat	D	DA
TraR	O7x(tra box)-PhCMVmin	p65-TraR	3-Oxo-C8-HSL	B	A
VAC-OFF	PIVanO2 (OVanO2-PhCMVmin)	VanA1 (VanR-VP16)	Vanillic acid	D	DA
Transcriptional repressor-responsive promoters					
Cumate - repressor	PCuO (PCMV5-OCuO)	CymR	Cumate	D	DR
E-ON	PETRON8 (PSV40-OETR8)	E-KRAB	Erythromycin	D	DR
NICE-ON	PNIC (PSV40-ONIC8)	NS (HdnR-KRAB)	6-Hydroxy-nicotine	D	DR
PIP-ON	PPIRON (PSV40-OPIR3)	PIT3 (PIP-KRAB)	Pristinamycin I	D	DR
Q-ON	PSCAON8 (PSV40-OscbR8)	SCS (ScbR-KRAB)	SCB1	D	DR
TET-ON repressor-based	OtetO-PHPRT	tTS-H4 (TetR-HDAC4)	Doxycycline	D	DR
T-REX	PTetO (PhCMV-OtetO2)	TetR	Tetracycline	D	DR
UREX	PUREX8 (PSV40-OhucO8)	mUTS (KRAB-HucR)	Uric acid	D	DR
VAC-ON	PVanON8 (PhCMV-OVanO8)	VanA4 (VanR-KRAB)	Vanillic acid	D	DR
Hybrid promoters					
QuoRexPIP-ON(NOT IF gate)	OscbR8-OPIR3-PhCMVmin	SCAPIT3	SCB1Pristinamycin I	DD	DADR
QuoRexE-ON(NOT IF gate)	OscbR-OETR8-PhCMVmin	SCAE-KRAB	SCB1Erythromycin	DD	DADR
TET-OFFE-ON(NOT IF gate)	OtetO7-OETR8-PhCMVmin	tTAE-KRAB	TetracyclineErythromycin	DD	DADR
TET-OFFPIP-ONE-ON	OtetO7-OPIR3-OETR8-PhCMVmin	tTAPIT3E-KRAB	TetracyclinePristinamycin IErythromycin	DDD	DADRDR

Table 5B. Exemplary Components of Inducible Promoters

Name	DNA SEQUENCE
minimal promoter; minP	AGAGGGTATATAATGGAAGCTCGACTTCCAG (SEQ ID NO: 1)

Name	DNA SEQUENCE
NFkB response element protein promoter; 5x NFkB-RE	GGGAATTTCCGGGGACTTTCCGGGAATTTCCGGGGACTTTCCGGGAATTTCC (SEQ ID NO: 2)
CREB response element protein promoter; 4x CRE	CACCAGACAGTGACGTGACGTGCCAGATCCCATGGCCGTCATACTGTGACGTCTTTTCAGACACCCCCATTGACGTCAATGGGAGAA (SEQ ID NO: 3)
NFAT response element protein promoter; 3x NFAT binding sites	GGAGGAAAAAAGTGTTCATACAGAAGGCGTGGAGGAAAAAAGTGTTCATACAGAAGGCGT (SEQ ID NO: 4)
SRF response element protein promoter; 5x SRE	AGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCT (SEQ ID NO: 5)
SRF response element protein promoter 2; 5x SRF-RE	AGTATGTCCATATTAGGACATCTACCATGTCCATATTAGGACATCTATGTCCATATTAGGACATCTTGTATGTCCATATTAGGACATCTAAATGTCCATATTAGGACATCT (SEQ ID NO: 6)
AP1 response element protein promoter; 6x AP1-RE	TGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAG (SEQ ID NO: 7)
TCF-LEF response element promoter; 8x TCF-LEF-RE	AGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGTATAAGATCAAAGGGCCTAAGATCAAAGGGGACTAAGATCAAAGGGTTTAAATCAAAGGGCTTAAGATCAAAGGGCCTA (SEQ ID NO: 8)
SBEx4	GTCTAGACGTCTAGACGTCTAGACGTCTAGAC (SEQ ID NO: 9)
SMAD2/3 - CAGACA x4	CAGACACAGACACAGACACAGACA (SEQ ID NO: 10)
STAT3 binding site	Ggatccggtactcgagatctgcatctaaagtaagcttgccattccggtactgttgtaaagccac (SEQ ID NO: 11)
minCMV	taggcgtgtacggtggaggcctatataagcagagctcgtttagtgaaacggtcagatcgctgga (SEQ ID NO: 170)
YB_TATA	TCTAGAGGGTATATAATGGGGGCCA (SEQ ID NO: 171)
minTK	Ttcgcatattaagtgagcgtgtggcctgaacaccgagcgaccctgcagcgaccctgtaa (SEQ ID NO: 172)

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

Table 5C. Exemplary Constitutive Promoters

Name	DNA SEQUENCE
CMV	GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCA GTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCC CATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATG

Name	DNA SEQUENCE
	TCGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGG TCTATATAAGCAGAGCTC (SEQ ID NO: 12)
EF1a	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTT GGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAAC TGGGAAAGTGATGCCGTGTAAGTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGACAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAG AACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGG CCCTTGCGTGCCCTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCCGAG CTTCGGGTTGGAAGTGGGTGGGAGAGTTTCGAGGCCTTGCGCTTAAGGAGCCCCCTTCG CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCTGCGAATCTGG TGGCACCTTCGCGCCTGTCTCGTGTCTTCGATAAGTCTCTAGCCATTTAAATTTTT GATGACCTGTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAG ATCTGCACACTGGTATTTCCGTTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCCT CCCAGCGCACATGTTCCGCGAGGCGGGGCGCTGCGAGCGCGACCACCGAGAATCGGA CGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGTCTCGCGCCCGCTGT ATCGCCCCCGCCCGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCGTGAGCGGA AAGATGGCCGCTTCCCGGTCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCT CGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCTCA GCCGTGCTTCATGTGACTCCACGAGTACCGGGCGCCGTCCAGGCACCTCGATTAG TTCTCGAGCTTTTGAGTACGTGCTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATG GAGTTTTCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATG TAATTCCTTGGAATTTGCCCTTTTTGAGTTGGATCTTGGTTTCATTCTCAAGCCTC AGACAGTGGTTCAAAGTTTTTTTTCTTCCATTTTCAGGTGTCGTGA (SEQ ID NO: 13)
EFS	GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTC CCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGAAGGTGGC GCGGGGTAAACTGGGAAAGTGATGTCGTGTAAGTGGCTCCGCCCTTTTTCCCGAGGGTG GGGGAGAACCGTATATAAGTGACAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGT TTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTACACGCGCC GCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCG CCTGTGGTGCCTCCTGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTGCA GACCGGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCA CGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGC CGTTACAGATCCAAGCTGTGACCGGCGCCTAC (SEQ ID NO: 14)
MND	TTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCA AGCTAGGATCAAGGTTAGGAACAGAGACAGCAGAATATGGGCCAAACAGGATA TCTGTGGTAAGCAGTTCTTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGA ATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTTGCCCCGGCTCAGGGCCAA GAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAG ATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACAAACC AATCAGTTGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGA GCCCA (SEQ ID NO: 15)
PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGGCAGGGACGCGGCTG CTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTT CACGTCCGTTCGACGCTCACCCGATCTTCGCCGCTACCTTGTGGGCCCCCGGC GACGCTTCTGCTCCGCCCTAAGTCGGGAAGGTTCTTGCGGTTGCGGCGGTGCCG GACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCTTCGACAGACGGACAGCGCC AGGGAGCAATGGCAGCGCGCCGACCGCATGGGCTGTGGCCAATAGCGGCTGCTCA GCGGGGCGCGCCGAGAGCAGCGGCCGGAAGGGGCGGTGCGGGAGGCGGGGTGTG GGGCGGTAGTGTGGGCCCTGTTCTGCCCCGCGCGGTGTTCCGCATTCTGCAAGCCTC CGGAGCGCACGTGCGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCC AG (SEQ ID NO: 16)
SFFV	GTAACGCCATTTTGCAAGGCATGGAAAAATACCAACCAAGAATAGAGAAGTTCAG ATCAAGGGCGGGTACATGAAAAAGTAACTGGGCCAAACAGGATATCTGCGGT GAGCAGTTTCGGCCCCGGCCCGGGGCAAGAACAGATGGTCACCGCAGTTTCGGCC CCGGCCCCGAGGCCAAGAACAGATGGTCCCCAGATATGGCCCAACCTCAGCAGTTT CTTAAGACCCATCAGATGTTTCCAGGCTCCCCAAGGACCTGAAATGACCCTGCGCC TTATTTGAATTAACCAATCAGCCTGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTTCCC GAGCTCTATAAAAGAGCTCACAACCCCTCACTCGGCGCGCCAGTCTCCGACAGACT GAGTCGCCCCGGG (SEQ ID NO: 17)

Name	DNA SEQUENCE
SV40	CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAG AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAG GCTCCCCAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATA GTCCCGCCCCTAACTCCGCCCATCCCCCCCCTAACTCCGCCCAAGTCCGCCCAATTCTC CGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCTGCCTC TGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA GCT (SEQ ID NO: 18)
UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCTCCTCACGGCGAGCGCTGCCAC GTCAGACGAAGGGCGCAGGAGCGTTCCTGATCCTTCCGCCCCGACGCTCAGGACAG CGGCCCGCTGCTCATAAGACTCGGCCCTAGAACCCAGTATCAGCAGAAGGACATTT TAGGACGGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCCAGAGAGCGGAACA GGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGGCGG TGAACGCCGATGATTATATAAGGACGCGCCGGGTGTGGCACAGCTAGTTCCGTGCGC AGCCGGGATTTGGGTGCGCGTTCTTGTGTGGATCGCTGTGATCGTCACTTGGTGA GTTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGTGGCCCGCGGCCGCTCGGTGGGA CGGAAGCGTGTGGAGAGACCGCCAAGGGCTGTAGTCTGGGTCCGCGAGCAAGGTTG CCCTGAACTGGGGGTTGGGGGAGCGCACAAAATGGCGGCTGTTCCCGAGTCTTGA ATGGAAGACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAAACAAGGTGGGGGGCATG GTGGGCGGCAAGAACCAGGTCTTGAGGCCCTTCGCTAATGCGGGAAGCTCTTAT TCGGGTGAGATGGGCTGGGGCACCATCTGGGGACCTGACGTGAAGTTTGTCACTG ACTGGAGAACTCGGTTTGTCTGTCTGGTTGCGGGGGCGGCAGTTATGCGGTGCCGTT GGGCAGTGACCCGTACCTTTGGGAGCGCGCGCTCGTCTGTGTCGTGACGTCACCCG TTCTGTTGGCTTATAATGCAGGGTGGGGCCACCTGCCGGTAGGTGTGCGGTAGGCTT TTCTCCGTGCGCAGGACGCAGGGTTCGGGCCTAGGGTAGGCTCTCCTGAATCGACAGG CGCCCGACCTCTGGTGAGGGGAGGGATAAGTGAGGCGTCAGTTTCTTTGTCGTTT TATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGTTTTTGAATATGCGCTCGGGGT GGCGAGTGTGTTTTGTGAAGTTTTTATGGCACCTTTTGAATGTAATCATTTGGGTCA ATATGTAATTTTCAGTGTTAGACTAGTAAAGCTTCTGCAGGTCGAGTCAAAAAATT GTCCGCTAAATCTGGCCGTTTTTGGCTTTTTTGTAGAC (SEQ ID NO: 19)
hEF1aV1	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTT GGGGGGAGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGTAAAC TGGGAAAGTGATGTCGTGTAAGTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC TATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGA ACACAGGTAAGTGCCGTGTGTGTTCCCGCGGGCCTGGCCTCTTTACGGTTTATGGC CCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGC TTCGGGTTGGAAGTGGGTGGGAGAGTTTCGAGGCCCTTGCCTTAAGGAGCCCCCTCGC CTCGTGCTTGAAGTTCAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCATCTGGT GGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAATTTTTG ATGACCTGCTGCGACGCTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGA TCTGCACACTGGTATTTTCGGTTTTTGGGGCCCGGGCGGCGACGGGGCCCGTGCCTC CCAGCGACATGTTCCGGCAGGCGGGGCTGCGAGCGCGGCCACCGAATCGGAC GGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGTCTCGCGCCGCCGTGTA TCGCCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCGTGAGCGGAA AGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTC GGGAGAGCGGGCGGGTGAGTACCCACACAAAGGAAAAGGGCCTTTCCGTCTCTAG CCGTGCTTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGT TCTCGAGCTTTTGGAGTACGTCTGTTTAAAGTTGGGGGGAGGGGTTTTATGCGATGG AGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGT AATTCCTCTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCAATCTCAAGCCTCA GACAGTGGTTCAAAGTTTTTTCTTCCATTTCAAGGTGTCGTGA (SEQ ID NO: 20)
hCAGG	ACTAGTTATTAATAGTAATCAATTACGGGGTCAATTAGTTCATAGCCCATATATGGAG TTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC CGCCCATGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTC CATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCC TGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTAC GTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCC CCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTAAATTATTTGT GCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGG

[illegible]

Name	DNA SEQUENCE
	accggcgctgttgaaccggcgaggcggggctggcgcccggttgggaggggttggggcctggcttctgccgcgcgcgcggg gacgctccgaccagtgttgcctttatgtaataacgcggccggcccgcttcttgcctcccaatctgggcgcgcgcgcggccctg gcggcctaaggactcggcgcccggaagtggccaggcgggggcgacctcggctcacagcgcgcccgctat (SEQ ID NO: 23)
heIF4A1	GTTGATTTCTTCATCCCTGGCACACGTCCAGGCAGTGTCTGAATCCATCTCTGCTACA GGGGAAAACAAATAACATTTGAGTCCAGTGGAGACCGGGAGCAGAAGTAAAGGGA AGTGATAACCCCCAGAGCCCCGAAGCCTCTGGAGGCTGAGACCTCGCCCCCTTGC GTGATAGGGCCTACGGAGCCACATGACCAAGGCACTGTCCCTCCGCACGTGTGAG AGTGCAGGGCCCCAAGATGGCTGCCAGGCCCTCAGGCCTGACTCTTCTATGTCACTT CCGTACCGGCGAGAAAAGCGGGGCCCTCCAGCCAATGAGGCTGCGGGGCGGGCCTTC ACCTTGATAGGCACTCGAGTTATCCAATGGTGCCTGCGGGCCGGAGCGACTAGGAA CTAACGTTCATGCCGAGTTGCTGAGCGCCGGCAGGCGGGGCGGGGCGGCCAAACCA ATGCGATGGCCGGGGCGGAGTCGGGCGCTCTATAAGTTGTGATAGGCGGGCACTC CGCCCTAGTTTCTAAGGACCATG (SEQ ID NO: 24)
hGAPD H	AGTTCCCCAACTTTCCCGCCTCTCAGCCTTTGAAAGAAAGAAAGGGGAGGGGGCAG GCCGCGTGCAGTCGCGAGCGGTGCTGGGCTCCGGCTCCAATTCCCCATCTCAGTCGC TCCCAAAGTCCTTCTGTTTCATCCAAGCGTGTAAGGGTCCCCGTCTTGACTCCCTAG TGTCTGCTGCCCACAGTCCAGTCCCTGGGAACCAGCACCGATCACCTCCCATCGGGC CAATCTCAGTCCCTTCCCCCTACGTTCGGGGCCCCACACGCTCGGTGCGTGCCAGTT GAACCAGGCGGTGCGGAAAAAAGAGCGGGGAGAAAGTAGGGCCCGGCTACTA GCGGTTTTACGGGCGCACGTAGCTCAGGCCTCAAGACCTTGGGCTGGGACTGGCTG AGCCTGGCGGGAGGCGGGGTCCGAGTCAACGCTGCCGCGCGCCCCCGGTTTCTAT AAATTGAGCCCGCAGCCTCCCGCTTCGCTCTCTGCTCCTCTGTTGACAGTCAGCC GCATCTTCTTTGCGTCGCCAGtgaagacggcgagagaaacccgggaggctaggacggcctgaaggcggc agggcgggcgagcggcgatgtgttcgcccgtcggggtgggcccggcgccctccgattgcaggggcgggcgaggacgt gatgcggcgcggtggcatggagcctgtgggggaggggaggggagggctgggtgtcggcggggaccactaggcgctcactgt tctctccctccgcgagCCGAGCCACATCGCTGAGACAC (SEQ ID NO: 25)
hGRP78	AGTGCAGTTACACGCGAAATGCCTCGGGGTCAGAAGTCGAGGAGAGATAGACAG TCTGCTGAACCAATGGGACACGCGGATGGGGCGGATGTTATCTACCAATTGGTGAAACG TTAGAAACGAATAGCAGCCAATGAATCAGCTGGGGGGGCGGAGCAGTGACGTTTAT TGCGGAGGGGGCCGCTTCGAATCGGCGCGGCCAGCTTGGTGGCCTGGGCCAATGA ACGGCCTCCAACGAGCAGGGCCTTCACCAATCGGCGGCCTCCACGACGGGGCTGGG GGAGGGTATATAAGCCGAGTAGGCGACGGTGAGGTGACGCCGCGCAAGACAGCA CAGACAGATTGACCTATTGGGGTGTTCGCGAGTGTGAGAGGGAAGCGCCGCGGCC TGATTCTTAGACCTGCCCTTCGCCTGTTCTGTCGCGCCTTGTGACCCCGGCCCTG CCGCCTGCAAGTCGGAATTCGCTGTGCTCCTGTGCTACGGCCTGTGGCTGGACTG CCTGCTGCTGCCCCAACTGGCTGGCAC (SEQ ID NO: 26)
hGRP94	TAGTTTCATCACCACCGCCACCCCCCGCCCCCGCCATCTGAAAGGGTTCTAGGG GATTTGCAACCTCTCTCGTGTGTTTCTTTCCGAGAAGCGCCGCCACACGAGAAA GCTGGCCGCGAAAGTCGTGCTGGAATCACTTCCAACGAAACCCAGGCATAGATGG GAAAGGGTGAAGAACACGTTGCCATGGCTACCGTTTCCCCGGTACGGAATAAACG CTCTCTAGGATCCGGAAGTAGTTCGCGCGGACCTCTCTAAAAGGATGGATGTGTTT TCTGCTTACATTCAATTGGACGTTTTCCCTTAGAGGCCAAGGCCGCCAGGCAAAGGG GCGGTCCACGCGTGAGGGGCGCGGAGCCATTTGATTGGAGAAAAGCTGCAAAAC CCTGACCAATCGGAAGGAGCCACGCTTCGGGCATCGGTACCGCACCTGGACAGCT CCGATTGGTGACTTCCGCCCCCCTCACGAATCCTCATTGGGTGCCGTGGGTGCGT GGTGCGGCGGATTGGTGGGTTCATGTTTCCCGTCCCCCGCCGCGAGAAGTGGGGG TGAAAAGCGGCCCGACCTGCTTGGGGTGTAAGTGGGCGGACCGCGCGGCTGGAGGTG TGAGGATCCGAACCCAGGGGTGGGGGTGGAGGCGGCTCCTGCGATCGAAGGGGA CTTGAGACTACCGGCCGCACGTC (SEQ ID NO: 27)
hHSP70	GGGCCGCCCCTCCCCCTCCTCTCAGGGTCCCTGTCCCTCCAGTGAATCCCAGAA GACTCTGGAGAGTTCTGAGCAGGGGGCGGCACTCTGGCCTCTGATTGGTCCAAGGA AGGCTGGGGGGCAGGACGGGAGGCGAAAACCTGGAATATTTCCCGACCTGGCAGCC TCATCGAGCTCGGTGATTGGCTCAGAAGGGGAAAAGGCGGGTCTCCGTGACGACTTA TAAAAGCCAGGGGCAAGCGGTCCGGATAACGGCTAGCCTGAGGAGCTGCTGCGAC AGTCCACTACCTTTTTCGAGAGTGAAGTCCCGTTGTCCAAGGCTTCCCAGAGCGAAC CTGTGCGGCTGCAGGACCGGCGCGTTCGAGTTTCGGGCTCCGGAAGGACCGAGCT CTTCTCGCGGATCCAGTGTTCGGTTTCAGCCCCCAATCTCAGAGCGGAGCCGACAG AGAGCAGGGAAACCC (SEQ ID NO: 28)

Name	DNA SEQUENCE
hKINb	GCCCCACCCCCGTCCGCGTTACAACCGGGAGGCCCCGCTGGGTCTGCACCGTCACCC TCCTCCCTGTGACCGCCACCTGATACCCAAACAACCTTTCTCGCCCCCTCCAGTCCCCA GCTCGCCGAGCGCTTTCGCGGGAGCCACCCAGCCTCAGTTTCCCCAGCCCCGGGCGG GGCGAGGGGCGATGACGTCATGCCGCGCGCGGCATTGTGGGGCGGGGCGAGGCG GGGCGCCGGGGGAGCAACACTGAGACGCCATTTTCGGCGGGCGGGAGCGGCGCAG GCGGCCGAGCGGGACTGGCTGGGTGGCTGGGCTGCTGGTGCAGGAGCCGCGGGG CTGTGCTCGGCGGCCAAGGGGACAGCGCGTGGGTGGCCGAGGATGCTGCGGGGCGG TAGCTCCGGCGCCCCCTCGCTGGTGACTGCTGCGCCGTGCCTCACACAGCCGAGGCGG GCTCGGCGCACAGTCGCTGCTCCGCGCTCGCGCCCGGCGGCGCTCCAGGTGCTGACA GCGCGAGAGAGCGCGGCCCTCAGGAGCAACAC (SEQ ID NO: 29)
hUBIb	TTCCAGAGCTTTTCGAGGAAGGTTTCTTCAACTCAAATTCATCCGCCTGATAATTTTCT TATATTTTCTTAAAGAAGGAAGAGAAGCGCATAGAGGAGAAGGGAAATAATTTTTT AGGAGCCTTTCTTACGGCTATGAGGAATTTGGGGCTCAGTTGAAAAGCCTAAACTGC CTCTCGGGAGGTTGGGCGCGGCGAACTACTTTCAGCGGCGCACGGAGACGGCGTCT ACCGTAGGGGTGATAAGTGACGCAACACTCGTTGCATAAATTTGCGTCCGCCAGC CCGGAGCATTAGGGGCGGTTGGCTTTGTTGGGTGAGCTTGTGTGCTCCCTGTGGG TGGACGTGGTTGGTGATTGGCAGGATCCTGGTATCCGCTAACAGgtactggccacagccgtaa agacctcgggggcgtgagaggggggaatgggtgaggtcaagctggaggcttctgggggtgggtgggcccgtgaggggaggggag ggcgaggtgacgcgacacccggccttctgggagagtgggcttgttgacctaagggggcgagggcagttggcacgcgcacgcgc gacagaaactaacagacattaaccaacagcgatccgctgcgtttactgggaggaaggcgaaagagtagttgtgtgctcttgaa accctaatttgaatccagatgagaatgggtgctccttctgtgttcaatgggatttttacttcgagcttctgggttgggttttgcagtt gcctaaccagctgcttaggtttaggcagattggagttcggtcggggagttgaatatccggaacagttagttggggaagctgtggacgt tgtaagagagcgctctggatttccgctgttgacgttgaaccttgaatgacgaatttcgtattaagtgacttagccttgaataatgagggga ggcttgcggaatattaacgtatttaaggcatttgaaggaaatgtgctaatttgaagaatattaggtgtaaaagcaagaatacaatgatcctg aggtgacacgctatgttttacttttaactagGTCACC (SEQ ID NO: 30)
CAG	gacattgattattgactagtattataatagtaaatcaattacgggggtcattagtcatagcccatatatggagttccgcgtta cataactacggtaaatggcccgctggctgacgcgccaacgacccccgccattgacgtcaataatgacgtatgtt cccatagtaacgcaatagggaactttcattgacgtcaatgggtggagtagttacggtaaaactgccacttgccagta catcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgctggcattatgccca gtacatgacctatgggactttcctactggcagtagcatctacgtattagtcacgtattaccatggctgaggtgagcc ccacgttctgcttactctccccatctccccccctccccaccccaatttgtattatttttaattattttgtgcagc gatggggcgggggggggggggggcgcgccagggcgggcgggcgggcgggcgagggcgggcgggcggg gagggcgagaggtgcgcgccagccaatcagagcgcgcgctccgaaagtcttctttatggcgagggcggc ggcgggcgggcgccctataaaaagcgaagcgcgcgggcgggcg (SEQ ID NO: 173)
HLP	Tgtttgctgcttgaatgtttgccattttagggtggacacaggacgctgtgtttctgagccagggggcgactcag atcccagcagtggaacttagccctgtttgctcctccgataactggggtagccttggttaattaccagcagcctc ccccgttccccctctggatccactgcttaataacggacgaggacagggccctgtcctcagcttcagcaccacc actgacctgggacagtgaat (SEQ ID NO: 174)

- The promoter can be a tissue-specific promoter. In general, a tissue-specific promoter directs transcription of a nucleic acid, (e.g., the engineered nucleic acids encoding the chimeric proteins, such as membrane-cleavable chimeric proteins having the formula **S – C –**
- 5 **MT** or **MT – C – S**) such that expression is limited to a specific cell type, organelle, or tissue. Tissue-specific promoters include, but are not limited to, albumin (liver specific, Pinkert et al., (1987)), lymphoid specific promoters (Calame and Eaton, 1988), particular promoters of T-cell receptors (Winoto and Baltimore, (1989)) and immunoglobulins; Banerji et al., (1983); Queen and Baltimore, 1983), neuron specific promoters (e.g. the neurofilament promoter;
- 10 Byrne and Ruddle, 1989), pancreas specific promoters (Edlund et al., (1985)) or mammary

gland specific promoters (milk whey promoter, U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166) as well as developmentally regulated promoters such as the murine hox promoters (Kessel and Gruss, Science 249:374-379 (1990)) or the α -fetoprotein promoter (Campes and Tilghman, Genes Dev. 3:537-546 (1989)), the contents of

5 each of which are fully incorporated by reference herein. The promoter can be constitutive in the respective specific cell type, organelle, or tissue. Tissue-specific promoters and/or regulatory elements can also include promoters from the liver fatty acid binding (FAB) protein gene, specific for colon epithelial cells; the insulin gene, specific for pancreatic cells; the transphyretin, α .1- antitrypsin, plasminogen activator inhibitor type 1 (PAI-I),

10 apolipoprotein AI and LDL receptor genes, specific for liver cells; the myelin basic protein (MBP) gene, specific for oligodendrocytes; the glial fibrillary acidic protein (GFAP) gene, specific for glial cells; OPSIN, specific for targeting to the eye; and the neural-specific enolase (NSE) promoter that is specific for nerve cells. Examples of tissue-specific promoters include, but are not limited to, the promoter for creatine kinase, which has been used to direct

15 expression in muscle and cardiac tissue and immunoglobulin heavy or light chain promoters for expression in B cells. Other tissue specific promoters include the human smooth muscle α -actin promoter. Exemplary tissue-specific expression elements for the liver include but are not limited to HMG-COA reductase promoter, sterol regulatory element 1, phosphoenol pyruvate carboxy kinase (PEPCK) promoter, human C- reactive protein (CRP) promoter,

20 human glucokinase promoter, cholesterol L 7- α hydroylase (CYP-7) promoter, beta-galactosidase α -2,6 sialyltransferase promoter, insulin-like growth factor binding protein (IGFBP-I) promoter, aldolase B promoter, human transferrin promoter, and collagen type I promoter. Exemplary tissue-specific expression elements for the prostate include but are not limited to the prostatic acid phosphatase (PAP) promoter, prostatic secretory protein of 94

25 (PSP 94) promoter, prostate specific antigen complex promoter, and human glandular kallikrein gene promoter (hgt-1). Exemplary tissue- specific expression elements for gastric tissue include but are not limited to the human H⁺/K⁺-ATPase α subunit promoter. Exemplary tissue-specific expression elements for the pancreas include but are not limited to pancreatitis associated protein promoter (PAP), elastase 1 transcriptional enhancer, pancreas

30 specific amylase and elastase enhancer promoter, and pancreatic cholesterol esterase gene promoter. Exemplary tissue-specific expression elements for the endometrium include, but are not limited to, the uteroglobin promoter. Exemplary tissue-specific expression elements for adrenal cells include, but are not limited to, cholesterol side-chain cleavage (SCC) promoter. Exemplary tissue-specific expression elements for the general nervous system

include, but are not limited to, gamma-gamman enolase (neuron- specific enolase, NSE) promoter. Exemplary tissue-specific expression elements for the brain include, but are not limited to, the neurofilament heavy chain (NF-H) promoter. Exemplary tissue-specific expression elements for lymphocytes include, but are not limited to, the human CGL-
 5 l/granzyme B promoter, the terminal deoxy transferase (TdT), lambda 5, VpreB, and lck (lymphocyte specific tyrosine protein kinase p56lck) promoter, the humans CD2 promoter and its 3 ' transcriptional enhancer, and the human NK and T cell specific activation (NKG5) promoter. Exemplary tissue-specific expression elements for the colon include, but are not limited to, pp60c-src tyrosine kinase promoter, organ-specific neoantigens (OSNs) promoter,
 10 and colon specific antigen-P promoter. Tissue-specific expression elements for breast cells are for example, but are not limited to, the human alpha-lactalbumin promoter. Exemplary tissue-specific expression elements for the lung include, but are not limited to, the cystic fibrosis transmembrane conductance regulator (CFTR) gene promoter.

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

In some embodiments, the activity of the promoter is increased or decreased by at least 2 fold (*e.g.*, 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold), relative to activity of the promoter in the absence of the tumor microenvironment. For example, the activity of the promoter is increased or decreased by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at
 30 least 50 fold, or at least 100 fold, relative to activity of the promoter in the absence of the tumor microenvironment. In some embodiments, the activity of the promoter is increased or decreased by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold, relative to activity of the promoter in the absence of the tumor microenvironment.

In some embodiments, a promoter of the present disclosure is activated under a hypoxic condition. A “hypoxic condition” is a condition where the body or a region of the body is deprived of adequate oxygen supply at the tissue level. Hypoxic conditions can cause inflammation (*e.g.*, the level of inflammatory cytokines increase under hypoxic conditions).

- 5 In some embodiments, the promoter that is activated under hypoxic condition is operably linked to a nucleotide encoding a chimeric proteins that decreases the expression of activity of inflammatory cytokines, thus reducing the inflammation caused by the hypoxic condition. In some embodiments, the promoter that is activated under hypoxic conditions comprises a hypoxia responsive element (HRE). A “hypoxia responsive element (HRE)” is a response
10 element that responds to hypoxia-inducible factor (HIF). The HRE, in some embodiments, comprises a consensus motif NCGTG (where N is either A or G).

Activation-Conditional Control Polypeptide (ACP) Promoter Systems

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

- The promoters of the ACP promoter system, *e.g.*, either a promoter driving expression of the ACP or the promoter sequence of the ACP-responsive promoter, can include any of the promoter sequences described herein (*see* “Promoters” above). The ACP-responsive
30 promoter can be derived from minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, minCMV, YB_TATA, minTK, inducer molecule

responsive promoters, and tandem repeats thereof. In some embodiments, the ACP-responsive promoter includes a minimal promoter.

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

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

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

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

domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

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

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

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

Multicistronic and Multiple Promoter Systems

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

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

In some embodiments, engineered nucleic acids can be multicistronic, *i.e.*, more than one separate polypeptide (*e.g.*, multiple chimeric proteins) can be produced from a single mRNA transcript. Engineered nucleic acids can be multicistronic through the use of various
15 linkers, *e.g.*, a polynucleotide sequence encoding a first chimeric proteins can be linked to a nucleotide sequence encoding a second chimeric protein, such as in a first gene:linker:second gene 5' to 3' orientation. A linker can encode a 2A ribosome skipping element, such as T2A. Other 2A ribosome skipping elements include, but are not limited to, E2A, P2A, and F2A. 2A ribosome skipping elements allow production of separate polypeptides encoded by the first
20 and second genes are produced during translation. A linker can encode a cleavable linker polypeptide sequence, such as a Furin cleavage site or a TEV cleavage site, wherein following expression the cleavable linker polypeptide is cleaved such that separate polypeptides encoded by the first and second genes are produced. A cleavable linker can include a polypeptide sequence, such as such a flexible linker (*e.g.*, a Gly-Ser-Gly sequence),
25 that further promotes cleavage.

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

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

In some embodiments, a linker of the present disclosure is a Furin-Gly-Ser-Gly-T2A fusion polypeptide.

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

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

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

Engineered Cells

Provided herein are engineered cells, and methods of producing the engineered cells, that produce membrane-cleavable chimeric proteins. In general, engineered cells of the present disclosure may be engineered to express the chimeric proteins provided for herein, such as the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein. These cells are referred to herein as “engineered cells.” These cells, which typically contain engineered nucleic acid, do not occur in nature. In some embodiments, the cells are engineered to include a nucleic acid comprising a promoter operably linked to a nucleotide sequence encoding a chimeric protein, for example, a membrane-cleavable chimeric protein. An engineered cell can comprise an engineered nucleic acid integrated into the cell’s genome. An engineered cell can comprise an engineered nucleic acid capable of expression without integrating into the cell’s genome, for example, engineered with a transient expression system such as a plasmid or mRNA.

The present disclosure also encompasses additivity and synergy between a chimeric protein(s) and the engineered cell from which they are produced. In some embodiments, cells are engineered to produce at least two (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) chimeric

proteins, for example at least two membrane-cleavable chimeric proteins. In other embodiments, cells are engineered to produce at least one chimeric proteins having an effector molecule that is not natively produced by the cells. Such an effector molecule may, for example, complement the function of effector molecules natively produced by the cells.

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

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

In some embodiments, engineered cells comprise one or more engineered nucleic acids encoding a promoter operably linked to a nucleotide sequence encoding a chimeric protein. In some embodiments, cells are engineered to include a plurality of engineered nucleic acids, e.g., at least two engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (e.g., 1, 2 or 3) chimeric protein. For example, cells may be engineered to comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10, engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (e.g., 1, 2 or 3) chimeric protein. In some embodiments, the cells are engineered to comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, or more engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (e.g., 1, 2 or 3) chimeric protein. Engineered cells

can comprise an engineered nucleic acid encoding at least one of the linkers described above, such as polypeptides that link a first polypeptide sequence and a second polypeptide sequence, one or more multicistronic linker described above, one or more additional promoters operably linked to additional ORFs, or a combination thereof.

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

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

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

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

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

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

10 In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce one or more integrins. Cells of the present disclosure may be engineered to produce any combination of integrin α and β subunits. The α subunit of an integrin may be, without limitation: ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, ITGA7, ITGA8, ITGA9, IGTA10, IGTA11, ITGAD, ITGAE, ITGAL, ITGAM, ITGAV, ITGA2B, ITGAX. The β
15 subunit of an integrin may be, without limitation: ITGB1, ITGB2, ITGB3, ITGB4, ITGB5, ITGB6, ITGB7, and ITGB8.

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

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

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

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

least one (*e.g.*, 1, 2, 3, 4, 5, or more) chimeric protein includes an effector molecule that inhibits at least one immunosuppressive mechanism in the tumor microenvironment, and at least one chimeric protein (*e.g.*, 1, 2, 3, 4, 5, or more) inhibits at least one immunosuppressive mechanism in the tumor microenvironment. In yet other embodiments, at least two (*e.g.*, 2, 3, 4, 5, or more) chimeric proteins includes an effector molecule that stimulate at least one immunostimulatory mechanism in the tumor microenvironment. In still other embodiments, at least two (*e.g.*, 1, 2, 3, 4, 5, or more) chimeric proteins includes an effector molecule that inhibit at least one immunosuppressive mechanism in the tumor microenvironment.

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

embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits tumor checkpoint molecules. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that activates stimulator of interferon genes (STING) signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that degrades immunosuppressive factors/metabolites. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits vascular endothelial growth factor signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that directly kills tumor cells (*e.g.*, granzyme, perforin, oncolytic viruses, cytolytic peptides and enzymes, anti-tumor antibodies, *e.g.*, that trigger ADCC).

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

In some embodiments, a cell (*e.g.*, an immune cell or a stem cell) is engineered to produce at least one chimeric protein including an effector molecule selected from IL-12, IFN- β , IFN- γ , IL-2, IL-15, IL-7, IL-36 γ , IL-18, IL-1 β , OX40-ligand, and CD40L; and/or at least a checkpoint inhibitor. Illustrative immune checkpoint molecules that can be targeted for blocking or inhibition include, but are not limited to, CTLA-4, 4-1BB (CD137), 4-1BBL (CD137L), PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, $\gamma\delta$ T, and memory CD8 $^{+}$ ($\alpha\beta$) T cells), CD160 (also referred to as BY55), and

CGEN-15049. Immune checkpoint inhibitors include antibodies, or antigen binding fragments thereof, or other binding proteins, that bind to and block or inhibit the activity of one or more of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4, CD160, and CGEN-15049. Exemplary

5 checkpoint inhibitors include, but are not limited to, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27

10 antibodies, anti-TNFA antibodies, anti-TREMI antibodies, and anti-TREM2 antibodies. Illustrative immune checkpoint inhibitors include pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumab (anti-PD-1; Opdivo® - BMS), pidilizumab (anti-PD-1 antibody; CT-011 – Teva/CureTech), AMP224 (anti-PD-1; NCI), avelumab (anti-PD-L1; Bavencio® - Pfizer), durvalumab (anti-PD-L1; MEDI4736/Imfinzi® -

15 Medimmune/AstraZeneca), atezolizumab (anti-PD-L1; Tecentriq® - Roche/Genentech), BMS-936559 (anti-PD-L1 - BMS), tremelimumab (anti-CTLA-4; Medimmune/AstraZeneca), ipilimumab (anti-CTLA-4; Yervoy® - BMS), lirilumab (anti-KIR; BMS), monalizumab (anti-NKG2A; Innate Pharma/AstraZeneca).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to

20 produce at least one chimeric protein including an effector molecule selected from IL-12, IFN- β , IFN- γ , IL-2, IL-15, IL-7, IL-36 γ , IL-18, IL-1 β , OX40-ligand, and CD40L; and/or at least one checkpoint inhibitor selected from anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-CTLA-4 antibodies, and anti-IL-35 antibodies; and/or at least one chimeric protein including an effector molecule selected from MIP1 α (CCL3), MIP1 β (CCL5), and CCL21;

25 and/or at least one chimeric protein including an effector molecule selected from CpG oligodeoxynucleotides; and/or at least one chimeric protein including an effector molecule selected from microbial peptides.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce IFN- β and at least one chimeric protein including an effector molecule selected from

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

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

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (e.g., “S” in the formula $S - C - MT$ or $MT - C - S$) is IL-1-beta, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, an IL-12p70 fusion protein, IL-15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, or TNF-alpha. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, or XCL1. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is anti-integrin alpha4,beta7, anti-MAdCAM, SDF1, or MMP-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is 4-1BBL or

CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is adenosine deaminase, a TGFbeta inhibitor, an immune checkpoint inhibitor, a VEGF inhibitor, or HPGE2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric

5 protein where the secretable effector molecule is an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, or combinations thereof. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-VEGF antibody, an anti-VEGF peptide, or a combination thereof. In some

10 embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-LAG-3 antibody, an anti-TIM-3 antibody, an anti-TIGIT antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-GAL9 antibody, an anti-A2AR antibody, an anti-phosphatidylserine

15 antibody, an anti-CD27 antibody, an anti-TNFa antibody, an anti-TREMI antibody, or an anti-TREM2 antibody.

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, “S” in the formula S – C – MT or MT – C – S) comprises IL-15. In some embodiments, a cell is engineered to

20 produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a fusion of IL-15 and the sushi domain of IL-15R α . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector consists of IL-15. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-

25 15 and the cell is further engineered to produce one or more additional effector molecules. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce one or more additional secretable effector molecules. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable

30 effector molecule is IL-15 and the cell is further engineered to produce a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a polynucleotide, a peptide, or an enzyme. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further

engineered to produce IL-12, IFN- γ , IL-2, IL-7, IL-36 γ , IL-18, IL-1 β , OX40-ligand, or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-12. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IFN- γ . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-7. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-36 γ . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-18. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-1 β . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce OX40-ligand. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce CD40L.

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

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

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-12, IFN- γ , IL-2, IL-7, IL-36 γ , IL-18, IL-1 β , OX40-ligand, or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-12. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IFN- γ . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-7. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-36 γ . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-18. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-1 β . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is OX40-ligand. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is CD40L.

A cell can also be further engineered to express additional proteins in addition to the chimeric proteins (*e.g.*, the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein), proteins of interest, or effector molecules described herein. A cell can be further engineered to express one or more antigen recognizing receptors.

- 5 Examples of antigens that may be targeted by one or more antigen recognizing receptors include, but are not limited to, 5T4, ADAM9, AFP, AXL, B7-H3, B7-H4, B7-H6, BCMA, C4.4, CA6, Cadherin 3, Cadherin 6, CCR4, CD19, CD20, CD22, CD123, CD133, CD138, CD142, CD166, CD25, CD30, CD33, CD352, CD37, CD38, CD44, CD56, CD66e, CD70, CD71, CD74, CD79b, CD80, CEA, CEACAM5, Claudin18.2, cMet, CSPG4, CTLA, DLK1, 10 DLL3, DR5, EGFR, ENPP3, EpCAM, EphA2, Ephrin A4, ETBR, FGFR2, FGFR3, FLT3, FRalpha, FRb, GCC, GD2, GFRa4, gpA33, GPC2, GPC3, gpNBM, GPRC5, HER2, IL-13R, IL-13Ra, IL-13Ra2, IL-8, IL-15, IL1RAP, Integrin aV, KIT, L1CAM, LAMP1, Lewis Y, LeY, LIV-1, LRRC, LY6E, MCSP, Mesothelin, MUC1, MUC16, MUC1C, NaPi2B, Nectin 4, NKG2D, NOTCH3, NY ESO 1, Ovarin, P-cadherin, pan-Erb2, PSCA, PSMA, PTK7, 15 ROR1, S Aures, SCT, SLAMF7, SLITRK6, SSTR2, STEAP1, Survivin, TDGF1, TIM1, TROP2, and WT1.

- An antigen recognizing receptor can include an antigen-binding domain, such as an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). An antigen 20 recognizing receptors can include an scFv. An scFv can include a heavy chain variable domain (VH) and a light chain variable domain (VL), which can be separated by a peptide linker. For example, an scFv can include the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

- 25 An antigen recognizing receptor can be a chimeric antigen receptor (CAR). A CAR can have one or more intracellular signaling domains, such as a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a 30 CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, fragments

thereof, combinations thereof, or combinations of fragments thereof. A CAR can have a transmembrane domain, such as a CD8 transmembrane domain, a CD28 transmembrane domain, a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, a BTLA transmembrane domain, fragments thereof, combinations thereof, or combinations of fragments thereof. A CAR can have a spacer region between the antigen-binding domain and the transmembrane domain.

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

Engineered Cell Types

Also provided herein are engineered cells. Cells can be engineered to comprise any of the engineered nucleic acids described herein (*e.g.*, any of the engineered nucleic acids encoding the membrane-cleavable chimeric proteins described herein). Cells can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are cells engineered to produce one or more chimeric proteins, where the one or more chimeric proteins are the membrane-cleavable chimeric proteins having the formula $S - C - MT$ or $MT - C - S$ described herein. In a particular aspect, provided herein are cells engineered to produce two or more chimeric proteins. In a particular aspect, provided herein are cells engineered to produce two or more of the chimeric proteins described herein, where each chimeric protein is a different protein of interest or effector molecule. In a particular aspect, provided herein are cells engineered to produce any of the chimeric proteins described herein and engineered to separately produce a different effector molecule or protein of interest (*e.g.*, a homing molecule, antigen receptor, etc.).

The engineered cells can be an immune cell, including but not limited to, a T cell, a CD8⁺ T cell, a CD4⁺ T cell, a gamma-delta ($\gamma\delta$) T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, or a dendritic cell. The engineered cells can be a T cell. The engineered cells can be an NK cell.

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

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

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

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

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

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

Also provided herein are engineered platelet cells. Platelet cells can be engineered to comprise any of the engineered nucleic acids described herein. Platelet cells can be

engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are platelet cells engineered to produce one or more of the chimeric proteins described herein. In a particular aspect, provided herein are platelet cells engineered to produce two or more of the chimeric proteins described herein.

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

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

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

cell, such as a primary cell isolated from a subject. A cultured cell can be cultured with one or more cytokines.

Also provided herein are methods that include culturing the engineered cells of the present disclosure. Methods of culturing the engineered cells described herein are known.

- 5 One skilled in the art will recognize that culturing conditions will depend on the particular engineered cell of interest. One skilled in the art will recognize that culturing conditions will depend on the specific downstream use of the engineered cell, for example, specific culturing conditions for subsequent administration of the engineered cell to a subject.

10 ***Methods of Engineering Cells***

Also provided herein are compositions and methods for engineering cells to produce one or more proteins of interest or effector molecules (*e.g.*, the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein).

- 15 In general, cells are engineered to produce proteins of interest or effector molecules through introduction (*i.e.*, delivery) of polynucleotides encoding the one or more proteins of interest or effector molecules, *e.g.*, the chimeric proteins described herein including the protein of interest or effector molecule, into the cell's cytosol and/or nucleus. For example, the polynucleotides encoding the one or more chimeric proteins can be any of the engineered nucleic acids encoding the membrane-cleavable chimeric proteins having the formula **S – C –**
20 **MT** or **MT – C – S** described herein. Delivery methods include, but are not limited to, viral-mediated delivery, lipid-mediated transfection, nanoparticle delivery, electroporation, sonication, and cell membrane deformation by physical means. One skilled in the art will appreciate the choice of delivery method can depend on the specific cell type to be engineered.

25

Viral-Mediated Delivery

- Viral vector-based delivery platforms can be used to engineer cells. In general, a viral vector-based delivery platform engineers a cell through introducing (*i.e.*, delivering) into a host cell. For example, a viral vector-based delivery platform can engineer a cell through
30 introducing any of the engineered nucleic acids described herein (*e.g.*, any of the exogenous polynucleotide sequences encoding the chimeric proteins described herein, such as the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein, and/or any of the expression cassettes described herein containing a

promoter and an exogenous polynucleotide sequence encoding the chimeric proteins, oriented from N-terminal to C-terminal). A viral vector-based delivery platform can be a nucleic acid, and as such, an engineered nucleic acid can also encompass an engineered virally-derived nucleic acid. Such engineered virally-derived nucleic acids can also be referred to as

5 recombinant viruses or engineered viruses.

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

A viral vector-based delivery platform can comprise more than one viral vector, such as separate viral vectors encoding the engineered nucleic acids, genes, or transgenes
20 described herein, and referred to as trans-acting elements or genes. For example, a helper-dependent viral vector-based delivery platform can provide additional genes needed for viral infectivity and/or viral production on one or more additional separate vectors in addition to the vector encoding the one or more chimeric proteins and/or other protein of interest. One viral vector can deliver more than one engineered nucleic acids, such as one vector that
25 delivers engineered nucleic acids that are configured to produce two or more chimeric proteins and/or other protein of interest. More than one viral vector can deliver more than one engineered nucleic acids, such as more than one vector that delivers one or more engineered nucleic acid configured to produce one or more chimeric proteins and/or other protein of interest. The number of viral vectors used can depend on the packaging capacity of the above
30 mentioned viral vector-based vaccine platforms, and one skilled in the art can select the appropriate number of viral vectors.

In general, any of the viral vector-based systems can be used for the *in vitro* production of molecules, such as the chimeric proteins, effector molecules, and/or other protein of interest described herein, or used *in vivo* and *ex vivo* gene therapy procedures, *e.g.*,

for *in vivo* delivery of the engineered nucleic acids encoding one or more chimeric proteins and/or other protein of interest. The selection of an appropriate viral vector-based system will depend on a variety of factors, such as cargo/payload size, immunogenicity of the viral system, target cell of interest, gene expression strength and timing, and other factors

5 appreciated by one skilled in the art.

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

25 The sequences may be preceded with one or more sequences targeting a subcellular compartment. Upon introduction (*i.e.* delivery) into a host cell, infected cells (*i.e.*, an engineered cell) can express the chimeric proteins and/or other protein of interest. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Pat. No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in 30 Stover *et al.* (Nature 351:456-460 (1991)). A wide variety of other vectors useful for the introduction (*i.e.*, delivery) of engineered nucleic acids, *e.g.*, Salmonella typhi vectors, and the like will be apparent to those skilled in the art from the description herein.

The viral vector-based delivery platforms can be a virus that targets a cell, herein referred to as an oncolytic virus. Examples of oncolytic viruses include, but are not limited to,

an oncolytic herpes simplex virus, an oncolytic adenovirus, an oncolytic measles virus, an oncolytic influenza virus, an oncolytic Indiana vesiculovirus, an oncolytic Newcastle disease virus, an oncolytic vaccinia virus, an oncolytic poliovirus, an oncolytic myxoma virus, an oncolytic reovirus, an oncolytic mumps virus, an oncolytic Maraba virus, an oncolytic rabies virus, an oncolytic rotavirus, an oncolytic hepatitis virus, an oncolytic rubella virus, an oncolytic dengue virus, an oncolytic chikungunya virus, an oncolytic respiratory syncytial virus, an oncolytic lymphocytic choriomeningitis virus, an oncolytic morbillivirus, an oncolytic lentivirus, an oncolytic replicating retrovirus, an oncolytic rhabdovirus, an oncolytic Seneca Valley virus, an oncolytic sindbis virus, and any variant or derivative thereof. Any of the oncolytic viruses described herein can be a recombinant oncolytic virus comprising one more transgenes (*e.g.*, an engineered nucleic acid) encoding one or more chimeric proteins and/or other protein of interest. The transgenes encoding the one or more chimeric proteins and/or other protein of interest can be configured to express the chimeric proteins and/or other protein of interest.

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

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

7,250,299; 7,226,780; 7,220,578; 7,211,247; 7,160,721; 7,078,031; 7,070,993; 7,056,699; 6,955,919, each herein incorporated by reference for all purposes.

The viral vector-based delivery platform can be adenovirus-based. In general, adenoviral based vectors are capable of very high transduction efficiency in many cell types, do not require cell division, achieve high titer and levels of expression, and can be produced in large quantities in a relatively simple system. In general, adenoviruses can be used for transient expression of a transgene within an infected cell since adenoviruses do not typically integrate into a host's genome. Adenovirus-based delivery platforms are described in more detail in Li *et al.*, Invest Ophthalmol Vis Sci 35:2543-2549, 1994; Borrás *et al.*, Gene Ther 6:515-524, 1999; Li and Davidson, PNAS 92:7700-7704, 1995; Sakamoto *et al.*, H Gene Ther 5:1088-1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655, each herein incorporated by reference for all purposes. Other exemplary adenovirus-based delivery platforms are described in more detail in U.S. Pat. Nos. 5,585,362; 6,083,716; 7,371,570; 7,348,178; 7,323,177; 7,319,033; 7,318,919; and 7,306,793 and International Patent Application WO96/13597, each herein incorporated by reference for all purposes.

The viral vector-based delivery platform can be adeno-associated virus (AAV)-based. Adeno-associated virus ("AAV") vectors may be used to transduce cells with engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein). AAV systems can be used for the *in vitro* production of proteins of interest, such as the chimeric proteins described herein and/or effector molecules, or used *in vivo* and *ex vivo* gene therapy procedures, *e.g.*, for *in vivo* delivery of the engineered nucleic acids encoding one or more chimeric proteins and/or other protein of interest (see, *e.g.*, West *et al.*, Virology 160:38-47 (1987); U.S. Pat. Nos. 4,797,368; 5,436,146; 6,632,670; 6,642,051; 7,078,387; 7,314,912; 6,498,244; 7,906,111; US patent publications US 2003-0138772, US 2007/0036760, and US 2009/0197338; Gao, *et al.*, J. Virol, 78(12):6381-6388 (June 2004); Gao, *et al.*, Proc Natl Acad Sci USA, 100(10):6081-6086 (May 13, 2003); and International Patent applications WO 2010/138263 and WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994), each herein incorporated by reference for all purposes). Exemplary methods for constructing recombinant AAV vectors are described in more detail in U.S. Pat. No. 5,173,414; Tratschin *et al.*, Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, *et al.*, Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samuiski *et al.*, J. Virol. 63:3822-3828 (1989), each herein incorporated by reference for all purposes. In general, an AAV-based vector comprises a

capsid protein having an amino acid sequence corresponding to any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV.Rh10, AAV11 and variants thereof. In particular examples, an AAV-based vector has a capsid protein having an amino acid sequence corresponding to AAV2. In particular examples, an AAV-based vector has a capsid protein having an amino acid sequence corresponding to AAV8.

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

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

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

Lipid Structure Delivery Systems

Engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein) can be introduced into a cell using a lipid-mediated delivery system. In general, a lipid-

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

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

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

liposome such that it can be delivered to a target entity. Lipophilic molecules or molecules with lipophilic regions may also dissolve in or associate with the lipid bilayer.

A liposome used according to the present embodiments can be made by different methods, as would be known to one of ordinary skill in the art. Preparations of liposomes are
5 described in further detail in WO 2016/201323, International Applications PCT/US85/01161 and PCT/US89/05040, and U.S. Patents 4,728,578, 4,728,575, 4,737,323, 4,533,254, 4,162,282, 4,310,505, and 4,921,706; each herein incorporated by reference for all purposes.

Liposomes can be cationic liposomes. Examples of cationic liposomes are described in more detail in U.S. Patent No. 5,962,016; 5,030,453; 6,680,068, U.S. Application
10 2004/0208921, and International Patent Applications WO03/015757A1, WO04029213A2, and WO02/100435A1, each hereby incorporated by reference in their entirety.

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

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

As used herein, the term "extracellular vesicle" or "EV" refers to a cell-derived vesicle comprising a membrane that encloses an internal space. In general, extracellular vesicles comprise all membrane-bound vesicles that have a smaller diameter than the cell from which they are derived. Generally extracellular vesicles range in diameter from 20 nm to 1000 nm, and can comprise various macromolecular cargo either within the internal space,
25 displayed on the external surface of the extracellular vesicle, and/or spanning the membrane. The cargo can comprise nucleic acids (*e.g.*, any of the engineered nucleic acids described herein), proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. By way of example and without limitation, extracellular vesicles include apoptotic bodies, fragments of cells, vesicles derived from cells by direct or indirect manipulation (*e.g.*, by
30

serial extrusion or treatment with alkaline solutions), vesiculated organelles, and vesicles produced by living cells (*e.g.*, by direct plasma membrane budding or fusion of the late endosome with the plasma membrane). Extracellular vesicles can be derived from a living or dead organism, explanted tissues or organs, and/or cultured cells.

5 As used herein the term “exosome” refers to a cell-derived small (between 20-300 nm in diameter, more preferably 40-200 nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from the cell by direct plasma membrane budding or by fusion of the late endosome with the plasma membrane. The exosome comprises lipid or fatty acid and polypeptide and optionally comprises a payload (*e.g.*, a
10 therapeutic agent), a receiver (*e.g.*, a targeting moiety), a polynucleotide (*e.g.*, a nucleic acid, RNA, or DNA, such as any of the engineered nucleic acids described herein), a sugar (*e.g.*, a simple sugar, polysaccharide, or glycan) or other molecules. The exosome can be derived from a producer cell, and isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof. An exosome is a species of extracellular
15 vesicle. Generally, exosome production/biogenesis does not result in the destruction of the producer cell. Exosomes and preparation of exosomes are described in further detail in WO 2016/201323, which is hereby incorporated by reference in its entirety.

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

Lipid nanoparticles (LNPs), in general, are synthetic lipid structures that rely on the amphiphilic nature of lipids to form membranes and vesicle like structures (Riley 2017). In general, these vesicles deliver cargo/payloads, such as any of the engineered nucleic acids or viral systems described herein, by absorbing into the membrane of target cells and releasing the cargo into the cytosol. Lipids used in LNP formation can be cationic, anionic, or neutral. The lipids can be synthetic or naturally derived, and in some instances biodegradable. Lipids can include fats, cholesterol, phospholipids, lipid conjugates including, but not limited to, polyethyleneglycol (PEG) conjugates (PEGylated lipids), waxes, oils, glycerides, and fat soluble vitamins. Lipid compositions generally include defined mixtures of materials, such as the cationic, neutral, anionic, and amphipathic lipids. In some instances, specific lipids are included to prevent LNP aggregation, prevent lipid oxidation, or provide functional chemical groups that facilitate attachment of additional moieties. Lipid composition can influence overall LNP size and stability. In an example, the lipid composition comprises dilinoleylmethyl- 4-dimethylaminobutyrate (MC3) or MC3-like molecules. MC3 and MC3-like lipid compositions can be formulated to include one or more other lipids, such as a PEG or PEG-conjugated lipid, a sterol, or neutral lipids. In addition, LNPs can be further engineered or functionalized to facilitate targeting of specific cell types. Another consideration in LNP design is the balance between targeting efficiency and cytotoxicity.

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

Nucleic-acid vectors, such as expression vectors, exposed directly to serum can have several undesirable consequences, including degradation of the nucleic acid by serum nucleases or off-target stimulation of the immune system by the free nucleic acids. Similarly, viral delivery systems exposed directly to serum can trigger an undesired immune response and/or neutralization of the viral delivery system. Therefore, encapsulation of an engineered nucleic acid and/or viral delivery system can be used to avoid degradation, while also avoiding potential off-target affects. In certain examples, an engineered nucleic acid and/or viral delivery system is fully encapsulated within the delivery vehicle, such as within the aqueous interior of an LNP. Encapsulation of an engineered nucleic acid and/or viral delivery system within an LNP can be carried out by techniques well-known to those skilled in the art,

- such as microfluidic mixing and droplet generation carried out on a microfluidic droplet generating device. Such devices include, but are not limited to, standard T-junction devices or flow-focusing devices. In an example, the desired lipid formulation, such as MC3 or MC3-like containing compositions, is provided to the droplet generating device in parallel with an
- 5 engineered nucleic acid or viral delivery system and any other desired agents, such that the delivery vector and desired agents are fully encapsulated within the interior of the MC3 or MC3-like based LNP. In an example, the droplet generating device can control the size range and size distribution of the LNPs produced. For example, the LNP can have a size ranging from 1 to 1000 nanometers in diameter, *e.g.*, 1, 10, 50, 100, 500, or 1000 nanometers.
- 10 Following droplet generation, the delivery vehicles encapsulating the cargo/payload (*e.g.*, an engineered nucleic acid and/or viral delivery system) can be further treated or engineered to prepare them for administration.

Nanoparticle Delivery

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

Genomic Editing Systems

- A genomic editing systems can be used to engineer a host genome to encode an
- 25 engineered nucleic acid, such as an engineered nucleic acid encoding one or more of the chimeric proteins (*e.g.*, any of the membrane-cleavable chimeric proteins having the formula $S - C - MT$ or $MT - C - S$ described herein). In general, a “genomic editing system” refers to any system for integrating an exogenous gene into a host cell’s genome. Genomic editing systems include, but are not limited to, a transposon system, a nuclease genomic editing
- 30 system, and a viral vector-based delivery platform.

A transposon system can be used to integrate an engineered nucleic acid, such as an engineered nucleic acid encoding one or more of the chimeric proteins (*e.g.*, any of the membrane-cleavable chimeric proteins having the formula $S - C - MT$ or $MT - C - S$

described herein), into a host genome. Transposons generally comprise terminal inverted repeats (TIR) that flank a cargo/payload nucleic acid and a transposase. The transposon system can provide the transposon in *cis* or in *trans* with the TIR-flanked cargo. A transposon system can be a retrotransposon system or a DNA transposon system. In general, transposon systems integrate a cargo/payload (*e.g.*, an engineered nucleic acid) randomly into a host genome. Examples of transposon systems include systems using a transposon of the Tc1/mariner transposon superfamily, such as a Sleeping Beauty transposon system, described in more detail in Hudecek *et al.* (Crit Rev Biochem Mol Biol. 2017 Aug;52(4):355-380), and U.S. Patent Nos. 6,489,458, 6,613,752 and 7,985,739, each of which is herein incorporated by reference for all purposes. Another example of a transposon system includes a PiggyBac transposon system, described in more detail in U.S. Patent Nos. 6,218,185 and 6,962,810, each of which is herein incorporated by reference for all purposes.

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

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

5 The identical, or substantially identical, sequences found at the 5' and 3' ends of the HR template, with respect to the exogenous sequence to be introduced, are generally referred to as arms (HR arms). HR arms can be identical to regions of the endogenous genomic target locus (*i.e.*, 100% identical). HR arms in some examples can be substantially identical to regions of the endogenous genomic target locus. While substantially identical HR arms can
10 be used, it can be advantageous for HR arms to be identical as the efficiency of the HDR pathway may be impacted by HR arms having less than 100% identity.

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

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

A CRISPR-mediated gene editing system can be used to engineer a host genome to encode an engineered nucleic acid, such as an engineered nucleic acid encoding one or more
30 of the chimeric proteins (*e.g.*, any of the degron-fusion chimeric proteins described herein or the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein). CRISPR systems are described in more detail in M. Adli ("The CRISPR tool kit for genome editing and beyond" Nature Communications; volume 9 (2018), Article number: 1911), herein incorporated by reference for all that it teaches. In general, a CRISPR-

mediated gene editing system comprises a CRISPR-associated (Cas) nuclease and a RNA(s) that directs cleavage to a particular target sequence. An exemplary CRISPR-mediated gene editing system is the CRISPR/Cas9 systems comprised of a Cas9 nuclease and a RNA(s) that has a CRISPR RNA (crRNA) domain and a trans-activating CRISPR (tracrRNA) domain.

- 5 The crRNA typically has two RNA domains: a guide RNA sequence (gRNA) that directs specificity through base-pair hybridization to a target sequence (“a defined nucleotide sequence”), *e.g.*, a genomic sequence; and an RNA domain that hybridizes to a tracrRNA. A tracrRNA can interact with and thereby promote recruitment of a nuclease (*e.g.*, Cas9) to a genomic locus. The crRNA and tracrRNA polynucleotides can be separate polynucleotides.
- 10 The crRNA and tracrRNA polynucleotides can be a single polynucleotide, also referred to as a single guide RNA (sgRNA). While the Cas9 system is illustrated here, other CRISPR systems can be used, such as the Cpf1/Cas12 or Cas13 systems. Nucleases can include derivatives thereof, such as Cas9 functional mutants, *e.g.*, a Cas9 “nickase” mutant that in general mediates cleavage of only a single strand of a defined nucleotide sequence as
- 15 opposed to a complete double-stranded break typically produced by Cas9 enzymes.

In general, the components of a CRISPR system interact with each other to form a Ribonucleoprotein (RNP) complex to mediate sequence specific cleavage. In some CRISPR systems, each component can be separately produced and used to form the RNP complex. In some CRISPR systems, each component can be separately produced *in vitro* and contacted

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

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

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

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

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

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

In general, the features of a CRISPR-mediated editing system described herein can apply to other nuclease-based genomic editing systems. TALEN is an engineered site-specific

nuclease, which is composed of the DNA- binding domain of TALE (transcription activator-like effectors) and the catalytic domain of restriction endonuclease FokI. By changing the amino acids present in the highly variable residue region of the monomers of the DNA binding domain, different artificial TALENs can be created to target various nucleotides sequences. The DNA binding domain subsequently directs the nuclease to the target sequences and creates a double-stranded break. TALEN-based systems are described in more detail in U.S. Ser. No. 12/965,590; U.S. Pat. No. 8,450,471; U.S. Pat. No. 8,440,431; U.S. Pat. No. 8,440,432; U.S. Pat. No. 10,172,880; and U.S. Ser. No. 13/738,381, all of which are incorporated by reference herein in their entirety. ZFN-based editing systems are described in more detail in U.S. Patent Nos. 6,453,242; 6,534,261; 6,599,692; 6,503,717; 6,689,558; 7,030,215; 6,794,136; 7,067,317; 7,262,054; 7,070,934; 7,361,635; 7,253,273; and U.S. Patent Publication Nos. 2005/0064474; 2007/0218528; 2005/0267061, all incorporated herein by reference in their entirety for all purposes.

15 Other Engineering Delivery Systems

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

Electroporation can be used to deliver polynucleotides to recipient entities.

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

and protocols can be used for electroporation. Examples include, but are not limited to, Neon[®] Transfection System, MaxCyte[®] Flow Electroporation[™], Lonza[®] Nucleofector[™] systems, and Bio-Rad[®] electroporation systems.

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

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

Delivery Vehicles

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

The cargo can comprise nucleic acids (*e.g.*, any of the engineered nucleic acids described herein, such as any of the engineered nucleic acids described herein encoding the chimeric proteins including the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein), as described above. The cargo can comprise proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. The cargo can be any of the chimeric proteins provided for herein (*e.g.*, any of the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein). The cargo can be a combination of the chimeric proteins described herein, *e.g.*, two or more of the chimeric proteins described herein. The cargo can be a combination of the chimeric protein described herein and another cargo of interest, such as another protein, carbohydrate, lipid, small molecule, and/or combination thereof.

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

any of the nanoparticles described herein, such as nanoparticles comprising lipids (as previously described), inorganic nanomaterials, and other polymeric materials.

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

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

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Methods of Treatment

Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) engineered cells as provided herein to produce *in vivo* at least one protein of interest produced by the engineered cells (*e.g.*, any of the chimeric proteins provided for herein, such as the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein, or the secreted effector molecules provided for herein following protease cleavage of the chimeric protein). Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) engineered cells as provided herein to produce *in vivo* at least two proteins of interest, *e.g.*, at least two of the chimeric proteins provided for herein, such as the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein, produced by the engineered cells.

Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) any of the delivery vehicles described herein, such as any of the delivery vehicles described herein comprising any of the proteins of interest described herein, *e.g.*, any of the chimeric proteins provided for herein, such as the membrane-cleavable

5 chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein. Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) any of the delivery vehicles described herein, such as any of the delivery vehicles described herein comprising two or more proteins of, *e.g.*, at least two of the chimeric proteins provided for herein, such as the membrane-cleavable chimeric proteins

10 having the formula **S – C – MT** or **MT – C – S** described herein.

In some embodiments, the engineered cells or delivery vehicles are administered via intravenous, intraperitoneal, intratracheal, subcutaneous, intratumoral, oral, anal, intranasal (*e.g.*, packed in a delivery particle), or arterial (*e.g.*, internal carotid artery) routes. Thus, the engineered cells or delivery vehicles may be administered systemically or locally (*e.g.*, to a

15 TME or via intratumoral administration). An engineered cell can be isolated from a subject, such as a subject known or suspected to have cancer. An engineered cell can be allogenic with reference to the subject being administered a treatment. Allogenic modified cells can be HLA-matched to the subject being administered a treatment. Delivery vehicles can be any of the lipid structure delivery systems described herein. Delivery vehicles can be any of the

20 nanoparticles described herein.

Engineered cells or delivery vehicles can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. For example, engineered cells or delivery vehicles can be administered in combination with one or more IMiDs described herein. FDA-approved IMiDs can be

25 administered in their approved fashion. In another example, engineered cells or delivery vehicles can be administered in combination with a checkpoint inhibitor therapy. Exemplary checkpoint inhibitors include, but are not limited to, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-

30 B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNF α antibodies, anti-TREM1 antibodies, and anti-TREM2 antibodies. Illustrative immune checkpoint inhibitors include pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumab (anti-PD-1; Opdivo® - BMS), pidilizumab (anti-PD-

1 antibody; CT-011 – Teva/CureTech), AMP224 (anti-PD-1; NCI), avelumab (anti-PD-L1; Bavencio® - Pfizer), durvalumab (anti-PD-L1; MEDI4736/Imfinzi® - Medimmune/AstraZeneca), atezolizumab (anti-PD-L1; Tecentriq® - Roche/Genentech), BMS-936559 (anti-PD-L1 - BMS), tremelimumab (anti-CTLA-4; Medimmune/AstraZeneca),
 5 ipilimumab (anti-CTLA-4; Yervoy® - BMS), lirilumab (anti-KIR; BMS), monalizumab (anti-NKG2A; Innate Pharma/AstraZeneca). In other examples, engineered cells or delivery vehicles can be administered in combination with TGFbeta inhibitors, VEGF inhibitors, or HPGE2. In another example, engineered cells or delivery vehicles can be administered in combination with an anti-CD40 antibody.

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

The engineered cells or delivery vehicles of the present disclosure may be used, in some instances, to treat cancer, such as ovarian cancer. Other cancers are described herein.

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

The methods provided herein also include delivering a preparation of engineered cells or delivery vehicles. A preparation, in some embodiments, is a substantially pure preparation, containing, for example, less than 5% (*e.g.*, less than 4%, 3%, 2%, or 1%) of cells other than engineered cells. A preparation may comprise 1×10^5 cells/kg to 1×10^7 cells/kg cells.

25 Preparation of engineered cells or delivery vehicles can include pharmaceutical compositions having one or more pharmaceutically acceptable carriers. For example, preparations of engineered cells or delivery vehicles can include any of the engineered viruses, such as an engineered AAV virus, or any of the engineered viral vectors, such as AAV vector, described herein.

30

***In vivo* Expression**

The methods provided herein also include delivering a composition *in vivo* capable of producing the engineered cells described herein, *e.g.*, capable of delivering any of the

engineered nucleic acids described herein to a cell *in vivo*. Such compositions include any of the viral-mediated delivery platforms, any of the lipid structure delivery systems, any of the nanoparticle delivery systems, any of the genomic editing systems, or any of the other engineering delivery systems described herein capable of engineering a cell *in vivo*.

- 5 The methods provided herein also include delivering a composition *in vivo* capable of producing any of the proteins of interest described herein, *e.g.*, any of the chimeric proteins provided for herein, such as the membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S** described herein. The methods provided herein also include delivering a composition *in vivo* capable of producing two or more of the proteins of interest
- 10 described herein. Compositions capable of *in vivo* production of proteins of interest include, but are not limited to, any of the engineered nucleic acids described herein. Compositions capable of *in vivo* production proteins of interest can be a naked mRNA or a naked plasmid.

Additional Embodiments

- 15 1. An engineered nucleic acid comprising an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula:

$$\mathbf{S - C - MT \text{ or } MT - C - S}$$

wherein

- 20 S comprises a secretable effector molecule,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

wherein the promoter is operably linked to the exogenous polynucleotide sequence,

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

- 25 polypeptide.

2. The engineered nucleic acid of embodiment 1, wherein the promoter is a constitutive promoter.

3. The engineered nucleic acid of embodiment 2, wherein the constitutive promoter is selected from the group consisting of: CAG, HLP, CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, hEF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.
- 5 4. The engineered nucleic acid of any one of embodiments 1-3, wherein the promoter is an inducible promoter.
5. The engineered nucleic acid of embodiment 4, wherein the inducible promoter comprises a response element selected from the group consisting of: an NFkB response element, a CREB response element, an NFAT response element, SRF
10 response element 1, SRF response element 2, an AP1 response element, a TCF-LEF response element promoter fusion, a Hypoxia responsive element, a SMAD binding element, a STAT3 binding site, a inducer molecule responsive element, and tandem repeats thereof.
6. The engineered nucleic acid of any one of embodiments 1-5, wherein the promoter is
15 a synthetic promoter.
7. The engineered nucleic acid of embodiment 6, wherein the synthetic promoter comprises a minimal promoter and at least one regulatory element that is heterologous to the minimal promoter.
8. The engineered nucleic acid of embodiment 7, wherein the at least one regulatory
20 element comprises an activation-conditional control polypeptide- (ACP-) binding domain sequence and a promoter sequence.
9. The engineered nucleic acid of embodiment 8, wherein the ACP-binding domain comprises one or more zinc finger binding sites.
10. The engineered nucleic acid of embodiment 8 or embodiment 9, wherein the synthetic
25 promoter is regulatable by an activation-conditional control polypeptide (ACP) that binds to the ACP-binding domain of the synthetic promoter.
11. The engineered nucleic acid of embodiment 10, wherein the ACP is a transcriptional modulator.

12. The engineered nucleic acid of embodiment 10 or embodiment 11, wherein the ACP is a transcriptional repressor.
13. The engineered nucleic acid of embodiment 10 or embodiment 11, wherein the ACP is a transcriptional activator.
- 5 14. The engineered nucleic acid of any one of embodiments 10-13, wherein the ACP further comprises a repressible protease and one or more cognate cleavage sites of the repressible protease.
15. The engineered nucleic acid of any one of embodiments 10-14, wherein the ACP further comprises a hormone-binding domain of estrogen receptor (ERT2 domain).
- 10 16. The engineered nucleic acid of any one of embodiments 10-14, wherein the ACP is a transcription factor.
17. The engineered nucleic acid of embodiment 16, wherein the transcription factor is a zinc-finger-containing transcription factor.
- 15 18. The engineered nucleic acid of any one of embodiments 10-17, wherein the ACP comprises a DNA-binding zinc finger protein domain (ZF protein domain) and a transcriptional effector domain.
19. The engineered nucleic acid of embodiment 18, wherein the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA).
20. The engineered nucleic acid of embodiment 19, wherein the ZF protein domain
20 comprises one to ten ZFA.
21. The engineered nucleic acid of any one of embodiments 18-20, wherein the effector
25 domain is selected from the group consisting of: a Herpes Simplex Virus Protein 16 (VP16) activation domain; an activation domain comprising four tandem copies of VP16, a VP64 activation domain; a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator (Rta) activation domain; a tripartite activator comprising the VP64, the p65, and the Rta activation domains (VPR activation domain); a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain); a Krüppel associated box (KRAB) repression

- domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.
- 5
22. The engineered nucleic acid of any one of embodiments 14-21, wherein the one or more cognate cleavage sites of the repressible protease are localized between the ZF protein domain and the effector domain.
23. The engineered nucleic acid of any one of embodiments 14-22, wherein the repressible protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).
- 10
24. The engineered nucleic acid of embodiment 23, wherein the cognate cleavage site comprises an NS3 protease cleavage site.
25. The engineered nucleic acid of embodiment 24, wherein the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.
- 15
26. The engineered nucleic acid of any one of embodiments 23-25, wherein the NS3 protease can be repressed by a protease inhibitor.
27. The engineered nucleic acid of embodiment 26, wherein the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.
- 20
28. The engineered nucleic acid of any one of embodiments 15-27, wherein the ACP is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof.
- 25
29. The engineered nucleic acid of embodiment 28, wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

30. The engineered nucleic acid of any one of embodiments 10-29, wherein the ACP further comprises a degron domain, and wherein the degron domain is operably linked to the ACP.
31. The engineered nucleic acid of embodiment 30, wherein the degron domain is derived from a degron selected from the group consisting of HCV NS4 degron, PEST (two copies of residues 277-307 of human IkB α), GRR (residues 352-408 of human p105), DRR (residues 210-295 of yeast Cdc34), SNS (tandem repeat of SP2 and NB (SP2-NB-SP2 of influenza A or influenza B), RPB (four copies of residues 1688-1702 of yeast RPB), SPmix (tandem repeat of SP1 and SP2 (SP2-SP1-SP2-SP1-SP2 of influenza A virus M2 protein), NS2 (three copies of residues 79-93 of influenza A virus NS protein), ODC (residues 106-142 of ornithine decarboxylase), Nek2A, mouse ODC (residues 422-461), mouse ODC_DA (residues 422-461 of mODC including D433A and D434A point mutations), an APC/C degron, a COP1 E3 ligase binding degron motif, a CRL4-Cdt2 binding PIP degron, an actinfilin-binding degron, a KEAP1 binding degron, a KLHL2 and KLHL3 binding degron, an MDM2 binding motif, an N-degron, a hydroxyproline modification in hypoxia signaling, a phytohormone-dependent SCF-LRR-binding degron, an SCF ubiquitin ligase binding phosphodegron, a phytohormone-dependent SCF-LRR-binding degron, a DSGxxS phospho-dependent degron, an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box.
32. The engineered nucleic acid of embodiment 30, wherein the degron domain comprises a cereblon (CRBN) polypeptide substrate domain capable of binding CRBN in response to an immunomodulatory drug (IMiD) thereby promoting ubiquitin pathway-mediated degradation of the ACP.
33. The engineered nucleic acid of embodiment 32, wherein the CRBN polypeptide substrate domain is selected from the group consisting of: IKZF1, IKZF3, CK1a, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN.
34. The engineered nucleic acid of embodiment 32, wherein the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences.

35. The engineered nucleic acid of embodiment 32, wherein the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of
FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNY
5 ACQRRDAL (SEQ ID NO: 175).
36. The engineered nucleic acid of any one of embodiments 32-35, wherein the IMiD is an FDA-approved drug.
37. The engineered nucleic acid of any one of embodiments 32-36, wherein the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.
- 10 38. The engineered nucleic acid of any one of embodiments 30-37, wherein the degron domain is N-terminal of the repressible protease, C-terminal of the repressible protease, N-terminal of the ZF protein domain, C-terminal of the ZF protein domain, N-terminal of the effector domain, or C-terminal of the effector domain.
- 15 39. The engineered nucleic acid of any one of embodiments 1-38, wherein the promoter is a tissue-specific promoter.
40. The engineered nucleic acid of any one of embodiments 1-39, wherein the secretable effector molecule comprises a signal peptide or a signal-anchor sequence.
41. The engineered nucleic acid of embodiment 40, wherein the signal peptide comprises a native signal peptide native to the secretable effector molecule.
- 20 42. The engineered nucleic acid of embodiment 40, wherein the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule.
43. The engineered nucleic acid of embodiment 42, 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, 25 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.

44. The engineered nucleic acid of any one of embodiments 1-43, 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.
45. The engineered nucleic acid of embodiment 44, 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.
46. The engineered nucleic acid of any one of embodiments 1-43, wherein the secretable effector molecule comprises IL-15, IL-12, or an IL-12p70 fusion protein.
47. The engineered nucleic acid of any one of embodiments 1-43, wherein the secretable effector molecule comprises IL-15.
48. The engineered nucleic acid of claim 47, wherein the secretable effector molecule comprises the amino acid sequence of SEQ ID NO: 199.
49. The engineered nucleic acid of any one of embodiments 1-43, wherein the secretable effector molecule comprises IL-15 and IL-15R α sushi domain or an IL15/IL-15R α sushi domain fusion protein.
50. The engineered nucleic acid of embodiment 49, wherein the secretable effector molecule comprises the amino acid sequence of SEQ ID NO: 202.
51. The engineered nucleic acid of any one of embodiments 1-43, wherein the secretable effector molecule comprises IL-12 or an IL-12p70 fusion protein.
52. The engineered nucleic acid of embodiment 51, wherein the secretable effector molecule comprises the amino acid sequence of SEQ ID NO: 203.
53. The engineered nucleic acid of embodiment 44, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

54. The engineered nucleic acid of embodiment 44, wherein the homing molecule is selected from the group consisting of: anti-integrin alpha4,beta7; anti-MAdCAM; SDF1; and MMP-2.
55. The engineered nucleic acid of embodiment 44, wherein the growth factor is selected from the group consisting of: FLT3L and GM-CSF.
56. The engineered nucleic acid of embodiment 44, wherein the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L.
57. The engineered nucleic acid of embodiment 44, wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, a TGFbeta inhibitor, an immune checkpoint inhibitor, a VEGF inhibitor, and HPGE2.
58. The engineered nucleic acid of embodiment 57, wherein the TGFbeta inhibitor is selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and a combination thereof.
59. The engineered nucleic acid of embodiment 57, 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-TNFa antibody, an anti-TREMI antibody, and an anti-TREM2 antibody.
60. The engineered nucleic acid of embodiment 57, wherein the VEGF inhibitor comprises an anti-VEGF antibody, an anti-VEGF peptide, or a combination thereof.
61. The engineered nucleic acid of any one of embodiments 1-60, wherein the secretable effector molecule is a human-derived effector molecule.

62. The engineered nucleic acid of any one of embodiments 1-61, wherein the protease cleavage site is cleavable by a protease selected from the group consisting of: a Type I 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.
63. The engineered nucleic acid of any one of embodiments 1-62, wherein the protease cleavage site is cleavable by an ADAM17 protease.
64. The engineered nucleic acid of any one of embodiments 1-63, wherein the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176).
65. The engineered nucleic acid of embodiment 64, wherein the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177).
66. The engineered nucleic acid of embodiment 65, wherein the first region is located N-terminal to the second region.
67. The engineered nucleic acid of any one of embodiments 1-66, 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, T, or A.
68. The engineered nucleic acid of any one of embodiments 1-66, 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.

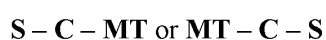
69. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179).
- 5 70. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180).
71. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181).
- 10 72. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182).
73. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183).
- 15 74. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184).
75. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185).
- 20 76. The engineered nucleic acid of any one of embodiments 1-68, wherein the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186).
77. The engineered nucleic acid of any one of embodiments 1-66, wherein the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187).
- 25

78. The engineered nucleic acid of any one of embodiments 1-66, wherein the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188).
- 5 79. The engineered nucleic acid of any one of embodiments 1-66, wherein the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189).
80. The engineered nucleic acid of any one of embodiments 1-66, wherein the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190).
- 10 81. The engineered nucleic acid of any one of embodiments 1-66, wherein the protease cleavage site comprises the amino acid sequence of VTPEPIFSLI (SEQ ID NO: 191).
82. The engineered nucleic acid of any one of embodiments 1-66, wherein the protease cleavage site comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198).
- 15 83. The engineered nucleic acid of any one of embodiments 1-82, wherein the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain.
84. The engineered nucleic acid of embodiment 83, wherein the transmembrane-intracellular domain and/or transmembrane domain is derived from PDGFR-beta, 20 CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, or BTLA.
85. The engineered nucleic acid of any one of embodiments 1-84, 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 25 post-translational modification tag, where the post-translational modification tag is capable of association with a cell membrane.
86. The engineered nucleic acid of embodiment 85, 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.

87. The engineered nucleic acid of any one of embodiments 1-84, wherein the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof.
88. The engineered nucleic acid of any one of embodiments 1-87, wherein when expressed in a cell, the secretable effector molecule is tethered to a cell membrane of the cell.
89. The engineered nucleic acid of embodiment 88, wherein 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.
90. The engineered nucleic acid of embodiment 89, wherein the protease expressed on the cell membrane is endogenous to the cell.
91. The engineered nucleic acid of embodiment 90, wherein the protease is selected from the group consisting of: a Type I 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.
92. The engineered nucleic acid of embodiment 91, wherein the protease is an ADAM17 protease.
93. The engineered nucleic acid of embodiment 89, wherein the protease expressed on the cell membrane is heterologous to the cell.
94. The engineered nucleic acid of embodiment 93, wherein the protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).

95. The engineered nucleic acid of embodiment 94, wherein the protease cleavage site comprises an NS3 protease cleavage site.
96. The engineered nucleic acid of embodiment 95, wherein the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B
5 junction cleavage site.
97. The engineered nucleic acid of any one of embodiments 89-96, wherein the protease can be repressed by a protease inhibitor.
98. The engineered nucleic acid of embodiment 97, wherein the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir,
10 boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.
99. The engineered nucleic acid of any one of embodiments 89-98, wherein expression and/or localization of the protease is capable of regulation.
100. The engineered nucleic acid of embodiment 99, wherein the expression and/or
15 localization is regulated by a cell state of the cell.
101. The engineered nucleic acid of any one of embodiments 1-100, wherein the engineered nucleic acid is a single-stranded or double-stranded nucleic acid selected from the group consisting of: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid.
- 20 102. An expression vector comprising the engineered nucleic acid of any one of embodiments 1-101.
103. The expression vector of embodiment 102, wherein the expression vector is a viral vector.
104. The expression vector of embodiment 103, wherein the viral vector is a retroviral
25 vector.
105. 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,

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

106. The chimeric protein of embodiment 105, wherein the secretable effector molecule comprises a signal peptide or a signal-anchor sequence.

107. The chimeric protein of embodiment 106, wherein the signal peptide comprises a
10 native signal peptide native to the secretable effector molecule.

108. The chimeric protein of embodiment 106, wherein the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule.

109. The chimeric protein of embodiment 108, wherein the non-native signal peptide or the
15 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.

20 110. The chimeric protein of any one of embodiments 105-109, 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.

25 111. The chimeric protein of embodiment 110, 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.

112. The chimeric protein of any one of embodiments 105-111, wherein the secretable effector molecule comprises IL-15, IL-12, or an IL-12p70 fusion protein.
113. The chimeric protein of embodiment 112, wherein the secretable effector molecule comprises IL-15.
- 5 114. The chimeric protein of embodiment 113, wherein the secretable effector molecule comprises the amino acid sequence of SEQ ID NO: 199.
115. The chimeric protein of embodiment 113, wherein the secretable effector molecule comprises IL-15 and IL-15R α sushi domain or an IL15/IL-15R α sushi domain fusion protein.
- 10 116. The chimeric protein of any one of embodiment 115, wherein the secretable effector molecule comprises the amino acid sequence of SEQ ID NO: 202.
117. The chimeric protein of embodiment 112, wherein the secretable effector molecule comprises IL-12 or an IL-12p70 fusion protein.
118. The chimeric protein of embodiment 117, wherein the secretable effector molecule
15 comprises the amino acid sequence of SEQ ID NO: 203.
119. The chimeric protein of embodiment 110, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.
120. The chimeric protein of embodiment 110, wherein the homing molecule is selected
20 from the group consisting of: anti-integrin α 4, β 7; anti-MAdCAM; SDF1; and MMP-2.
121. The chimeric protein of embodiment 110, wherein the growth factor is selected from the group consisting of: FLT3L and GM-CSF.
122. The chimeric protein of embodiment 110, wherein the co-activation molecule is
25 selected from the group consisting of: 4-1BBL and CD40L.

123. The chimeric protein of embodiment 110, wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, a TGFbeta inhibitor, an immune checkpoint inhibitor, a VEGF inhibitor, and HPGE2.
124. The chimeric protein of embodiment 123, wherein the TGF-beta inhibitor is selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and a combination thereof.
125. The chimeric protein of embodiment 123, 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-TNFa antibody, an anti-TREMI antibody, and an anti-TREM2 antibody.
126. The chimeric protein of embodiment 123, wherein the VEGF inhibitor comprises an anti-VEGF antibody, an anti-VEGF peptide, or a combination thereof.
127. The chimeric protein of any one of embodiments 105-126, wherein the secretable effector molecule is a human-derived effector molecule.
128. The chimeric protein of any one of embodiments 105-127, 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.
129. The chimeric protein of any one of embodiments 105-128, wherein the protease cleavage site is cleavable by an ADAM17 protease.

130. The chimeric protein of any one of embodiments 105-129, wherein the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176).
- 5 131. The chimeric protein of embodiment 130, wherein the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177).
132. The chimeric protein of embodiment 131, wherein the first region is located N-terminal to the second region.
- 10 133. The chimeric protein of any one of embodiments 105-132, 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, T, or A.
- 15 134. The chimeric protein of any one of embodiments 105-132, 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.
- 20 135. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179).
136. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180).
- 25 137. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181).
138. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182).

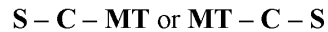
139. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183).
- 5 140. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184).
141. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185).
- 10 142. The chimeric protein of any one of embodiments 105-134, wherein the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186).
143. The chimeric protein of any one of embodiments 105-132, wherein the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187).
- 15 144. The chimeric protein of any one of embodiments 105-132, wherein the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188).
145. The chimeric protein of any one of embodiments 105-132, wherein the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189).
- 20 146. The chimeric protein of any one of embodiments 105-132, wherein the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190).
- 25 147. The chimeric protein of any one of embodiments 105-132, wherein the protease cleavage site comprises the amino acid sequence of VTPEIFSLI (SEQ ID NO: 191).

148. The chimeric protein of any one of embodiments 105-132, wherein the protease cleavage site comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198).
149. The chimeric protein of any one of embodiments 105-148, wherein the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain.
150. The chimeric protein of embodiment 149, 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.
151. The chimeric protein of any one of embodiments 105-150, 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.
152. The chimeric protein of embodiment 151, 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.
153. The chimeric protein of any one of embodiments 105-152, wherein the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof.
154. The chimeric protein of any one of embodiments 105-153, wherein when expressed in a cell, the secretable effector molecule is tethered to a cell membrane of the cell.
155. The chimeric protein of embodiment 154, wherein 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.
156. The chimeric protein of embodiment 155, wherein the protease expressed on the cell membrane is endogenous to the cell.

157. The chimeric protein of embodiment 156, wherein 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.
158. The chimeric protein of embodiment 156, wherein the protease is an ADAM17 protease.
159. The chimeric protein of embodiment 155, wherein the protease expressed on the cell membrane is heterologous to the cell.
160. The chimeric protein of embodiment 159, wherein the protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).
161. The chimeric protein of embodiment 160, wherein the protease cleavage site comprises an NS3 protease cleavage site.
162. The chimeric protein of embodiment 161, wherein the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.
163. The chimeric protein of any one of embodiments 155-162, wherein the protease can be repressed by a protease inhibitor.
164. The chimeric protein of embodiment 163, wherein the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.
165. The chimeric protein of any one of embodiments 155-164, wherein the protease further comprises a degron.

166. The chimeric protein of any one of embodiments 155-165, wherein expression and/or localization of the protease is capable of regulation.
167. The chimeric protein of embodiment 166, wherein the expression and/or localization is regulated by a cell state of the cell.
- 5 168. A composition comprising the engineered nucleic acid of any one of embodiments 1-101, or the expression vector of any one of embodiments 102-104, or the membrane-cleavable chimeric protein of any one of embodiments 105-167 and a pharmaceutically acceptable carrier.
- 10 169. An isolated cell comprising the engineered nucleic acid of any one of embodiments 1-101, or the expression vector of any one of embodiments 102-104, or the membrane-cleavable chimeric protein of any one of embodiments 105-167.
- 15 170. An isolated cell comprising an engineered nucleic acid, wherein the engineered nucleic acid comprises an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula:
- $$\mathbf{S - C - MT \text{ or } MT - C - S}$$
- wherein
- S comprises a secretable effector molecule,
- C comprises a protease cleavage site, and
- 20 MT comprises a cell membrane tethering domain,
- wherein the promoter is operably linked to the exogenous polynucleotide sequence, and wherein $\mathbf{S - C - MT}$ or $\mathbf{MT - C - S}$ is configured to be expressed as a single polypeptide.
- 25 171. The isolated cell of embodiment 169 or embodiment 170, wherein the engineered nucleic acid is recombinantly expressed.
172. The isolated cell of any one of embodiments 169-171, wherein the engineered nucleic acid is expressed from a vector or a selected locus from the genome of the cell.

173. An isolated cell comprising a membrane-cleavable chimeric protein, wherein the membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula:



5 wherein

S comprises a secretable effector molecule,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

10 and wherein $\mathbf{S - C - MT}$ or $\mathbf{MT - C - S}$ is configured to be expressed as a single polypeptide.

174. The isolated cell of any one of embodiments 169-173, 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.

- 20 175. The isolated cell of any one of embodiments 169-173, wherein the cell is a Natural Killer (NK) cell.

176. The isolated cell of any one of embodiments 169-175, wherein the cell is autologous.

177. The isolated cell of any one of embodiments 169-175, wherein the cell is allogeneic.

- 25 178. The isolated cell of any one of embodiments 169-173, wherein the cell is a tumor cell selected from the group consisting of: a bladder tumor cell, a brain tumor cell, a breast tumor cell, a cervical tumor cell, a colorectal tumor cell, an esophageal tumor cell, a glioma cell, a kidney tumor cell, a liver tumor cell, a lung tumor cell, a melanoma cell, an ovarian tumor cell, a pancreatic tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

179. The isolated cell of embodiment 178, wherein the cell was engineered via transduction with an oncolytic virus.
180. The isolated cell of any one of embodiments 169-179, wherein the cell further comprises a protease capable of cleaving the protease cleavage site.
- 5 181. The isolated cell of embodiment 180, wherein the protease is an endogenous protease.
182. The isolated cell of embodiment 181, 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
10 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.
- 15 183. The isolated cell of embodiment 181, wherein the endogenous protease is an ADAM17 protease.
184. The isolated cell of embodiment 180, wherein the protease is a heterologous protease.
185. The isolated cell of embodiment 184, wherein the heterologous protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).
- 20 186. The isolated cell of any one of embodiments 180-185, wherein the protease is expressed on the cell membrane of the cell.
187. The isolated cell of embodiment 186, wherein the protease is capable of cleaving the protease cleavage site.
188. The isolated cell of embodiment 187, wherein cleavage of the protease cleavage site
25 releases the secretable effector molecule from the cell membrane of the cell.
189. The isolated cell of any one of embodiments 169-188, wherein the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176).

190. The isolated cell of embodiment 189, wherein the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177).
191. The isolated cell of embodiment 190, wherein the first region is located N-terminal to the second region.
- 5 192. The isolated cell of any one of embodiments 169-191, 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, T, or A.
- 10 193. The isolated cell of any one of embodiments 169-191, 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.
194. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179).
- 15 195. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180).
196. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181).
197. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182).
- 20 198. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183).
199. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184).
- 25 200. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185).

201. The isolated cell of any one of embodiments 169-193, wherein the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186).
202. The isolated cell of any one of embodiments 169-191, wherein the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187).
- 5 203. The isolated cell of any one of embodiments 169-191, wherein the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188).
204. The isolated cell of any one of embodiments 169-191, wherein the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189).
- 10 205. The isolated cell of any one of embodiments 169-191, wherein the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190).
206. The isolated cell of any one of embodiments 169-191, wherein the protease cleavage site comprises the amino acid sequence of VTPEIFSLI (SEQ ID NO: 191).
207. The isolated cell of any one of embodiments 169-191, wherein the protease cleavage site comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198).
- 15 208. The isolated cell of any one of embodiments 169-207, wherein the cell further comprises an antigen recognizing receptor.
209. The isolated cell of embodiment 208, wherein the antigen recognizing receptor comprises an antigen-binding domain.
- 20 210. The isolated cell of embodiment 209, wherein the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb).
211. The isolated cell of embodiment 209, wherein the antigen-binding domain comprises a single chain variable fragment (scFv).
- 25 212. The isolated cell of embodiment 211, wherein the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

213. The isolated cell of embodiment 212, wherein the VH and VL are separated by a peptide linker.
214. The isolated cell of embodiment 213, wherein the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.
215. The isolated cell of any one of embodiments 208-214, wherein the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR).
216. The isolated cell of any one of embodiments 208-214, wherein the antigen recognizing receptor is a CAR.
217. The isolated cell of embodiment 216, wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.
218. The isolated cell of embodiment 216 or embodiment 217, wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain, a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

219. The isolated cell of any one of embodiments 216-218, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.
220. A composition comprising the isolated cell of any one of embodiments 169-219, and a pharmaceutically acceptable carrier.
- 5 221. A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells of any one of embodiments 169-219 or the composition of embodiment 220.
222. The method of embodiment 221, wherein the isolated cell is derived from the subject.
223. The method of embodiment 221 or embodiment 222, wherein the isolated cell is
10 allogeneic with reference to the subject.
224. A lipid-based structure comprising the engineered nucleic acid of any one of embodiments 1-101, or the expression vector of any one of embodiments 102-104, or the membrane-cleavable chimeric protein of any one of embodiments 105-167.
225. The lipid-based structure of embodiment 224, wherein the lipid-based structure
15 comprises a extracellular vesicle, a lipid nanoparticle, a micelle, or a liposome.
226. A composition comprising the lipid-based structure of embodiment 224 or embodiment 225, and a pharmaceutically acceptable carrier.
227. A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the lipid-based structures of embodiment 224
20 or embodiment 225, or the composition of embodiment 226.
228. The method of embodiment 227, wherein the administering comprises systemic administration.
229. The method of embodiment 227 or embodiment 228, wherein the lipid-based structure is capable of engineering a cell in the subject.
- 25 230. The method of any one of embodiments 227-229, wherein the method further comprises administering a checkpoint inhibitor.

231. The method of embodiment 230, wherein the 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-
5 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.
232. The method of any one of embodiments 227-231, wherein the method further
10 comprises administering an anti-CD40 antibody.
233. A nanoparticle comprising the engineered nucleic acid of any one of embodiments 1-101 or the membrane-cleavable chimeric protein of any one of embodiments 105-167.
234. The nanoparticle of embodiment 233, wherein the nanoparticle comprises an inorganic material.
- 15 235. A composition comprising the nanoparticle of embodiment 233 or embodiment 234.
236. A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the nanoparticles of embodiment 233 or embodiment 234, or the composition of embodiment 235.
237. The method of embodiment 236, wherein the administering comprises systemic
20 administration.
238. The method of embodiment 236 or embodiment 237, wherein the nanoparticle is capable of engineering a cell in the subject.
239. The method of any one of embodiments 236-238, wherein the method further comprises administering a checkpoint inhibitor.
- 25 240. A virus engineered to comprise the engineered nucleic acid of any one of embodiments 1-101 or the expression vector of any one of embodiments 102-104.

241. The engineered virus of embodiment 240, wherein the virus is selected from the group consisting of: a lentivirus, a retrovirus, an oncolytic virus, an adenovirus, an adeno-associated virus (AAV), and a virus-like particle (VLP).
242. A composition comprising the engineered virus of embodiment 240 or embodiment 241, and a pharmaceutically acceptable carrier.
243. A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of the engineered virus of embodiment 240 or embodiment 241, or the composition of embodiment 242.
244. The method of embodiment 243, wherein the administering comprises systemic administration.
245. The method of embodiment or embodiment 244, wherein the engineered virus infects a cell in the subject and expresses the expression cassette.
246. The method of any one of embodiments 243-245, wherein the method further comprises administering a checkpoint inhibitor.
247. A method of inducing release of a membrane-tethered effector molecule, comprising:
- providing a cell, wherein the cell comprises a membrane-bound protease and a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula:
- $$\mathbf{S - C - MT \text{ or } MT - C - S}$$
- wherein
- S comprises a secretable effector molecule,
- C comprises a cognate protease cleavage site of the membrane-bound protease, and
- MT comprises a cell membrane tethering domain,
- wherein $\mathbf{S - C - MT}$ or $\mathbf{MT - C - S}$ is configured to be expressed as a single polypeptide; 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
 5 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.
248. The method of embodiment 247, wherein the secretable effector molecule comprises a
 10 signal peptide or a signal-anchor sequence.
249. The method of embodiment 248, wherein the signal peptide comprises a native signal peptide native to the secretable effector molecule.
250. The method of embodiment 248, wherein the signal peptide comprises a non-native
 15 signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule.
251. The method of embodiment 250, 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,
 20 osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF.
252. The method of any one of embodiments 247-250, 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
 25 factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.
253. The method of embodiment 252, 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,
 30 and TNF-alpha.

254. The method of any one of embodiments 247-253, wherein the secretable effector molecule comprises IL-12, an IL-12p70 fusion protein, or IL-15.
255. The method of embodiment 254, wherein the secretable effector molecule comprises IL-15.
- 5 256. The method of embodiment 255, wherein the secretable effector molecule comprises the amino acid sequence of SEQ ID NO: 199.
257. The method of embodiment 255, wherein the secretable effector molecule comprises IL-15 and IL-15R α sushi domain or an IL15/IL-15R α sushi domain fusion protein.
258. The method of embodiment 257, wherein the secretable effector molecule comprises
10 the amino acid sequence of SEQ ID NO: 202.
259. The method of embodiment 254, wherein the secretable effector molecule comprises IL-12 or an IL-12p70 fusion protein.
260. The method of embodiment 259, wherein the secretable effector molecule comprises the amino acid sequence of SEQ ID NO: 203.
- 15 261. The method of embodiment 252, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.
262. The method of embodiment 252, wherein the homing molecule is selected from the group consisting of: anti-integrin α 4, β 7; anti-MAdCAM; SDF1; and MMP-2.
- 20 263. The method of embodiment 252, wherein the growth factor is selected from the group consisting of: FLT3L and GM-CSF.
264. The method of embodiment 252, wherein the co-activation molecule is selected from the group consisting of: 4-1BBL and CD40L.
265. The method of embodiment 252, wherein the tumor microenvironment modifier is
25 selected from the group consisting of: adenosine deaminase, a TGF β inhibitor, an immune checkpoint inhibitor, a VEGF inhibitor, and HPGE2.

266. The method of embodiment 265, wherein the TGFbeta inhibitor is selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and a combination thereof.
267. The method of embodiment 265, 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-TNFa antibody, an anti-TREMI antibody, and an anti-TREM2 antibody.
268. The method of embodiment 265, wherein the VEGF inhibitor comprises an anti-VEGF antibody, an anti-VEGF peptide, or a combination thereof.
269. The method of any one of embodiments 243-268, wherein the secretable effector molecule is a human-derived effector molecule.
270. The method of any one of embodiments 243-269, wherein the cognate 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.
271. The method of any one of embodiments 243-270, wherein the cognate protease cleavage site is cleavable by an ADAM17 protease.
272. The method of any one of embodiments 243-271, wherein the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176).

273. The method of embodiment 272, wherein the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177).
274. The method of embodiment 273, wherein the first region is located N-terminal to the second region.
- 5 275. The method of any one of embodiments 243-274, 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, T, or A.
- 10 276. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179).
277. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180).
278. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181).
- 15 279. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182).
280. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183).
- 20 281. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184).
282. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185).
283. The method of any one of embodiments 243-275, wherein the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186).
- 25 284. The method of any one of embodiments 243-274, wherein the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187).

285. The method of any one of embodiments 243-274, wherein the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188).
286. The method of any one of embodiments 243-274, wherein the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189).
- 5 287. The method of any one of embodiments 243-274, wherein the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190).
288. The method of any one of embodiments 243-274, wherein the protease cleavage site comprises the amino acid sequence of VTPEPIFSLI (SEQ ID NO: 191).
289. The method of any one of embodiments 243-274, wherein the protease cleavage site
10 comprises the amino acid sequence of ITQGLAVSTISSFF (SEQ ID NO: 198).
290. The method of any one of embodiments 243-289, wherein the cell membrane tethering domain comprises a transmembrane-intracellular domain or a transmembrane domain.
291. The method of embodiment 290, wherein the transmembrane-intracellular domain
15 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.
292. The method of any one of embodiments 243-291, wherein the cell membrane tethering domain comprises a post-translational modification tag, or motif capable of
20 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.
293. The method of embodiment 292, wherein the post-translational modification tag
25 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.

294. The method of any one of embodiments 243-291, wherein the cell membrane tethering domain comprises a cell surface receptor, or a cell membrane-bound portion thereof.
295. The method of any one of embodiments 243-294 wherein the protease is endogenous to the cell.
296. The method of embodiment 295, wherein the protease is selected from the group consisting of: a Type I 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.
297. The method of embodiment 296, wherein the protease is an ADAM17 protease.
298. The method of any one of embodiments 243-294 298, wherein the protease is heterologous to the cell.
299. The method of embodiment 298, wherein the protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).
300. The method of embodiment 299, wherein the protease cleavage site comprises an NS3 protease cleavage site.
301. The method of embodiment 300, wherein the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.
302. The method of any one of embodiments 243-301, wherein the protease can be repressed by a protease inhibitor.
303. The method of embodiment 302, wherein the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.

304. The method of any one of embodiments 243-303, wherein the protease further comprises a degron.
305. The method of any one of embodiments 243-303, wherein expression and/or localization of the protease is capable of regulation.
- 5 306. The method of embodiment 305, wherein the expression and/or localization is regulated by a cell state of the cell.

EXAMPLES

Below are examples of specific embodiments for carrying out the present invention.

- 10 The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. For example, the experiments described and performed below demonstrate the general utility of engineering cells to secrete payloads (*e.g.*, effector molecules) and delivering those cells to induce an immunogenic response against tumors.
- 15 Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1 - Regulated Cytokine Secretion Using Membrane-Cleavable System

- A Membrane-Cleavable system is assessed for the ability to regulate the secretion of
20 cytokines.

Methods

Engineering of IL-15 secreting cells:

- Lentiviral vectors encoding membrane-cleavable IL-15 constructs are generated.
- 25 Lentivirus is produced using: Lenti-X 293T packaging cell line (Clontech, Cat# 632180); LX293T Complete growth medium, without antibiotics; DMEM, hi-glucose; 1mM Sodium Pyruvate; 10% FBS, heat-inactivated; Opti-Mem I Reduced Serum Media (Gibco/Thermo Fisher; Cat# 31985); FuGene HD (Promega, Cat#E2311); Envelope, Packaging, and Transfer Vector plasmids; VSV-G-pseudotyped envelope vector (pMD2.G);
- 30 Packaging vector that contains Gag, Pol, Rev, and Tat that can be used with 2nd and 3rd generation transfer vectors (psMAX2). 293T(FT) cells from 90% confluent 10cm dishes are

lifted and dispensed at 1:3 dilution late in the afternoon the day before transfection and incubated cells as normal overnight at 37°C, 5% CO₂ (cells should be 60-85% confluent the next day at time of transfection).

A transfection reaction is prepped for each 10cm dish according to the protocol

5 below:

1. Prep transfection reaction for each 10cm dish in a separate 1.7mL tube.
2. Add 900uL Opti-Mem I at RT.
3. Add 9ug vector backbone (containing gene of interest) per reaction.
4. Add 8ug packaging vector per reaction.
- 10 5. Add 1ug envelope vector per reaction (pMD2.G).
6. Mix thoroughly by quickly vortexing for 3 seconds.
7. Add 55uL Fugene HD per reaction.
8. Mix by quickly pipetting up and down 20-30 times.
9. Let sit at RT for 10 min (allowing DNA complexes to form).
- 15 10. Slowly add mixture in dropwise manner around the dish, then mix by gently rocking back-forth and up-down for 5-10 seconds (do not swirl).
11. Place dish into virus incubator.

Viral supernatants are harvested on days 2 and 3 using a serological pipette. Cellular debris is removed using a Millipore steriflip 0.45um filters. A Lenti-X Concentrator (Cat. Nos. 631231 & 631232) is used according to the protocol: 1) Combine 1 volume of Lenti-X Concentrator with 3 volumes of clarified supernatant. Mix by gentle inversion; 2) Incubate mixture on ice or at 4°C for 30 minutes to overnight; (3) Centrifuge sample at 1,500 x g for 45 minutes at 4°C; (4) Carefully remove and discard supernatant, taking care not to disturb the pellet; (5) Gently resuspend the pellet in 1/10 to 1/100th of the original volume using sterile PBS + 0.1% BSA.

25 Primary T cells are transduced using the viral supernatants according to the protocol below:

1. Activate T cells using Gibco (Thermo Fisher) Dynabeads Human T-Activator anti-CD3/anti-CD28 beads.
- 30 a) Count the T cells – 1e6 cells per well in a 24 well plate
- b) Wash dynabeads with OpTmizer media
- c) Vortex Dynabeads thoroughly immediately before use
- d) Transfer the desired volume of Dynabeads to a tube

- e) Add a 10x volume of PBS, and mix (vortex for 5 seconds, or keep on a roller for at least 5 min)
- f) Place the tube on a magnet for 1 min and discard the supernatant by aspiration
- 5 g) Repeat washing of beads - for second wash, use OpTmizer media without IL-2
- h) Remove the tube from the magnet and resuspend the washed Dynabeads in the needed volume of OpTmizer Media with 100U/mL IL-2 to reach 6e6 beads/mL
- 10 i) Add T cell suspension to beads so that 1 mL contains 1e6 T cells and 3e6 Dynabeads per mL
- j) Plate 1 mL of mixture into each well of a 24-well plate
- k) Place the 24-well plate in a 37°C incubator
- l) After 20 hrs, T cells are ready to be transduced
- 15 2) T cell transduction:
- a) Remove 500 ul of media by carefully pipetting from the top, ensuring not to disturb the settled T cells and beads
- b) Add the lentivirus mixture onto the cells drop by drop to each corresponding well
- 20 c) Mix virus and cells by pipetting up and down gently
- d) Place the plate in a 37°C incubator
- e) Add 500 ul of media back the next day
- f) Remove the CD3 dynabeads from each well after day 3 of transduction
- 25 i) Vigorously mix the cells-dynabeads mixture by pipetting up and down
- ii) Transfer cells-dynabeads mixture into an eppendorf tube (1.5mL)
- iii) Place the eppendorf tube to a magnet holder
- iv) Keep the eppendorf tube in a magnet holder and remove the media containing cells from eppendorf tube to another eppendorf tube

30

Primary NK cells are transduced using the viral supernatants according to the protocol below:

1. Retronectin Coating of non-TC Treated 12 well plates (Day1). For 13 mL of total volume to coat one plate (final Retronectin concentration per well is 32ug/mL volume corresponding to 8ug/cm² plate area):
 - a. Thaw retronectin
 - 5 b. Take 0.416 mL from the original stock bottle (1ug/ul concentration).
 - c. Add into 15 mL falcon tube and complete the volume to 13 mL with DPBS(1X)
 - d. Mix gently. Do not mix the solution vigorously. Do not vortex
 - e. Transfer 1 mL retronectin solution into each well
 - 10 f. Incubate at 4°C overnight. (1 day before Transduction)
2. Blocking Plate with 1% BSA solution (Day 2)
 - a. Collect Retronectin solution from each well and store at -20°C
 - b. Block with 1% sterile BSA solution in PBS (1mL /well) for 5-30 min at RT
 - c. Aspirate the BSA solution and wash with DPBS once
- 15 3. NK transduction (Day 2)
 - a. Add 1mL (0.5e6/mL) NK cells and add desired amount of viral titer to each on a 12-well plate
 - b. Spin down the plate at 1200g, 20C, for 90 min
 - c. Add 1 mL MACS NK media with cytokines into the wells
 - 20 d. Then transfer the plate to a 37°C incubator

Measurement of surface-bound IL-15:

- Engineered cells are stained 7 days after transduction first with Polyclonal Rabbit anti-Human IL-15 primary antibody (LS-C487337) for 1.5 hours at room temperature, and
- 25 second with an anti-rabbit secondary antibody (labeled with Alexa Fluor 488) for 30 minutes. Surface-bound IL-15 is assessed by flow cytometry on a Beckman Coulter Flow Cytometer.

Measurement of secreted IL-15:

- Engineered cells (10⁶) are plated 7 days after transduction in 1mL fresh media. After
- 30 48 hours of incubation at 37 degrees, IL-15 secreted in the cell media is quantified by ELISA (Human IL-15 Quantikine ELISA Kit cat# D1500).

Determination of Protein Expression and Cleavage Efficiency

A Membrane-Cleavable system is designed that uses a chimeric protein having the formula S – C – MT or MT – C – S (oriented from N-terminal to C-terminal), in which S refers to a secretable payload (*e.g.*, an effector molecule), C refers to a protease cleavage site, and MT refers to a cell membrane tethering domain. **FIG. 1A** illustrates a schematic of the Membrane-Cleavable system described herein in which a desired payload is expressed as a chimeric protein in which a protease cleavage site is inserted between the payload and membrane-tethering domain. The left panel illustrates a schematic of the Membrane-Cleavable system using a transmembrane spanning architecture (*e.g.*, the chimeric protein contains a transmembrane domain). The right panel illustrates a schematic of the Membrane-Cleavable system using a membrane-associated architecture (*e.g.*, the chimeric protein contains a post-translational modification tag allowing association with a cell membrane). **FIG. 1B** illustrates a representative Membrane-Cleavable system for the regulated secretion of IL-15 in which IL-15 is expressed as a chimeric protein having a cleavage site capable of cleavage by TACE inserted between the IL-15 payload and membrane-tethering domain. The left panel illustrates a Type I transmembrane architecture (*e.g.*, S – C – MT) through use of Type I transmembrane domains, such as PDGFR-beta and CD8. The right panel a Type II transmembrane architecture (*e.g.*, MT – C – S) through use of Type II transmembrane domains, such as NKG2D and TNFR2.

Various protease cleavage site sequences based on a putative ADAM17 motif are assessed for expression and cleavage properties. Specifically, candidate protease cleavage site sequences presented in Table A with substitutions at the -1 and +1 position relative to the cleavage site (candidates #1-5) or with substitutions throughout the sequence (candidates #6-10) are examined. Various membrane tethering domain sequences are also assessed in the system, including Type I transmembrane architectures (*e.g.*, S – C – MT) through use of Type I transmembrane domains, such as PDGFR-beta and CD8 transmembrane domain sequences, as well as inverting the orientation of the chimeric protein, such as constructing Type II transmembrane architectures (*e.g.*, MT – C – S) through use of Type II transmembrane domains, such as NKG2D and TNFR2.

Table A – Candidate protease cleavage site sequences

Construct	P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'	P5'	FULL SEQ	SEQ ID NO
Pos Cntrl #1	P	R	A	E	A	V	K	G	G		PRAEAVKGG	179
Pos Cntrl #2	P	R	A	E	A	L	K	G	G		PRAEALKGG	180
Candidate #1	P	R	A	E	Y	S	K	G	G		PRAEYSKGG	181
Candidate #2	P	R	A	E	P	I	K	G	G		PRAEPIKGG	182
Candidate #3	P	R	A	E	A	Y	K	G	G		PRAEAYKGG	183
Candidate #4	P	R	A	E	S	S	K	G	G		PRAESSKGG	184
Candidate #5	P	R	A	E	F	T	K	G	G		PRAEFTKGG	185
Candidate #6	D	E	P	H	Y	S	Q	R	R		DEPHYSQRR	187
Candidate #7	P	P	L	G	P	I	F	N	P	G	PPLGPIFNPG	188
Candidate #8	P	L	A	Q	A	Y	R	S	S		PLAQAYRSS	189
Candidate #9	T	P	I	D	S	S	F	N	P	D	TPIDSSFNPD	190
Candidate #10	V	T	P	E	P	I	F	S	L	I	VTPEPIFSLI	191
Neg Cntrl	P	R	A	E	A	A	K	G	G		PRAEAAKGG	186

The secretion properties for various Membrane-Cleavable systems featuring various combinations of protease cleavage sites and membrane tethering domains are assessed through analysis of surface-bound payload (*e.g.*, surface-bound IL-15 by flow-cytometry) and secreted payload (*e.g.*, secreted IL-15 by supernatant ELISA).

Properties assessed include:

- Total protein expression (secreted + membrane bound)
- Cleavage efficiency (ratio of secreted vs membrane bound cytokines, *i.e.*, membrane release efficiency), including in various cell types of interest, such as T cells, NK cells, and HEK 293 cells

A Membrane-Cleavable system demonstrating optimal total protein expression and the desired cleavage efficiency is determined.

15 Determination of Cell Type and Cell State Secretion Properties

Various protease cleavage site sequences based on a putative ADAM17 motif are assessed for secretion properties including protease specificity and secretion kinetics in different cell types and cell states. Specifically, candidate protease cleavage site sequences include those presented in Table A, as shown above. Various membrane tethering domain sequences are also assessed in the system, including Type I transmembrane architectures (*e.g.*, S – C – MT) through use of Type I transmembrane domains, such as PDGFR-beta and

CD8 transmembrane domain sequences, as well as inverting the orientation of the chimeric protein, such as constructing Type II transmembrane architectures (e.g., MT – C – S) through use of Type II transmembrane domains, such as NKG2D and TNFR2.

The secretion properties for various Membrane-Cleavable systems featuring various combinations of protease cleavage sites and membrane tethering domains are assessed through analysis of surface-bound payload (e.g., surface-bound IL-15 by flow-cytometry) and secreted payload (e.g., secreted IL-15 by supernatant ELISA).

Properties assessed include:

- Secretion properties (e.g., specificity and kinetics), in cell types of interest, such as NK cells and T cells
- Secretion properties (e.g., specificity and kinetics), in various cell states, in particular cell states that may regulate protease expression and/or localization (e.g., +/- PMA or other activation states)

A Membrane-Cleavable system demonstrating the desired regulated secretion properties is determined. Specifically, a protease cleavage site and membrane tethering domain combination is determined demonstrating secretion kinetics for regulated secretion (membrane release) of the payload at a desired rate in cell types and cell states of interest.

Example 2 - Regulated IL-15 Secretion with Various Linker/Cleavage Site/Transmembrane Domain Combinations

Variations of Membrane-Cleavable systems, including three of the protease cleavage sites shown in **Table A** (SEQ ID NOs: 180, 187, and 191), various polypeptide linkers, various polypeptide linker orientations (i.e., a polypeptide linker N-terminal to the cleavage site and/or a polypeptide linker C-terminal to the cleavage site), and two transmembrane domains (a CD8 transmembrane domain and a B7-1 transmembrane domain) were assessed for the ability to regulate the secretion of a secretable payload, IL-15. The Membrane-Cleavable systems of this example include an orientation of, in the N-terminal to C-terminal direction, S – C – MT, in which S refers to the secretable payload, C refers to a protease cleavage site, and MT refers to a cell membrane tethering domain.

Engineering of IL-15 secreting cells:

Lentiviral vectors encoding membrane-cleavable IL-15 constructs, with varied linkers, linker orientations, and transmembrane domains were generated. Descriptions of the constructs are provided in **Table B**.

Table B – Description of Various Membrane-Cleavable IL-15 Constructs

Construct	N Term Linker	Cleavage Site (SEQ ID NO)	C-Terminal Linker	TM Domain
SB03534	None	180	None	B7-1
SB03530	None	191	None	B7-1
SB03514	None	187	None	B7-1
SB03513	None	180	SGGGGSGGGGSGGGGSGGGGS GGGSLQ (SEQ ID NO: 195)	B7-1
SB03531	None	191	SGGGGSGGGGSGGGGSGGGGS GGGSLQ (SEQ ID NO: 195)	B7-1
SB03516	None	187	SGGGGSGGGGSGGGGSGGGGS GGGSLQ (SEQ ID NO: 195)	B7-1
SB03515	None	180	TTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACD (SEQ ID NO: 196)	B7-1
SB03535	None	191	TTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACD (SEQ ID NO: 196)	CD8
SB03537	None	187	TTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACD (SEQ ID NO: 196)	CD8
SB03536	SGGGGSGGGGSG (SEQ ID NO: 194)	180	GGGSGGGGSGGGSLQ (SEQ ID NO: 197)	CD8
SB03532	SGGGGSGGGGSG (SEQ ID NO: 194)	191	GGGSGGGGSGGGSLQ (SEQ ID NO: 197)	B7-1
SB03518	SGGGGSGGGGSG (SEQ ID NO: 194)	187	GGGSGGGGSGGGSLQ (SEQ ID NO: 197)	B7-1
SB03517	SGGGGSGGGGSGGG GSGGGGSGGGSLQ (SEQ ID NO: 195)	180	None	B7-1
SB03520	SGGGGSGGGGSGGG GSGGGGSGGGSLQ (SEQ ID NO: 195)	191	None	B7-1
SB03519	SGGGGSGGGGSGGG GSGGGGSGGGSLQ (SEQ ID NO: 195)	187	None	B7-1

Lentivirus was produced as described in **Example 1**, encoding the constructs described in **Table B**. Primary NK cells were transduced with the lentiviruses by transduction methods described in **Example 1**. IL-15 membrane-bound expression and secretion were assayed as described in **Example 1**. A graph of the relationship of membrane-bound

5 expression and secretion for each construct is provided in **FIG. 2**. Constructs SB03514 and SB03536 are not shown in the graph due to low expression levels. As shown in **FIG. 2**, the secretion levels for each construct widely vary. SB03516, SB03515, SB03533, SB03518, SB03517, SB03531, SB03532, and SB03520 have higher IL-15 secretion levels compared to a no virus control (NV). Of these constructs, SB03515, SB03517, SB03532, SB03531, and

10 SB03518 demonstrated the highest secretion levels. As can be seen in **FIG. 2**, the linker type and orientation correlate with secretion level. For example, constructs including a single gly-ser linker (SEQ ID NO: 195) C-terminal to the cleavage site (see data points with a square shape), and constructs including a gly-ser linker (SEQ ID NO: 195) N-terminal to the cleavage site and a second gly-ser linker (SEQ ID NO: 197) C-terminal to the cleavage site

15 (see data points with a diamond shape) had the highest secretion levels. Furthermore, use of a single gly-ser linker (SEQ ID NO: 195) N-terminal to the cleavage site (without a second gly-ser linker C-terminal to the cleavage site) correlated with an intermediate secretion level. Thus, the variation in linkers and linker orientation as shown in **Table B** provide the opportunity to select for a desired ratio of cell bound to secreted IL-15.

20

Example 3 - Regulated IL-12 Secretion

Variations of Membrane-Cleavable systems, including three of the protease cleavage sites shown in **Table A** (SEQ ID NOs: 180, 187, and 191) were assessed for the ability to regulate the secretion of a secretable payload, IL-12, by NK cells and T cells. The

25 Membrane-Cleavable systems of this example included an orientation of, in the N-terminal to C-terminal direction, S – C – MT, in which S refers to the secretable payload, C refers to a protease cleavage site, and MT refers to a cell membrane tethering domain.

Engineering of IL-12 secreting cells:

Lentiviral vectors encoding membrane-cleavable IL-12 constructs were generated.

30 Descriptions of the constructs are provided in **Table C**.

Table C – Description of Various Membrane-Cleavable IL-12 Constructs

Construct	N Term Linker	Cleavage Site	C-Terminal Linker	TM Domain
SB04182	None	VTPEPIFSLI (SEQ ID NO: 191)	SGGGGSGGGGSGGGG SGGGGSGGGSLQ (SEQ ID NO: 195)	B7-1
SB04183	SGGGGSGGGGSG (SEQ ID NO: 194)	VTPEPIFSLI (SEQ ID NO: 191)	GGGSGGGGSGGGSLQ (SEQ ID NO: 197)	B7-1
SB04184	SGGGGSGGGGSG (SEQ ID NO: 194)	(SEQ ID NO: 187)	GGGSGGGGSGGGSLQ (SEQ ID NO: 197)	B7-1
SB04185	SGGGGSGGGGSG (SEQ ID NO: 194)	ITQGLAVSTI SSFF (SEQ ID NO: 198)	GGGSGGGGSGGGSLQ (SEQ ID NO: 197)	B7-1

Lentivirus was produced as described in **Example 1**, encoding the constructs described in **Table C**. Constructs SB04183, SB04183, and SB04183 include cleavage sites described in **Example 1**. Construct SB04184 includes an ADAM17 cleavage site that is native to CD16. Primary NK cells were expanded and then transduced with the lentiviruses by transduction methods described in **Example 1**. IL-12 membrane-bound expression and secretion were assayed as described in **Example 1**. As shown **FIG. 3A**, a high level of membrane-bound IL-12 expression (at least 90% of cells positive) was achieved in NK cells with constructs SB04812, SB04184, and SB04185. Additionally, primary T cells were transduced with the lentiviruses by transduction methods described in **Example 1**. As shown in **FIG. 3B**, low to moderate membrane-bound IL-12 expression (about 20% to about 15% positive cells) was achieved in T cells with all constructs. As shown in **FIG. 3C**, a high level of IL-12 secretion (at least 50,000pg/ml) was achieved in NK cells at 3 days post transduction and 4 days post-transduction for constructs SB04182 and SB04184. For construct SB04185, greater than 50,000pg/ml of secreted IL-12 was measured at 4 days post-transduction. Additionally, a measurable amount of IL-12 was secreted from T cells with constructs SB04182 and SB04184.

Example 4 - Regulated IL-15 Secretion

Variations of Membrane-Cleavable systems, including the protease cleavage sites of SEQ ID NO: 191, were assessed for the ability to regulate secretion of a secretable IL-15 payload. The Membrane-Cleavable systems of this example included an orientation of, in the

N-terminal to C-terminal direction, S – C – MT, in which S refers to the secretable payload, C refers to a protease cleavage site, and MT refers to a cell membrane tethering domain.

- Dual expression constructs were generated, which included a first promoter operably linked to the Membrane-Cleavable system, and a second promoter operably linked to a chimeric antigen receptor. Descriptions of the constructs are provided in **Table D**.

Table D – Description of Various Membrane-Cleavable IL-15 Constructs

Construct	Promoter	Secretable Effector (S)	Cleavage Site (C)	TM Domain (MT)
CAR/NFAT crIL15	5X NFAT minADEp	IL-15	VTPEPIFSLI (SEQ ID NO: 191)	B7-1
CAR/NFAT cr sushi IL15	5X NFAT minADEp	IL-15/IL-15Rsushi	VTPEPIFSLI (SEQ ID NO: 191)	B7-1
CAR/SV40 crIL15	SV40	IL-15	VTPEPIFSLI (SEQ ID NO: 191)	B7-1

- Lentivirus was produced as described in **Example 1**, encoding the constructs described in **Table D**. Primary, donor derived NK cells (expanded with IL-15/IL-21-expressing feeder cells) were transduced with each virus (or untransduced as a control). Next, 18 days after transduction, the NK cells were activated by co-culture with target cells that express the target antigen of the CAR (two different target cell lines were used, “target cell 1” and “target cell 2”). 24 hours after activation, IL-15 membrane-bound expression and secretion were assayed as described in **Example 1**. As shown **FIG. 4A**, membrane-bound IL-15 expression was detectable 24 hours after activation. Next, secretion levels were assayed as described in **Example 1** at 24 hours, 48 hours, and 72 hours post-activation. As shown in **FIG. 4B**, all constructs achieved an increase in secreted IL-15 following the 24 hour time-point. Additionally, of the three constructs, the IL-15/IL-15Rsushi construct achieved the highest levels of secretion.

Other Sequences

HIV-1 protease (SEQ ID NO: 144):

PQVTLWQRPLVTIKIGGQLKEALLDTGADDTVLEEMSLPGRWPKMKMIGGIGGFIKVR
QYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQIGCTLNF

Angiotensin converting enzyme (ACE) (SEQ ID NO: 156):

MGAASGRRGPGLLLLPLLLLLPPQPALALDPGLQPGNFSADEAGAQLFAQSYNSSA
EQVLFQSVAAASWAHDTNITAENARRQEEAALLSQEFAEAWGQKAKELYEPIWQNFT
DPQLRRIIGAVRTLGSANLPLAKRQQYNALLSNMSRIYSTAKVCLPNKTATCWSLDP
DLTNILASSRSYAMLLFAWEGWHNAAGIPLKPLYEDFTALSNEAYKQDGFTDTGAY
5 WRSWYNSPTFEDDLEHLYQQLEPLYLNLHAFVRRALHRRYGDRYINLRGPIAHLG
DMWAQSWENIYDMVVPFDPKPNLDVTSTMLQQGWNATHMFRVAEEFFTSLELSPM
PPEFWEGSMLEKPADGREVVCHASAWDFYNRKDFRIKQCTRVTMDQLSTVHHEMG
HIQYYLQYKDLPVSLRRGANPGFHEAIGDVLALSVSTPEHLHKIGLLDRVTNDTESDI
NYLLKMALEKIAFLPFGYLDQWRWGVFSGRTPPSRYNFDWWYLRTKYQGICPPVT
10 RNETHFDAGAKFHVPNVTPYIRYFVSFVLQFQFHEALCKEAGYEGPLHQCDIYRSTK
AGAKLRKVLQAGSSRPWQEVVKDMVGLDALDAQPLLKYFQPVQTQWLQEQQNQNG
EVLGWPEYQWHPPLPDNYPEGIDLVTDEAEASKFVEEYDRTSQVWVNEAYAEANWN
YNTNITTETSKILLQKNMQIANHTLKYGTQARKFDVNQLQNTTIKRIKKVQDLERAA
LPAQELEEYNKILLDMETTYSVATVCHPNGSCLQLEPDLTNVMATSRKYEDLLWAW
15 EGWRDKAGRAILQFYPKYVELINQAARLNGYVDAGDSWRSMYETPSLEQDLERLFQ
ELQPLYLNLHAYVRRALHRHYGAQHINLEGPIAHLGGMWAQTWSNIYDLVVPFP
SAPSMDTTEAMLKQGWTPRRMFKEADDDFTSLGLLPVPPEFWNKSMLEKPTDGREV
VCHASAWDFYNGKDFRIKQCTTVNLEDLVVAHHEMGHIQYFMQYKDLPVALREGA
NPGFHEAIGDVLALSVSTPKHLHSLNLLSSEGGSDHDLINFLMKMALDKIAFIPFSYL
20 VDQWRWRVFDGSITKENYNQEWWSRLKYQGLCPPVPRTQGDGDPGAKFHIPSSVP
YIRYFVSFIIQFQFHEALCQAAGHTGPLHKCDIYQSKEAGQRLATAMKLGFSRPWPE
AMQLITGQPNMSASAMLSYFKPLLDWLRTENELHGEKLGWPQYNWTPNSARSEGP
LPDSGRVSFLGLDLDAQARVGQWLLLFLGIALLVATLGLSQRLFSIRHRSLSHRSH
GPQFGSEVELRHS

25

DENV NS3pro (NS2B/NS3) >sp|P33478|1475-2093 (SEQ ID NO: 157):
SGVLWDTPSPPEVERAVLDDGIYRIMQRGLGRSQVGVGVFQDGVFHTMWHVTRG
AVLMYQGKRLEPSWASVKKDLISYGGGWRFQGSWNTGEEVQVIAVEPGKNPNVQ
TAPGTFKTPEGEVGAIALDFKPGTSGSPIVNREGKIVGLYGNGVVTTSPTYVSAIAQA
30 KASQEGPLPEIEDEVFRKRNLTIMDLHPGSGKTRRYLPAIVREAIRRVRTLILAPTRV
VASEMAEALKGMPIRYQTTAVKSEHTGKEIVDLMCHATFTMRLLSPVRVPNYNMII
MDEAHFTDPASIARRGYISTRVGMGEAAAFMTATPPGSVEAFPQSNAVIQDEERDIP
ERSWNSGYEWITDFPGKTVWVPSIKSGNDIANCLRKNGKRVIQLSRKTFDTEYQKT
KNNDWDYVVTDDISEMGANFRADRVIDPRRCLKPVILKDGPERVILAGPMPVTVAS

AAQRRGRIGRNQNKEGDQYVYMGQPLNNDHHAHWTEAKMLLDNINTPEGIIPALF
EPEREKSAAIDGEYRLRGEARKTFVELMRRGDLPVWLSYKVASEGFQYSDDRRWCDF
GERNNQVLEENMDVEMWTKEGERKKLRPRWLDARTYSDPLALREFKEFAAGR

- 5 DENV NS3pro (NS2B/NS3) >sp|P14340|1476-2093 (SEQ ID NO: 158):
AGVLWDVPSPPPVGKAELEDGAYRIKQKQILGYSQIGAGVYKEGTFHTMWHVTRG
AVLMHKGKRIEPSWADVKKDLISYGGGWKLEGEWKEGEEVQVLALEPGKNPRAVQ
TKPGLFKTNAGTIGAVSLDFSPGTSGSPIIDKKGKVVGLYGNGVVTRSGAYVSAIAQT
EKSIEDNPEIEDDIFRKRKLTIMDLHPGAGKTKRYLPAIVREAIKRGLRTLILAPTRVV
10 AAEMEEALRGLPIRYQTPAIRAEHTGREIVDLMCHATFTMRLSPVRVPNYNLIIMDE
AHFTDPASIAARGYISTRVEMGEAAGIFMTATPPGSRDPFPQSNAPIMDEEREIPERSW
SSGHEWVTFDKGKTVWFVPSIKAGNDIAACLRKNGKKVIQLSRKTFDSEYVKTRTN
DWDFVVTDDISEMGANFKAERVIDPRRCMKPVILTDGEERVILAGPMPVTHSSAAQR
RGRIGRNPKNENDQYIYMGEPLENDECAHWKEAKMLLDNINTPEGIIPSMFEPERE
15 KVDAIDGEYRLRGEARKTFVDLMRRGDLPVWLAIRVAAEGINYADRRWCDFGIKN
NQILEENVEVEIWTKEGERKKLKPRWLDKIYSDPLALKEFKFAAGR

- DENV NS3pro (NS2B/NS3) >sp|Q99D35|1474-2092 (SEQ ID NO: 159):
SGVLWDVPSPPETQKAELEEGVYRIKQQGIFGKTQVGVGQKEGVFHTMWHVTRG
20 AVLTHNGKRLEPNWASVKKDLISYGGGWRLSAQWQKGEEVQVIAVEPGKNPKNFQ
TMPGIFQTTTGEIGAIALDFKPGTSGSPIINREGKVVGLYGNGVVTKNGGYVSGIAQT
NAEPDGPTPELEEEMFKKRNLTIMDLHPGSGKTRKYLPAIVREAIKRRLRTLILAPTR
VVAAEMEEALKGLPIRYQTTATKSEHTGREIVDLMCHATFTMRLSPVRVPNYNLIIM
MDEAHFTDPASIAARGYISTRVGMGEAAAFMTATPPGTADAFPQSNAPIQDEERDIP
25 ERSWNSGNEWITDFVGKTVWFVPSIKAGNDIANCLRKNGKKVIQLSRKTFDTEYQK
TKLNDWDFVVTDDISEMGANFKADRVIDPRRCLKPVILTDGPERVILAGPMPVTVAS
AAQRRGRVGRNPQKENDQYIFMGQPLNKDEHHAHWTEAKMLLDNINTPEGIIPALF
EPEREKSAAIDGEYRLKGESRKTFVELMRRGDLPVWLAHKVASEGIKYTDRKWCFD
GERNNQILEENMDVEIWTKEGEKKLRPRWLDARTYSDPLALKEFKDFAAGR

- 30 DENV NS3pro (NS2B/NS3) >sp|Q5UCB8|1475-2092 (SEQ ID NO: 160):
SGALWDVPSPAATQKAALSEGVRIMQRGLFGKTQVGVGIIHIEGVFHTMWHVTRGS
VICHETGRLEPSWADVVRNDMISYGGGWRLGDKWDKEEDVQVLAIEPGKNPKHVQT
KPGLFKTLTGEIGAVTLDFKPGTSGSPIINRKKGKVIGLYGNGVVTKSGDYVSAITQAE
35 RIGEPDYEVEDDIFRKKRLTIMDLHPGAGKTKRILPSIVREALKRRLRTLILAPTRVVA

AEMEEALRGLPIRYQTPAVKSEHTGREIVDLMCHATFTTRLLSSTRVPNYNLIVMDE
 AHFTDPSSVAARGYISTRVEMGEAAAFMTATPPGTTPFPQSNSPIEDIEREIPERSW
 NTGFDWITDYQGKTVWFVPSIKAGNDIANCLRKSGKKVIQLSRKTFDTEYPKTKLTD
 WDFVVTTDISEMGANFRAGRVIDPRRCLKPVILPDGPERVILAGPIVTPASAAQRRG
 5 RIGRNP AQEDDQYVFSGDPLKNDEDHAHWTEAKMLLDNIYTPEGIITLFGPEREKT
 QAIDGEFRLRGEQRKTFVELMRRGDLPVWLSYKVASAGISYKDREWCFTGERNNQI
 LEENMEVEIWTREGEKKLRPKWLDARVYADPMALKDFKEFASGRK

PEST (two copies of residues 277-307 of human I κ B α ; SEQ ID NO: 161):
 10 LQMLPESEDEESYDTESEFTEFTEDEL PYDDGSLQMLPESEDEESYDTESEFTEFTEDE
 LPYDD

GRR (residues 352-408 of human p105; SEQ ID NO: 162):
 EIKDKKEEVQRKRQKLMPNFSDSFGGGSGAGAGGGGMFGSGGGGGGTGSTGPGYSFP
 15 H

DRR (residues 210-295 of yeast Cdc34; SEQ ID NO: 163):
 IDDENGSVILQDDDYDDGNNHIPFEDDDVYNYNDNDDDDERIEFEDDDDDDDSID
 NDSVMDRKQPHKAEDESEDVEDVERVSKKD

20 SNS (tandem repeat of SP2 and NB (SP2-NB-SP2 of influenza A or influenza B; e.g., SEQ
 ID NO: 164):
 PESMREEYRKEGSKRIKCPDCEPFCNKRGSPESMREEYRKE

25 RPB (four copies of residues 1688-1702 of yeast RPB; SEQ ID NO: 165):
 RSYSP TSPNYSPTSPSGSYSP TSPNYSPTSPSGGSRSYSP TSPNYSPTSPSGSYSP TSPNY
 SPTSPSG

30 SPmix (tandem repeat of SP1 and SP2 (SP2-SP1-SP2-SP1-SP2 of influenza A virus M2
 protein; SEQ ID NO: 166):
 PESMREEYRKEGSSLLTEVETPGSPESMREEYRKEGSSLLTEVETPGSPESMREEYRK
 E

35 NS2 (three copies of residues 79-93 of influenza A virus NS protein; SEQ ID NO: 167):
 LIEEVRHRLKTTENSGSLIEEVRHRLKTTENSGSLIEEVRHRLKTTENSGS

ODC (residues 106-142 of ornithine decarboxylase; SEQ ID NO: 168):
 FPPEVEEQDDGTLPMSCAQESGMDRHPAACASARINV

mouse ODC (residues 422–461; SEQ ID NO: 169):
SHGFPPEVEEQAAGTLPMSCAQESGMDRHPAACASARINV

5 SB03513 (SEQ ID NO: 210)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLLLELQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTSDEPHYSQRRLLPSWAITLISVNGIFVICCLTYCFAPRCRE
RRRNERLRRESVRPV

10

SB03514 (SEQ ID NO: 211)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLLLELQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTSVTPEPIFSLILLPSWAITLISVNGIFVICCLTYCFAPRCRER

15 RRNERLRRESVRPV

SB03515 (SEQ ID NO: 212)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLLLELQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEK
20 NIKEFLQSFVHIVQMFINTSDEPHYSQRRSGGGGSGGGGSGGGGSGGGGSGGGSLQL
LPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03516 (SEQ ID NO: 213)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
25 KVTAMKCFLLLELQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTSVTPEPIFSLISGGGGSGGGGSGGGGSGGGGSGGGSLQLL
PSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03517 (SEQ ID NO: 214)

30 AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLLLELQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTSSGGGGSGGGGSGDEPHYSQRRGGGSGGGGSGGGSLQL
LPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03518 (SEQ ID NO: 215)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLELQVISLESGDASIHDVTENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTSSGGGGSGGGSGSVTPEPIFSLIGGGSGGGSGGGSLQLL
5 PSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03519 (SEQ ID NO: 216)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLELQVISLESGDASIHDVTENLILANNSLSSNGNVTESGCKECEEELEEK
10 NIKEFLQSFVHIVQMFINTSSGGGGSGGGSGGGSGGGSGGGSLQDEPHYSQRRL
LPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

SB03520 (SEQ ID NO: 217)

AATMDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
15 KVTAMKCFLELQVISLESGDASIHDVTENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTSSGGGGSGGGSGGGSGGGSGGGSLQVTPEPIFSLILL
PSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV

5X NFAT minADEp promoter (SEQ ID NO: 218)

20 GGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACT
GGGGACTTTCCACTCCTGCAGGagctGGCGGCCAGACGCTAGCGGGGGGCTATAA
AAGGGGGTGGGGGCGTTCGTCTCACTCT

Interpretations

25 All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

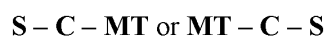
30 It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, *i.e.*, to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of”
5 shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03

CLAIMS

What is claimed is:

1. A 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 $\mathbf{S} - \mathbf{C} - \mathbf{MT}$ or $\mathbf{MT} - \mathbf{C} - \mathbf{S}$ 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, trypsiongen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF.
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 I 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 TPIDSSFNPDP (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 I 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 I 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 isolated cells of any one of claims 14-17 or the composition of claim 18.
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.