METHOD AND COMPOSITIONS FOR REGULATED ARMORING OF CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos: 62/947,427 filed December 12, 2019 and 63/116,103 filed November 19, 2020, each of which is hereby incorporated in its entirety by reference for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 3, 2021, is named STB-018WO_SL.txt, and is 191,375 bytes in size.

BACKGROUND

[0003] Tumors employ a range of direct and indirect suppression strategies to avoid recognition and clearance by the immune system. These escape strategies can effectively shut down cell therapies. Combinatorial armoring, expression of combinations of effectors, can impact the entire cancer immunity cycle and boost the activity of cell therapies as unarmored therapies have poor efficacy in solid tumors. However, current cell and gene therapy products have no control. Uncontrolled armored therapies can have toxicity in subjects. Thus, additional methods of controlling and regulating the expression of combinations of effector molecules are required.

SUMMARY

[0004] In one aspect, the present disclosure provides an engineered expression system comprising: (a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding a transcriptional modulator, wherein the first promoter is operably linked to the first exogenous polynucleotide, wherein the transcriptional modulator comprises (i) a DNA-binding domain, (ii) a transcriptional effector domain, and (iii) a hormone-binding domain of estrogen receptor (ERT2 domain); and wherein the ERT2 domain is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof; and (b) a second expression cassette comprising a transcriptional modulator -responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)x** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, and X = 1 to 20, and wherein the effector molecule is a cytokine, wherein the transcriptional modulator -responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the transcriptional modulator is capable of inducing expression of the second expression cassette by binding to the transcriptional modulator -responsive promoter

wherein (i) the first expression cassette is comprised within a first nucleic acid and the second expression cassette is comprised within a second nucleic acid, or (ii) wherein the first expression cassette and the second expression cassette are comprised within a single nucleic acid; and (c) an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor comprising an antigen-binding domain, wherein the first expression cassette and/or the second expression cassette further comprises the additional exogenous polynucleotide sequence, or an additional expression cassette comprising an additional promoter and an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor comprising an antigen-binding domain, wherein the engineered expression system further comprises the additional expression cassette.

[0004a] In another aspect, the present disclosure provides one or more expression vectors comprising the first expression cassette, the second expression cassette, and/or the additional expression cassette if present of any one of the engineered expression systems as described herein, and wherein (a) a first vector comprises the first expression cassette and the additional expression cassette if present, and a second vector comprises the second expression cassette, (b) a first vector comprises the first expression cassette, and a second vector comprises the second expression cassette and the the additional expression cassette if present, (c) a first vector comprises the first expression cassette and the second expression cassette, and a second vector comprises the additional expression cassette if present, or (d) a vector comprises the first expression cassette, the second expression cassette, and the additional expression cassette if present.

[0004b] In another aspect, the present disclosure provides an isolated cell comprising the engineered expression system as described herein or the one or more expression vectors as described herein, wherein the engineered expression system is recombinantly expressed, wherein the engineered expression system is expressed from a one or more vectors or one or more selected loci from the genome of the cell, 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, and/or wherein the cell is autologous or the cell is allogeneic.

[0004c] In another aspect, the present disclosure provides a pharmaceutical composition comprising the engineered expression system as described herein, the one or more expression vectors as described herein, or the isolated cell as described herein, and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

[0004d] In another aspect, the present disclosrure provides for the isolated cell as described herein or the composition as described herein for use in therapy. In another aspect, the present disclosure provides a use of the isolated cell as described herein or the composition as described herein in the manufacture of a medicament for treating cancer in a subject.

[0004e] In another aspect, the present disclosure provides a kit comprising the isolated cell as described herein or the pharmaceutical composition as described herein, wherein the kit further comprises written instructions for using the cell or composition for treating and/or preventing cancer in a subject.

[0004f] In one example, provided herein are engineered nucleic acids comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)x** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the

[0005] In another example, provided herein are engineered expression systems comprising: (a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide; and (b) a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)x** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter. In some examples, the first expression cassette and the second expression cassette are encoded by separate polynucleotide sequences. In some examples, the first expression cassette and the second expression cassette are encoded by the same polynucleotide sequence. In some examples, the first expression cassette and/or the second expression cassette further comprises an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor. In some examples, the first expression cassette further comprises an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor. In some examples, the second expression cassette further comprises an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor. In some examples, the engineered expression system further comprises an additional expression cassette including an additional promoter and an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor, wherein the additional promoter is operably linked to the additional exogenous polynucleotide. In some examples, the additional exogenous polynucleotide sequence is encoded by the same polynucleotide as the first expression cassette or the second expression cassette. In some examples, the additional exogenous polynucleotide sequence is encoded by the same polynucleotide as the first expression cassette. In some examples, the additional exogenous polynucleotide sequence is encoded by the same polynucleotide as the second expression cassette. In some examples, a first vector comprises the first expression cassette and the additional expression cassette if present, and a second vector comprises the second expression cassette. In some examples, a first vector comprises the first expression cassette, and a second vector comprises the second expression cassette and the the additional expression cassette if present. In some examples, a first vector comprises the first expression cassette and the second expression cassette, and a second vector comprises the additional expression cassette if present. In some examples, the

engineered expression system comprises any of the examples, features, or examples of the engineered nucleic acids described herein, including, but not limited to, any of the examples, features, or examples described in enumerated examples 1-359.

[0006] In some examples, when the second expression cassette comprises two or more units of **(L – E)**x, each linker polynucleotide sequence is operably associated with the translation of each molecule as a separate polypeptide.

[0007] In some examples, the linker polynucleotide sequence encodes a 2A ribosome skipping tag.

[0008] In some examples, the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

[0009] In some examples, the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

[0010] In some examples, the linker polynucleotide sequence encodes a cleavable polypeptide.

[0011] In some examples, the cleavable polypeptide comprises a furin polypeptide sequence.

[0012] In some examples, the second expression cassette comprising one or more units of **(L – E)**x further comprises a polynucleotide sequence encoding a secretion signal peptide.

[0013] In some examples, for each X the corresponding secretion signal peptide is operably associated with the effector molecule.

[0014] In some examples, each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule.

[0015] In some examples, each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule.

[0016] In some examples, the non-native secretion signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

[0017] In some examples, the ACP-responsive promoter comprises an ACP-binding domain and a promoter sequence.

[0018] In some examples, the promoter sequence is derived from a promoter selected from the group consisting of: 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.

[0019] In some examples, the ACP-responsive promoter is a synthetic promoter.

[0020] In some examples, the ACP-responsive promoter comprises a minimal promoter.

[0021] In some examples, the ACP-binding domain comprises one or more zinc finger binding sites.

[0022] In some examples, the first promoter is a constitutive promoter, an inducible promoter, or a synthetic promoter.

[0023] In some examples, the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

[0024] In some examples, each effector molecule is independently 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, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

[0025] In some examples, the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

[0026] In some examples, the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

[0027] In some examples, the homing molecule is selected from the group consisting of: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; CCR4; and GPR15.

[0028] In some examples, the growth factor is selected from the group consisting of: FLT3L and GM-CSF.

[0029] In some examples, the co-activation molecule is selected from the group consisting of: c-Jun, 4-1BBL and CD40L.

[0030] In some examples, the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

[0031] In some examples, the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

[0032] In some examples, the immune checkpoint inhibitors are selected from the group consisting of: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

[0033] In some examples, the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

[0034] In some examples, each effector molecule is a human-derived effector molecule.

[0035] In some examples, the first exogenous polynucleotide sequence further encodes an antigen recognizing receptor.

[0036] In certain examples, provided herein are engineered nucleic acids comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: $\mathbf{(L-E)_X}$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

[0037] In certain examples, provided herein are engineered nucleic acids comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an activation-conditional control polypeptide-responsive (ACP-responsive) promoter and a second exogenous polynucleotide sequence having the formula: $\mathbf{(L-E)_X}$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably

linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent.

[0038] In some examples, the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

[0039] In some examples, the ACP is the antigen recognizing receptor and the ACP is capable of inducing expression of the second expression cassette following binding of the ACP to a cognate antigen. In some examples, the ACP-responsive promoter is an inducible promoter that is capable of being induced by the ACP binding to the cognate antigen. In some examples, the ACP-responsive promoter is derived from a promoter region of a gene upregulated following binding of the ACP to the cognate antigen.

[0040] In some examples, the ACP-responsive promoter is selected from the group consisting of a constitutive promoter, an inducible promoter, and a synthetic promoter.

[0041] In some examples, the ACP-responsive promoter comprises a minimal promoter.

[0042] In some examples, the ACP-binding domain comprises one or more zinc finger binding sites.

[0043] In some examples, further comprising a linker polynucleotide sequence localized between the first expression cassette and the second expression cassette.

[0044] In some examples, wherein the linker polynucleotide sequence is operably associated with the translation of the ACP and each effector molecule as separate polypeptides.

[0045] In some examples, the first exogenous polynucleotide sequence further comprises a linker polynucleotide sequence localized between the region of the first exogenous polynucleotide sequence encoding the ACP and the region of the first exogenous polynucleotide sequence encoding the antigen recognizing receptor. In some examples, the linker polynucleotide sequence is operably associated with the translation of the ACP and the antigen recognizing receptor as separate polypeptides.

[0046] In some examples, the engineered nucleic acids further comprise a linker polynucleotide sequence localized between the first expression cassette and the second expression cassette. In some examples, the linker polynucleotide sequence is operably associated with the translation of the antigen receptor and each effector molecule as separate polypeptides.

[0047] In some examples, the first promoter is operably linked to the first exogenous polynucleotide sequence encoding the ACP, linker polynucleotide sequence, and antigen recognizing receptor.

[0048] In some examples, the linker polynucleotide sequence encodes a 2A ribosome skipping tag. In some examples, the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A. In some examples, the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES). In some examples, the linker polynucleotide sequence encodes a cleavable polypeptide. In some examples, the cleavable polypeptide comprises a furin polypeptide sequence.

[0049] In some examples, 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 examples, the antigen recognizing receptor recognizes GPC3. In some examples, the antigen recognizing receptor recognizes mesothelin (MSLN).

[0050] In some examples, the antigen recognizing receptor comprises an antigen-binding domain.

[0051] In some examples, the antigen-binding domain that binds to GPC3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (**SEQ ID NO: 119**), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (**SEQ ID NO: 120**), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (**SEQ ID NO: 121**), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (**SEQ ID NO: 122**), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (**SEQ ID NO: 123**), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (**SEQ ID NO: 124**).

[0052] In some examples, the VH region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of EVQLVETGGGMVQPEGSLKLSCAASGFTFNKNAMNWVRQAPGKGLEWVARIRNKT NNYATYYADSVKARFTISRDDSQSMLYLQMNNLKIEDTAMYYCVAGNSFA YWGQGTLVTVSA (**SEQ ID NO: 125**) or EVQLVESGGGLVQPGGSLRLSCAASGFTFNKNAMNWVRQAPGKGLEWVGRIRNKT NNYATYYADSVKARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQG TLVTVSA (**SEQ ID NO: 126**).

[0053] In some examples, the VL region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of DIVMSQSPSSLVVSIGEKVTMTCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWA SSRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYNYPLTFGAGTKLELK (**SEQ ID NO: 127**), or DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKLLIYWA SSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFGQGTKLEIK (**SEQ ID NO: 128**).

[0054] In some examples, the antigen-binding domain that binds to MSLN comprises the three complementarity determining regions (CDRs) of a single-domain monoclonal antibody having the amino acid sequence of: QVQLVESGGGTVQAGGSLKLACAASGLPRTYNVMGWFRQAPGKEREGVAIIYTTTG ATYYRDSVKGRATISQDNAKKSVSLQMNSLRPEDTAIYYCVARQPNSGPWEYWGQ GTQVTVSS (**SEQ ID NO: 129**), or QVKLEESGGGSVQAGGSLRLSCTTSGYTNSYKWMGWFRQAPGQEREGVAVIYTGN DRTYYSDSVKGRFTISRDNAKNMIYLDMTRLRPEDSAVYECAIGHDGAWRYWGQG TQVTVSS (**SEQ ID NO: 130**).

[0055] In some examples, 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).

[0056] In some examples, the antigen-binding domain comprises a single chain variable fragment (scFv).

[0057] In some examples, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

[0058] In some examples, the VH and VL are separated by a peptide linker.

[0059] In some examples, 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.

[0060] In some examples, the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR).

[0061] In some examples, the antigen recognizing receptor is a CAR.

[0062] In some examples, the CAR comprises one or more intracellular signaling domains, and each of the one or more intracellular signaling domains is 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, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, a CD16a intracellular signaling domain, a DNAM-1 intracellular signaling domain, a KIR2DS1 intracellular signaling domain, a KIR3DS1 intracellular signaling domain, a NKp44 intracellular signaling domain, a NKp46 intracellular signaling domain, a FceRlg intracellular signaling domain, a NKG2D intracellular signaling domain, and an EAT-2 intracellular signaling domain.

[0063] In some examples, 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, a BTLA transmembrane domain, an OX40 transmembrane domain, a DAP10 transmembrane domain, a DAP12 transmembrane domain, a CD16a transmembrane domain, a DNAM-1 transmembrane domain, a KIR2DS1 transmembrane domain, a KIR3DS1 transmembrane domain, an NKp44 transmembrane domain, an NKp46 transmembrane domain, an FceRlg transmembrane domain, and an NKG2D.

[0064] In some examples, the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

[0065] In some examples, the ACP is a transcriptional modulator.

[0066] In some examples, the ACP is a transcriptional repressor.

[0067] In some examples, the ACP is a transcriptional activator.

[0068] In some examples, the ACP further comprises a repressible protease and one or more cognate cleavage sites of the repressible protease.

[0069] In some examples, the ACP further comprises a hormone-binding domain of estrogen receptor (ERT2 domain).

[0070] In some examples, the ACP is a transcription factor.

[0071] In some examples, the transcription factor is a zinc-finger-containing transcription factor.

[0072] In some examples, the ACP comprises a DNA-binding zinc finger protein domain (ZF protein domain) and a transcriptional effector domain.

[0073] In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA).

[0074] In some examples, the ZF protein domain comprises one to ten ZFA.

[0075] In some examples, the effector domain is selected from the group consisting of: a Herpes Simplex Virus Protein 16 (VP16) activation domain; an activation domain consisting of four tandem copies of VP16, a VP64 activation domain; a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator (Rta) activation domain; a tripartite activator comprising the VP64, the p65, and the Rta activation domains, the tripartite activator is known as a VPR activation domain; a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300, known as a p300 HAT core activation domain; a Krüppel associated box (KRAB) repression domain; a truncated Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif (SEQ ID NO: 162) of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain ("WRPW" disclosed as SEQ ID NO: 162); a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

[0076] In some examples, the one or more cognate cleavage sites of the repressible protease are localized between the ZF protein domain and the effector domain.

[0077] In some examples, the repressible protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).

[0078] In some examples, the cognate cleavage site comprises an NS3 protease cleavage site.

[0079] In some examples, the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.

[0080] In some examples, the NS3 protease can be repressed by a protease inhibitor.

[0081] In some examples, the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir. In some examples, the protease inhibitor is grazoprevir. In some examples, the protease inhibitor is grazoprevir and and elbasvir. In some examples, wherein the grazoprevir and the elbasvir is co-formulated in a pharmaceutical composition. In some examples, the pharmaceutical composition is a tablet. In some examples, the grazoprevir and the elbasvir are at a 2 to 1 weight ratio. In some examples, the grazoprevir is 100 mg per unit dose and the elbasvir is 50 mg per unit dose.

[0082] In some examples, the ACP is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof.

[0083] In some examples, the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

[0084] In some examples, the ACP further comprises a degron, and wherein the degron is operably linked to the ACP.

[0085] In some examples, the degron is selected from the group consisting of HCV NS4 degron, PEST (two copies of residues 277-307 of human IκBα), 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 (SEQ ID NO: 163), an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box.

[0086] In some examples, the degron 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.

[0087] In some examples, the CRBN polypeptide substrate domain is selected from the group consisting of: IKZF1, IKZF3, CKla, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN.

[0088] In some examples, the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences.

[0089] In some examples, the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRR DAL (**SEQ ID NO: 131**).

[0090] In some examples, the IMiD is an FDA-approved drug.

[0091] In some examples, the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

[0092] In some examples, the degron is localized 5' of the repressible protease, 3' of the repressible protease, 5' of the ZF protein domain, 3' of the ZF protein domain, 5' of the effector domain, or 3' of the effector domain.

[0093] In some examples, the engineered nucleic acid further comprises an insulator.

[0094] In some examples, the insulator is localized between the first expression cassette and the second expression cassette.

[0095] In some examples, the first expression cassette is localized in the same orientation relative to the second expression cassette.

[0096] In some examples, the first expression cassette is localized in the opposite orientation relative to the second expression cassette.

[0097] In some examples, the engineered nucleic acid is selected from the group consisting of: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid.

[0098] In another example, provided herein are expression vectors comprising the engineered nucleic acid, the expression sysem, or the first expression cassette, the second expression cassette, and/or the additional expression cassettes disclosed herein.

[0099] In another example, provided herein are compositions comprising the engineered nucleic acid, the expression sysem, or the first expression cassette, the second expression

cassette, and/or the additional expression cassette described herein, and a pharmaceutically acceptable carrier.

[00100] In another example, provided herein are isolated cells comprising the engineered nucleic acid, the expression sysem, or the first expression cassette, the second expression cassette, and/or the additional expression cassette described herein or the vector as described herein.

[00101] In some examples, the engineered nucleic acid is recombinantly expressed.

[00102] In some examples, the engineered nucleic acid is expressed from a vector or a selected locus from the genome of the cell.

[00103] In some examples, 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. In some examples, the cell is a Natural Killer (NK) cell.

[00104] In some examples, the cell is autologous.

[00105] In some examples, the cell is allogeneic.

[00106] In some examples, the cell is a tumor cell selected from the group consisting of: an adenocarcinoma cell, 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, a mesothelioma cell, an ovarian tumor cell, a pancreatic tumor cell, a gastric tumor cell, a testicular yolk sac tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

[00107] In some examples, the cell is engineered via transduction with an oncolytic virus.

[00108] In some examples, the oncolytic virus is selected from the group consisting of: 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.

[00109] In some examples, the oncolytic virus is a recombinant oncolytic virus comprising the first expression cassette and the second expression cassette.

[00110] In some examples, the cell is a bacterial cell selected from the group consisting of: *Clostridium beijerinckii, Clostridium sporogenes, Clostridium novyi, Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella typhimurium*, and *Salmonella choleraesuis*.

[00111] In another example, provided herein are compositions comprising the isolated cell described herein, and a pharmaceutically acceptable carrier.

[00112] In another example, provided herein are methods of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells or the compositions described herein.

[00113] In another example, provided herein are methods of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells or the compositions described herein.

[00114] In another example, provided herein are methods of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the isolated cells or the compositions described herein.

[00115] In another example, provided herein are methods of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the isolated cells or the compositions described herein.

[00116] In another example, provided herein are methods of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the isolated cells or the compositions described herein.

[00117] In some examples, the administering comprises systemic administration.

[00118] In some examples, the administering comprises intratumoral administration.

[00119] In some examples, the isolated cell is derived from the subject.

[00120] In some examples, the isolated cell is allogeneic with reference to the subject.

[00121] In some examples, the method further comprises administering a checkpoint inhibitor.

[00122] In some examples, 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-TNFa antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody.

[00123] In some examples, the method further comprises administering an anti-CD40 antibody.

[00124] In some examples, the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

[00125] In another example, provided herein are lipid-based structures the engineered nucleic acid, the expression sysem, or the first expression cassette, the second expression cassette, and/or the additional expression cassette described herein.

[00126] In some examples, the lipid-based structure comprises a extracellular vesicle.

[00127] In some examples, the extracellular vesicle is selected from the group consisting of: a nanovesicle and an exosome.

[00128] In some examples, the lipid-based structure comprises a lipid nanoparticle or a micelle.

[00129] In some examples, the lipid-based structure comprises a liposome.

[00130] In another example, provided herein are compositions comprising the lipid-based structure described herein, and a pharmaceutically acceptable carrier.

[00131] In another example, provided herein are methods of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the lipid-based structures or compositions described herein.

[00132] In another example, provided herein are methods of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the lipid-based structures or compositions described herein.

[00133] In another example, provided herein are methods of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the lipid-based structures or compositions described herein.

[00134] In another example, provided herein are methods of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the lipid-based structures or compositions described herein.

[00135] In another example, provided herein are methods of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the lipid-based structures or compositions described herein.

[00136] In some examples, the administering comprises systemic administration.

[00137] In some examples, the administering comprises intratumoral administration.

[00138] In some examples, the lipid-based structure is capable of engineering a cell in the subject.

[00139] In some examples, the method further comprises administering a checkpoint inhibitor.

[00140] In some examples, 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-TNFa antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody.

[00141] In some examples, the method further comprises administering an anti-CD40 antibody.

[00142] In some examples, the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

[00143] In another example, provided herein are nanoparticles the engineered nucleic acid, the expression sysem, or the first expression cassette, the second expression cassette, and/or the additional expression cassette described herein.

[00144] In some examples, the nanoparticle comprises an inorganic material.

[00145] In another example, provided herein are compositions comprising the nanoparticles described herein.

[00146] In another example, provided herein are methods of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the nanoparticles or the compositions described herein

[00147] In another example, provided herein are methods of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the nanoparticles or the compositions described herein.

[00148] In another example, provided herein are methods of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the nanoparticles or the compositions described herein.

[00149] In another example, provided herein are methods of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the nanoparticles or the compositions described herein.

[00150] In another example, provided herein are methods of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the nanoparticles or the compositions described herein.

[00151] In some examples, the administering comprises systemic administration.

[00152] In some examples, the administering comprises intratumoral administration.

[00153] In some examples, the nanoparticle is capable of engineering a cell in the subject.

[00154] In some examples, the method further comprises administering a checkpoint inhibitor.

[00155] In some examples, 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-TNFa antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody.

[00156] In some examples, the method further comprises administering an anti-CD40 antibody.

[00157] In some examples, the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

[00158] In another example, provided herein are viruses engineered to comprise the engineered nucleic acid described herein.

[00159] In some examples, 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).

[00160] In some examples, the virus is an oncolytic virus.

[00161] In some examples, the first expression cassette and the second expression cassette are capable of being expressed in a tumor cell.

[00162] In some examples, the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

[00163] In some examples, the oncolytic virus is selected from the group consisting of: 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.

[00164] In another example, provided herein are compositions comprising the engineered virus or the compositions.

[00165] In another example, provided herein are methods of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered viruses or the compositions.

[00166] In another example, provided herein are methods of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the engineered viruses or the compositions.

[00167] In another example, provided herein are methods of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the engineered viruses or the compositions.

[00168] In another example, provided herein are methods of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the engineered viruses or the compositions.

[00169] In some examples, the administering comprises systemic administration.

[00170] In some examples, the administering comprises intratumoral administration.

[00171] In some examples, the engineered virus infects a cell in the subject and expresses the first expression cassette and the second expression cassette.

[00172] In some examples, the method further comprises administering a checkpoint inhibitor.

[00173] In some examples, 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-TNFa antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody.

[00174] In some examples, the method further comprises administering an anti-CD40 antibody.

[00175] In some examples, the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

[00176] In another example, provided herein are engineered cells comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)x** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

[00177] In some examples, the first expression cassette and the second expression cassette are encoded by separate polynucleotide sequences.

[00178] In some examples, the first expression cassette and the second expression cassette are encoded by a single polynucleotide sequence.

[00179] The engineered cell of any one of claims 153-155, wherein when the second expression cassette comprises two or more units of **$(L_1 – E)_x$**, each L_1 linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide.

[00180] In some examples, the engineered cell further comprises a second linker polynucleotide sequence, wherein the second linker polynucleotide links the first expression cassette to the second expression cassette.

[00181] In some examples, the second linker polynucleotide sequence is operably associated with the translation of each effector molecule and the ACP as separate polypeptides.

[00182] In some examples, each linker polynucleotide sequence encodes a 2A ribosome skipping tag.

[00183] In some examples, the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

[00184] In some examples, each linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

[00185] In some examples, the linker polynucleotide sequence encodes a cleavable polypeptide.

[00186] In some examples, the cleavable polypeptide comprises a furin polypeptide sequence.

[00187] In some examples, the second expression cassette comprising one or more units of $(L_1 – E)_X$ further comprises a polynucleotide sequence encoding a secretion signal peptide.

[00188] In some examples, for each X the corresponding secretion signal peptide is operably associated with the effector molecule.

[00189] In some examples, each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule.

[00190] In some examples, each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule.

[00191] In some examples, the non-native secretion signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

[00192] In some examples, the ACP-responsive promoter comprises an ACP-binding domain and a promoter sequence.

[00193] In some examples, the promoter sequence is derived from a promoter selected from the group consisting of: 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.

[00194] In some examples, the ACP-responsive promoter is a synthetic promoter.

[00195] In some examples, the ACP-responsive promoter comprises a minimal promoter.

[00196] In some examples, the ACP-binding domain comprises one or more zinc finger binding sites.

[00197] In some examples, the first promoter is a constitutive promoter, an inducible promoter, or a synthetic promoter.

[00198] In some examples, the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

[00199] In some examples, each effector molecule is independently 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, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

[00200] In some examples, the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

[00201] In some examples, the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

[00202] In some examples, the homing molecule is selected from the group consisting of: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; and GPR15.

[00203] In some examples, the growth factor is selected from the group consisting of: FLT3L and GM-CSF.

[00204] In some examples, the co-activation molecule is selected from the group consisting of: c-Jun, 4-1BBL, and CD40L.

[00205] In some examples, the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

[00206] In some examples, the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

[00207] In some examples, the immune checkpoint inhibitors are selected from the group consisting of: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

[00208] In some examples, the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

[00209] In some examples, each effector molecule is a human-derived effector molecule.

[00210] In some examples, the cell further comprises a third expression cassette comprising a third promoter and a third exogenous polynucleotide sequence encoding an

antigen recognizing receptor, wherein the third promoter is operably linked to the third exogenous polynucleotide.

[00211] In some examples, the first exogenous polynucleotide sequence further encodes an antigen recognizing receptor.

[00212] In another example, provided herein are engineered cells comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)$_X$** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

[00213] In another example, provided herein are engineered cells comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an activation-conditional control polypeptide-responsive (ACP-responsive) promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)$_X$** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent.

[00214] In some examples, the cell further comprises a third expression cassette comprising a third promoter and a third exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the third promoter is operably linked to the third exogenous polynucleotide.

[00215] In some examples, the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

[00216] In some examples, the ACP is the antigen recognizing receptor and the ACP is capable of inducing expression of the second expression cassette following binding of the ACP to a cognate antigen. In some examples, the ACP-responsive promoter is an inducible

promoter that is capable of being induced by the ACP binding to the cognate antigen. In some examples, the ACP-responsive promoter is derived from a promoter region of a gene upregulated following binding of the ACP to the cognate antigen.

[00217] In some examples, the ACP is the antigen recognizing receptor and the ACP is capable of inducing expression of the second expression cassette by binding to its cognate antigen.

[00218] In some examples, the ACP-responsive promoter is an inducible promoter that is capable of being induced by the ACP binding to its cognate antigen.

[00219] In some examples, the ACP-responsive promoter is selected from the group consisting of a constitutive promoter, an inducible promoter, and a synthetic promoter.

[00220] In some examples, the ACP-responsive promoter comprises a minimal promoter.

[00221] In some examples, the ACP-binding domain comprises one or more zinc finger binding sites.

[00222] In some examples, the first exogenous polynucleotide sequence further comprises a third linker polynucleotide sequence localized between the region of the first exogenous polynucleotide sequence encoding the ACP and the region of the first exogenous polynucleotide sequence encoding the antigen recognizing receptor. In some examples, the third linker polynucleotide sequence is operably associated with the translation of the ACP and the antigen recognizing receptor as separate polypeptides. In some examples, the first promoter is operably linked to the first exogenous polynucleotide sequence encoding the ACP, third linker polynucleotide sequence, and antigen recognizing receptor.

[00223] In some examples, the cells further comprise a third linker polynucleotide sequence localized between the first expression cassette and the second expression cassette.

[00224] In some examples, the third linker polynucleotide sequence is operably associated with the translation of the antigen receptor and each effector molecule as separate polypeptides.

[00225] In some examples, the third linker polynucleotide sequence encodes a 2A ribosome skipping tag. In some examples, the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A. In some examples, the third linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES). In some examples, the third linker polynucleotide sequence encodes a cleavable polypeptide. In some examples, the cleavable polypeptide comprises a furin polypeptide sequence.

[00226] In some examples, the third linker polynucleotide sequence is operably associated with the translation of the ACP and the antigen recognizing receptor as separate polypeptides.

[00227] In some examples, 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 examples, the antigen recognizing receptor recognizes GPC3. In some examples, the antigen recognizing receptor recognizes mesothelin (MSLN).

[00228] In some examples, the antigen recognizing receptor comprises an antigen-binding domain.

[00229] In some examples, the antigen-binding domain that binds to GPC3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (**SEQ ID NO: 119**), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (**SEQ ID NO: 120**), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (**SEQ ID NO: 121**), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (**SEQ ID NO: 122**), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (**SEQ ID NO: 123**), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (**SEQ ID NO: 124**).

[00230] In some examples, the VH region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of EVQLVETGGGMVQPEGSLKLSCAASGFTFNKNAMNWVRQAPGKGLEWVARIRNKTNNYATYYADSVKARFTISRDDSQSMLYLQMNNLKIEDTAMYYCVAGNSFAYWGQGTLVTVSA (**SEQ ID NO: 125**) or EVQLVESGGGLVQPGGSLRLSCAASGFTFNKNAMNWVRQAPGKGLEWVGRIRNKT

NNYATYYADSVKARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQG TLVTVSA (**SEQ ID NO: 126**).

[00231] In some examples, the VL region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of DIVMSQSPSSLVVSIGEKVTMTCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWA SSRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYNYPLTFGAGTKLELK (**SEQ ID NO: 127**), or
DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKLLIYWA SSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFGQGTKLEIK (**SEQ ID NO: 128**).

[00232] In some examples, the antigen-binding domain that binds to MSLN comprises the three complementarity determining regions (CDRs) of a single-domain monoclonal antibody having the amino acid sequence of:
QVQLVESGGGTVQAGGSLKLACAASGLPRTYNVMGWFRQAPGKEREGVAIIYTTTG ATYYRDSVKGRATISQDNAKKSVSLQMNSLRPEDTAIYYCVARQPNSGPWEYWGQ GTQVTVSS (**SEQ ID NO: 129**), or
).

[00233] In some examples, 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).

[00234] In some examples, the antigen-binding domain comprises a single chain variable fragment (scFv).

[00235] QVKLEESGGGSVQAGGSLRLSCTTSGYTNSYKWMGWFRQAPGQEREGV AVIYTGNDRTYYSDSVKGRFTISRDNAKNMIYLDMTRLRPEDSAVYECAIGHDGAW RYWGQGTQVTVSS (**SEQ ID NO: 130**In some examples, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

[00236] In some examples, the VH and VL are separated by a peptide linker.

[00237] In some examples, 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.

[00238] In some examples, the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR).

[00239] In some examples, the antigen recognizing receptor is a CAR.

[00240] In some examples, 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.

[00241] In some examples, 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, a BTLA transmembrane domain, an OX40 transmembrane domain, a DAP10 transmembrane domain, a DAP12 transmembrane domain, a CD16a transmembrane domain, a DNAM-1 transmembrane domain, a KIR2DS1 transmembrane domain, a KIR3DS1 transmembrane domain, an NKp44 transmembrane domain, an NKp46 transmembrane domain, an FceRlg transmembrane domain, and an NKG2D transmembrane domain.

[00242] In some examples, the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

[00243] In some examples, the ACP is a transcriptional modulator.

[00244] In some examples, the ACP is a transcriptional repressor.

[00245] In some examples, the ACP is a transcriptional activator.

[00246] In some examples, the ACP further comprises a repressible protease and one or more cognate cleavage sites of the repressible protease.

[00247] In some examples, the ACP further comprises a hormone-binding domain of estrogen receptor (ERT2 domain).

[00248] In some examples, the ACP is a transcription factor.

[00249] In some examples, the ACP is a zinc-finger-containing transcription factor.

[00250] In some examples, the transcription factor comprises a DNA-binding zinc finger protein domain (ZF protein domain) and an effector domain.

[00251] In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA).

[00252] In some examples, the ZF protein domain comprises one to ten ZFA.

[00253] In some examples, the effector domain is selected from the group consisting of: 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 consisting of the VP64, the p65, and the Rta activation domains, the tripartite activator is known as a VPR activation domain; a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300, known as a p300 HAT core activation domain; a Krüppel associated box (KRAB) repression domain; a truncated Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif (SEQ ID NO: 162) of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain ("WRPW" disclosed as SEQ ID NO: 162); a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

[00254] In some examples, the one or more cognate cleavage sites of the repressible protease are localized between the ZF protein domain and the effector domain.

[00255] In some examples, the repressible protease is a hepatitis C virus (HCV) nonstructural protein 3 (NS3).

[00256] In some examples, the cognate cleavage site comprises an NS3 protease cleavage site.

[00257] In some examples, the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.

[00258] In some examples, the NS3 protease can be repressed by a protease inhibitor.

[00259] In some examples, the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir. In some examples, the protease inhibitor is grazoprevir. In some examples, the protease inhibitor is grazoprevir and and elbasvir. In some examples, wherein the grazoprevir and the elbasvir is co-formulated in a pharmaceutical composition. In some examples, the pharmaceutical composition is a tablet.

In some examples, the grazoprevir and the elbasvir are at a 2 to 1 weight ratio. In some examples, the grazoprevir is 100 mg per unit dose and the elbasvir is 50 mg per unit dose.

[00260] In some examples, the ACP is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof.

[00261] In some examples, the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

[00262] In some examples, the ACP further comprises a degron, and wherein the degron is operably linked to the ACP.

[00263] In some examples, the degron is selected from the group consisting of HCV NS4 degron, PEST (two copies of residues 277-307 of human IκBα), 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 (SEQ ID NO: 163), an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box.

[00264] In some examples, the degron 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.

[00265] In some examples, the CRBN polypeptide substrate domain is selected from the group consisting of: IKZF1, IKZF3, CKla, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN.

[00266] In some examples, the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences.

[00267] In some examples, the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of

FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRR DAL (**SEQ ID NO: 131**).

[00268] In some examples, the IMiD is an FDA-approved drug.

[00269] In some examples, the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

[00270] In some examples, the degron is localized 5' of the repressible protease, 3' of the repressible protease, 5' of the ZF protein domain, 3' of the ZF protein domain, 5' of the effector domain, or 3' of the effector domain.

[00271] In some examples, the engineered nucleic acid further comprises an insulator.

[00272] In some examples, the insulator is localized between the first expression cassette and the second expression cassette.

[00273] In some examples, the first expression cassette is localized in the same orientation relative to the second expression cassette.

[00274] In some examples, the first expression cassette is localized in the opposite orientation relative to the second expression cassette.

[00275] In some examples, the cell further comprises a third expression cassette comprising a third promoter and a third exogenous polynucleotide sequence having the formula: **(L – E)**x wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the third promoter is operably linked to the third exogenous polynucleotide, and wherein for the first iteration of the (L – E) unit, L is absent.

[00276] In some examples, when the third expression cassette comprises two or more units of **(L – E)**x, each L linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide.

[00277] In some examples, each linker polynucleotide sequence encodes a 2A ribosome skipping tag.

[00278] In some examples, the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

[00279] In some examples, each linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

[00280] In some examples, the linker polynucleotide sequence encodes a cleavable polypeptide.

[00281] In some examples, the cleavable polypeptide comprises a furin polypeptide sequence.

[00282] In some examples, the third expression cassette comprising one or more units of **(L – E)**x further comprises a polynucleotide sequence encoding a secretion signal peptide.

[00283] In some examples, for each X the corresponding secretion signal peptide is operably associated with the effector molecule.

[00284] In some examples, each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule.

[00285] In some examples, each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule.

[00286] In some examples, the non-native secretion signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

[00287] In some examples, the additional promoter is a constitutive promoter, an inducible promoter, or a synthetic promoter.

[00288] In some examples, the additional promoter is a constitutive promoter selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

[00289] In some examples, each effector molecule is independently 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, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

[00290] In some examples, the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

[00291] In some examples, the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

[00292] In some examples, the homing molecule is selected from the group consisting of: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; and GPR15.

[00293] In some examples, the growth factor is selected from the group consisting of: FLT3L and GM-CSF.

[00294] In some examples, the co-activation molecule is selected from the group consisting of: c-Jun, 4-1BBL, and CD40L.

[00295] In some examples, the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

[00296] In some examples, the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

[00297] In some examples, the immune checkpoint inhibitors are selected from the group consisting of: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

[00298] In some examples, the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

[00299] In some examples, each effector molecule is a human-derived effector molecule.

[00300] In some examples, 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 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 examples, the cell is a Natural Killer (NK) cell.

[00301] In some examples, the cell is autologous.

[00302] In some examples, the cell is allogeneic.

[00303] In some examples, the cell is a tumor cell selected from the group consisting of: an adenocarcinoma cell, 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, a mesothelioma cell, an

ovarian tumor cell, a pancreatic tumor cell, a gastric tumor cell, a testicular yolk sac tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

[00304] In some examples, the cell was engineered via transduction with an oncolytic virus.

[00305] In some examples, the oncolytic virus is selected from the group consisting of: 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.

[00306] In some examples, the oncolytic virus is a recombinant oncolytic virus comprising the first expression cassette and the second expression cassette.

[00307] In some examples, the cell is a bacterial cell selected from the group consisting of: *Clostridium beijerinckii*, *Clostridium sporogenes*, *Clostridium novyi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Salmonella choleraesuis*.

[00308] In another example, provided herein are compositions comprising the engineered cells, and a pharmaceutically acceptable carrier.

[00309] In another example, provided herein are methods of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the engineered cells or the compositions.

[00310] In another example, provided herein are methods of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells or the compositions.

[00311] In another example, provided herein are methods of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the engineered cells or the compositions.

[00312] In another example, provided herein are methods of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the engineered cells or the compositions.

[00313] In another example, provided herein are methods of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the engineered cells or the compositions.

[00314] In some examples, the administering comprises systemic administration.

[00315] In some examples, the administering comprises intratumoral administration.

[00316] In some examples, the engineered cell is derived from the subject.

[00317] In some examples, the engineered cell is allogeneic with reference to the subject.

[00318] In some examples, the method further comprises administering a checkpoint inhibitor.

[00319] In some examples, 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-TNFa antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody.

[00320] In some examples, the method further comprises administering an anti-CD40 antibody.

[00321] In some examples, the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

[00322] In some examples, the method further comprises administering a protease inhibitor. In some examples, the protease inhibitor is administered in a sufficient amount to repress a repressible protease. In some examples, the protease inhibitor is administered prior to, concurrently with, subsequent to administration of the engineered cells or the composition comprising the engineered cells. In some examples, the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir. In some examples, the

protease inhibitor is grazoprevir. In some examples, the protease inhibitor is grazoprevir and and elbasvir. In some examples, wherein the grazoprevir and the elbasvir is co-formulated in a pharmaceutical composition. In some examples, the pharmaceutical composition is a tablet. In some examples, the grazoprevir and the elbasvir are at a 2 to 1 weight ratio. In some examples, the grazoprevir is 100 mg per unit dose and the elbasvir is 50 mg per unit dose.

[00323] In some examples, the method further comprises administering tamoxifen or a metabolite thereof. In some examples, the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[00324] These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, and accompanying drawings.

[00325] **FIG. 1A** provides a diagram of an exemplary regulatable TF protein and TF inducible gene with a minCMV promoter and an mCherry gene

[00326] **FIG. 1B** shows expression of the mCherry protein in cells expressing both the regulatable TF and the mCherry vector in the presence or absence of asunaprevir.

[00327] **FIG. 1C** provides a diagram of an exemplary regulatable TF protein and regulatable TF inducible gene with a minYB_TATA promoter and an mCherry gene.

[00328] **FIG. 1D** shows expression of the mCherry protein in cells expressing both the regulatable TF and the mCherry vector in the presence or absence of asunaprevir.

[00329] **FIG. 2A** provides a diagram of an exemplary single vector expressing a regulatable TF and a regulatable TF inducible gene with a minTK promoter and an IL-10 gene.

[00330] **FIG. 2B** shows IL-10 production in cells expressing the regulatable TF and IL-10 vector in the presence or absence of asunaprevir.

[00331] **FIG. 3A** provides a diagram of an exemplary single vector expressing a regulatable TF and a regulatable TF inducible gene with a minTK promoter and an IL-12 gene.

[00332] **FIG. 3B** shows IL-12 production in cells expressing the regulatable TF and IL-12 vector in the presence or absence of asunaprevir.

[00333] **FIG. 4A** provides a diagram of an exemplary single vector expressing a regulatable TF linked to a myc-tagged CAR gene and a regulatable TF inducible gene with a minYB_TATA promoter and an mCherry gene.

[00334] **FIG. 4B** shows expression of the CAR in cells expressing the regulatable TF and mCherry vector in the presence and absence of asunaprevir.

[00335] **FIG. 4C** shows expression of mCherry in cells expressing the regulatable TF and mCherry vector in the presence or absence of asunaprevir.

[00336] **FIG. 5** provides additional exemplary vectors for regulatable TF and effector gene expression in a single vector system.

[00337] **FIG. 6A** shows a schematic of a GPC3 CAR construct ("1106").

[00338] **FIG. 6B** shows the CAR transduction profile in combination with various constructs as assessed by flow cytometry.

[00339] **FIG. 7** shows the transduction profile of various constructs as assessed YFP MFI (top panel) and percentage (bottom panel).

[00340] **FIG. 8A** shows IL-12 production of various constructs with (right) or without (left) co-expression of a CAR.

[00341] **FIG. 8B** shows IL-15 production of various constructs with (right) or without (left) co-expression of a CAR.

[00342] **FIG. 8C** shows IL-21 production of various constructs with (right) or without (left) co-expression of a CAR.

[00343] **FIG. 9A** shows IL-12 production of various constructs with (bottom) or without (top) co-culturing with target HepG2 cells.

[00344] **FIG. 9B** shows IL-15 production of various constructs with (bottom) or without (top) co-culturing with target HepG2 cells.

[00345] **FIG. 9C** shows IL-21 production of various constructs with (bottom) or without (top) co-culturing with target HepG2 cells.

[00346] **FIG. 10A** shows TNFa production of various constructs with (bottom) or without (top) co-culturing with target HepG2 cells.

[00347] **FIG. 10B** shows IFNg production of various constructs with (bottom) or without (top) co-culturing with target HepG2 cells.

[00348] **FIG. 10C** shows IL-2 production of various constructs with (bottom) or without (top) co-culturing with target HepG2 cells.

[00349] **FIG. 11A** shows a schematic of a GPC3 CAR construct ("1108").

[00350] **FIG. 11B** shows the CAR transduction profile in combination with various constructs as assessed by flow cytometry.

[00351] **FIG. 12** shows tumor sizes as assessed by BLI measurement on days 11, 14, 21, and 24 for mice treated with T cells transduced with various constructs. **FIG. 13C** – IL-15; **FIG. 13D** – IL-12; **FIG. 13E** – IL-21)

[00352] **FIG. 13A** shows individual mice treated T cells without virus (**FIG. 13A** – left panel) or GPC3-CAR T alone without a cytokine (**FIG. 13A** – right panel).

[00353] **FIG. 13B** shows individual mice treated with GPC3-CAR T engineered with an IL-12/IL-21 co-expression armoring.

[00354] **FIG. 13C** shows individual mice treated with GPC3-CAR T engineered with an IL-15 armoring.

[00355] **FIG. 13D** shows individual mice treated with GPC3-CAR T engineered with an IL-12 armoring.

[00356] **FIG. 13E** shows individual mice treated with GPC3-CAR T engineered with an IL-21 armoring.

[00357] **FIG. 14A** shows production of TNFα as assessed in plasma of mice treated with various constructs at Day 3 (top panel) and Day 13 (bottom panel) post treatment.

[00358] **FIG. 14B** shows production of IFNγ as assessed in plasma of mice treated with various constructs at Day 3 (top panel) and Day 13 (bottom panel) post treatment.

[00359] **FIG. 14C** shows production of IL-2 as assessed in plasma of mice treated with various constructs at Day 3 (top panel) and Day 13 (bottom panel) post treatment.

[00360] **FIG. 15A** shows production of IL-12 as assessed in plasma of mice treated with various constructs at Day 3 (top panel) and Day 10 (bottom panel) post treatment.

[00361] **FIG. 15B** shows production of IL-15 as assessed in plasma of mice treated with various constructs at Day 3 (top panel) and Day 10 (bottom panel) post treatment.

[00362] **FIG. 15C** shows production of IL-21 as assessed in plasma of mice treated with various constructs at Day 3 (top panel) and Day 10 (bottom panel) post treatment.

[00363] **FIG. 16A** shows tumor size (left panel) and human T cell persistence (right panel) for T cells engineered with various constructs at day 14 post tumor injection (Day 3 post T cell treatment).

[00364] **FIG. 16B** shows tumor size (left panel) and human T cell persistence (right panel) for T cells engineered with various constructs at day 21 post tumor injection (Day 13 post T cell treatment)

[00365] **FIG. 17A** shows schematics of various payload expression systems using a Tamoxifen-based regulatable TF expression system.

[00366] **FIG. 17B** shows reporter expression using various Tamoxifen-based regulatable TF expression systems following treatment with different amounts of 4-OHT.

[00367] **FIG. 17C** shows reporter expression using various Tamoxifen-based regulatable TF expression systems following treatment with different amounts of N-desmethyltamoxifen.

[00368] **FIG. 17D** shows reporter expression using various Tamoxifen-based regulatable TF expression systems following treatment with different amounts of endoxifen.

[00369] **FIG. 17E** shows a summary of reporter expression using various Tamoxifen-based regulatable TF expression systems following treatment.

[00370] **FIG. 18** shows a summary of CAR expression using various Tamoxifen-based regulatable TF expression systems following treatment.

[00371] **FIG. 19** shows flow cytometry plots of CAR expression using various Tamoxifen-based regulatable TF expression systems following treatment

[00372] **FIG. 20** shows schematics of various payload expression systems using drug-inducible ACP-based regulatable TF expression systems.

[00373] **FIG. 21** shows schematics of various payload expression systems using drug-inducible ACP-based regulatable TF expression systems.

[00374] **FIG. 22** shows CAR expression using various drug-inducible ACP-based regulatable TF expression systems.

[00375] **FIG. 23A** shows reporter expression using a specific drug-inducible ACP-based regulatable TF expression system.

[00376] **FIG. 23B** shows CAR expression using a specific drug-inducible ACP-based regulatable TF expression system.

[00377] **FIG. 24A** shows reporter expression using a specific drug-inducible ACP-based regulatable TF expression system.

[00378] **FIG. 24B** shows CAR expression using a specific drug-inducible ACP-based regulatable TF expression system.

[00379] **FIG. 25** shows a schematic of an ACP for drug-inducible formats (also referred to as “synTF”) using an NS3/NS4 protease cleavage site and a VPR transcriptional effector domain (Construct “1845”) as well as the expression cassette using a 4x BS minYB-TATA ACP-responsive promoter driving hIL-12 effector molecule payload.

[00380] **FIG. 26** shows i*n vitro* production of hIL-12 using an ACP-based regulatable expression system. Columns are no drug, 0.1μM GRZ, and 0.5μM GRZ from left to right, respectively.

[00381] **FIG. 27** shows the experimental design used to assessed ACP-based regulatable expression systems *in vivo*.

[00382] **FIG. 28** shows fold-expansion of T cells engineered with a constitutive hIL-12 expression system or an ACP-based regulatable expression system *in vivo*.

[00383] **FIG. 29** shows the glucose profile of T cells engineered with a constitutive hIL-12 expression system or an ACP-based regulatable expression system *in vivo*.

[00384] **FIG. 30** shows the percentage of circulating T cells engineered with a constitutive hIL-12 expression system or an ACP-based regulatable expression system *in vivo.*

[00385] **FIG. 31** shows the production of hIL-12 in plasma produced by T cells engineered with a constitutive hIL-12 expression system or an ACP-based regulatable expression system *in vivo*.

[00386] **FIG. 32** shows schematics of various payload expression systems using drug-inducible ACP-based regulatable TF expression systems.

[00387] **FIG. 33** shows i*n vitro* production of hIL-12 by T cells transduced with various ACP-based regulatable expression systems following treatment with various concentrations of grazoprevir.

[00388] **FIG. 34** shows i*n vitro* production of hIL-15 by T cells transduced with various ACP-based regulatable expression systems following treatment with various concentrations of grazoprevir.

[00389] **FIG. 35** shows CAR expression in T cells transduced with various drug-inducible ACP-based regulatable TF expression systems.

[00390] **FIG. 36** shows CAR activity using various drug-inducible ACP-based regulatable TF expression systems as assessed by target cell killing (LDH release).

[00391] **FIG. 37** shows i*n vitro* production of hIL-12 by NK cells transduced with various ACP-based regulatable expression systems following treatment with various concentrations of grazoprevir.

[00392] **FIG. 38** shows schematics of various payload expression systems using drug-inducible ACP-based regulatable TF expression systems.

[00393] **FIG. 39** shows i*n vitro* production of hIL-12 by T cells transduced with various ACP-based regulatable expression systems following treatment with various concentrations of grazoprevir.

[00394] **FIG. 40** shows i*n vitro* production of hIL-15 by T cells transduced with various ACP-based regulatable expression systems following treatment with various concentrations of grazoprevir.

[00395] **FIG. 41** shows i*n vitro* production of hIL-15 by T cells transduced with various ACP-based regulatable expression systems following treatment with various concentrations of grazoprevir.

[00396] **FIG. 42** shows the T cells in the blood (hCD3+:hCD45+ shown as % of live cells) engineered with a constitutive hIL-12 expression system or a drug-inducible ACP-based regulatable expression system *in vivo* over a time course (Day 4 top panel; Day 8 middle panel; Day 12 bottom panel) for various grazoprevir dosing regimens. *Samples of 2 groups were lost and not included in the analysis.

[00397] **FIG. 43** shows the production of hIL-12 in plasma produced by T cells engineered with a constitutive hIL-12 expression system or a drug-inducible ACP-based regulatable expression system *in vivo* for various grazoprevir dosing regimens at Day 4.

[00398] **FIG. 44** shows the production of hIL-12 in plasma produced by T cells engineered with a constitutive hIL-12 expression system or a drug-inducible ACP-based regulatable expression system *in vivo* for various grazoprevir dosing regimens at Days 8 (left panel) and 12 (right panel).

[00399] **FIG. 45** shows an “on/off/on” Grazoprevir (Grz) dosing regimen.

[00400] **FIG. 46** shows the T cells in the blood (hCD3+:hCD45+ shown as % of live cells) engineered with a constitutive hIL-12 expression system or a drug-inducible ACP-based regulatable expression system *in vivo* over a time course at the indicated days for an “on/off/on” Grazoprevir (Grz) dosing regimen.

[00401] **FIG. 47** shows the production of hIL-12 in plasma produced by T cells engineered with a constitutive hIL-12 expression system or a drug-inducible ACP-based regulatable expression system *in vivo* over a time course at the indicated days for an “on/off/on” Grazoprevir (Grz) dosing regimen.

[00402] **FIG. 48** shows body weight of mice administered T cells engineered with a constitutive hIL-12 expression system or a drug-inducible ACP-based regulatable expression system *in vivo* over a time course at the indicated days for an “on/off/on” Grazoprevir (Grz) dosing regimen.

[00403] **FIG. 49** presents a workflow for a screen directed to assessing promoters that turn on transcription when CAR cells are activated by target cells.

[00404] **FIG. 50** presents the constructs and candidate promoters assessed in a screen directed to promoters that turn on transcription when CAR cells are activated by target cells.

[00405] **FIG. 51** shows quantified mKate expression by flow-cytometry for constructs and candidate promoters assessed in a screen directed to promoters that turn on transcription when CAR cells are activated by target cells at 24 hours following culturing with (right column) or without (left column) HepG2 target cells.

[00406] **FIG. 52** shows quantified mKate expression by flow-cytometry for constructs and candidate promoters assessed in a screen directed to promoters that turn on transcription when CAR cells are activated by target cells at 48 hours following culturing with (right column) or without (left column) HepG2 target cells

[00407] **FIG. 53** shows histograms of mKate expression by flow-cytometry for identified promoters that turn on transcription when CAR cells are activated by target cells at 24 hours (top panel) and 48 hours (bottom panel) following culturing with HepG2 target cells ("Promoter+Target"). Also shown is CAR only cultured without HepG2 targets ("CAR only"), CAR only cultured with HepG2 targets ("CAR+Target"), CAR + promoter cultured without HepG2 targets ("Promoter Only").

[00408] **FIG. 54** shows i*n vitro* production of hIL-12 by T cells transduced with various ACP-based regulatable expression systems following treatment with various concentrations of grazoprevir with or without elbasvir.

DETAILED DESCRIPTION

Definitions

[00409] Terms used in the claims and specification are defined as set forth below unless otherwise specified.

[00410] The term "ameliorating" refers to any therapeutically beneficial result in the treatment of a disease state, e.g., a cancer disease state, including prophylaxis, lessening in the severity or progression, remission, or cure thereof.

[00411] The term "in situ" refers to processes that occur in a living cell growing separate from a living organism, e.g., growing in tissue culture.

[00412] The term "in vivo" refers to processes that occur in a living organism.

[00413] The term "mammal" as used herein includes both humans and non-humans and include but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

[00414] The term percent "identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

[00415] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[00416] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., infra).

[00417] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).

[00418] The term “sufficient amount” means an amount sufficient to produce a desired effect, e.g., an amount sufficient to modulate protein aggregation in a cell.

[00419] The term “therapeutically effective amount” is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a “prophylactically effective amount” as prophylaxis can be considered therapy.

[00420] It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

Engineered Nucleic Acids and Polypeptides

[00421] Regulation of drug expression in cell therapies is required to hit therapeutic efficacy windows. Such methods are described herein using a regulatable transcription factor that can drive expression of any desirable effector molecule or combination of effector molecules. This system is versatile as it can regulate intracellular or membrane bound proteins by using, for example, a modular protease system that enables ON or OFF configurations. It can use protease switch drugs that are FDA approved and can be administered via oral delivery with a favorable pharmacokinetic profile. In addition, the methods and compositions described herein may be used, e.g., for regulated immunomodulatory effector expression in cell or gene therapies. The regulatable transcription factor can be used in conjunction with, e.g., CAR T cells, CAR NK cells, TCR T cells, TIL therapies, viral-specific T cells, or any other appropriate immune cell therapy.

[00422] In one aspect, provided herein are engineered nucleic acids comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and/or an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an activation-conditional control polypeptide-responsive (ACP-responsive) promoter and a second exogenous polynucleotide sequence having the formula: $\mathbf{(L-E)_X}$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (**L – E**) unit, L is absent, and optionally wherein the ACP is capable of inducing expression of the first expression cassette by binding to the ACP-responsive promoter. In some embodiments, the ACP includes a drug-inducible domain, such as a tetracycline responsive domain (*e.g.*, a TetR domain) or a repressible protease domain (*e.g.*, an NS3 protease). In some embodiments, the ACP is an antigen recognizing receptor and the receptor is capable of inducing expression of the second expression cassette following binding to its cognate antigen ("activation inducible system"), such as a CAR binding to a cognate antigen and the ACP-responsive promoter includes a promoter sequence capable of driving expression of the second expression cassette in response to CAR signaling.

[00423] In one aspect, provided herein are engineered nucleic acids comprising: a first expression cassette comprising: (a) a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and an antigen

recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)$_X$** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

[00424] In one aspect, provided herein are engineered nucleic acids comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an activation-conditional control polypeptide-responsive (ACP-responsive) promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)$_X$** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent. Expression of the second expression cassette can be induced by an ACP binding to the ACP-responsive promoter. An ACP can be a receptor, such as the antigen recognizing receptor, can induce expression of the second expression cassette upon ACP binding to a cognate ligand (*e.g.*, a cognate antigen), such as downstream signaling following ligand binding inducing expression from an ACP-responsive promoter. In a non-limiting illustrative example, an ACP can be a chimeric antigen receptor (CAR), and upon CAR binding to a cognate receptor, downstream signaling (*e.g.*, T cell or NK cell receptor signaling) can induce expression of a cytokine payload (*e.g.*, cytokine armoring) from an ACP-responsive promoter that is specific to CAR binding of a target antigen. Examples of ACP-responsive promoters useful for in activation inducible systems are described below (*see* "Promoters").

[00425] In some embodiments, when the second expression cassette comprises two or more units of **(L – E)$_X$**, each linker polynucleotide sequence is operably associated with the translation of each molecule as a separate polypeptide.

[00426] X can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more.

[00427] In some embodiments, a single engineered nucleic acid comprises at least one, two, three four, five, or more expression cassettes. In general, each expression cassette refers

to a promoter operably linked to a polynucleotide sequence encoding protein of interest. For example, each of an ACP, an effector molecule, and an antigen recognizing receptor can be encoded by a separate expression cassette on the same engineered nucleic acid (*e.g.*, vector). The expression cassettes can be oriented in any direction relative to each other (e.g., the cassettes can be in the same orientation or the opposite orientation). In exemplary engineered nucleic acids with three or more expression cassettes the cassettes can be in the same orientation or a mixed orientation (e.g., the first and second cassette can be in the same orientation while the third cassette is in the opposite orientation). In some embodiments, the first expression cassette is localized in the same orientation relative to the second expression cassette. In some embodiments, the first expression cassette is localized in the opposite orientation relative to the second expression cassette.

[00428] In some embodiments, one or more engineered nucleic acids can comprise at least one, two, three four, five, or more expression cassettes. Stratagies for regulated armoring including two or more engineered nucleic acids can be referred to as an "engineered expression system." In one aspect, engineered expression systems are provided herein that include (a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide; and (2) a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)x** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter. In some embodiments of the expression system, the first expression cassette and the second expression cassette are encoded by separate polynucleotide sequences. In some embodiments of the expression system, the first expression cassette and the second expression cassette are encoded by the same polynucleotide sequence. In some embodiments of the expression system, the first expression cassette and/or the second expression cassette further includes an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor. In some embodiments of the expression system, the first expression cassette further includes an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor. In some embodiments of the expression system, the second expression cassette further includes an additional

exogenous polynucleotide sequence encoding an antigen recognizing receptor. In some embodiments of the expression system, the engineered expression system further includes an additional expression cassette including an additional promoter and an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor, wherein the additional promoter is operably linked to the additional exogenous polynucleotide. In some embodiments of the expression system, the additional exogenous polynucleotide sequence is encoded by the same polynucleotide as the first expression cassette or the second expression cassette. In some embodiments of the expression system, the additional exogenous polynucleotide sequence is encoded by the same polynucleotide as the first expression cassette. In some embodiments of the expression system, the additional exogenous polynucleotide sequence is encoded by the same polynucleotide as the second expression cassette. In some embodiments of the expression system, a first vector includes the first expression cassette and the additional expression cassette if present, and a second vector includes the second expression cassette. In some embodiments of the expression system, a first vector includes the first expression cassette, and a second vector includes the second expression cassette and the the additional expression cassette if present. In some embodiments of the expression system, a first vector includes the first expression cassette and the second expression cassette, and a second vector includes the additional expression cassette if present.

[00429] As illustrative non-limiting examples of expression systems, (1) an antigen recognizing receptor expression cassette and an effector molecule expression cassette can be encoded by a first engineered nucleic acid, and an ACP expression cassette can be encoded by a second engineered nucleic acid; (2) an ACP expression cassette and an effector molecule expression cassette can be encoded by a first engineered nucleic acid, and an antigen recognizing receptor expression cassette can be encoded by a second engineered nucleic acid; (3) an ACP expression cassette and an antigen recognizing receptor expression cassette can be encoded by a first engineered nucleic acid, and an effector molecule expression cassette can be encoded by a second engineered nucleic acid. In an additional illustrative non-limiting example, an effector molecule expression cassette can be encoded by a first engineered nucleic acid, and an ACP expression cassette can be encoded by a second engineered nucleic acid.

[00430] In some embodiments, expression cassettes can be multicistronic, i.e., more than one separate polypeptide (e.g., multiple exogenous polynucleotides or effector molecules) can be produced from a single mRNA transcript. For example, a multicistronic expression

cassette can encode both an ACP and antigen recognizing receptor, *e.g.*, both expressed from a single expression cassette driven by a constitutive promoter. In another example, a multicistronic expression cassette can encode both an effector molecule and an antigen recognizing receptor, *e.g.*, both expressed from a single expression cassette driven by an ACP-responsive promoter. Expression cassettes can be multicistronic through the use of various linkers, e.g., a polynucleotide sequence encoding a first protein of interest can be linked to a nucleotide sequence encoding a second protein of interest, such as in a first gene:linker:second gene 5' to 3' orientation. Multicistronic features and options are described in the section "Multicistronic and Multiple Promoter Systems."

[00431] In some embodiments, the engineered nucleic acid is selected from: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid. Also provided herein is an expression vector comprising the engineered nucleic acid.

[00432] In some embodiments, the engineered nucleic acid further comprises an insulator. The insulator can be localized between the first expression cassette and the second expression cassette. The insulator can be localized between the first expression cassette and the second expression cassette where both cassettes are in the same orientation relative to one another. The insulator can be localized between the first expression cassette and the second expression cassette where the cassettes are in the opposite orientation relative to one another. An insulator is a cis-regulatory element that has enhancer-blocking or barrier function. Enhancer-blocker insulators block enhancers from acting on the promoter of nearby genes. Barrier insulators prevent euchromatin silencing. An example of a suitable insulator of the present disclosure is the A2 insulator as described in Liu M, *et al*., *Nat Biotechnol*. 2015 Feb;33(2):198-203. Additional insulators are described in West et al, *Genes & Dev*, 002. 16: 271-288, both of which are incorporated by reference in their entirety. Other examples of suitable insulators include, without limitation, an A1 insulator, a CTCF insulator, a *gypsy* insulator, an *HS5* insulator, and a β-globin locus insulator, such as *cHS4.* In some embodiments, the insulator is an A2 insulator, an A1 insulator, a CTCF insulator, an HS5 insulator, a *gypsy* insulator, a β-globin locus insulator, or a cHS4 insulator. The insulator can be an A2 insulator.

Activation-conditional control polypeptide (ACP)

[00433] In some embodiments, the ACP is a transcriptional modulator. In some embodiments, the ACP is a transcriptional repressor. In some embodiments, the ACP is a transcriptional activator. In some embodiments, the ACP is a transcription factor. In some

embodiments, the ACP comprises a DNA-binding domain and a transcriptional effector domain. In some embodiments, the transcription factor is a zinc-finger-containing transcription factor. In some embodiments, the zinc-finger-containing transcription factor may be a synthetic transcription factor. In some embodiments, the ACP DNA-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain) and an effector domain. In some embodiments, the DNA-binding domain comprises a tetracycline (or derivative thereof) repressor (TetR) domain. In some embodiments, the ACP is an antigen recognizing receptor of the present disclosure.

Zinc Finger Protein Domain

[00434] 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. The ZF motifs can be directly adjacent to each other, or separated by a flexible linker sequence. In some embodiments, a ZFA is an array, string, or chain of ZF motifs arranged in tandem. A ZFA can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,1 3, 14, or 15 zinc finger motifs. The ZFA can have from 1-10, 1-15, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5 3-6, 3-7, 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, or 5-15 zinc finger motifs.

[00435] 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.

[00436] An exemplary ZF protein domain is shown in the sequence SRPGERPFQCRICMRNFSRRHGLDRHTRTHTGEKPFQCRICMRNFSDHSSLKRHLRTHTGSQKPFQCRICMRNFSVRHNLTRHLRTHTGEKPFQCRICMRNFSDHSNLSRHLKTH

TGSQKPFQCRICMRNFSQRSSLVRHLRTHTGEKPFQCRICMRNFSESGHLKRHLRTHL
RGS (SEQ ID NO: 88).

ACP Effector Domain

[00437] The ACP can also further comprise an effector domain, such as a transcriptional effector domain. For instance, a transcriptional effector domain can be the effector or activator domain of a transcription factor. Transcription factor activation domains are also known as transactivation domains, and act as scaffold domains for proteins such as transcription coregulators that act to activate or repress transcription of genes. Any suitable transcriptional effector domain can be used in the ACP including, but not limited to, a Herpes Simplex Virus Protein 16 (VP16) activation domain; an activation domain consisting of four tandem copies of VP16, a VP64 activation domain; a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator (Rta) activation domain; a tripartite activator comprising the VP64, the p65, and the Rta activation domains, the tripartite activator is known as a VPR activation domain; a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300, known as a p300 HAT core activation domain; a Krüppel associated box (KRAB) repression domain; a truncated Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif (SEQ ID NO: 162) of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain ("WRPW" disclosed as SEQ ID NO: 162); a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain, or any combination thereof.

[00438] 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 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 (SEQ ID NO: 162) of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain ("WRPW" disclosed as SEQ ID NO: 162); a DNA (cytosine-5)-

methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

[00439] Exemplary transcription effector domain protein sequences are shown in **Table 8**. Exemplary transcription effector domain nucleotide sequences are shown in **Table 9**.

Table 8 – Transcriptional Effector Domain (Protein)

Amino Acid Sequence	SEQ ID NO:	Description
RTLVTFKDVFVDFTREEWKLLDTAQQIVYRNV MLENYKNLVSLGYQLTKPDVILRLEKGEEPWL V	96	KRAB
RTLVTFKDVFVDFTREEWKLLDTAQQIVYRNV MLENYKNLVSLGY	97	truncated KRAB (min KRAB)
EASGSGRADALDDFDLDMLGSDALDDFDLDM LGSDALDDFDLDMLGSDALDDFDLDMLINSRS SGSPKKKRKVGSQYLPDTDDRHRIEEKRKRTY ETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSAS VPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQIS QASALAPAPPQVLPQAPAPAPAPAMVSALAQA PAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEA LLQLQFDDEDLGALLGNSTDPAVFTDLASVDN SEFQQLLNQGIPVAPHTTEPMLMEYPEAITRLV TGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSI ADMDFSALLGSGSGSRDSREGMFLPKPEAGSA ISDVFEGREVCQPKRIRPFHPPGSPWANRPLPAS LAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTP EASHLLEDPDEETSQAVKALREMADTVIPQKE EAAICGQMDLSHPPPRGHLDELTTTLESMTEDL NLDSPLTPELNEILDTFLNDECLLHAMHISTGLS IFDTSLF	90	VPR activation domain

Table 9 - Transcriptional Effector Domain (Nucleotide)

Nucleic Acid Sequence	SEQ ID NO:	Description
AGAACCCTGGTCACCTTCAAGGACGTGTTCG TGGACTTCACCCGGGAAGAGTGGAAGCTGCT GGATACAGCCCAGCAGATCGTGTACCGGAA CGTGATGCTGGAAAACTACAAGAATCTGGTG TCCCTGGGCTACCAGCTGACCAAGCCTGACG TGATCCTGCGGCTGGAAAAGGGCGAAGAAC CTTGGCTGGTG	98	KRAB
AGAACCCTGGTCACCTTCAAGGACGTGTTCG TGGACTTCACCCGGGAAGAGTGGAAGCTGCT GGATACAGCCCAGCAGATCGTGTACCGGAA CGTGATGCTGGAAAACTACAAGAATCTGGTG TCCCTGGGCTAC	99	truncated KRAB (min KRAB)

Drug-inducible domains

[00440] 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 may be induced by tamoxifen, or a metabolite thereof, such as 4-hydroxy-tamoxifen (4-OHT), and comprises an estrogen receptor variant, such as ERT2. In

some embodiments, the ACP is a small molecule (e.g., drug) inducible polypeptide that comprises a repressible protease and one or more cognate cleavage sites of the repressible protease.

[00441] The term "repressible protease" as used herein, refers to a protease that can be inactivated by the presence or absence of a specific agent (e.g., that binds to the 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.

[00442] Non-limiting examples of repressible proteases include hepatitis C virus proteases (e.g., NS3 and NS2-3); signal peptidase; proprotein convertases of the subtilisin/kexin family (furin, PCI, PC2, PC4, PACE4, PC5, PC); proprotein convertases cleaving at hydrophobic residues (e.g., Leu, Phe, Val, or Met); proprotein convertases cleaving at small amino acid residues such as Ala or Thr; proopiomelanocortin converting enzyme (PCE); chromaffin granule aspartic protease (CGAP); prohormone thiol protease; carboxypeptidases (e.g., carboxypeptidase E/H, carboxypeptidase D and carboxypeptidase Z); aminopeptidases (e.g., arginine aminopeptidase, lysine aminopeptidase, aminopeptidase B); prolyl endopeptidase; aminopeptidase N; insulin degrading enzyme; calpain; high molecular weight protease; and, caspases 1, 2, 3, 4, 5, 6, 7, 8, and 9. Other proteases include, but are not limited to, aminopeptidase N; puromycin sensitive aminopeptidase; angiotensin converting enzyme; pyroglutamyl peptidase II; dipeptidyl peptidase IV; N-arginine dibasic convertase;endopeptidase 24.15; endopeptidase 24.16; amyloid precursor protein secretases alpha, beta and gamma; angiotensin converting enzyme secretase; TGF alpha secretase; T F alpha secretase; FAS ligand secretase; TNF receptor-I and -II secretases; CD30 secretase; KL1 and KL2 secretases; IL6 receptor secretase; CD43, CD44 secretase; CD 16-1 and CD 16-11 secretases; L-selectin secretase; Folate receptor secretase; MMP 1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 14, and 15; urokinase plasminogen activator; tissue plasminogen activator; plasmin; thrombin; BMP-l (procollagen C-peptidase); ADAM 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11; and, granzymes A, B, C, D, E, F, G, and H. For a discussion of proteases, see, e.g., V. Y. H. Hook, Proteolytic and cellular mechanisms in prohormone and proprotein processing, RG Landes Company, Austin, Tex., USA (1998); N. M. Hooper et al., Biochem. J. 321 : 265-279 (1997); Z. Werb, Cell 91 : 439-442 (1997); T. G. Wolfsberg et al., J. Cell Biol. 131 : 275-278 (1995); K. Murakami and J. D. Etlinger, Biochem. Biophys. Res. Comm. 146: 1249-1259 (1987); T.

Berg et al., Biochem. J. 307: 313-326 (1995); M. J. Smyth and J. A. Trapani, Immunology Today 16: 202-206 (1995); R. V. Talanian et al., J. Biol. Chem. 272: 9677-9682 (1997); and N. A. Thomberry et al., J. Biol. Chem. 272: 17907-17911 (1997), the disclosures of which are incorporated herein.

[00443] The term "cognate cleavage site" as used herein, refers to a specific sequence or sequence motif recognized by and cleaved by the repressible protease. A cleavage site for a protease includes the specific amino acid sequence or motif recognized by the protease during proteolytic cleavage and typically includes the surrounding one to six amino acids on either side of the scissile bond, which bind to the active site of the protease and are used for recognition as a substrate.

[00444] Other proteases, including those listed above and in Table 1, can be used. When a protease is selected, its cognate cleavage site and protease inhibitors known in the art to bind and inhibit the protease can be used in a combination. Exemplary combinations for the use are provided below in **Table 1**. Representative sequences of the proteases are available from public database including UniProt through the uniprot.org website. UniProt accession numbers for the proteases are also provided below in Table 1.

Table 1

Protease (UniProt Accession Number or SEQ ID NO.)	**Cognate cleavage site**	**Protease inhibitors**
HCV NS4A/4B	DEMEECSQHL (SEQ ID NO: 81) EDVVPCSMG (SEQ ID NO: 82)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS5A/5B	DEMEECSQHL (SEQ ID NO: 81) EDVVPCSMG (SEQ ID NO: 82)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS3	DEMEECSQHL (SEQ ID NO: 81) EDVVPCSMG (SEQ ID NO: 82)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HCV NS2-3	DEMEECSQHL (SEQ ID NO: 81) EDVVPCSMG (SEQ ID NO: 82)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir
HIV-1 protease		Amprenavir, Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir,

Table 1		
Protease (UniProt Accession Number or SEQ ID NO.)	**Cognate cleavage site**	**Protease inhibitors**
		Saquinavir, Tipranavir
Signal peptidase (P67812, P15367, P00804, P0803)	preference of eukaryotic signal peptidase for cleavage after residue 20 ($Xaa^{20\downarrow}$) of pre(Δpro)apoA-II: Ala, Cys > Gly > Ser, Thr > Pro > Asn, Val, Ile, Leu, Tyr, His, Arg, Asp.	
proprotein convertases cleaving at hydrophobic residues (e.g., Leu, Phe, Val, or Met) (Q16549, 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 or Thr (Q16549, Q8NBP7, Q92824, P29120, Q6UW60, P29122)	(K/R)-(X)n-(K/R)↓, where n is 0, 2, 4 or 6 and X is any amino acid	
proopiomelanocortin converting enzyme (PCE) (Q9UO77615, O776133)	Cleavage at paired basic residues in certain prohormones, either between them, or on the carboxyl side	
chromaffin granule aspartic protease (CGAP)	tends to cleave dipeptide bonds that have hydrophobic residues as well as a beta-methylene group	
prohormone thiol protease (cathepsin L1) (P07154, P07711, P06797, P25975, Q28944)		
carboxypeptidases (e.g., carboxypeptidase E/H, carboxypeptidase D and carboxypeptidase Z) (Q9M099, P15169, Q04609, P08819, P08818, O77564, P70627, O35409, P07519, Q8VZU3, P22792, P15087, P16870, Q9JHH6, Q96IY4, Q7L8A9)	cleaves a peptide bond at the carboxy-terminal (C-terminal) end of a protein or peptide	
aminopeptidases (e.g., arginine aminopeptidase, lysine aminopeptidase, aminopeptidase B)	cleaves a peptide bond at the amino-terminal (N-terminal) end of a protein or peptide	
prolyl endopeptidase (Q12884, P48147, P97321, Q4J6C6)	Hydrolysis of Pro-|-Xaa >> Ala-|-Xaa in oligopeptides. Release of an N-terminal dipeptide, Xaa-Yaa-|-Zaa-, from a polypeptide, preferentially when Yaa is Pro, provided Zaa is neither Pro nor hydroxyproline	
aminopeptidase N (P97449, P15144, P15145, P15684)	Release of an N-terminal amino acid, Xaa-|-Yaa- from a peptide, amide or arylamide. Xaa is preferably Ala, but may be most amino acids including Pro (slow action). When a terminal hydrophobic residue is followed by a prolyl residue, the two may be released as an intact Xaa-Pro dipeptide	
insulin degrading enzyme (P14735, P35559, Q9JHR7, P22817, Q24K02)	Degradation of insulin, glucagon and other polypeptides. No action on proteins. Cleaves multiple short polypeptides that vary considerably in sequence	
Calpain (O08529, P17655, Q07009, Q27971, P20807, P07384, O35350, O14815,	No specific amino acid sequence is uniquely recognized by calpains. Amongst protein substrates, tertiary structure elements rather than primary amino	

Table 1		
Protease (UniProt Accession Number or SEQ ID NO.)	**Cognate cleavage site**	**Protease inhibitors**
P04632, Q9Y6Q1, O15484, Q9HC96, A6NHC0, Q9UMQ6)	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) (SEQ ID NO: 164), 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-|- (SEQ ID NO: 165).	
caspase 2 (P42575, P29594)	Strict requirement for an Asp residue at P1, with 316-asp being essential for proteolytic activity and has a preferred cleavage sequence of Val-Asp-Val-Ala-Asp-|- (SEQ ID NO: 166).	
caspase 3 (P42574, P70677)	Strict requirement for an Asp residue at positions P1 and P4. It has a preferred cleavage sequence of Asp-Xaa-Xaa-Asp-|- with a hydrophobic amino-acid residue at P2 and a hydrophilic amino-acid 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-|- (SEQ ID NO: 165) but also cleaves at Asp-Glu-Val-Asp-|- (SEQ ID NO: 167).	
caspase 5 (P51878)	Strict requirement for Asp at the P1 position. It has a preferred cleavage sequence of Tyr-Val-Ala-Asp-|- (SEQ ID NO: 165) but also cleaves at Asp-Glu-Val-Asp-|- (SEQ ID NO: 167).	
caspase 6 (P55212)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of Val-Glu-His-Asp-|- (SEQ ID NO: 168).	
caspase 7 (P97864, P55210)	Strict requirement for an Asp residue at position P1 and has a preferred cleavage sequence of Asp-Glu-Val-Asp-|- (SEQ ID NO: 167).	
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 (SEQ ID NO: 169).	
caspase 10 (Q92851)	Strict requirement for Asp at position P1 and has a preferred cleavage sequence of Leu-Gln-Thr-Asp-|-Gly (SEQ ID NO: 170).	
puromycin sensitive aminopeptidase (P55786, Q11011)	Release of an N-terminal amino acid, preferentially alanine, from a wide range of peptides, amides and arylamides.	
angiotensin converting enzyme (ACE) (P12821, P09470, Q9BYF1)	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),

Table 1		
Protease (UniProt Accession Number or SEQ ID NO.)	**Cognate cleavage site**	**Protease inhibitors**
		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 (SEQ ID NO: 171) 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 (SEQ ID NO: 172) in the Swedish variant of AlzhFeimer's amyloid precursor protein	
amyloid precursor protein secretase gamma (P05067, P12023, Q9Y5Z0, P56817)	intramembrane cleavage of integral membrane proteins	
MMP 1 (P03956, Q9EPL5uy)	Cleavage of the triple helix of collagen at about three-quarters of the length of the molecule from the N-terminus, at 775-Gly-|-Ile-776 in the alpha-1(I) chain. Cleaves synthetic substrates and alpha-macroglobulins at bonds where P1' is a hydrophobic residue.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 2 (P08253, P33434)	Cleavage of gelatin type I and collagen types IV, V, VII, X. Cleaves the collagen-like sequence Pro-Gln-Gly-|-Ile-Ala-Gly-Gln (SEQ ID NO: 173).	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 3 (P08254, P28862)	Preferential cleavage where P1', P2' and P3' are hydrophobic residues.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 7 (P09237, Q10738)	Cleavage of 14-Ala-|-Leu-15 and 16-Tyr-|-Leu-17 in B chain of insulin. No action on collagen types I, II, IV, V. Cleaves gelatin chain alpha-2(I) > alpha-1(I).	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 8 (P22894, O70138)	Can degrade fibrillar type I, II, and III collagens. Cleavage of interstitial collagens in the triple helical domain. Unlike EC 3.4.24.7, this enzyme cleaves type III collagen more slowly than type I.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 9 (P14780, P41245)	Cleavage of gelatin types I and V and collagen types IV and V. Cleaves KiSS1 at a Gly-|-Leu bond.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1

Table 1		
Protease (UniProt Accession Number or SEQ ID NO.)	**Cognate cleavage site**	**Protease inhibitors**
	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.	
MMP 10 (P09238, O55123)	Can degrade fibronectin, gelatins of type I, III, IV, and V; weakly collagens III, IV, and V.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 11 (P24347, Q02853)	A(A/Q)(N/A)↓(L/Y)(T/V/M/R)(R/K) G(G/A)E↓LR (SEQ ID NO: 174) ↓ denotes the cleavage site	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 12 (P39900, P34960)	Hydrolysis of soluble and insoluble elastin. Specific cleavages are also produced at 14-Ala-|-Leu-15 and 16-Tyr-|-Leu-17 in the B chain of insulin Has significant elastolytic activity. Can accept large and small amino acids at the P1' site, but has a preference for leucine. Aromatic or hydrophobic residues are preferred at the P1 site, with small hydrophobic residues (preferably alanine) occupying P3	SB-3CT *p*-OH SB-3CT *O*-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 *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
MMP 14 (P50281, P53690)	Activates progelatinase A by cleavage of the propeptide at 37-Asn-|-Leu-38. Other bonds hydrolyzed include 35-Gly-|-Ile-36 in the propeptide of collagenase 3, and 341-Asn-|-Phe-342, 441-Asp-|-Leu-442 and 354-Gln-|-Thr-355 in the aggrecan interglobular domain.	SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1
urokinase plasminogen activator (uPA) (P00749, P06869)	Specific cleavage of Arg-|-Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
tissue plasminogen activator (tPA) (P00750, P11214)	Specific cleavage of Arg-|-Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
tissue plasminogen activator (tPA) (P00750, P11214)	Specific cleavage of Arg-|-Val bond in plasminogen to form plasmin.	Plasminogen activator inhibitors (PAI)
Plasmin (P00747, P20918)	Preferential cleavage: Lys-|-Xaa > Arg-|-Xaa, higher selectivity than trypsin. Converts fibrin into soluble products.	α-2-antiplasmin (AP)
Thrombin (P00734, P19221)	Cleaves bonds after Arg and Lys Converts fibrinogen to fibrin and activates factors V, VII, VIII, XIII, and, in complex with thrombomodulin, protein C.	
BMP-1 (procollagen C-peptidase) (P13497, P98063)	Cleavage of the C-terminal propeptide at Ala-|-Asp in type I and II procollagens and at Arg-|-Asp in type III.	
ADAM (Q9P0K1, Q9UKQ2, Q9JLN6, O14672, Q13444, P78536, Q13443, O43184, P78325, Q9UKF5, Q9BZ11, Q9H2U9, Q99965, O75077, Q9H013, O43506)		SB-3CT *p*-OH SB-3CT *O*-phosphate SB-3CT RXP470.1

Table 1		
Protease (UniProt Accession Number or SEQ ID NO.)	**Cognate cleavage site**	**Protease inhibitors**
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: 175)	
chymotrypsin-like serine protease (P08217, Q9UNI1, Q91X79, P08861, P09093, P08218)		-*Thermobifida fusca* Thermopin -*Pyrobaculum aerophilum* Aeropin -*Thermococcus kodakaraensis* Tk-serpin -*Alteromonas sp*. Marinostatin -*Streptomyces misionensis* SMTI -*Streptomyces sp*. chymostatin
alphavirus proteases (P08411, P03317, P13886, Q8JUX6, Q86924, Q4QXJ8, Q8QL53, P27282, Q5XXP4)		
chymotrypsin-like cysteine proteases (Q86TL0, Q14790, Q99538, O15553)		-*Thermobifida fusca* Thermopin -*Pyrobaculum aerophilum* Aeropin -*Thermococcus kodakaraensis* Tk-serpin -*Alteromonas sp*. Marinostatin -*Streptomyces misionensis* SMTI -*Streptomyces sp*. chymostatin
papain-like cysteine proteases (P25774, P53634, Q96K76)		
picornavirus leader proteases (P03305, P03311, P13899)		
HIV proteases (P04585, P03367, P04584, P03369, P12497, P03366, P04587)		
Herpesvirus proteases (P10220, Q2HRB6, O40922, Q69527)		
adenovirus proteases (P03252, P24937, Q83906, P68985, P09569, P11825, P10381)		
Streptomyces griseus protease A (SGPA) (P00776)		
Streptomyces griseus protease B (SGPB) (P00777)		
alpha-lytic protease (P85142, P00778)		

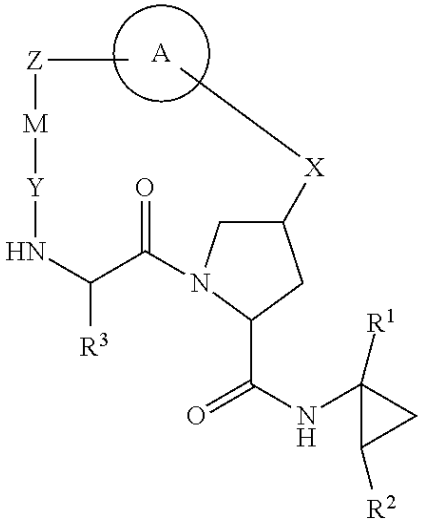
Table 1		
Protease (UniProt Accession Number or SEQ ID NO.)	**Cognate cleavage site**	**Protease inhibitors**
serine proteases (P48740, P98064, Q9UL52, P05981, O60235)		
cysteine proteases (Q86TL0, Q14790, Q8WYN0, Q96DT6, P55211)		
aspartic proteases (Q9Y5Z0, P56817, Q00663, Q53RT3, P0CY27)		
threonine proteases (Q9UI38, Q16512, Q9H6P5, Q8IWU2)		
Mast cell (MC) chymase (CMA1) (NM_001836)	Abz-HPFHL-Lys(Dnp)-NH2 (SEQ ID NO: 176)	BAY 1142524 SUN13834
Rat mast cell protease-1, -2, -3, -4, -5 (NM_017145, NM_172044, NM_001170466, NM_019321, NM_013092)	Abz-HPFHL-Lys(Dnp)-NH2 (SEQ ID NO: 176)	TY-51469
Rat vascular chymase (RVCH) (O70500)	Abz-HPFHL-Lys(Dnp)-NH2 (SEQ ID NO: 176)	
DENV NS3pro (NS2B/NS3)	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
NS3/NS4 protease cleavage site	EDVVCCHSIY (SEQ ID NO: 159) LYQEFDEMEECSQH (SEQ ID NO: 160)	Simeprevir, Danoprevir, Asunaprevir, Ciluprevir, Boceprevir, Sovaprevir, Paritaprevir, Telaprevir, Grazoprevir

[00445] In some embodiments, the one or more cognate cleavage sites of the repressible protease are localized between the DNA-binding domain and the effector domain of the ACP. In some embodiments, the repressible protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3). In some embodiments, the cognate cleavage site comprises an NS3 protease cleavage site. In some embodiments, the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.

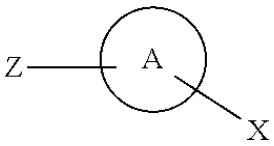
[00446] In some embodiments, the NS3 protease can be repressed by a protease inhibitor. Any suitable protease inhibitor can be used, including, but not limited to, simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir, or any combination thereof. In some embodiments, the protease inhibitor is selected from: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and

voxiloprevir. In some embodiments, the protease inhibitor is grazoprevir. In some embodiments, the protease inhibitor is a combination of grazoprevir and elbasvir (a NS5A inhibitor of the hepatitis C virus NS5A replication complex). Grazoprevir and elbasvir can be co-formulated as a pharmaceutical composition, such as in tablet form (*e.g.*, the tablet available under the tradename Zepatier®). Grazoprevir and elbasvir can be co-formulated at a 2:1 weight ratio, respectively, such as at a unit dose of 100 mg grazoprevir 50 mg elbasvir (*e.g.*, as in the tablet available under the tradename Zepatier®). Protease inhibitors that are structurally similar to grazoprevir can be used, such as any with the general formula (I) below

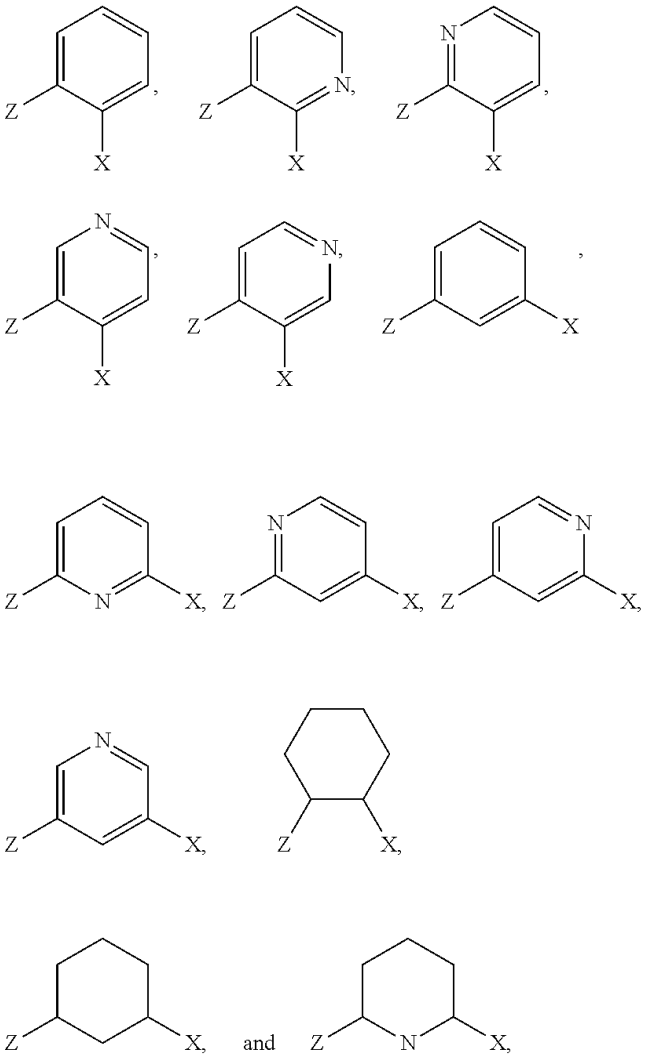
(I)



where



is one or more rings selected from the group consisting of:



and

R^1 is selected from the group consisting of —CO_2R^{10} and —$CONR^{10}SO_2R^6$; R^2 is —$CH{=}CH_2$; R^3 is C_1-C_6 alkyl; R^6 is C_3 cycloalkyl; Y is selected from the group consisting of —OC(O)—; Z is a direct bond; M is selected from the group consisting of C_1-C_{12} alkylenes and C_2-C_{12} alkenylenes, M is substituted with 1 to 2 substituents F independently selected from the group consisting of C_1-C_8 alkyl and $=CH_2$; X is selected from the group consisting of —$(CH_2)_{0\text{-}3}O$—, where Ⓐ is attached to —$(CH_2)_{0\text{-}3}$ if present; and each R^{10} is independently H. Grazoprevir, elbasvir, and combinations thereof are described in U.S. Pat. Nos. 9,738,661; 7,973,040; and 8,871,759 and U.S. Pat. Pub No. US20160243128, each herein incorporated by reference for all purposes.

[00447] In some embodiments, an ACP of the present disclosure comprises a small molecule (e.g., drug) inducible hormone-binding domain of estrogen receptor (ERT2 domain). In some embodiments, the ERT2 domain is an estrogen receptor variant that binds to tamoxifen, and metabolites thereof, but not to estradiol. Non-limiting examples of

tamoxifen metabolites may include 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen. In some embodiments, when expressed in a cell and in the absence of the small molecule (e.g., tamoxifen or a metabolite thereof) the ACP comprising the ERT2 domain binds to HSP90 and is maintained in the cytoplasm of the cell. In some embodiments, upon introduction of the small molecule (e.g., tamoxifen or a metabolite thereof), the small molecule displaces HSP90 bound to the ERT2 domain, which allows the ACP comprising the ERT2 domain to translocate to the nucleus of the cell.

[00448] Accordingly, in some embodiments an ACP of the present disclosure comprising an ERT2 domain is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof. In some embodiments, the tamoxifen metabolite is selected from 4-hydroxy-tamoxifen (4-OHT), N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

[00449]

Degredation Sequences and Degrons

[00450] In some embodiments, the ACP further comprises a degron, wherein the degron is operably linked to the ACP. In some embodiments, the degron is localized 5' of the repressible protease, 3' of the repressible protease, 5' of the DNA-binding domain, 3' of the DNA-binding domain, 5' of the effector domain, or 3' of the effector domain.

[00451] The terms "degron" "degron domain," as used herein, refers to a protein or a part thereof that is important in regulation of protein degradation rates. Various degrons known in the art, including but not limited to short amino acid sequences, structural motifs, and exposed amino acids, can be used in various embodiments of the present disclosure. Degrons identified from a variety of organisms can be used. Degrons and degron pathways are generally known, see, e.g., Varshazsky A., PNAS 2019 Jan 8;116(2):358-366, hereby incorporated by reference.

[00452] The term "degradation sequence" as used herein, refers to a sequence that promotes degradation of an attached protein through either the proteasome or autophagy-lysosome pathways. Degradation sequences known in the art can be used for various embodiments of the present disclosure. In some embodiments, a degradation sequence comprises a degron identified from an organism, or a modification thereof. In some embodiments, a degradation sequence is a polypeptide that destabilize a protein such that half-life of the protein is reduced at least two-fold, when fused to the protein. Many different degradation sequences/signals (e.g., of the ubiquitin- proteasome system) are known in the

art, any of which may be used as provided herein. A degradation sequence may be operably linked to a cell receptor, but need not be contiguous with it as long as the degradation sequence still functions to direct degradation of the cell receptor. In some embodiments, the degradation sequence induces rapid degradation of the cell receptor. For a discussion of degradation sequences and their function in protein degradation, see, e.g., Kanemaki et al. (2013) Pflugers Arch. 465(3):4l9-425, Erales et al. (2014) Biochim Biophys Acta 1843(l):216-221 , Schrader et al. (2009) Nat. Chem. Biol. 5(11): 815-822, Ravid et al. (2008) Nat. Rev. Mol. Cell. Biol. 9(9):679-690, Tasaki et al. (2007)Trends Biochem Sci. 32(1 1):520-528, Meinnel et al. (2006) Biol. Chem. 387(7):839- 851, Kim et al. (2013) Autophagy 9(7): 1100-1103, Varshavsky (2012) Methods Mol. Biol. 832: 1-11, and Fayadat et al. (2003) Mol Biol Cell. 14(3): 1268-1278; herein incorporated by reference.

[00453] In some embodiments, the degron or degradation sequence is selected from: HCV NS4 degron, PEST (two copies of residues 277-307 of human IκBα), 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 (SEQ ID NO: 163), an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box.

[00454] In some embodiments, the degron 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 embodiments, the CRBN polypeptide substrate domain is selected from: IKZF1, IKZF3, CKla, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN. In some embodiments, the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences. In some embodiments, the CRBN

polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRDAL (**SEQ ID NO: 131**).

[00455] In some embodiments, the immunomodulatory drug (IMiD) is an FDA-approved drug. In some embodiments, the IMiD is selected from: thalidomide, lenalidomide, and pomalidomide.

Promoters

[00456] In some embodiments, an engineered nucleic acid of the present disclosure comprises a first expression cassette comprising a first promoter operably linked to an exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP). In some embodiments, an engineered nucleic acid of the present disclosure comprises a second expression cassette comprising an ACP-responsive promoter operably linked to a second exogenous polynucleotide sequence encoding one or more effector molecules. In some embodiments, the first expression cassette and second expression cassette are each encoded by a separate engineered nucleic acid of the present disclosure. In other embodiments, the first expression cassette and the second expression cassette are encoded by the same engineered nucleic acid of the present disclosure.

[00457] In some embodiments, an ACP-responsive promoter of the present disclosure comprises an ACP-binding domain and a promoter sequence. In some embodiments, the ACP-responsive promoter is operable linked to a nucleotide sequence encoding an effector molecule.

[00458] In some embodiments, an engineered nucleic acid comprises an ACP-responsive promoter operably linked to a nucleotide sequence encoding an effector molecule. In some embodiments, an engineered nucleic acid comprises an ACP-responsive promoter operably linked to a nucleotide sequence encoding at least 2 effector molecules. For example, the engineered nucleic acid may comprise an ACP-responsive 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 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 effector molecules. In some embodiments, an engineered nucleic acid comprises an ACP-responsive promoter operably linked to a nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more effector molecules.

[00459] 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.

[00460] 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).

[00461] Promoters of an engineered nucleic acid of the present disclosure 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.

[00462] 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.

[00463] Non-limiting examples of responsive promoters (also referred to as "inducible promoters") (*e.g.*, TGF-beta responsive promoters) are listed in Table 2, 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). Other non-limiting examples of inducible promoters include those shown in Table 3.

Table 2. Exemplary Inducible 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-	BIT (BirA-VP16)	Biotin	B	A

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
	PhCMVmin)				
Cumate – activator	PCR5 (OCuO6-PhCMVmin)	cTA (CymR-VP16)	Cumate	D	DA
Cumate – reverse activator	PCR5 (OCuO6-PhCMVmin)	rcTA (rCymR-VP16)	Cumate	B	A
E-OFF	PETR (OETR-PhCMVmin)	ET (E-VP16)	Erythromycin	D	DA
NICE-OFF	PNIC (ONIC-PhCMVmin)	NT (HdnoR-VP16)	6-Hydroxy-nicotine	D	DA
PEACE	PTtgR1 (OTtgR-PhCMVmin)	TtgA1 (TtgR-VP16)	Phloretin	D	DA
PIP-OFF	PPIR (OPIR-Phsp70min)	PIT (PIP-VP16)	Pristinamycin I	D	DA
QuoRex	PSCA (OscbR-PhCMVmin)PSPA (OpapRI-PhCMVmin)	SCA (ScbR-VP16)	SCB1	D	DA
Redox	PROP (OROP-PhCMVmin)	REDOX (REX-VP16)	NADH	D	DA
TET-OFF	PhCMV∗-1 (OtetO7-PhCMVmin)	tTA (TetR-VP16)	Tetracycline	D	DA
TET-ON	PhCMV∗-1 (OtetO7-PhCMVmin)	rtTA (rTetR-VP16)	Doxycycline	B	A
TIGR	PCTA (OrheO-PhCMVmin)	CTA (RheA-VP16)	Heat	D	DA
TraR	O7x(tra box)-PhCMVmin	p65-TraR	3-Oxo-C8-HSL	B	A
VAC-OFF	P1VanO2 (OVanO2-PhCMVmin)	VanA1 (VanR-VP16)	Vanillic acid	D	DA
Transcriptional repressor-responsive promoters					
Cumate - repressor	PCuO (PCMV5-OCuO)	CymR	Cumate	D	DR
E-ON	PETRON8 (PSV40-OETR8)	E-KRAB	Erythromycin	D	DR
NICE-ON	PNIC (PSV40-ONIC8)	NS (HdnoR-KRAB)	6-Hydroxy-nicotine	D	DR
PIP-ON	PPIRON (PSV40-OPIR3)	PIT3 (PIP-KRAB)	Pristinamycin I	D	DR
Q-ON	PSCAON8 (PSV40-OscbR8)	SCS (ScbR-KRAB)	SCB1	D	DR
TET-ON<comma> 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					

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
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	Tetracycline Erythromycin	DD	DADR
TET-OFFPIP-ONE-ON	OtetO7-OPIR3-OETR8-PhCMVmin	tTAPIT3E-KRAB	Tetracycline Pristinamycin IErythromycin	DDD	DADRDR

Table 3. Exemplary Inducible Promoters

Name	DNA SEQUENCE	Source
minimal promoter; minP	AGAGGGTATATAATGGAAGCTCGACTTCCAG (SEQ ID NO: 1)	EU581860.1 (Promega)
NFkB response element protein promoter; 5x NFkB-RE	GGGAATTTCCGGGGACTTTCCGGGAATTTCCGGGGACTTTCCGGGAATTTCC (SEQ ID NO: 2)	EU581860.1 (Promega)
CREB response element protein promoter; 4x CRE	CACCAGACAGTGACGTCAGCTGCCAGATCCCATGGCCGTCATACTGTGACGTCTTTCAGACACCCCATTGACGTCAATGGGAGAA (SEQ ID NO: 3)	DQ904461.1 (Promega)
NFAT response element protein promoter; 3x NFAT binding sites	GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGT (SEQ ID NO: 4)	DQ904462.1 (Promega)
SRF response element protein promoter; 5x SRE	AGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCT (SEQ ID NO: 5)	FJ773212.1 (Promega)
SRF response element protein promoter 2; 5x SRF-RE	AGTATGTCCATATTAGGACATCTACCATGTCCATATTAGGACATCTACTATGTCCATATTAGGACATCTTGTATGTCCATATTAGGACATCTAAAATGTCCATATTAGGACATCT (SEQ ID NO: 6)	FJ773213.1 (Promega)
AP1 response element protein promoter; 6x AP1-RE	TGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAG (SEQ ID NO: 7)	JQ858516.1 (Promega)
TCF-LEF response element promoter; 8x TCF-LEF-RE	AGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGTATAAGATCAAAGGGCCTAAGATCAAAGGGACTAAGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGCCTA (SEQ ID NO: 8)	JX099537.1 (Promega)
SBEx4	GTCTAGACGTCTAGACGTCTAGACGTCTAGAC (SEQ ID NO: 9)	Addgene Cat No: 16495
SMAD2/3 - CAGACA x4	CAGACACAGACACAGACACAGACA (SEQ ID NO: 10)	Jonk *et al.* (J Biol Chem. 1998 Aug 14;273(33):21145-52.
STAT3 binding site	Ggatccggtactcgagatctgcgatctaagtaagcttggcattccggtactgttggtaaagccac (SEQ ID NO: 11)	Addgene Sequencing Result #211335

Name	DNA SEQUENCE	Source
5x NFAT	GGGACTTTCCACTGGGGACTTTCCACTG GGGACTTTCCACTGGGGACTTTCCACTG GGGACTTTCC (SEQ ID NO: 152)	
min AdeP	AGACGCTAGCGGGGGGCTATAAAAGGG GGTGGGGGCGTTCGTCCTCACTCT (SEQ ID NO: 153)	
5x NFAT_minAdeP	GGGACTTTCCACTGGGGACTTTCCACTG GGGACTTTCCACTGGGGACTTTCCACTG GGGACTTTCCACTCCTGCAGGagctGGCG CGCCAGACGCTAGCGGGGGGCTATAAA AGGGGGTGGGGGCGTTCGTCCTCACTCT (SEQ ID NO: 154)	
YB-TATA	TCTAGAGGGTATATAATGGGGGCCA (SEQ ID NO: 155)	

[00464] Other 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. In some embodiments, the promoter is a constitutive promoter. Exemplary constitutive promoters are shown in Table 4.

Table 4. Exemplary Constitutive Promoters

Name	DNA SEQUENCE
CMV	GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTT CATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG CTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAG TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAAC TGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACG TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGA CTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGC GGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCC AAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGG GACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGC GTGTACGGTGGGAGGTCTATATAAGCAGAGCTC (SEQ ID NO: 12)
EF1a	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGT TGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA ACTGGGAAAGTGATGCCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGA ACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCG CCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGG TTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTG ATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGG AGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGC GTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGT AAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGC GACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGC GACCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCC TGTCCTCGCGCCGCCGTGTATCGCCCCGCCCCGGGCGGCAAGGCTGGCCCGGTCG

Name	DNA SEQUENCE
	GCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGTCCTGCTGCAGGGAGCT CAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAA GGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCG GGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAG GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGAC TGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTC CATTTCAGGTGTCGTGA (SEQ ID NO: 13)
EFS	GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGT CCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTG GCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAG GGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCA ACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTC ACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGC CGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAG CTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAG CCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTT TTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTAC (SEQ ID NO: 14)
MND	TTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGC AAGCTAGGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGA TATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGC AGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGG CCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACC ATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAA CTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCAA TAAAAGAGCCCA (SEQ ID NO: 15)
PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACGCGGC TGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACAT TCTTCACGTCCGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTGGGCCCC CCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGCGGTTCGCGGC GTGCCGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGA CAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGCCAATAGC GGCTGCTCAGCGGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGCGGTGCGGGAG GCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCCTGCCCGCGCGGTGTTCCGCATT CTGCAAGCCTCCGGAGCGCACGTCGGCAGTCGGCTCCCTCGTTGACCGAATCACC GACCTCTCTCCCCAG (SEQ ID NO: 16)
SFFV	GTAACGCCATTTTGCAAGGCATGGAAAAATACCAAACCAAGAATAGAGAAGTTCA GATCAAGGGCGGGTACATGAAAATAGCTAACGTTGGGCCAAACAGGATATCTGCG GTGAGCAGTTTCGGCCCCGGCCCGGGGCCAAGAACAGATGGTCACCGCAGTTTCG GCCCCGGCCCGAGGCCAAGAACAGATGGTCCCCAGATATGGCCCAACCCTCAGCA GTTTCTTAAGACCCATCAGATGTTTCCAGGCTCCCCCAAGGACCTGAAATGACCCT GCGCCTTATTTGAATTAACCAATCAGCCTGCTTCTCGCTTCTGTTCGCGCGCTTCTG CTTCCCGAGCTCTATAAAAGAGCTCACAACCCCTCACTCGGCGCGCCAGTCCTCCG ACAGACTGAGTCGCCCGGG (SEQ ID NO: 17)
SV40	CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCA GAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCC AGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACC ATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCA TTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCT CTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTT TGCAAAAAGCT (SEQ ID NO: 18)
UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCACGGCGAGCGCTGCC ACGTCAGACGAAGGGCGCAGGAGCGTTCCTGATCCTTCCGCCCGGACGCTCAGGA CAGCGGCCCGCTGCTCATAAGACTCGGCCTTAGAACCCCAGTATCAGCAGAAGGA CATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCCAGAGAGCG

Name	DNA SEQUENCE
	GAACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTG GGGCGGTGAACGCCGATGATTATATAAGGACGCGCCGGGTGTGGCACAGCTAGTT CCGTCGCAGCCGGGATTTGGGTCGCGGTTCTTGTTTGTGGATCGCTGTGATCGTCA CTTGGTGAGTTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGTGGCCGCCGGGCCGC TCGGTGGGACGGAAGCGTGTGGAGAGACCGCCAAGGGCTGTAGTCTGGGTCCGCG AGCAAGGTTGCCCTGAACTGGGGGTTGGGGGGAGCGCACAAAATGGCGGCTGTTC CCGAGTCTTGAATGGAAGACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAAACAAG GTGGGGGGCATGGTGGGCGGCAAGAACCCAAGGTCTTGAGGCCTTCGCTAATGCG GGAAAGCTCTTATTCGGGTGAGATGGGCTGGGGCACCATCTGGGGACCCTGACGT GAAGTTTGTCACTGACTGGAGAACTCGGGTTTGTCGTCTGGTTGCGGGGGCGGCA GTTATGCGGTGCCGTTGGGCAGTGCACCCGTACCTTTGGGAGCGCGCGCCTCGTCG TGTCGTGACGTCACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCCACCTGCCGG TAGGTGTGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCGGGCCTAGGGTA GGCTCTCCTGAATCGACAGGCGCCGGACCTCTGGTGAGGGGAGGGATAAGTGAGG CGTCAGTTTCTTTGGTCGGTTTTATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGG TTTTGAACTATGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCACC TTTTGAAATGTAATCATTTGGGTCAATATGTAATTTTCAGTGTTAGACTAGTAAAG CTTCTGCAGGTCGACTCTAGAAAATTGTCCGCTAAATTCTGGCCGTTTTTGGCTTTT TTGTTAGAC (SEQ ID NO: 19)
hEF1aV1	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGT TGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGA ACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCG CCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGG TTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTG ATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGG AGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGC GTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGT AAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGC GACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGC GGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCC TGGTCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCG GCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCT CAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAA GGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCG GGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAG GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGAC TGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTC CATTTCAGGTGTCGTGA (SEQ ID NO: 20)
hCAGG	ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGA GTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGAC CCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGA CTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTA CATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAAT GGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAG TACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTG CTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTT TAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGC GGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGC AGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGG CGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGGAGTCGCTGCGACGCT GCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGA CTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCT GTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGC CTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGT

Name	DNA SEQUENCE
	GCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGCCCGGCGGCTG TGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGG AGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGGCTGCGAGGGGAACAAA GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCG GTCGGGCTGCAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGG CTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGG GGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGA GGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGC GGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTT CCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCT CTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGG AGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGC TGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC TTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTT CTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGG CAAAGAATTC (SEQ ID NO: 21)
hEF1aV2	GGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCA ATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGT GTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTA GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAG (SEQ ID NO: 22)
hACTb	CCACTAGTTCCATGTCCTTATATGGACTCATCTTTGCCTATTGCGACACACACTCA ATGAACACCTACTACGCGCTGCAAAGAGCCCCGCAGGCCTGAGGTGCCCCCACCT CACCACTCTTCCTATTTTTGTGTAAAAATCCAGCTTCTTGTCACCACCTCCAAGGA GGGGGAGGAGGAGGAAGGCAGGTTCCTCTAGGCTGAGCCGAATGCCCCTCTGTGG TCCCACGCCACTGATCGCTGCATGCCCACCACCTGGGTACACACAGTCTGTGATTC CCGGAGCAGAACGGACCCTGCCCACCCGGTCTTGTGTGCTACTCAGTGGACAGAC CCAAGGCAAGAAAGGGTGACAAGGACAGGGTCTTCCCAGGCTGGCTTTGAGTTCC TAGCACCGCCCCGCCCCCAATCCTCTGTGGCACATGGAGTCTTGGTCCCCAGAGTC CCCCAGCGGCCTCCAGATGGTCTGGGAGGGCAGTTCAGCTGTGGCTGCGCATAGC AGACATACAACGGACGGTGGGCCCAGACCCAGGCTGTGTAGACCCAGCCCCCCCG CCCCGCAGTGCCTAGGTCACCCACTAACGCCCCAGGCCTGGTCTTGGCTGGGCGT GACTGTTACCCTCAAAAGCAGGCAGCTCCAGGGTAAAAGGTGCCCTGCCCTGTAG AGCCCACCTTCCTTCCCAGGGCTGCGGCTGGGTAGGTTTGTAGCCTTCATCACGGG CCACCTCCAGCCACTGGACCGCTGGCCCCTGCCCTGTCCTGGGGAGTGTGGTCCTG CGACTTCTAAGTGGCCGCAAGCCACCTGACTCCCCCAACACCACACTCTACCTCTC AAGCCCAGGTCTCTCCCTAGTGACCCACCCAGCACATTTAGCTAGCTGAGCCCCAC AGCCAGAGGTCCTCAGGCCCTGCTTTCAGGGCAGTTGCTCTGAAGTCGGCAAGGG GGAGTGACTGCCTGGCCACTCCATGCCCTCCAAGAGCTCCTTCTGCAGGAGCGTA CAGAACCCAGGGCCCTGGCACCCGTGCAGACCCTGGCCCACCCCACCTGGGCGCT CAGTGCCCAAGAGATGTCCACACCTAGGATGTCCCGCGGTGGGTGGGGGGCCCGA GAGACGGGCAGGCCGGGGGCAGGCCTGGCCATGCGGGGCCGAACCGGGCACTGC CCAGCGTGGGGCGCGGGGGCCACGGCGCGCGCCCCCAGCCCCCGGGCCCAGCAC CCCAAGGCGGCCAACGCCAAAACTCTCCCTCCTCCTCTTCCTCAATCTCGCTCTCG CTCTTTTTTTTTTTCGCAAAAGGAGGGGAGAGGGGGTAAAAAAATGCTGCACTGT GCGGCGAAGCCGGTGAGTGAGCGGCGCGGGGCCAATCAGCGTGCGCCGTTCCGA AAGTTGCCTTTTATGGCTCGAGCGGCCGCGGCGGCGCCCTATAAAACCCAGCGGC GCGACGCGCCACCACCGCCGAGACCGCGTCCGCCCCGCGAGCACAGAGCCTCGCC TTTGCCGATCCGCCGCCCGTCCACACCCGCCGCCAGGTAAGCCCGGCCAGCCGAC CGGGGCAGGCGGCTCACGGCCCGGCCGCAGGCGGCCGCGGCCCCTTCGCCCGTGC AGAGCCGCCGTCTGGGCCGCAGCGGGGGGCGCATGGGGGGGGAACCGGACCGCC GTGGGGGGCGCGGGAGAAGCCCCTGGGCCTCCGGAGATGGGGGACACCCCACGC CAGTTCGGAGGCGCGAGGCCGCGCTCGGGAGGCGCGCTCCGGGGGTGCCGCTCTC GGGGCGGGGGCAACCGGCGGGGTCTTTGTCTGAGCCGGGCTCTTGCCAATGGGGA TCGCAGGGTGGGCGCGGCGGAGCCCCCGCCAGGCCCGGTGGGGGCTGGGGCGCC ATTGCGCGTGCGCGCTGGTCCTTTGGGCGCTAACTGCGTGCGCGCTGGGAATTGGC GCTAATTGCGCGTGCGCGCTGGGACTCAAGGCGCTAACTGCGCGTGCGTTCTGGG

Name	DNA SEQUENCE
	GCCCGGGGTGCCGCGGCCTGGGCTGGGGCGAAGGCGGGCTCGGCCGGAAGGGGT GGGGTCGCCGCGGCTCCCGGGCGCTTGCGCGCACTTCCTGCCCGAGCCGCTGGCC GCCCGAGGGTGTGGCCGCTGCGTGCGCGCGCGCCGACCCGGCGCTGTTTGAACCG GGCGGAGGCGGGGCTGGCGCCCGGTTGGGAGGGGGTTGGGGCCTGGCTTCCTGCC GCGCGCCGCGGGGACGCCTCCGACCAGTGTTTGCCTTTTATGGTAATAACGCGGC CGGCCCGGCTTCCTTTGTCCCCAATCTGGGCGCGCGCCGGCGCCCCCTGGCGGCCT AAGGACTCGGCGCGCCGGAAGTGGCCAGGGCGGGGGCGACCTCGGCTCACAGCG CGCCCGGCTAT (SEQ ID NO: 23)
heIF4A1	GTTGATTTCCTTCATCCCTGGCACACGTCCAGGCAGTGTCGAATCCATCTCTGCTA CAGGGGAAAACAAATAACATTTGAGTCCAGTGGAGACCGGGAGCAGAAGTAAAG GGAAGTGATAACCCCCAGAGCCCGGAAGCCTCTGGAGGCTGAGACCTCGCCCCCC TTGCGTGATAGGGCCTACGGAGCCACATGACCAAGGCACTGTCGCCTCCGCACGT GTGAGAGTGCAGGGCCCCAAGATGGCTGCCAGGCCTCGAGGCCTGACTCTTCTAT GTCACTTCCGTACCGGCGAGAAAGGCGGGCCCTCCAGCCAATGAGGCTGCGGGGC GGGCCTTCACCTTGATAGGCACTCGAGTTATCCAATGGTGCCTGCGGGCCGGAGC GACTAGGAACTAACGTCATGCCGAGTTGCTGAGCGCCGGCAGGCGGGGCCGGGG CGGCCAAACCAATGCGATGGCCGGGGCGGAGTCGGGCGCTCTATAAGTTGTCGAT AGGCGGGCACTCCGCCCTAGTTTCTAAGGACCATG (SEQ ID NO: 24)
hGAPDH	AGTTCCCCAACTTTCCCGCCTCTCAGCCTTTGAAAGAAAGAAAGGGGAGGGGGCA GGCCGCGTGCAGTCGCGAGCGGTGCTGGGCTCCGGCTCCAATTCCCCATCTCAGTC GCTCCCAAAGTCCTTCTGTTTCATCCAAGCGTGTAAGGGTCCCCGTCCTTGACTCC CTAGTGTCCTGCTGCCCACAGTCCAGTCCTGGGAACCAGCACCGATCACCTCCCAT CGGGCCAATCTCAGTCCCTTCCCCCCTACGTCGGGGCCCACACGCTCGGTGCGTGC CCAGTTGAACCAGGCGGCTGCGGAAAAAAAAAAGCGGGGAGAAAGTAGGGCCCG GCTACTAGCGGTTTTACGGGCGCACGTAGCTCAGGCCTCAAGACCTTGGGCTGGG ACTGGCTGAGCCTGGCGGGAGGCGGGGTCCGAGTCACCGCCTGCCGCCGCGCCCC CGGTTTCTATAAATTGAGCCCGCAGCCTCCCGCTTCGCTCTCTGCTCCTCCTGTTCG ACAGTCAGCCGCATCTTCTTTTGCGTCGCCAGGTGAAGACGGGCGGAGAGAAACC CGGGAGGCTAGGGACGGCCTGAAGGCGGCAGGGGCGGGCGCAGGCCGGATGTGT TCGCGCCGCTGCGGGGTGGGCCCGGGCGGCCTCCGCATTGCAGGGGCGGGCGGAG GACGTGATGCGGCGCGGGCTGGGCATGGAGGCCTGGTGGGGGAGGGGAGGGGAG GCGTGGGTGTCGGCCGGGGCCACTAGGCGCTCACTGTTCTCTCCCTCCGCGCAGCC GAGCCACATCGCTGAGACAC (SEQ ID NO: 25)
hGRP78	AGTGCGGTTACCAGCGGAAATGCCTCGGGGTCAGAAGTCGCAGGAGAGATAGAC AGCTGCTGAACCAATGGGACCAGCGGATGGGGCGGATGTTATCTACCATTGGTGA ACGTTAGAAACGAATAGCAGCCAATGAATCAGCTGGGGGGGCGGAGCAGTGACG TTTATTGCGGAGGGGGCCGCTTCGAATCGGCGGCGGCCAGCTTGGTGGCCTGGGC CAATGAACGGCCTCCAACGAGCAGGGCCTTCACCAATCGGCGGCCTCCACGACGG GGCTGGGGGAGGGTATATAAGCCGAGTAGGCGACGGTGAGGTCGACGCCGGCCA AGACAGCACAGACAGATTGACCTATTGGGGTGTTTCGCGAGTGTGAGAGGGAAGC GCCGCGGCCTGTATTTCTAGACCTGCCCTTCGCCTGGTTCGTGGCGCCTTGTGACC CCGGGCCCCTGCCGCCTGCAAGTCGGAAATTGCGCTGTGCTCCTGTGCTACGGCCT GTGGCTGGACTGCCTGCTGCTGCCCAACTGGCTGGCAC (SEQ ID NO: 26)
hGRP94	TAGTTTCATCACCACCGCCACCCCCCCGCCCCCCCGCCATCTGAAAGGGTTCTAGG GGATTTGCAACCTCTCTCGTGTGTTTCTTCTTTCCGAGAAGCGCCGCCACACGAGA AAGCTGGCCGCGAAAGTCGTGCTGGAATCACTTCCAACGAAACCCCAGGCATAGA TGGGAAAGGGTGAAGAACACGTTGCCATGGCTACCGTTTCCCCGGTCACGGAATA AACGCTCTCTAGGATCCGGAAGTAGTTCCGCCGCGACCTCTCTAAAAGGATGGAT GTGTTCTCTGCTTACATTCATTGGACGTTTTCCCTTAGAGGCCAAGGCCGCCCAGG CAAAGGGGCGGTCCCACGCGTGAGGGGCCCGCGGAGCCATTTGATTGGAGAAAA GCTGCAAACCCTGACCAATCGGAAGGAGCCACGCTTCGGGCATCGGTCACCGCAC CTGGACAGCTCCGATTGGTGGACTTCCGCCCCCCCTCACGAATCCTCATTGGGTGC CGTGGGTGCGTGGTGCGGCGCGATTGGTGGGTTCATGTTTCCCGTCCCCCGCCCGC GAGAAGTGGGGGTGAAAAGCGGCCCGACCTGCTTGGGGTGTAGTGGGCGGACCG CGCGGCTGGAGGTGTGAGGATCCGAACCCAGGGGTGGGGGGTGGAGGCGGCTCC

Name	DNA SEQUENCE
	TGCGATCGAAGGGGACTTGAGACTCACCGGCCGCACGTC (SEQ ID NO: 27)
hHSP70	GGGCCGCCCACTCCCCCTTCCTCTCAGGGTCCCTGTCCCCTCCAGTGAATCCCAGA AGACTCTGGAGAGTTCTGAGCAGGGGGCGGCACTCTGGCCTCTGATTGGTCCAAG GAAGGCTGGGGGGCAGGACGGGAGGCGAAAACCCTGGAATATTCCCGACCTGGC AGCCTCATCGAGCTCGGTGATTGGCTCAGAAGGGAAAAGGCGGGTCTCCGTGACG ACTTATAAAAGCCCAGGGGCAAGCGGTCCGGATAACGGCTAGCCTGAGGAGCTGC TGCGACAGTCCACTACCTTTTTCGAGAGTGACTCCCGTTGTCCCAAGGCTTCCCAG AGCGAACCTGTGCGGCTGCAGGCACCGGCGCGTCGAGTTTCCGGCGTCCGGAAGG ACCGAGCTCTTCTCGCGGATCCAGTGTTCCGTTTCCAGCCCCCAATCTCAGAGCGG AGCCGACAGAGAGCAGGGAACCC (SEQ ID NO: 28)
hKINb	GCCCCACCCCCGTCCGCGTTACAACCGGGAGGCCCGCTGGGTCCTGCACCGTCAC CCTCCTCCCTGTGACCGCCCACCTGATACCCAAACAACTTTCTCGCCCCTCCAGTC CCCAGCTCGCCGAGCGCTTGCGGGGAGCCACCCAGCCTCAGTTTCCCCAGCCCCG GGCGGGGCGAGGGGCGATGACGTCATGCCGGCGCGCGGCATTGTGGGGCGGGGC GAGGCGGGGCGCCGGGGGGAGCAACACTGAGACGCCATTTTCGGCGGCGGGAGC GGCGCAGGCGGCCGAGCGGGACTGGCTGGGTCGGCTGGGCTGCTGGTGCGAGGA GCCGCGGGGCTGTGCTCGGCGGCCAAGGGGACAGCGCGTGGGTGGCCGAGGATG CTGCGGGGCGGTAGCTCCGGCGCCCCTCGCTGGTGACTGCTGCGCCGTGCCTCAC ACAGCCGAGGCGGGCTCGGCGCACAGTCGCTGCTCCGCGCTCGCGCCCGGCGGCG CTCCAGGTGCTGACAGCGCGAGAGAGCGCGGCCTCAGGAGCAACAC (SEQ ID NO: 29)
hUBIb	TTCCAGAGCTTTCGAGGAAGGTTTCTTCAACTCAAATTCATCCGCCTGATAATTTT CTTATATTTTCCTAAAGAAGGAAGAGAAGCGCATAGAGGAGAAGGGAAATAATTT TTTAGGAGCCTTTCTTACGGCTATGAGGAATTTGGGGCTCAGTTGAAAAGCCTAAA CTGCCTCTCGGGAGGTTGGGCGCGGCGAACTACTTTCAGCGGCGCACGGAGACGG CGTCTACGTGAGGGGTGATAAGTGACGCAACACTCGTTGCATAAATTTGCGCTCC GCCAGCCCGGAGCATTTAGGGGCGGTTGGCTTTGTTGGGTGAGCTTGTTTGTGTCC CTGTGGGTGGACGTGGTTGGTGATTGGCAGGATCCTGGTATCCGCTAACAGGTAC TGGCCCACAGCCGTAAAGACCTGCGGGGGCGTGAGAGGGGGGAATGGGTGAGGT CAAGCTGGAGGCTTCTTGGGGTTGGGTGGGCCGCTGAGGGGAGGGGAGGGCGAG GTGACGCGACACCCGGCCTTTCTGGGAGAGTGGGCCTTGTTGACCTAAGGGGGGC GAGGGCAGTTGGCACGCGCACGCGCCGACAGAAACTAACAGACATTAACCAACA GCGATTCCGTCGCGTTTACTTGGGAGGAAGGCGGAAAAGAGGTAGTTTGTGTGGC TTCTGGAAACCCTAAATTTGGAATCCCAGTATGAGAATGGTGTCCCTTCTTGTGTT TCAATGGGATTTTTACTTCGCGAGTCTTGTGGGTTTGGTTTTGTTTTCAGTTTGCCT AACACCGTGCTTAGGTTTGAGGCAGATTGGAGTTCGGTCGGGGGAGTTTGAATAT CCGGAACAGTTAGTGGGGAAAGCTGTGGACGCTTGGTAAGAGAGCGCTCTGGATT TTCCGCTGTTGACGTTGAAACCTTGAATGACGAATTTCGTATTAAGTGACTTAGCC TTGTAAAATTGAGGGGAGGCTTGCGGAATATTAACGTATTTAAGGCATTTTGAAG GAATAGTTGCTAATTTTGAAGAATATTAGGTGTAAAAGCAAGAAATACAATGATC CTGAGGTGACACGCTTATGTTTTACTTTTAAACTAGGTCACC (SEQ ID NO: 30)

[00465] In some embodiments, the promoter sequence is derived from a promoter selected 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.

[00466] In some embodiments, the first promoter is a constitutive promoter, an inducible promoter, or a synthetic promoter. In some embodiments, the constitutive promoter is selected from: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

[00467] In some embodiments, the ACP-responsive promoter is a synthetic promoter. In some embodiments, the ACP-responsive promoter comprises a minimal promoter. In some embodiments, the ACP-binding domain comprises one or more zinc finger binding sites. The ACP-binding domain can comprise 1, 2, 3, 4,5 ,6 7, 8, 9, 10, or more zinc finger binding sites. In some embodiments, the ACP-binding domain comprises one zinc finger binding site. In some embodiments, the ACP-binding domain comprises two zinc finger binding sites. In some embodiments, the ACP-binding domain comprises three zinc finger binding sites. In some embodiments, the ACP-binding domain comprises four zinc finger binding sites. An exemplary ACP-binding domain comprising zinc finger binding sites is shown in the sequence

cgggtttcgtaacaatcgcatgaggattcgcaacgccttcGGCGTAGCCGATGTCGCGctcccgtctcagtaaaggtc GGCGTAGCCGATGTCGCGcaatcggactgccttcgtacGGCGTAGCCGATGTCGCGcgtatcagtcg cctcggaacGGCGTAGCCGATGTCGCGcattcgtaagaggctcactctcccttacacggagtggataACTAGTT CTAGAGGGTATATAATGGGGGCCA (SEQ ID NO: 92).

[00468] In some embodiments, the ACP-responsive promoter comprises an enhancer that promotes transcription when an antigen recognizing receptor engages a cognate antigen, *e.g.*, an antigen expressed on a target cell. Enhancers can include, but are not limited to, enhancers enriched in the ATAC-seq of activated T cells (Gate et al. Nat Genet. Author manuscript; available in PMC 2019 Jan 9; herein incorporated by reference for all purposes) or enhancers associated with upregulated genes in single-cell RNA seq data (Xhangolli et al. Genomics Proteomics Bioinformatics. 2019 Apr;17(2):129-139. doi: 10.1016/j.gpb.2019.03.002; herein incorporated by reference for all purposes). An enhancer can be a synthetic enhancer, such as a pair of transcription factors known or suspected to be upregulated in activated T cells or NK cells. Synthetic enhancers can include multiple iterations of transcription factor binding sites, such 4 iterations of two distinct transcription factor binding sites in an aaaabbbb or abababab organization. Illustrative non-limiting examples of genes from which enhancers can be derived include, but are not limited to, ATF2, ATF7, BACH1, BATF, Bcl-6, Blimp-1, BMI1, CBFB, CREB1, CREM, CTCF, E2F1, EBF1, EGR1, ETV6, FOS, FOXA1, FOXA2, GATA3, HIF1A, IKZF1, IKZF2, IRF4, JUN, JUNB, JUND, Lef1, NFAT, NFIA, NFIB,

NFKB, NR2F1, Nur77, PU.1, RELA, RUNX3, SCRT1, SCRT2, SP1, STAT4, STAT5A, T-Bet, Tcf7, ZBED1, ZNF143, or ZNF217.

[00469] In some embodiments, the ACP-responsive promoter comprises a promoter that promotes transcription when a receptor engages a cognate ligand, such as in a activation inducible system. In some embodiments, the ACP-responsive promoter comprises a promoter that promotes transcription when an antigen recognizing receptor engages a cognate antigen, *e.g.*, an antigen expressed on a target cell. For example, when the ACP is an antigen receptor (*e.g.*, a CAR), the ACP-responsive promoter can include promoters that are induced by signal transduction following antigen receptor binding to a cognate antigen. ACP-responsive promoters can include promoters with increased transcriptional activity in activated T cells and/or NK cells. ACP-responsive promoters can include promoters derived from genes that are upregulated in activated cells, such as T cells and/or NK cells. ACP-responsive promoters can include promoters derived from genes that have increased transcription factor binding in activated cells, such T cells and/or NK cells. Derived promoters can include the genomic region 2kb upstream of the gene. Derived promoters can include the genomic region -100bp downstream of the transcription initiation site the gene. Derived promoters can include the genomic region 2kb upstream of the gene to -100bp downstream of the transcription initiation site the gene. Derived promoters can include the genomic region upstream of the translation initiation site the gene. Derived promoters can include the genomic region 2kb upstream to the translation initiation site the gene. Derived promoters can include one or more enhancers identified in a promoter region. ACP-responsive promoters can include, but are not limited to, promoters derived from CCL3, CCL4, or MTA2 genes. ACP-responsive promoters can include, but are not limited to, a CCL3 promoter region (*e.g.*, SEQ ID NO: 156), a CCL4 promoter region (*e.g.*, SEQ ID NO: 157), and/or a MTA2 promoter region (*e.g.*, SEQ ID NO: 158). ACP-responsive promoters can include enhancers present in a CCL3 promoter region (*e.g.*, SEQ ID NO: 156), a CCL4 promoter region (*e.g.*, SEQ ID NO: 157), and/or a MTA2 promoter region (*e.g.*, SEQ ID NO: 158). ACP-responsive promoters can include synthetic promoters. For example, ACP-responsive promoters can include antigen induced enhancers or promoter sequences combined with other promoters, such as minimal promoters (*e.g.*, min AdeP or YB-TATA). ACP-responsive promoters can include synthetic enhancers, such as promoters including multiple iterations of transcription factor binding sites. In an illustrative non-limiting example, ACP-responsive promoter including a synthetic promoter can include 5 iterations of NFAT transcription factor binding sites in combination with a minimal Ade promoter (5x NFAT_minAdeP).

Multicistronic and Multiple Promoter Systems

[00470] In some embodiments, engineered nucleic acids are configured to produce multiple effector molecules. For example, nucleic acids may be configured to produce 2-20 different effector molecules. In some embodiments, nucleic acids are configured to produce 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14, 9-13, 9-12, 9-11, 9-10, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-20, 15-19, 15-18, 15-17, 15-16, 16-20, 16-19, 16-18, 16-17, 17-20, 17-19, 17-18, 18-20, 18-19, or 19-20 effector molecules. 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 effector molecules.

[00471] In some embodiments, engineered nucleic acids can be multicistronic, i.e., more than one separate polypeptide (e.g., multiple exogenous polynucleotides or effector molecules) can be produced from a single mRNA transcript. Engineered nucleic acids can be multicistronic through the use of various linkers, e.g., a polynucleotide sequence encoding a first exogenous polynucleotide or effector molecule can be linked to a nucleotide sequence encoding a second exogenous polynucleotide or effector molecule, such as in a first gene:linker:second gene 5’ to 3’ orientation. A linker polynucleotide sequence can encode a 2A ribosome skipping element, such as T2A. Other 2A ribosome skipping elements include, but are not limited to, E2A, P2A, and F2A. 2A ribosome skipping elements allow production of separate polypeptides encoded by the first and second genes are produced during translation. A linker can encode a cleavable linker polypeptide sequence, such as a Furin cleavage site or a TEV cleavage site, wherein following expression the cleavable linker polypeptide is cleaved such that separate polypeptides encoded by the first and second genes are produced. A cleavable linker can include a polypeptide sequence, such as such a flexible linker (e.g., a Gly-Ser-Gly sequence), that further promotes cleavage.

[00472] In some embodiments, when the second expression cassette comprises two or more units of **$(L_1 – E)_x$**, each L_1 linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide.

[00473] 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.

[00474] A linker can be a combination of linkers, such as a Furin-2A linker that can produce separate polypeptides through 2A ribosome skipping followed by further cleavage of the Furin site to allow for complete removal of 2A residues. In some embodiments, a combination of linkers can include a Furin sequence, a flexible linker, and 2A linker. Accordingly, in some embodiments, the linker is a Furin-Gly-Ser-Gly-2A fusion polypeptide. In some embodiments, a linker is a Furin-Gly-Ser-Gly-T2A fusion polypeptide.

[00475] 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 effector molecule, each separated by linkers such that separate polypeptides encoded by the first, second, and third effector molecules are produced).

[00476] "Linkers," as used herein can refer to polypeptides that link a first polypeptide sequence and a second polypeptide sequence or the multicistronic linkers described above.

Effector molecules

[00477] Any suitable effector molecule known in the art can be encoded by the engineered nucleic acid or expressed by the engineered cell. Suitable effector molecules can be grouped into therapeutic classes based on structure similarity, sequence similarity, or function. Effector molecule therapeutic classes include, but are not limited to, cytokines, chemokines, homing molecules, growth factors, co-activation molecules, tumor microenvironment modifiers, receptors, ligands, antibodies, polynucleotides, peptides, and enzymes.

[00478] In some embodiments, each effector molecule is independently selected from a therapeutic class, wherein the therapeutic class is selected from: a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier a, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

[00479] In some embodiments, a effector molecule is 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. Non-limiting examples of chemokines that may be encoded by the engineered nucleic acids of the present disclosure include: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1, or any combination thereof. In some embodiments, the chemokine is selected from: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

[00480] In some embodiments, a effector molecule is a cytokine. Non-limiting examples of cytokines that may be encoded by the engineered nucleic acids of the present disclosure include: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha, or any combination thereof. In some embodiments, the cytokine is selected from: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

[00481] In some embodiments, engineered nucleic acids are configured to produce at least one homing molecule. "Homing," refers to active navigation (migration) of a cell to a target site (*e.g.*, a cell, tissue (*e.g.*, tumor), or organ). A "homing molecule" refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate interaction of an engineered cell to a target site. Non-limiting examples of homing molecules include CXCR1, CCR9, CXCR2, CXCR3, CXCR4, CCR2, CCR4, FPR2, VEGFR, IL6R, CXCR1, CSCR7, PDGFR, anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; CCR4; and GPR15, or any combination thereof. In some embodiments, the homing molecule is selected from: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; CCR4; and GPR15.

[00482] In some embodiments, engineered nucleic acids are configured to produce at least one growth factor. Suitable growth factors for use as an effector molecule include, but are not limited to, FLT3L and GM-CSF, or any combination thereof. In some embodiments, the growth factor is selected from: FLT3L and GM-CSF.

[00483] In some embodiments, engineered nucleic acids are configured to produce at least one co-activation molecule. Suitable co-activation molecules for use as an effector molecule include, but are not limited to, c-Jun, 4-1BBL and CD40L, or any combination thereof. In some embodiments, the co-activation molecule is selected from: c-Jun, 4-1BBL and CD40L.

[00484] A “tumor microenvironment” is the cellular environment in which a tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix (ECM) (see, *e.g.*, Pattabiraman, D.R. & Weinberg, R.A. *Nature Reviews Drug Discovery* 13, 497–512 (2014); Balkwill, F.R. *et al. J Cell Sci* 125, 5591-5596, 2012; and Li, H. *et al. J Cell Biochem* 101(4), 805-15, 2007). Suitable tumor microenvironment modifiers for use as an effector molecule include, but are not limited to, adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2, or any combination thereof. In some embodiments, the tumor microenvironment modifier is selected from: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

[00485] In some embodiments, engineered nucleic acids are configured to produce at least one TGFbeta inhibitor. Suitable TGFbeta inhibitors for use as an effector molecule include, but are not limited to, an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, or combinations thereof. In some embodiments, the TGFbeta inhibitors are selected from: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

[00486] In some embodiments, engineered nucleic acids are configured to produce at least one immune checkpoint inhibitor. Suitable immune checkpoint inhibitors for use as an effector molecule include, but are not limited to, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies, or any combination thereof. In some embodiments, the immune checkpoint inhibitors are selected from: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

[00487] Illustrative immune checkpoint inhibitors include pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumamb (anti-PD-1; Opdivo® - BMS), pidilizumab (anti-PD-1 antibody; CT-011 – Teva/CureTech), AMP224 (anti-PD-1; NCI), avelumab (anti-PD-L1; Bavencio® - Pfizer), durvalumab (anti-PD-L1; MEDI4736/Imfinzi® -

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

[00488] In some embodiments, engineered nucleic acids are configured to produce at least one VEGF inhibitor. Suitable VEGF inhibitors for use as an effector molecule include, but are not limited to, anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof. In some embodiments, the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

[00489] In some embodiments, each effector molecule is a human-derived effector molecule.

Secretion Signals

[00490] In general, the one or more effector molecules comprise a secretion signal peptide (also referred to as a signal peptide or signal sequence) at the effector molecule's N-terminus that direct newly synthesized proteins destined for secretion or membrane insertion to the proper protein processing pathways. In embodiments with two or more effector molecules, each effector molecule can comprise a secretion signal (S). In embodiments with two or more effector molecules, each effector molecule can comprise a secretion signal such that each effector molecule is secreted from an engineered cell. In embodiments, the second expression cassette comprising one or more units of **(L – E)x** further comprises a polynucleotide sequence encoding a secretion signal peptide (S). In embodiments, for each X the corresponding secretion signal peptide is operably associated with the effector molecule. In embodiments, the second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L -S- E)x.**

[00491] The secretion signal peptide operably associated with a effector molecule can be a native secretion signal peptide native secretion signal peptide (*e.g.*, the secretion signal peptide generally endogenously associated with the given effector molecule). The secretion signal peptide operably associated with a 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 5.

Table 5. Exemplary Signal Secretion Peptides

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
IL-12	MCHQQLVISWFSL VFLASPLVA (SEQ ID NO: 56)	P29460	ATGTGTCACCAGCAGCTCGTTAT ATCCTGGTTTAGTTTGGTGTTTCT CGCTTCACCCCTGGTGGCA (SEQ ID NO: 31)
IL-12 (Codon Optimized)	MCHQQLVISWFSL VFLASPLVA (SEQ ID NO: 57)	-	ATGTGCCATCAGCAACTCGTCAT CTCCTGGTTCTCCCTTGTGTTCCT CGCTTCCCCTCTGGTCGCC (SEQ ID NO: 32)
IL-2 (Optimized)	MQLLSCIALILALV (SEQ ID NO: 58)	-	ATGCAACTGCTGTCATGTATCGC ACTCATCCTGGCGCTGGTA (SEQ ID NO: 33)
IL-2 (Native)	MYRMQLLSCIALSL ALVTNS (SEQ ID NO: 59)	P60568	ATGTATCGGATGCAACTTTTGAG CTGCATCGCATTGTCTCTGGCGCT GGTGACAAATTCC (SEQ ID NO: 34)
Trypsinogen-2	MNLLLILTFVAAAV A (SEQ ID NO: 60)	P07478	ATGAATCTCTTGCTCATACTTACG TTTGTCGCTGCTGCCGTTGCG (SEQ ID NO: 35)
Gaussia Luciferase	MGVKVLFALICIAV AEA (SEQ ID NO: 61)	-	ATGGGCGTGAAGGTCTTGTTTGC CCTTATCTGCATAGCTGTTGCGG AGGCG (SEQ ID NO: 36)
CD5	MPMGSLQPLATLY LLGMLVASCLG (SEQ ID NO: 62)	P06127	ATGCCGATGGGGAGCCTTCAACC TTTGGCAACGCTTTATCTTCTGGG GATGTTGGTTGCTAGTTGCCTTGG G (SEQ ID NO: 37)
IgKVII (mouse)	METDTLLLWVLLL WVPGSTGD (SEQ ID NO: 63)		ATGGAAACTGACACGTTGTTGCT GTGGGTATTGCTCTTGTGGGTCCC AGGATCTACGGGCGAC (SEQ ID NO: 38)
IgKVII (human)	MDMRVPAQLLGLL LLWLRGARC (SEQ ID NO: 64)	P01597	ATGGATATGAGGGTTCCCGCCCA GCTTTTGGGGCTGCTTTTGTTGTG GCTTCGAGGGGCTCGGTGT (SEQ ID NO: 39)
VSV-G	MKCLLYLAFLFIGV NC (SEQ ID NO: 65)	-	ATGAAGTGTCTGTTGTACCTGGC GTTTCTGTTCATTGGTGTAAACTG T (SEQ ID NO: 40)
Prolactin	MNIKGSPWKGSLL LLLVSNLLLCQSVA P (SEQ ID NO: 66)	P01236	ATGAATATCAAAGGAAGTCCGTG GAAGGGTAGTCTCCTGCTGCTCC TCGTATCTAACCTTCTCCTTTGTC AATCCGTGGCACCC (SEQ ID NO: 41)
Serum albumin preproprotein	MKWVTFISLLFLFS SAYS (SEQ ID NO: 67)	P02768	ATGAAATGGGTAACATTCATATC ACTTCTCTTTCTGTTCAGCTCTGC GTATTCT (SEQ ID NO: 42)
Azurocidin preproprotein	MTRLTVLALLAGL LASSRA (SEQ ID NO: 68)	20160	ATGACAAGGCTTACTGTTTTGGC TCTCCTCGCTGGACTCTTGGCTTC CTCCCGAGCA (SEQ ID NO: 43)
Osteonectin (BM40)	MRAWIFFLLCLAG RALA (SEQ ID NO: 69)	P09486	ATGAGGGCTTGGATTTTTTTTCTG CTCTGCCTTGCCGGTCGAGCCCT GGCG (SEQ ID NO: 44)
CD33	MPLLLLLPLLWAG ALA (SEQ ID NO: 70)	P20138	ATGCCTCTTCTGCTTTTGCTTCCT CTTTTGTGGGCAGGTGCCCTCGC A (SEQ ID NO: 45)

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
IL-6	MNSFSTSAFGPVAF SLGLLLVLPAAFPA P (SEQ ID NO: 71)	P05231	ATGAACTCTTTCTCAACCTCTGCG TTTGGTCCGGTCGCTTTCTCCCTT GGGCTCCTGCTTGTCTTGCCAGC AGCGTTTCCTGCGCCA (SEQ ID NO: 46)
IL-8	MTSKLAVALLAAF LISAALC (SEQ ID NO: 72)	P10145	ATGACAAGTAAACTGGCGGTAGC CTTGCTCGCGGCCTTTTTGATTTC CGCAGCCCTTTGT (SEQ ID NO: 47)
CCL2	MKVSAALLCLLLIA ATFIPQGLA (SEQ ID NO: 73)	P13500	ATGAAGGTAAGTGCAGCGTTGCT TTGCCTTCTCCTCATTGCAGCGAC CTTTATTCCTCAAGGGCTGGCC (SEQ ID NO: 48)
TIMP2	MGAAARTLRLALG LLLLATLLRPADA (SEQ ID NO: 74)	P16035	ATGGGAGCGGCAGCTAGAACACT TCGACTTGCCCTTGGGCTCTTGCT CCTTGCAACCCTCCTTAGACCTGC CGACGCA (SEQ ID NO: 49)
VEGFB	MSPLLRRLLLAALL QLAPAQA (SEQ ID NO: 75)	P49765	ATGTCACCGTTGTTGCGGAGATT GCTGTTGGCCGCACTTTTGCAACT GGCTCCTGCTCAAGCC (SEQ ID NO: 50)
Osteoprotegerin	MNNLLCCALVFLDI SIKWTTQ (SEQ ID NO: 76)	O00300	ATGAATAACCTGCTCTGTTGTGC GCTCGTGTTCCTGGACATTTCTAT AAAATGGACAACGCAA (SEQ ID NO: 51)
Serpin E1	MQMSPALTCLVLG LALVFGEGSA (SEQ ID NO: 77)	P05121	ATGCAAATGTCTCCTGCCCTTACC TGTCTCGTACTTGGTCTTGCGCTC GTATTTGGAGAGGGATCAGCC (SEQ ID NO: 52)
GROalpha	MARAALSAAPSNP RLLRVALLLLLLVA AGRRAAG (SEQ ID NO: 78)	P09341	ATGGCAAGGGCTGCACTCAGTGC TGCCCCGTCTAATCCCAGATTGCT TCGAGTTGCATTGCTTCTTCTGTT GCTGGTTGCAGCTGGTAGGAGAG CAGCGGGT (SEQ ID NO: 53)
CXCL12	MNAKVVVVLVLV LTALCLSDG (SEQ ID NO: 79)	P48061	ATGAATGCAAAAGTCGTGGTCGT GCTGGTTTTGGTTCTGACGGCGTT GTGTCTTAGTGATGGG (SEQ ID NO: 54)
IL-21 (Codon Optimized)	MERIVICLMVIFLG TLVHKSSS (SEQ ID NO: 80)	Q9HBE4	ATGGAACGCATTGTGATCTGCCT GATGGTCATCTTCCTGGGCACCTT AGTGCACAAGTCGAGCAGC (SEQ ID NO: 55)
CD8a	MALPVTALLLPLAL LLHAARP (SEQ ID NO: 83)	P01732	ATGGCTCTCCCTGTAACTGCCCTG CTTCTTCCCCTTGCCTTGCTTCTC CATGCCGCTAGACCC (SEQ ID NO: 84)
GMCSFR	MLLLVTSLLLCELP HPAFLLIP (SEQ ID NO: 85)	P15509	ATGCTGCTGCTGGTCACATCTCTG CTGCTGTGCGAGCTGCCCCATCC TGCCTTTCTGCTGATCCCT (SEQ ID NO: 86) ATGCTGCTGCTGGTTACATCTCTG CTGCTGTGCGAGCTGCCCCATCC TGCCTTTCTGCTGATCCCT (SEQ ID NO: 87)

Antigen recognizing receptors

[00492] Certain aspects of the present disclosure relate to an engineered nucleic comprising an antigen recognizing receptor. In some embodiments, an engineered nucleic acid of the present disclosure comprises a first expression cassette that further comprises an antigen recognizing receptor. In some embodiments, the first expression cassette comprises a polynucleotide sequence encoding the antigen recognizing receptor that is operably linked to the first exogenous polynucleotide sequence encoding the ACP and to the first promoter. Suitable antigen recognizing receptors for use as an effector molecule recognize antigens that include, but are not limited to, 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 (MSLN), 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, or any combination thereof.

[00493] In some embodiments, the antigen recognizing receptor recognizes an antigen selected from: 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.

[00494] In some embodiments, the antigen recognizing receptor recognizes GPC3. An antigen recognizing receptor that recognizes GPC3 can include an anti-binding domain that binds to GPC3. In some embodiments, the antigen-binding domain that binds to GPC3

includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH includes: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (**SEQ ID NO: 119**), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (**SEQ ID NO: 120**), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (**SEQ ID NO: 121**), and wherein the VL includes: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (**SEQ ID NO: 122**), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (**SEQ ID NO: 123**), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (**SEQ ID NO: 124**). In some embodiments, the antigen-binding domain that binds to GPC3 includes a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (**SEQ ID NO: 119**). In some embodiments, the antigen-binding domain that binds to GPC3 includes a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (**SEQ ID NO: 120**). In some embodiments, the antigen-binding domain that binds to GPC3 includes a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (**SEQ ID NO: 121**). In some embodiments, the antigen-binding domain that binds to GPC3 includes a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (**SEQ ID NO: 122**). In some embodiments, the antigen-binding domain that binds to GPC3 includes a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (**SEQ ID NO: 123**). In some embodiments, the antigen-binding domain that binds to GPC3 includes a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (**SEQ ID NO: 124**).

[00495] In some embodiments, the antigen-binding domain that binds to GPC3 includes a VH region having an amino acid sequence with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of EVQLVETGGGMVQPEGSLKLSCAASGFTFNKNAMNWVRQAPGKGLEWVARIRNKTNNYATYYADSVKARFTISRDDSQSMLYLQMNNLKIEDTAMYYCVAGNSFAYWGQGTLVTVSA (**SEQ ID NO: 125**) or EVQLVESGGGLVQPGGSLRLSCAASGFTFNKNAMNWVRQAPGKGLEWVGRIRNKT

NNYATYYADSVKARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQG TLVTVSA (**SEQ ID NO: 126**).

[00496] In some embodiments, the antigen-binding domain that binds to GPC3 includes a VL region having an amino acid sequence with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of DIVMSQSPSSLVVSIGEKVTMTCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWA SSRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYNYPLTFGAGTKLELK (**SEQ ID NO: 127**), or DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKLLIYWA SSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFGQGTKLEIK (**SEQ ID NO: 128**).

[00497] In some embodiments, the antigen recognizing receptor recognizes MSLN. An antigen recognizing receptor that recognizes MSLN can include an anti-binding domain that binds to MSLN. In some embodiments, the antigen-binding domain that binds to MSLN includes a single-domain binding domain having an amino acid sequence with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of QVQLVESGGGTVQAGGSLKLACAASGLPRTYNVMGWFRQAPGKEREGVAIIYTTTG ATYYRDSVKGRATISQDNAKKSVSLQMNSLRPEDTAIYYCVARQPNSGPWEYWGQ GTQVTVSS (**SEQ ID NO: 129**), or QVKLEESGGGSVQAGGSLRLSCTTSGYTNSYKWMGWFRQAPGQEREGVAVIYTGN DRTYYSDSVKGRFTISRDNAKNMIYLDMTRLRPEDSAVYECAIGHDGAWRYWGQG TQVTVSS (**SEQ ID NO: 130**). In some embodiments, the antigen-binding domain that binds to MSLN includes each of the CDR sequences from a single-domain binding domain having an amino acid sequence QVQLVESGGGTVQAGGSLKLACAASGLPRTYNVMGWFRQAPGKEREGVAIIYTTTG ATYYRDSVKGRATISQDNAKKSVSLQMNSLRPEDTAIYYCVARQPNSGPWEYWGQ GTQVTVSS (**SEQ ID NO: 129**), or QVKLEESGGGSVQAGGSLRLSCTTSGYTNSYKWMGWFRQAPGQEREGVAVIYTGN DRTYYSDSVKGRFTISRDNAKNMIYLDMTRLRPEDSAVYECAIGHDGAWRYWGQG TQVTVSS (**SEQ ID NO: 130**). In some embodiments, the antigen-binding domain that binds to MSLN includes one or more CDR sequences from a single-domain binding domain having an amino acid sequence

QVQLVESGGGTVQAGGSLKLACAASGLPRTYNVMGWFRQAPGKEREGVAIIYTTTG ATYYRDSVKGRATISQDNAKKSVSLQMNSLRPEDTAIYYCVARQPNSGPWEYWGQ GTQVTVSS (**SEQ ID NO: 129**), or QVKLEESGGGSVQAGGSLRLSCTTSGYTNSYKWMGWFRQAPGQEREGVAVIYTGN DRTYYSDSVKGRFTISRDNAKNMIYLDMTRLRPEDSAVYECAIGHDGAWRYWGQG TQVTVSS (**SEQ ID NO: 130**).

[00498] In some embodiments, the first expression cassette further comprises a linker polynucleotide sequence localized between the ACP and the antigen recognizing receptor.

[00499] In some embodiments, the antigen recognizing receptor comprises an antigen-binding domain. In some embodiments, 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 embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the VH and VL are separated by a peptide linker.

[00500] An scFv has a variable domain of light chain (VL) connected from its C-terminus to the N-terminal end of a variable domain of heavy chain (VH) by a polypeptide chain. Alternately the scFv comprises of polypeptide chain where in the C-terminal end of the VH is connected to the N-terminal end of VL by a polypeptide chain. In some embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[00501] An sdAb is a molecule in which one variable domain of an antibody specifically binds to an antigen without the presence of the other variable domain.

[00502] A F(ab) fragment contains the constant domain (CL) of the light chain and the first constant domain (CH1) of the heavy chain along with the variable domains VL and VH on the light and heavy chains respectively. F(ab′) fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. $F(ab')_2$ fragments contain two Fab' fragments joined, near the hinge region, by disulfide bonds.

[00503] In some embodiments, the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR). In some embodiments, the antigen recognizing receptor is a CAR. In some embodiments, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from: 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 embodiments, the CAR comprises a CD3zeta-chain intracellular signaling domain and one or more additional intracellular signaling domains (e.g., co-stimulatory domains) selected from a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, a CD16a intracellular signaling domain, a DNAM-1 intracellular signaling domain, a KIR2DS1 intracellular signaling domain, a KIR3DS1 intracellular signaling domain, a NKp44 intracellular signaling domain, a NKp46 intracellular signaling domain, a FceRlg intracellular signaling domain, a NKG2D intracellular signaling domain, and an EAT-2 intracellular signaling domain.

[00504] In some embodiments, the CAR further comprises a transmembrane domain, and the transmembrane domain is selected from: 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, an OX40 transmembrane domain, a DAP10 transmembrane domain, a DAP12 transmembrane domain, a CD16a transmembrane domain, a DNAM-1 transmembrane domain, a KIR2DS1 transmembrane domain, a KIR3DS1 transmembrane domain, an NKp44 transmembrane domain, an NKp46 transmembrane domain, an FceRlg transmembrane domain, and an NKG2D transmembrane domain.

[00505] In some embodiments, the CAR further comprises a spacer region (e.g., hinge domain) between the antigen-binding domain and the transmembrane domain. A spacer or hinge domain is any oligopeptide or polypeptide that functions to link the transmembrane domain to the extracellular domain and/or the intracellular signaling domain in the polypeptide chain. Spacer or hinge domains provide flexibility to the inhibitory chimeric receptor or tumor-targeting chimeric receptor, or domains thereof, or prevent steric hindrance of the inhibitory chimeric receptor or tumor-targeting chimeric receptor, or domains thereof. In some embodiments, a spacer domain or hinge domain may comprise up to 300 amino acids (e.g., 10 to 100 amino acids, or 5 to 20 amino acids). In some embodiments, one or more spacer domain(s) may be included in other regions of an inhibitory chimeric receptor or tumor-targeting chimeric receptor.

[00506] Exemplary spacer or hinge domains may include, without limitation an IgG domain (such as an IgG1 hinge, an IgG2 hinge, an IgG3 hinge, or an IgG4 hinge), an IgD hinge domain, a CD8α hinge domain, and a CD28 hinge domain. In some embodiments, the spacer or hinge domain is an IgG domain, an IgD domain, a CD8α hinge domain, or a CD28 hinge domain.

[00507] Exemplary spacer or hinge domain protein sequences are shown in **Table 6**. Exemplary spacer or hinge domain nucleotide sequences are shown in **Table 7**.

Table 6

Amino Acid Sequence	**SEQ ID NO:**	**Description**
AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPS PLFPGPSKP	100	CD28 hinge
ESKYGPPCPSCP	101	IgG4 minimal hinge
ESKYGPPAPSAP	102	IgG4 minimal hinge, no disulfides
ESKYGPPCPPCP	103	IgG4 S228P minimal hinge, enhanced disulfide formation
EPKSCDKTHTCP	104	IgG1 minimal hinge
AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLS LRPEACRPAAGGAVHTRGLDFACDIYIWAPLA GTCGVLLLSLVITLYCNHRN	105	Extended CD8a hinge
TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGA VHTRGLDFACD	106	CD8a hinge
ACPTGLYTHSGECCKACNLGEGVAQPCGANQ TVCEPCLDSVTFSDVVSATEPCKPCTECVGLQS MSAPCVEADDAVCRCAYGYYQDETTGRCEAC RVCEAGSGLVFSCQDKQNTVCEECPDGTYSDE ADAEC	107	LNGFR hinge
ACPTGLYTHSGECCKACNLGEGVAQPCGANQ TVC	108	Truncated LNGFR hinge (TNFR-Cys1)
AVGQDTQEVIVVPHSLPFKV	109	PDGFR-beta extracellular linker

Table 7		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GCAGCAGCTATCGAGGTGATGTATCCTCCGC CCTACCTGGATAATGAAAAGAGTAATGGGA CTATCATTCATGTAAAAGGGAAGCATCTTTG TCCTTCTCCCCTTTTCCCCGGTCCGTCTAAAC CT	110	CD28 hinge
GAA AGC AAG TAC GGT CCA CCT TGC CCT AGC TGT CCG	111	IgG4 minimal hinge
GAA TCC AAG TAC GGC CCC CCA GCG CCT AGT GCC CCA	112	IgG4 minimal hinge, no disulfides
GAA TCT AAA TAT GGC CCG CCA TGC CCG CCT TGC CCA	113	IgG4 S228P minimal hinge, enhanced disulfide formation
GAA CCG AAG TCT TGT GAT AAA ACT CAT ACG TGC CCG	114	IgG1 minimal hinge
GCT GCT GCT TTC GTA CCC GTG TTC CTC CCT GCT AAG CCT ACG ACT ACC CCC GCA CCG AGA CCA CCC ACG CCA GCA CCC ACG ATTGCT AGC CAG CCC CTT AGT TTG CGA CCA GAA GCT TGT CGG CCT GCT GCT GGT GGC GCG GTA CAT ACC CGC GGC CTT GAT TTT GCTTGC GAT ATA TAT ATC TGG GCG CCT CTG GCC GGA ACA TGC GGG GTC CTC CTC CTT TCT CTG GTT ATT ACT CTC TAC TGT AAT CACAGG AAT	115	Extended CD8a hinge
GCC TGC CCG ACC GGG CTC TAC ACT CAT AGC GGG GAA TGT TGT AAG GCA TGT AAC TTG GGT GAG GGC GTC GCA CAG CCC TGC GGAGCT AAC CAA ACA GTG TGC GAA CCC TGC CTC GAT AGT GTG ACG TTC TCT GAT GTT GTA TCA GCT ACA GAG CCT TGC AAA CCA TGTACT GAG TGC GTT GGA CTT CAG TCA ATG AGC GCT CCA TGT GTG GAG GCA GAT GAT GCG GTC TGT CGA TGT GCT TAC GGA TAC TACCAA GAC GAG ACA ACA GGG CGG TGC GAG GCC TGT AGA GTT TGT GAG GCG GGC TCC GGG CTG GTG TTT TCA TGT CAA GAC AAG CAAAAT ACG GTC TGT GAA GAG TGC CCT GAT GGC ACC TAC TCA GAC GAA GCA GAT GCA GAA TGC	116	LNGFR hinge
GCC TGC CCT ACA GGA CTC TAC ACG CAT AGC GGT GAG TGT TGT AAA GCA TGC AAC CTC GGG GAA GGT GTA GCC CAG CCA TGC GGG GCT AAC CAA ACC GTT TGC	117	Truncated LNGFR hinge (TNFR-Cys1)
GCTGTGGGCCAGGACACGCAGGAGGTCATC GTGGTGCCACACTCCTTGCCCTTTAAGGTG	118	PDGFR-beta extracellular linker

[00508] Suitable transmembrane domains, spacer or hinge domains, and intracellular domains for use in a CAR are generally described in Stoiber et al, Cells 2019, 8(5), 472; Guedan et al, Mol Therapy: Met & Clinic Dev, 2019 12:145-156; and Sadelain et al, Cancer Discov; 2013, 3(4); 388–98, each of which are hereby incorporated by reference in their entirety.

[00509] In some embodiments, the CAR further comprises a secretion signal peptide. Any suitable secretion signal peptide of the present disclosure may be used.

Post-Transcriptional Regulatory Elements

[00510] In some embodiments, an engineered nucleic acid of the present disclosure comprises a post-transcriptional regulatory element (PRE). PREs can enhance gene expression via enabling tertiary RNA structure stability and 3' end formation. Non-limiting examples of PREs include the Hepatitis B virus PRE (HPRE) and the Woodchuck Hepatitis Virus PRE (WPRE). In some embodiments, the post-transcriptional regulatory element is a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE). In some embodiments, the WPRE comprises the alpha, beta, and gamma components of the WPRE element. In some embodiments, the WPRE comprises the alpha component of the WPRE element.

Engineered Cells

[00511] Also provided herein are cells, and methods of producing cells, that comprise one or more engineered nucleic acids of the present disclosure. These cells are referred to herein as "engineered cells." These cells, which typically contain one or more engineered nucleic acids, do not occur in nature. In some embodiments, the cells are isolated cells that recombinantly express the one or more engineered nucleic acids. In some embodiments, the engineered one or more nucleic acids are expressed from one or more vectors or a selected locus from the genome of the cell. In some embodiments, the cells are engineered to include a first nucleic acid comprising a promoter operable linked to a nucleotide sequence encoding an activation-conditional control polypeptide (ACP), such as a transcription factor, and/or antigen recognizing receptor. In some embodiments, the transcription factor comprises a repressible protease and a cognate cleavage site. In some embodiments, the transcription factor comprises a degron. In some embodiments, the ACP is the antigen recognizing receptor.

[00512] In some embodiments, the engineered cells comprise a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and/or an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: $(L - E)_X$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the

ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter. ACP is the antigen recognizing receptor and the receptor is capable of inducing expression of the second expression cassette by binding to its cognate antigen.

[00513] In some embodiments, X can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more.

[00514] In some embodiments, the first expression cassette and the second expression cassette are encoded by separate polynucleotide sequences in engineered cells. For example, in some embodiments, the engineered cell comprises two engineered nucleic acids; a first engineered nucleic acid comprising a polynucleotide sequence encoding the first expression cassette and a second engineered nucleic acid comprising a polynucleotide sequence encoding the second expression cassette. In an illustrative example, an effector molecule expression cassette can be encoded by a first engineered nucleic acid in an engineered cell, and an ACP expression cassette can be encoded by a second engineered nucleic acid in the engineered cell. In another illustrative example, an effector molecule expression cassette can be encoded by a first engineered nucleic acid, an ACP expression cassette can be encoded by a second engineered nucleic acid, and an antigen recognizing receptor expression cassette can be encoded by a third engineered nucleic acid in an engineered cell.

[00515] In some embodiments, the first expression cassette and the second expression cassette are encoded by a single polynucleotide sequence in engineered cells. For example, in some embodiments, the engineered cells comprises a single engineered nucleic acid comprising a polynucleotide sequence encoding both the first expression cassette and the second expression cassette. Other illustrative examples include, but are not limited to, (1) an antigen recognizing receptor expression cassette and an effector molecule expression cassette can be encoded by a first engineered nucleic acid, and an ACP expression cassette can be encoded by a second engineered nucleic acid; (2) an ACP expression cassette and an effector molecule expression cassette can be encoded by a first engineered nucleic acid, and an antigen recognizing receptor expression cassette can be encoded by a second engineered nucleic acid; (3) an ACP expression cassette and an antigen recognizing receptor expression cassette can be encoded by a first engineered nucleic acid, and an effector molecule expression cassette can be encoded by a second engineered nucleic acid.

[00516] In some embodiments, expression cassettes of polynucleotide sequences in engineered cells can be multicistronic, i.e., more than one separate polypeptide (e.g., multiple exogenous polynucleotides or effector molecules) can be produced from a single mRNA

transcript. For example, a multicistronic expression cassette can encode both an ACP and antigen recognizing receptor, *e.g.*, both expressed from a single expression cassette driven by a constitutive promoter. In another example, a multicistronic expression cassette can encode both an effector molecule and an antigen recognizing receptor, *e.g.*, both expressed from a single expression cassette driven by an ACP-responsive promoter. Expression cassettes can be multicistronic through the use of various linkers, e.g., a polynucleotide sequence encoding a first protein of interest can be linked to a nucleotide sequence encoding a second protein of interest, such as in a first gene:linker:second gene 5' to 3' orientation. Multicistronic features and options are described in the section "Multicistronic and Multiple Promoter Systems."

[00517] In some embodiments, the second expression cassette comprises two or more units of $(L – E)_X$, each L linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide. In some embodiments, the second expression cassette comprising one or more units of $(L – E)_X$ further comprises a polynucleotide sequence encoding a secretion signal peptide. In some embodiments, for each X the corresponding secretion signal peptide is operably associated with the effector molecule. In some embodiments, each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule. In some embodiments, each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule. In some embodiments, the non-native secretion signal peptide is selected from: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

[00518] In some embodiments, the cells are engineered to include an additional expression cassette comprising an additional promoter operably linked to an additional exogenous nucleotide sequence encoding an additional effector molecule, for example, one that stimulates an immune response. In some embodiments, the engineered cell further comprises an additional expression cassette comprising an additional promoter and an additional exogenous polynucleotide sequence having the formula: $(L – E)_X$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the additional promoter is operably linked to the additional exogenous polynucleotide, and wherein for the first iteration of the (L – E) unit, L is absent.

[00519] X can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more.

[00520] In some embodiments, the additional expression cassette comprises two or more units of $(L – E)_X$, each L linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide. In some embodiments, the additional expression cassette comprises one or more units of $(L – E)_X$ further comprises a polynucleotide sequence encoding a secretion signal peptide. In some embodiments, for each X the corresponding secretion signal peptide is operably associated with the effector molecule. In some embodiments, each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule. In some embodiments, each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule. In some embodiments, the non-native secretion signal peptide is selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVIICD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

[00521] In some embodiments, the first promoter and/or the additional promoter is a constitutive promoter, an inducible promoter, or a synthetic promoter. In some embodiments, the first promoter and/or the additional promoter is a constitutive promoter selected from: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

[00522] An engineered cell of the present disclosure 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.

[00523] The present disclosure also encompasses additivity and synergy between an effector molecule(s) and the engineered cell from which they are produced. In some embodiments, cells are engineered to produce one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) effector molecules, each of which may modulate a different tumor-mediated immunosuppressive mechanism. In other embodiments, cells are engineered to produce at least one 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.

[00524] In some embodiments, a cell (*e.g.*, a tumor cell, an erythrocyte, a platelet cell, or a bacterial cell) is engineered to produce one or more effector molecules. For example, cells

may be engineered to produce 1-20 different effector molecules. In some embodiments, cells engineered to produce 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14, 9-13, 9-12, 9-11, 9-10, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-20, 15-19, 15-18, 15-17, 15-16, 16-20, 16-19, 16-18, 16-17, 17-20, 17-19, 17-18, 18-20, 18-19, or 19-20 effector molecules. 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 effector molecules.

[00525] In some embodiments, engineered cells comprise one or more engineered nucleic acids comprising a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: $(L – E)_X$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20 wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter. 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 first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an ACP and a second exogenous polynucleotide sequence having the formula: $(L – E)_X$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20. In some embodiments, the second exogenous polynucleotide sequence encodes at least one (*e.g.*, 1, 2 or 3) effector molecule. The second exogenous polynucleotide sequence can encode at least 1, at least 2, at

least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or more effector molecules. 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, at least 10, or more, engineered nucleic acids, each encoding a first expression cassette comprising a promoter operably linked to an ACP polynucleotide sequence, and a second expression cassette comprising an ACP-responsive promoter and an exogenous nucleotide sequence encoding at least one (*e.g.*, 1, 2, 3, or more) effector molecules. 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 first expression cassette comprising a promoter operably linked to an ACP polynucleotide sequence, and a second expression cassette comprising an ACP-responsive promoter and an exogenous nucleotide sequence encoding at least one (*e.g.*, 1, 2, 3, or more) effector molecules.

[00526] In some embodiments, the engineered cells further comprise a third expression cassette comprising a third promoter and a third exogenous polynucleotide sequence encoding an antigen recognizing receptor, wherein the third promoter is operably linked to the third exogenous polynucleotide. In some embodiments, the first exogenous polynucleotide sequence further encodes an antigen recognizing receptor.

[00527] In some embodiments, engineered cells comprise one or more engineered nucleic acids comprising a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and/or an antigen recognizing receptor, wherein the first promoter is operably linked to the first exogenous polynucleotide and a second expression cassette comprising an activation-conditional control polypeptide-responsive (ACP-responsive) promoter and a second exogenous polynucleotide sequence having the formula: $(L – E)_X$ wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20 wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter. In embodiments where the first exogenous polynucleotide sequence encodes an antigen recognizing receptor, the engineered cells may further comprise a third expression cassette comprising a third promoter and a third exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the third promoter is operably linked to the third exogenous polynucleotide.

Exemplary antigen recognizing receptors include chimeric antigen receptors (CARs) or T cell receptors (TCRs). In some embodiments, the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter. In some embodiments, the ACP is the antigen recognizing receptor and the ACP is capable of inducing expression of the second expression cassette by binding to its cognate antigen. In some embodiments, the ACP-responsive promoter is an inducible promoter that is capable of being induced by the ACP binding to its cognate antigen.

[00528] Engineered cells can comprise an engineered nucleic acid encoding at least one of the linkers described above, such as polypeptides that link a first polypeptide sequence and a second polypeptide sequence, one or more multicistronic linker described above, one or more additional promoters operably linked to additional ORFs, or a combination thereof. In some embodiments, the first expression cassette and the second expression cassette are encoded by separate polynucleotide sequences. In some embodiments, the first expression cassette and the second expression cassette are encoded by a single polynucleotide sequence. In some embodiments, when the second expression cassette comprises two or more units of **$(L_1 – E)_x$**, each L_1 linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide. In some embodiments, the engineered cell further comprises a second linker polynucleotide sequence, wherein the second linker polynucleotide links the first expression cassette to the second expression cassette. In some embodiments, the second linker polynucleotide sequence is operably associated with the translation of each effector molecule and the ACP as separate polypeptides.

[00529] In some embodiments, a cell (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce an effector molecule independently selected from a therapeutic class, wherein the therapeutic class is selected from: a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier a, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

[00530] In some embodiments, a cell of the present disclosure (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce a chemokine. In some embodiments, the chemokine is selected from: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

[00531] In some embodiments, a cell of the present disclosure (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce a cytokine. In some embodiments, the cytokine is selected from: IL1-beta, IL2, IL4, IL6, IL7,

IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

[00532] In some embodiments, a cell of the present disclosure (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce at least one homing molecule. "Homing," refers to active navigation (migration) of a cell to a target site (*e.g.*, a cell, tissue (*e.g.*, tumor), or organ). A "homing molecule" refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate interaction of an engineered cell to a target site. In some embodiments, the homing molecule is selected from: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; CCR4; and GPR15.

[00533] In some embodiments, a cell of the present disclosure (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce at least one growth factor. In some embodiments, the growth factor is selected from: FLT3L and GM-CSF.

[00534] In some embodiments, a cell of the present disclosure (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce at least one co-activation molecule. In some embodiments, the co-activation molecule is selected from: c-Jun, 4-1BBL and CD40L.

[00535] In some embodiments, a cell of the present disclosure (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce at least one TGFbeta inhibitor. In some embodiments, the TGFbeta inhibitors are selected from: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

[00536] In some embodiments, a cell of the present disclosure (*e.g.*, a T cell, an immune cell, a stem cell, a tumor cell, an erythrocyte, or a platelet cell) is engineered to produce at least one immune checkpoint inhibitor. In some embodiments, the immune checkpoint inhibitors are selected from: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

[00537] Illustrative immune checkpoint inhibitors include pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumamb (anti-PD-1; Opdivo® - BMS), pidilizumab

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

[00538] In some embodiments, a cell of the present disclosure (*e.g.*, a tumor cell, an erythrocyte, a platelet cell, or a bacterial cell) is engineered to produce at least one VEGF inhibitor. In some embodiments, the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

[00539] In some embodiments, each effector molecule is a human-derived effector molecule.

Engineereed Cell Types

[00540] An engineered cell or isolated cell of the present disclosure can be a human cell. An engineered cell or isolated 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 cell. An engineered primary cell can be any somatic cell. An engineered primary cell can be a MSC. In some embodiments, the engineered cell is derived from the subject. In some embodiments, the engineered cell is allogeneic with reference to the subject.

[00541] An engineered cell of the present disclosure can be isolated from a subject, such as a subject known or suspected to have cancer. Cell isolation methods are known to those skilled in the art and include, but are not limited to, sorting techniques based on cell-surface marker expression, such as FACS sorting, positive isolation techniques, and negative isolation, magnetic isolation, and combinations thereof. An engineered cell can be allogenic with reference to the subject being administered a treatment. Allogenic modified cells can be HLA-matched to the subject being administered a treatment. An engineered cell can be a cultured cell, such as an *ex vivo* cultured cell. An engineered cell can be an *ex vivo* cultured cell, such as a primary cell isolated from a subject. Cultured cell can be cultured with one or more cytokines.

[00542] In some embodiments, an engineered or isolated cell of the present disclosure is selected from: 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 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 embodiments, the engineered cell is a Natural Killer (NK) cell. In some embodiments, an engineered cell is autologous. In some embodiments, an engineered cell is allogeneic.

[00543] In some embodiments, an engineered cell of the present disclosure is a tumor cell selected from: an adenocarcinoma cell, 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, a mesothelioma cell, an ovarian tumor cell, a pancreatic tumor cell, a gastric tumor cell, a testicular yolk sac tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

[00544] In some embodiments, an engineered cell of the present disclosure is a bacterial cell selected from: *Clostridium beijerinckii*, *Clostridium sporogenes*, *Clostridium novyi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Salmonella choleraesuis*.

[00545] Also provided herein are methods that include culturing the engineered cells of the present disclosure. Methods of culturing the engineered cells described herein are known. One skilled in the art will recognize that culturing conditions will depend on the particular engineered cell of interest. One skilled in the art will recognize that culturing conditions will depend on the specific downstream use of the engineered cell, for example, specific culturing conditions for subsequent administration of the engineered cell to a subject.

Methods of Engineering Cells

[00546] Also provided herein are compositions and methods for engineering cells to produce the activation conditional control polypeptide (ACP) and one or more effectors molecules encoded by any engineered nucleic acid comprising the first and second expression cassettes as described herein, or.

[00547] In general, cells are engineered to produce ACPs and effector molecules through introduction (i.e., delivery) of one or more polynucleotides of the present disclosure comprising the first promoter and the exogenous polynucleotide sequence encoding the ACP

and the second expression cassette comprising an ACP-responsive promoter and the second exogenous sequence encoding one or more effector molecules into the cell's cytosol and/or nucleus. For example, the polynucleotide expression cassettes encoding the ACP polypeptide and the one or more effector molecules can be any of the engineered nucleic acids 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.

[00548] In some embodiments, the engineered 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. In some embodiments, the oncolytic virus is a recombinant oncolytic virus comprising the first expression cassette and the second expression cassette. In some embodiments, the oncolytic virus further comprises the third expression cassette.

[00549] The virus, including any of the oncolytic viruses described herein, can be a recombinant virus that encodes one more transgenes encoding one or more effector molecules, 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 effector molecules, such as any of the engineered nucleic acids described herein. In some embodiments, the cell is engineered via transduction with an oncolytic virus.

Viral-Mediated Delivery

[00550] Viral vector-based delivery platforms can be used to engineer cells. In general, a viral vector-based delivery platform engineers a cell through introducing (i.e., delivering) into a host cell. For example, a viral vector-based delivery platform can engineer a cell through

introducing any of the engineered nucleic acids described herein. A viral vector-based delivery platform can be a nucleic acid, and as such, an engineered nucleic acid can also encompass an engineered virally-derived nucleic acid. Such engineered virally-derived nucleic acids can also be referred to as recombinant viruses or engineered viruses.

[00551] A viral vector-based delivery platform can encode more than one engineered nucleic acid, gene, or transgene within the same nucleic acid. For example, an engineered virally-derived nucleic acid, e.g., a recombinant virus or an engineered virus, can encode one or more transgenes, including, but not limited to, any of the engineered nucleic acids described herein that encode one or more effector molecules. The one or more transgenes encoding the one or more effector molecules can be configured to express the one or more effector molecules. 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 effector molecules), such as viral genes needed for viral infectivity and/or viral production (e.g., capsid proteins, envelope proteins, viral polymerases, viral transcriptases, etc.), referred to as cis-acting elements or genes.

[00552] A viral vector-based delivery platform can comprise more than one viral vector, such as separate viral vectors encoding the engineered nucleic acids, genes, or transgenes described herein, and referred to as trans-acting elements or genes. For example, a helper-dependent viral vector-based delivery platform can provide additional genes needed for viral infectivity and/or viral production on one or more additional separate vectors in addition to the vector encoding the one or more effector molecules. One viral vector can deliver more than one engineered nucleic acids, such as one vector that delivers engineered nucleic acids that are configured to produce two or more effector molecules. 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 effector molecules. The number of viral vectors used can depend on the packaging capacity of the above mentioned viral vector-based vaccine platforms, and one skilled in the art can select the appropriate number of viral vectors.

[00553] In general, any of the viral vector-based systems can be used for the in vitro production of molecules, such as 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 effector molecules. The selection of an appropriate viral vector-based system will depend on a variety of factors, such as cargo/payload size, immunogenicity of the viral system, target

cell of interest, gene expression strength and timing, and other factors appreciated by one skilled in the art.

[00554] 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, Sakuma 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).

[00555] 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, and in some case secrete, the one or more effector molecules. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Pat. No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al. (Nature 351:456-460 (1991)). A wide variety of other vectors useful for the introduction (i.e., delivery) of engineered nucleic acids, e.g., Salmonella typhi vectors, and the like will be apparent to those skilled in the art from the description herein.

[00556] The viral vector-based delivery platforms can be a virus that targets a tumor 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 effector molecules. The transgenes encoding the one or more effector molecules can be configured to express the one or more effector molecules.

[00557] In some embodiments, the virus is selected from: a lentivirus, a retrovirus, an oncolytic virus, an adenovirus, an adeno-associated virus (AAV), and a virus-like particle (VLP).

[00558] 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 effector molecules) into the target cell to provide permanent transgene expression. Retroviral-based delivery systems include, but are not limited to, those based upon murine leukemia, virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency vims (SIV), human immuno deficiency vims (HIV), and combinations thereof (see, e.g., Buchscher et al., J. Virol. 66:2731-2739 (1992); Johann et ah, J. Virol. 66:1635-1640 (1992); Sommnerfelt et al., Virol. 176:58-59 (1990); Wilson et ah, J. Virol. 63:2374-2378 (1989); Miller et al, J, Virol. 65:2220-2224 (1991); PCT/US94/05700). Other retroviral systems include the Phoenix retrovirus system.

[00559] 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.

[00560] The viral vector-based delivery platform can be adenovirus-based. In general, adenoviral based vectors are capable of very high transduction efficiency in many cell types, do not require cell division, achieve high titer and levels of expression, and can be produced in large quantities in a relatively simple system. In general, adenoviruses can be used for transient expression of a transgene within an infected cell since adenoviruses do not typically integrate into a host's genome. Adenovirus-based delivery platforms are described in more detail in Li et al., Invest Opthalmol Vis Sci 35:2543 2549, 1994; Borras et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655, each herein incorporated by reference for all purposes. Other exemplary adenovirus-based delivery platforms are described in more detail in U.S. Pat. Nos. 5585362; 6,083,716, 7,371,570; 7,348,178; 7,323,177; 7,319,033; 7,318,919; and 7,306,793 and International Patent Application WO96/13597, each herein incorporated by reference for all purposes.

[00561] 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 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 effector molecules (see, e.g., West et al., Virology 160:38-47 (1987); U.S. Pat. Nos. 4,797,368; 5,436,146; 6,632,670; 6,642,051; 7,078,387; 7,314,912; 6,498,244; 7,906,111; US patent publications US 2003-0138772, US 2007/0036760, and US 2009/0197338; Gao, et al., J. Virol, 78(12):6381-6388 (June 2004); Gao, et al, Proc Natl Acad Sci USA, 100(10):6081-6086 (May 13, 2003); and International Patent applications WO 2010/138263 and WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994), each herein incorporated by reference for all purposes). Exemplary methods for constructing recombinant AAV vectors are described in more detail in U.S. Pat. No, 5,173,414; Tratschin et ah, Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, et ah, Mol. Cell, Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:64666470 (1984); and Samuiski et ah, J. Virol. 63:03822-3828 (1989), each herein incorporated by reference for all purposes. In general, an AAV-based vector comprises a capsid protein having an amino acid sequence corresponding to any one of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV.Rh10, AAV11 and variants thereof.

[00562] 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.

[00563] 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

[00564] Engineered nucleic acids of the present disclosure (e.g., any of the engineered nucleic acids described herein) can be introduced into a cell using a lipid-mediated delivery system. In general, a lipid-mediated delivery system uses a structure composed of an outer lipid membrane enveloping an internal compartment. Examples of lipid-based structures include, but are not limited to, a lipid-based nanoparticle, a liposome, a micelle, an exosome, a vesicle, an extracellular vesicle, a cell, or a tissue. Lipid structure delivery systems can deliver a cargo/payload (e.g., any of the engineered nucleic acids described herein) *in vitro*, *in vivo*, or *ex vivo*.

[00565] A lipid-based nanoparticle can include, but is not limited to, a unilamellar liposome, a multilamellar liposome, and a lipid preparation. As used herein, a “liposome” is a generic term encompassing in vitro preparations of lipid vehicles formed by enclosing a

desired cargo, e.g., an engineered nucleic acid, such as any of the engineered nucleic acids described herein, within a lipid shell or a lipid aggregate. Liposomes may be characterized as having vesicular structures with a bilayer membrane, generally comprising a phospholipid, and an inner medium that generally comprises an aqueous composition. Liposomes include, but are not limited to, emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. Liposomes can be unilamellar liposomes. Liposomes can be multilamellar liposomes. Liposomes can be multivesicular liposomes. Liposomes can be positively charged, negatively charged, or neutrally charged. In certain embodiments, the liposomes are neutral in charge. Liposomes can be formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of a desired purpose, e.g., criteria for in vivo delivery, such as liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka 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.

[00566] A multilamellar liposome is generated spontaneously when lipids comprising phospholipids are suspended in an excess of aqueous solution such that multiple lipid layers are separated by an aqueous medium. Water and dissolved solutes are entrapped in closed structures between the lipid bilayers following the lipid components undergoing self-rearrangement. A desired cargo (e.g., a polypeptide, a nucleic acid, a small molecule drug, an engineered nucleic acid, such as any of the engineered nucleic acids described herein, a viral vector, a viral-based delivery system, etc.) can be encapsulated in the aqueous interior of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the polypeptide/nucleic acid, interspersed within the lipid bilayer of a liposome, entrapped in a liposome, complexed with a liposome, or otherwise associated with the liposome such that it can be delivered to a target entity. Lipophilic molecules or molecules with lipophilic regions may also dissolve in or associate with the lipid bilayer.

[00567] A liposome used according to the present embodiments can be made by different methods, as would be known to one of ordinary skill in the art. Preparations of liposomes are described in further detail in WO 2016/201323, International Applications PCT/US85/01161 and PCT/US89/05040, and U.S. Patents 4,728,578, 4,728,575, 4,737,323, 4,533,254, 4,162,282, 4,310,505, and 4,921,706; each herein incorporated by reference for all purposes.

[00568] Liposomes can be cationic liposomes. Examples of cationic liposomes are described in more detail in U.S. Patent No. 5,962,016; 5,030,453; 6,680,068, U.S. Application 2004/0208921, and International Patent Applications WO03/015757A1, WO04029213A2, and WO02/100435A1, each hereby incorporated by reference in their entirety.

[00569] Lipid-mediated gene delivery methods are described, for instance, in WO 96/18372; WO 93/24640; Mannino & Gould-Fogerite, BioTechniques 6(7): 682-691 (1988); U.S. Pat. No. 5,279,833 Rose U.S. Pat. No. 5,279,833; WO91/06309; and Felgner et al., Proc. Natl. Acad. Sci. USA 84: 7413-7414 (1987), each herein incorporated by reference for all purposes.

[00570] Exosomes are small membrane vesicles of endocytic origin that are released into the extracellular environment following fusion of multivesicular bodies with the plasma membrane. The size of exosomes ranges between 30 and 100 nm in diameter. Their surface consists of a lipid bilayer from the donor cell's cell membrane, and they contain cytosol from the cell that produced the exosome, and exhibit membrane proteins from the parental cell on the surface. Exosomes useful for the delivery of nucleic acids are known to those skilled in the art, e.g., the exosomes described in more detail in U.S. Pat. No. 9,889,210, herein incorporated by reference for all purposes.

[00571] As used herein, the term “extracellular vesicle” or “EV” refers to a cell-derived vesicle comprising a membrane that encloses an internal space. In general, extracellular vesicles comprise all membrane-bound vesicles that have a smaller diameter than the cell from which they are derived. Generally extracellular vesicles range in diameter from 20 nm to 1000 nm, and can comprise various macromolecular cargo either within the internal space, displayed on the external surface of the extracellular vesicle, and/or spanning the membrane. The cargo can comprise nucleic acids (e.g., any of the engineered nucleic acids described herein), proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. By way of example and without limitation, extracellular vesicles include apoptotic bodies, fragments of cells, vesicles derived from cells by direct or indirect manipulation (e.g., by serial extrusion or treatment with alkaline solutions), vesiculated organelles, and vesicles produced by living cells (e.g., by direct plasma membrane budding or fusion of the late endosome with the plasma membrane). Extracellular vesicles can be derived from a living or dead organism, explanted tissues or organs, and/or cultured cells.

[00572] As used herein the term “exosome” refers to a cell-derived small (between 20-300 nm in diameter, more preferably 40-200 nm in diameter) vesicle comprising a membrane that

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

[00573] As used herein, the term "nanovesicle" (also referred to as a "microvesicle") refers to a cell-derived small (between 20-250 nm in diameter, more preferably 30-150 nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from the cell by direct or indirect manipulation such that said nanovesicle would not be produced by said producer cell without said manipulation. In general, a nanovesicle is a sub-species of an extracellular vesicle. Appropriate manipulations of the producer cell include but are not limited to serial extrusion, treatment with alkaline solutions, sonication, or combinations thereof. The production of nanovesicles may, in some instances, result in the destruction of said producer cell. Preferably, populations of nanovesicles are substantially free of vesicles that are derived from producer cells by way of direct budding from the plasma membrane or fusion of the late endosome with the plasma membrane. The nanovesicle comprises lipid or fatty acid and polypeptide, and optionally comprises a payload (e.g., a therapeutic agent), a receiver (e.g., a targeting moiety), a polynucleotide (e.g., a nucleic acid, RNA, or DNA, such as any of the engineered nucleic acids described herein), a sugar (e.g., a simple sugar, polysaccharide, or glycan) or other molecules. The nanovesicle, once it is derived from a producer cell according to said manipulation, may be isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof.

[00574] 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.

[00575] 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.

[00576] Nucleic-acid vectors, such as expression vectors, exposed directly to serum can have several undesirable consequences, including degradation of the nucleic acid by serum nucleases or off-target stimulation of the immune system by the free nucleic acids. Similarly, viral delivery systems exposed directly to serum can trigger an undesired immune response and/or neutralization of the viral delivery system. Therefore, encapsulation of an engineered nucleic acid and/or viral delivery system can be used to avoid degradation, while also avoiding potential off-target affects. In certain examples, an engineered nucleic acid and/or viral delivery system is fully encapsulated within the delivery vehicle, such as within the aqueous interior of an LNP. Encapsulation of an engineered nucleic acid and/or viral delivery system within an LNP can be carried out by techniques well-known to those skilled in the art, such as microfluidic mixing and droplet generation carried out on a microfluidic droplet generating device. Such devices include, but are not limited to, standard T-junction devices or flow-focusing devices. In an example, the desired lipid formulation, such as MC3 or MC3-like containing compositions, is provided to the droplet generating device in parallel with an engineered nucleic acid or viral delivery system and any other desired agents, such that the delivery vector and desired agents are fully encapsulated within the interior of the MC3 or

MC3-like based LNP. In an example, the droplet generating device can control the size range and size distribution of the LNPs produced. For example, the LNP can have a size ranging from 1 to 1000 nanometers in diameter, e.g., 1, 10, 50, 100, 500, or 1000 nanometers. Following droplet generation, the delivery vehicles encapsulating the cargo/payload (e.g., an engineered nucleic acid and/or viral delivery system) can be further treated or engineered to prepare them for administration.

Nanoparticle Delivery

[00577] Nanomaterials can be used to deliver engineered nucleic acids (e.g., any of the engineered nucleic acids described herein). Nanomaterial vehicles, importantly, can be made of non-immunogenic materials and generally avoid eliciting immunity to the delivery vector itself. These materials can include, but are not limited to, lipids (as previously described), inorganic nanomaterials, and other polymeric materials. Nanomaterial particles are described in more detail in Riley et al. (Recent Advances in Nanomaterials for Gene Delivery—A Review. Nanomaterials 2017, 7(5), 94), herein incorporated by reference for all purposes.

Genomic Editing Systems

[00578] A genomic editing systems can be used to engineer a host genome to encode an engineered nucleic acid, such as an engineered nucleic acid of the present disclosure. In general, a "genomic editing system" refers to any system for integrating an exogenous gene into a host cell's genome. Genomic editing systems include, but are not limited to, a transposon system, a nuclease genomic editing system, and a viral vector-based delivery platform.

[00579] A transposon system can be used to integrate an engineered nucleic acid, such as an engineered nucleic acid of the present disclosure, 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.

[00580] 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 of the present disclosure. 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 effector molecules).

[00581] 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.

[00582] The identical, or substantially identical, sequences found at the 5' and 3' ends of the HR template, with respect to the exogenous sequence to be introduced, are generally referred to as arms (HR arms). HR arms can be identical to regions of the endogenous genomic target locus (i.e., 100% identical). HR arms in some examples can be substantially identical to regions of the endogenous genomic target locus. While substantially identical HR arms can be used, it can be advantageous for HR arms to be identical as the efficiency of the HDR pathway may be impacted by HR arms having less than 100% identity.

[00583] Each HR arm, i.e., the 5' and 3' HR arms, can be the same size or different sizes. Each HR arm can each be greater than or equal to 50, 100, 200, 300, 400, or 500 bases in length. Although HR arms can, in general, be of any length, practical considerations, such as the impact of HR arm length and overall template size on overall editing efficiency, can also be taken into account. An HR arms can be identical, or substantially identical to, regions of an endogenous genomic target locus immediately adjacent to a cleavage site. Each HR arms can be identical to, or substantially identical to, regions of an endogenous genomic target locus immediately adjacent to a cleavage site. Each HR arms can be identical, or substantially identical to, regions of an endogenous genomic target locus within a certain distance of a cleavage site, such as 1 base-pair, less than or equal to 10 base-pairs, less than or equal to 50 base-pairs, or less than or equal to 100 base-pairs of each other.

[00584] A nuclease genomic editing system can use a variety of nucleases to cut a target genomic locus, including, but not limited to, a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease or derivative thereof, a Transcription activator-like effector nuclease (TALEN) or derivative thereof, a zinc-finger nuclease (ZFN) or derivative thereof, and a homing endonuclease (HE) or derivative thereof.

[00585] A CRISPR-mediated gene editing system can be used to engineer a host genome to encode an engineered nucleic acid, such as an engineered nucleic acid encoding one or more of the effector molecules described herein. CRISPR systems are described in more detail in M. Adli ("The CRISPR tool kit for genome editing and beyond" Nature Communications; volume 9 (2018), Article number: 1911), herein incorporated by reference for all that it teaches. In general, a CRISPR-mediated gene editing system comprises a CRISPR-associated (Cas) nuclease and a RNA(s) that directs cleavage to a particular target sequence. An exemplary CRISPR-mediated gene editing system is the CRISPR/Cas9 systems comprised of a Cas9 nuclease and a RNA(s) that has a CRISPR RNA (crRNA) domain and a trans-activating CRISPR (tracrRNA) domain. The crRNA typically has two RNA domains: a guide RNA sequence (gRNA) that directs specificity through base-pair hybridization to a target sequence ("a defined nucleotide sequence"), e.g., a genomic sequence; and an RNA domain that hybridizes to a tracrRNA. A tracrRNA can interact with and thereby promote recruitment of a nuclease (e.g., Cas9) to a genomic locus. The crRNA and tracrRNA polynucleotides can be separate polynucleotides. The crRNA and tracrRNA polynucleotides can be a single polynucleotide, also referred to as a single guide RNA (sgRNA). While the Cas9 system is illustrated here, other CRISPR systems can be used, such as the Cpf1 system. Nucleases can include derivatives thereof, such as Cas9 functional mutants, e.g., a Cas9

"nickase" mutant that in general mediates cleavage of only a single strand of a defined nucleotide sequence as opposed to a complete double-stranded break typically produced by Cas9 enzymes.

[00586] In general, the components of a CRISPR system interact with each other to form a Ribonucleoprotein (RNP) complex to mediate sequence specific cleavage. In some CRISPR systems, each component can be separately produced and used to form the RNP complex. In some CRISPR systems, each component can be separately produced in vitro and contacted (i.e., "complexed") with each other in vitro to form the RNP complex. The in vitro produced RNP can then be introduced (i.e., "delivered") into a cell's cytosol and/or nucleus, e.g., a T cell's cytosol and/or nucleus. The in vitro produced RNP complexes can be delivered to a cell by a variety of means including, but not limited to, electroporation, lipid-mediated transfection, cell membrane deformation by physical means, lipid nanoparticles (LNP), virus like particles (VLP), and sonication. In a particular example, in vitro produced RNP complexes can be delivered to a cell using a Nucleofactor/Nucleofection® electroporation-based delivery system (Lonza®). Other electroporation systems include, but are not limited to, MaxCyte electroporation systems, Miltenyi CliniMACS electroporation systems, Neon electroporation systems, and BTX electroporation systems. CRISPR nucleases, e.g., Cas9, can be produced in vitro (i.e., synthesized and purified) using a variety of protein production techniques known to those skilled in the art. CRISPR system RNAs, e.g., an sgRNA, can be produced in vitro (i.e., synthesized and purified) using a variety of RNA production techniques known to those skilled in the art, such as in vitro transcription or chemical synthesis.

[00587] 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.

[00588] 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.

[00589] 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.

[00590] 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.

[00591] 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.

[00592] 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 Fokl. By changing the amino acids present in the highly variable residue region of the monomers of the DNA binding domain, different artificial TALENs can be created to target various nucleotides sequences. The DNA binding domain subsequently directs the nuclease to the target sequences and creates a double-stranded break. TALEN-based systems are described in more detail in U.S. Ser. No. 12/965,590; U.S. Pat. No. 8,450,471; U.S. Pat. No. 8,440,431; U.S. Pat. No. 8,440,432; U.S. Pat. No. 10,172,880; and U.S. Ser. No. 13/738,381, all of which are incorporated by reference herein in their entirety. ZFN-based editing systems are described in more detail in U.S. Patent Nos. 6,453,242; 6,534,261; 6,599,692; 6,503,717; 6,689,558; 7,030,215; 6,794,136; 7,067,317; 7,262,054; 7,070,934; 7,361,635; 7,253,273;

and U.S. Patent Publication Nos. 2005/0064474; 2007/0218528; 2005/0267061, all incorporated herein by reference in their entireties for all purposes.

Other Engineering Delivery Systems

[00593] 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.

[00594] Electroporation can used to deliver polynucleotides to recipient entities. Electroporation is a method of internalizing a cargo/payload into a target cell or entity's interior compartment through applying an electrical field to transiently permeabilize the outer membrane or shell of the target cell or entity. In general, the method involves placing cells or target entities between two electrodes in a solution containing a cargo of interest (e.g., any of the engineered nucleic acids described herein). The lipid membrane of the cells is then disrupted, i.e., permeabilized, by applying a transient set voltage that allows the cargo to enter the interior of the entity, such as the cytoplasm of the cell. In the example of cells, at least some, if not a majority, of the cells remain viable. Cells and other entities can be electroporated in vitro, in vivo, or ex vivo. Electroporation conditions (e.g., number of cells, concentration of cargo, recovery conditions, voltage, time, capacitance, pulse type, pulse length, volume, cuvette length, electroporation solution composition, etc.) vary depending on several factors including, but not limited to, the type of cell or other recipient entity, the cargo to be delivered, the efficiency of internalization desired, and the viability desired. Optimization of such criteria are within the scope of those skilled in the art. A variety devices and protocols can be used for electroporation. Examples include, but are not limited to, Neon® Transfection System, MaxCyte® Flow Electroporation™, Lonza® Nucleofector™ systems, and Bio-Rad® electroporation systems.

[00595] 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.

[00596] 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.

Methods of Use

[00597] Methods for treatment of diseases are also encompassed by this disclosure. Said methods include administering a therapeutically effective amount of an engineered nucleic acid, engineered cell, or isolated cell as described above. In some aspects, provided herein are methods of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the engineered cells, isolated cells, or compositions disclosed herein.

[00598] In some aspects, provided herein are methods of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells, isolated cells, or compositions disclosed herein.

[00599] In some aspects, provided herein are methods of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the engineered cells, isolated cells, or compositions disclosed herein.

[00600] In some aspects, provided herein are methods of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the engineered cells, isolated cells, or compositions disclosed herein.

[00601] In some aspects, provided herein are methods of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the engineered cells, isolated cells, or compositions disclosed herein.

[00602] In some embodiments, the administering comprises systemic administration. In some embodiments, the administering comprises intratumoral administration. In some embodiments, the isolated cell is derived from the subject. In some embodiments, the isolated cell is allogeneic with reference to the subject.

[00603] In some embodiments, the method further comprises administering a checkpoint inhibitor. the checkpoint inhibitor is selected from: an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-LAG-3 antibody, an anti-TIM-3 antibody, an anti-TIGIT antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-GAL9 antibody, an anti-A2AR antibody, an anti-phosphatidylserine antibody, an anti-CD27 antibody, an anti-TNFa antibody, an anti-TREM1 antibody, and an anti-TREM2 antibody. In some embodiments, the method further comprises administering an anti-CD40 antibody.

[00604] In some embodiments, the tumor is selected from: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

[00605] 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.

[00606] The methods provided herein also include delivering a preparation of engineered cells or delivery vehicles. A preparation, in some embodiments, is a substantially pure preparation, containing, for example, less than 5% (*e.g.*, less than 4%, 3%, 2%, or 1%) of cells other than engineered cells. A preparation may comprise $1x10^5$ cells/kg to $1x10^7$ cells/kg cells.

[00607] The methods provided herein also include administering a drug or pharmaceutical composition in combination with a therapeutically effective dose of any of the engineered cells, isolated cells, or compositions disclosed herein such that the ACP is induced and/or that a repressible protease is repressed. For example, tamoxifen or a metabolite thereof (*e.g.*, 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, or endoxifen) can be administered to induce the ACP. The drug or pharmaceutical can be administered prior to, concurrently with, simultaneously with, and/or subsequent to administration of any of the engineered cells, isolated cells, or compositions disclosed herein. The drug or pharmaceutical can be administered serially. The drug or pharmaceutical can be administered concurrently or simultaneously with administration of any of the engineered cells, isolated cells, or compositions disclosed herein. The drug or pharmaceutical can be administered at separate intervals than (*e.g.*, prior to or subsequent to) administration of any of the engineered cells, isolated cells, or compositions disclosed herein. The drug or pharmaceutical can be administered both concurrently/simultaneously as well as at separate intervals than any of the engineered cells, isolated cells, or compositions disclosed herein. The drug or pharmaceutical composition and the engineered cells, isolated cells, or compositions can be administered via different routes, *e.g.*, the drug or pharmaceutical composition can be administered orally and the engineered cells, isolated cells, or compositions can be administered intraperitoneally, intravenously, subcutaneously, or any other route appropriate for administration, as will be appreciated by one skilled in the art.

[00608] The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

[00609] The methods provided herein include administering a protease inhibitor. In some embodiments, the NS3 protease can be repressed by a protease inhibitor. Any suitable protease inhibitor can be used, including, but not limited to, simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir, or any combination thereof. In some embodiments, the protease inhibitor is selected from: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.

[00610] In some embodiments, the protease inhibitor is grazoprevir. In some embodiments, the protease inhibitor is a combination of grazoprevir and elbasvir (a NS5A inhibitor of the hepatitis C virus NS5A replication complex). Grazoprevir and elbasvir can be co-formulated as a pharmaceutical composition, such as in tablet form (*e.g.*, the tablet available under the tradename Zepatier®). Grazoprevir and elbasvir can be co-formulated at a 2:1 weight ratio, respectively, such as at a unit dose of 100 mg grazoprevir 50 mg elbasvir (*e.g.*, as in the tablet available under the tradename Zepatier®). The protease inhibitor can be administered at a dose capable of repressing a repressible protease domain of an ACP. The protease inhibitor can be administered at an approved dose for another indication. As an illustrative non-limiting example, Zepatier can be administered at its approved dose for treatment of HCV.

[00611] Grazoprevir, including in combination with elbasvir, can be administered orally in a dosage range of 0.001 to 1000 mg/kg of mammal (e.g., human) body weight per day in a single dose or in divided doses. One dosage range is 0.01 to 500 mg/kg body weight per day orally in a single dose or in divided doses. Another dosage range is 0.1 to 100 mg/kg body weight per day orally in single or divided doses. For oral administration, grazoprevir, including in combination with elbasvir, can be provided in the form of tablets or capsules containing 1.0 to 500 mg of the active ingredient, particularly 1, 5, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 400, 500, and 750 mg of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. Generally, a total daily dosage of grazoprevir, including in combination with elbasvir, can range from about 1 to about 2500 mg per day, although variations will necessarily occur depending on the target of therapy, the

patient and the route of administration. In one embodiment, the dosage of grazoprevir, including in combination with elbasvir, is from about 10 to about 1000 mg/day, administered in a single dose or in 2-4 divided doses. In another embodiment, the dosage of grazoprevir, including in combination with elbasvir, is from about 1 to about 500 mg/day, administered in a single dose or in 2-4 divided doses. In still another embodiment, the dosage of grazoprevir, including in combination with elbasvir, is from about 1 to about 100 mg/day, administered in a single dose or in 2-4 divided doses. In yet another embodiment, the dosage of grazoprevir, including in combination with elbasvir, is from about 1 to about 50 mg/day, administered in a single dose or in 2-4 divided doses. In another embodiment, the dosage of grazoprevir, including in combination with elbasvir, is from about 500 to about 1500 mg/day, administered in a single dose or in 2-4 divided doses. In still another embodiment, the dosage of grazoprevir, including in combination with elbasvir, is from about 500 to about 1000 mg/day, administered in a single dose or in 2-4 divided doses. In yet another embodiment, the dosage of grazoprevir, including in combination with elbasvir, is from about 100 to about 500 mg/day, administered in a single dose or in 2-4 divided doses.

In vivo Expression

[00612] 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*.

[00613] The methods provided herein also include delivering a composition *in vivo* capable of producing any of the effector molecules described herein. The methods provided herein also include delivering a composition *in vivo* capable of producing two or more of the effector molecules described herein. Compositions capable of *in vivo* production of effector molecules include, but are not limited to, any of the engineered nucleic acids described herein. Compositions capable of in vivo production of effector molecules can be a naked mRNA or a naked plasmid.

Pharmaceutical Compositions

[00614] The engineered nucleic acid or engineered cell can be formulated in pharmaceutical compositions. These compositions can comprise, in addition to one or more

of the engineered nucleic acids or engineered cells, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material can depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

[00615] Pharmaceutical compositions for oral administration can be in tablet, capsule, powder or liquid form. A tablet can include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol can be included.

[00616] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required.

[00617] Whether it is a polypeptide, nucleic acid, small molecule or other pharmaceutically useful compound according to the present disclosure that is to be given to an individual, administration is preferably in a “therapeutically effective amount” or “prophylactically effective amount”(as the case can be, although prophylaxis can be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of protein aggregation disease being treated. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

[00618] A composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Additional Embodiments

[00619] Provided below are enumerated embodiments describing specific embodiments of the invention:

Embodiment 1: An engineered nucleic acid comprising:

a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide; and

b) a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula:

(L – E)$_X$

wherein

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20,

wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and

wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

Embodiment 2: The engineered nucleic acid of embodiment 1, wherein when the second expression cassette comprises two or more units of **(L – E)$_X$**, each linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide.

Embodiment 3: The engineered nucleic acid of embodiment 1 or embodiment 2, wherein the linker polynucleotide sequence encodes a 2A ribosome skipping tag.

Embodiment 4: The engineered nucleic acid of embodiment 3, wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

Embodiment 5: The engineered nucleic acid of any one of embodiments 1-2, the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

Embodiment 6: The engineered nucleic acid of any one of embodiments 1-5, wherein the linker polynucleotide sequence encodes a cleavable polypeptide.

Embodiment 7: The engineered nucleic acid of embodiment 6, wherein the cleavable polypeptide comprises a furin polypeptide sequence.

Embodiment 8: The engineered nucleic acid of any one of embodiments 1-7, wherein the second expression cassette comprising one or more units of **(L – E)**x further comprises a polynucleotide sequence encoding a secretion signal peptide for each X.

Embodiment 9: The engineered nucleic acid of embodiment 8, wherein for each X the corresponding secretion signal peptide is operably associated with the effector molecule.

Embodiment 10: The engineered nucleic acid of embodiment 8 or embodiment 9, wherein each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule.

Embodiment 11: The engineered nucleic acid of any one of embodiments 8-10, wherein each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule.

Embodiment 12: The engineered nucleic acid of embodiment 11, wherein the non-native secretion signal peptide is a secretion signal peptide of a molecule selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

Embodiment 13: The engineered nucleic acid of any one of embodiments 1-12, wherein the ACP-responsive promoter comprises an ACP-binding domain sequence and a promoter sequence.

Embodiment 14: The engineered nucleic acid of embodiment 13, wherein the promoter sequence is derived from a promoter selected from the group consisting of: 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.

Embodiment 15: The engineered nucleic acid of any one of embodiments 1-14, wherein the ACP-responsive promoter comprises a synthetic promoter.

Embodiment 16: The engineered nucleic acid of any one of embodiments 1-15, wherein the ACP-responsive promoter comprises a minimal promoter.

Embodiment 17: The engineered nucleic acid of any one of embodiments 12-16, wherein the ACP-binding domain comprises one or more zinc finger binding sites.

Embodiment 18: The engineered nucleic acid of any one of embodiments 1-17, wherein the first promoter comprises a constitutive promoter, an inducible promoter, or a synthetic promoter.

Embodiment 19: The engineered nucleic acid of embodiment 18, wherein the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

Embodiment 20: The engineered nucleic acid of any one of embodiments 1-19, wherein each effector molecule is independently 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, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

Embodiment 21: The engineered nucleic acid of embodiment 20, wherein the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

Embodiment 22: The engineered nucleic acid of embodiment 20, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

Embodiment 23: The engineered nucleic acid of embodiment 20, wherein the homing molecule is selected from the group consisting of: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; CCR4; and GPR15.

Embodiment 24: The engineered nucleic acid of embodiment 20, wherein the growth factor is selected from the group consisting of: FLT3L and GM-CSF.

Embodiment 25: The engineered nucleic acid of embodiment 20, wherein the co-activation molecule is selected from the group consisting of: c-Jun, 4-1BBL and CD40L.

Embodiment 26: The engineered nucleic acid of embodiment 20, wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

Embodiment 27: The engineered nucleic acid of embodiment 26, wherein the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

Embodiment 28: The engineered nucleic acid of embodiment 26, wherein the immune checkpoint inhibitors are selected from the group consisting of: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

Embodiment 29: The engineered nucleic acid of embodiment 26, wherein the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

Embodiment 30: The engineered nucleic acid of any one of embodiments 1-29, wherein each effector molecule is a human-derived effector molecule.

Embodiment 31: The engineered nucleic acid of any one of embodiments 1-30, wherein the first exogenous polynucleotide sequence further encodes an antigen recognizing receptor.

Embodiment 32: An engineered nucleic acid comprising:

a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and an antigen recognizing receptor,

wherein the first promoter is operably linked to the first exogenous polynucleotide; and

b) a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula:

$(L – E)_X$

wherein

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20,

wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and

wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

Embodiment 33: An engineered nucleic acid comprising:

a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an antigen recognizing receptor,

wherein the first promoter is operably linked to the first exogenous polynucleotide; and

b) a second expression cassette comprising an activation-conditional control polypeptide-responsive (ACP-responsive) promoter and a second exogenous polynucleotide sequence having the formula:

$(L – E)_X$

wherein

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20,

wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent.

Embodiment 34: The engineered nucleic acid of embodiment 33, wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

Embodiment 35: The engineered nucleic acid of embodiment 33, wherein the ACP is the antigen recognizing receptor and the ACP is capable of inducing expression of the second expression cassette following binding of the ACP to a cognate antigen.

Embodiment 36: The engineered nucleic acid of embodiment 35, wherein the ACP-responsive promoter is an inducible promoter that is capable of being induced by the ACP binding to the cognate antigen.

Embodiment 37: The engineered nucleic acid of embodiment 36, wherein the ACP-responsive promoter is derived from a promoter region of a gene upregulated following binding of the ACP to the cognate antigen.

Embodiment 38: The engineered nucleic acid of any one of embodiments 32-34, wherein the ACP-responsive promoter is selected from the group consisting of a constitutive promoter, an inducible promoter, and a synthetic promoter.

Embodiment 39: The engineered nucleic acid of any one of embodiments 32-38, wherein the ACP-responsive promoter comprises a minimal promoter.

Embodiment 40: The engineered nucleic acid of any one of embodiments 32-39, wherein the ACP-binding domain comprises one or more zinc finger binding sites.

Embodiment 41: The engineered nucleic acid of any one of embodiments 1-30, further comprising a linker polynucleotide sequence localized between the first expression cassette and the second expression cassette.

Embodiment 42: The engineered nucleic acid of embodiment 41, wherein the linker polynucleotide sequence is operably associated with the translation of the ACP and each effector molecule as separate polypeptides.

Embodiment 43: The engineered nucleic acid of embodiment 31 or embodiment 32, wherein the first exogenous polynucleotide sequence further comprises a linker polynucleotide sequence localized between the region of the first exogenous polynucleotide sequence encoding the ACP and the region of the first exogenous polynucleotide sequence encoding the antigen recognizing receptor.

Embodiment 44: The engineered nucleic acid of embodiment 43, wherein the linker polynucleotide sequence is operably associated with the translation of the ACP and the antigen recognizing receptor as separate polypeptides.

Embodiment 45: The engineered nucleic acid of any one of embodiments 33-36, further comprising a linker polynucleotide sequence localized between the first expression cassette and the second expression cassette.

Embodiment 46: The engineered nucleic acid of embodiment 45, wherein the linker polynucleotide sequence is operably associated with the translation of the antigen receptor and each effector molecule as separate polypeptides.

Embodiment 47: The engineered nucleic acid of any one of embodiments 41-46, wherein the linker polynucleotide sequence encodes a 2A ribosome skipping tag.

Embodiment 48: The engineered nucleic acid of embodiment 47, wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

Embodiment 49: The engineered nucleic acid of any one of embodiments 41-46, wherein the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

Embodiment 50: The engineered nucleic acid of any one of embodiments 41-49, wherein the linker polynucleotide sequence encodes a cleavable polypeptide.

Embodiment 51: The engineered nucleic acid of embodiment 50, wherein the cleavable polypeptide comprises a furin polypeptide sequence.

Embodiment 52: The engineered nucleic acid of any one of embodiments 31-51, wherein 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.

Embodiment 53: The engineered nucleic acid of any one of embodiments 31-52, wherein the antigen recognizing receptor recognizes GPC3.

Embodiment 54: The engineered nucleic acid of any one of embodiments 31-52, wherein the antigen recognizing receptor recognizes mesothelin (MSLN).

Embodiment 55: The engineered nucleic acid of any one of embodiments 31-54, wherein the antigen recognizing receptor comprises an antigen-binding domain.

Embodiment 56: The engineered nucleic acid of embodiment 53 or embodiment 55, wherein the antigen-binding domain that binds to GPC3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region,

wherein the VH comprises:

a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (**SEQ ID NO: 119**),

a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (**SEQ ID NO: 120**), and

a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (**SEQ ID NO: 121**), and

wherein the VL comprises:

a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (**SEQ ID NO: 122**),

a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (**SEQ ID NO: 123**), and

a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (**SEQ ID NO: 124**).

Embodiment 57: The engineered nucleic acid of embodiment 56, wherein the VH region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of

EVQLVETGGGMVQPEGSLKLSCAASGFTFNKNAMNWVRQAPGKGLEWVARIRNKT
NNYATYYADSVKARFTISRDDSQSMLYLQMNNLKIEDTAMYYCVAGNSFA

YWGQGTLVTVSA (**SEQ ID NO: 125**) or
EVQLVESGGGLVQPGGSLRLSCAASGFTFNKNAMNWVRQAPGKGLEWVGRIRNKT
NNYATYYADSVKARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQG
TLVTVSA (**SEQ ID NO: 126**).

Embodiment 58: The engineered nucleic acid of embodiment 56 or embodiment 57, wherein the VL region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of
DIVMSQSPSSLVVSIGEKVTMTCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWA
SSRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYNYPLTFGAGTKLELK
(**SEQ ID NO: 127**), or
DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKLLIYWA
SSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFGQGTKLEIK
(**SEQ ID NO: 128**).

Embodiment 59: The engineered nucleic acid of embodiment 54 or embodiment 55, wherein the antigen-binding domain that binds to MSLN comprises the three complementarity determining regions (CDRs) of a single-domain monoclonal antibody having the amino acid sequence of:
QVQLVESGGGTVQAGGSLKLACAASGLPRTYNVMGWFRQAPGKEREGVAIIYTTTG
ATYYRDSVKGRATISQDNAKKSVSLQMNSLRPEDTAIYYCVARQPNSGPWEYWGQ
GTQVTVSS (**SEQ ID NO: 129**), or
QVKLEESGGGSVQAGGSLRLSCTTSGYTNSYKWMGWFRQAPGQEREGVAVIYTGN
DRTYYSDSVKGRFTISRDNAKNMIYLDMTRLRPEDSAVYECAIGHDGAWRYWGQG
TQVTVSS (**SEQ ID NO: 130**).

Embodiment 60: The engineered nucleic acid of any one of embodiments 55-59, 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).

Embodiment 61: The engineered nucleic acid of any one of embodiments 55-59, wherein the antigen-binding domain comprises a single chain variable fragment (scFv).

Embodiment 62: The engineered nucleic acid of embodiment 61, wherein the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

Embodiment 63: The engineered nucleic acid of embodiment 62, wherein the VH and VL are separated by a peptide linker.

Embodiment 64: The engineered nucleic acid of embodiment 63, 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.

Embodiment 65: The engineered nucleic acid of any one of embodiments 31-64, wherein the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR).

Embodiment 66: The engineered nucleic acid of any one of embodiments 31-65, wherein the antigen recognizing receptor is a CAR.

Embodiment 67: The engineered nucleic acid of embodiment 66, wherein the CAR comprises one or more intracellular signaling domains, and each of the one or more intracellular signaling domains is 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, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, a CD16a intracellular signaling domain, a DNAM-1 intracellular signaling domain, a KIR2DS1 intracellular signaling domain, a KIR3DS1 intracellular signaling domain, a NKp44 intracellular signaling domain, a NKp46 intracellular signaling domain, a FceRlg intracellular signaling domain, a NKG2D intracellular signaling domain, and an EAT-2 intracellular signaling domain.

Embodiment 68: The engineered nucleic acid of embodiment 66 or embodiment 67, 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, a BTLA transmembrane domain, an OX40 transmembrane domain, a DAP10 transmembrane domain, a DAP12 transmembrane domain, a CD16a transmembrane domain, a DNAM-1 transmembrane domain, a KIR2DS1 transmembrane domain, a KIR3DS1 transmembrane domain, an NKp44

transmembrane domain, an NKp46 transmembrane domain, an FceRlg transmembrane domain, and an NKG2D transmembrane domain.

Embodiment 69: The engineered nucleic acid of any one of embodiments 66-68, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

Embodiment 70: The engineered nucleic acid of any one of embodiments 1-69, wherein the ACP is a transcriptional modulator.

Embodiment 71: The engineered nucleic acid of any one of embodiments 1-70, wherein the ACP is a transcriptional repressor.

Embodiment 72: The engineered nucleic acid of any one of embodiments 1-70, wherein the ACP is a transcriptional activator.

Embodiment 73: The engineered nucleic acid of any one of embodiments 1-72, wherein the ACP further comprises a repressible protease and one or more cognate cleavage sites of the repressible protease.

Embodiment 74: The engineered nucleic acid of any one of embodiments 1-73, wherein the ACP further comprises a hormone-binding domain of estrogen receptor (ERT2 domain).

Embodiment 75: The engineered nucleic acid of any one of embodiments 72-74, wherein the ACP is a transcription factor.

Embodiment 76: The engineered nucleic acid of embodiment 74, wherein the transcription factor is a zinc-finger-containing transcription factor.

Embodiment 77: The engineered nucleic acid of any one of embodiments 1-76, wherein the ACP comprises a DNA-binding zinc finger protein domain (ZF protein domain) and a transcriptional effector domain.

Embodiment 78: The engineered nucleic acid of embodiment 77, wherein the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA).

Embodiment 79: The engineered nucleic acid of embodiment 78, wherein the ZF protein domain comprises one to ten ZFA.

Embodiment 80: The engineered nucleic acid of any one of embodiments 77-79, wherein 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 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 truncated Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif (SEQ ID NO: 162) of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain ("WRPW" disclosed as SEQ ID NO: 162); a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

Embodiment 81: The engineered nucleic acid of any one of embodiments 77-80, wherein the one or more cognate cleavage sites of the repressible protease are localized between the ZF protein domain and the effector domain.

Embodiment 82: The engineered nucleic acid of any one of embodiments 73-81, wherein the repressible protease is hepatitis C virus (HCV) nonstructural protein 3 (NS3).

Embodiment 83: The engineered nucleic acid of embodiment 82, wherein the cognate cleavage site comprises an NS3 protease cleavage site.

Embodiment 84: The engineered nucleic acid of embodiment 83, wherein the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.

Embodiment 85: The engineered nucleic acid of any one of embodiments 82-84, wherein the NS3 protease can be repressed by a protease inhibitor.

Embodiment 86: The engineered nucleic acid of embodiment 85, wherein the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.

Embodiment 87: The engineered nucleic acid of embodiment 85, wherein the protease inhibitor is grazoprevir.

Embodiment 88: The engineered nucleic acid of embodiment 85, wherein the protease inhibitor comprises grazoprevir and elbasvir.

Embodiment 89: The engineered nucleic acid of embodiment 88, wherein the grazoprevir and the elbasvir is co-formulated in a pharmaceutical composition.

Embodiment 90: The engineered nucleic acid of embodiment 89, wherein the pharmaceutical composition is a tablet.

Embodiment 91: The engineered nucleic acid of embodiment 89 or 90, wherein the grazoprevir and the elbasvir are at a 2 to 1 weight ratio.

Embodiment 92: The engineered nucleic acid of embodiment 91, wherein the grazoprevir is 100 mg per unit dose and the elbasvir is 50 mg per unit dose.

Embodiment 93: The engineered nucleic acid of any one of embodiments 74-92, wherein the ACP is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof.

Embodiment 94: The engineered nucleic acid of embodiment 93, wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

Embodiment 95: The engineered nucleic acid of any one of embodiments 1-92, wherein the ACP further comprises a degron, and wherein the degron is operably linked to the ACP.

Embodiment 96: The engineered nucleic acid of embodiment 95, wherein the degron is selected from the group consisting of HCV NS4 degron, PEST (two copies of residues 277-307 of human IκBα), 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 (SEQ ID NO: 163), an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box.

Embodiment 97: The engineered nucleic acid of embodiment 93, wherein the degron 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.

Embodiment 98: The engineered nucleic acid of embodiment 97, wherein the CRBN polypeptide substrate domain is selected from the group consisting of: IKZF1, IKZF3, CKla, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN.

Embodiment 99: The engineered nucleic acid of embodiment 97, wherein the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences.

Embodiment 100: The engineered nucleic acid of embodiment 97, wherein the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRDAL (**SEQ ID NO: 131**).

Embodiment 101: The engineered nucleic acid of any one of embodiments 97-100, wherein the IMiD is an FDA-approved drug.

Embodiment 102: The engineered nucleic acid of any one of embodiments 97-101, wherein the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

Embodiment 103: The engineered nucleic acid of any one of embodiments 93-102, wherein the degron is localized 5' of the repressible protease, 3' of the repressible protease, 5' of the ZF protein domain, 3' of the ZF protein domain, 5' of the effector domain, or 3' of the effector domain.

Embodiment 104: The engineered nucleic acid of any one of embodiments 1-103, wherein the engineered nucleic acid further comprises an insulator.

Embodiment 105: The engineered nucleic acid of embodiment 104, wherein the insulator is localized between the first expression cassette and the second expression cassette.

Embodiment 106: The engineered nucleic acid of any one of embodiments 1-105, wherein the first expression cassette is localized in the same orientation relative to the second expression cassette.

Embodiment 107: The engineered nucleic acid of any one of embodiments 1-106, wherein the first expression cassette is localized in the opposite orientation relative to the second expression cassette.

Embodiment 108: The engineered nucleic acid of any one of embodiments 1-107, wherein the engineered nucleic acid is selected from the group consisting of: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid.

Embodiment 109: An expression vector comprising the engineered nucleic acid of any one of embodiments 1-108.

Embodiment 110: A composition comprising the engineered nucleic acid of any one of embodiments 1-108, and a pharmaceutically acceptable carrier.

Embodiment 111: An isolated cell comprising the engineered nucleic acid of any one of embodiments 1-108 or the vector of embodiment 109.

Embodiment 112: The isolated cell of embodiment 111, wherein the engineered nucleic acid is recombinantly expressed.

Embodiment 113: The isolated cell of embodiment 111 or embodiment 112, wherein the engineered nucleic acid is expressed from a vector or a selected locus from the genome of the cell.

Embodiment 114: The isolated cell of any one of embodiments 111-113, 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.

Embodiment 115: The isolated cell of any one of embodiments 111-114, wherein the cell is a Natural Killer (NK) cell.

Embodiment 116: The isolated cell of any one of embodiments 111-115, wherein the cell is autologous.

Embodiment 117: The isolated cell of any one of embodiments 111-115, wherein the cell is allogeneic.

Embodiment 118: The isolated cell of any one of embodiments 111-113, wherein the cell is a tumor cell selected from the group consisting of: an adenocarcinoma cell, 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, a mesothelioma cell, an ovarian tumor cell, a pancreatic tumor cell, a gastric tumor cell, a testicular yolk sac tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

Embodiment 119: The isolated cell of embodiment 118, wherein the cell was engineered via transduction with an oncolytic virus.

Embodiment 120: The isolated cell of embodiment 119, wherein the oncolytic virus is selected from the group consisting of: 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.

Embodiment 121: The isolated cell of embodiment 119 or embodiment 120 wherein the oncolytic virus is a recombinant oncolytic virus comprising the first expression cassette and the second expression cassette.

Embodiment 122: The isolated cell of any one of embodiments 111-113, wherein the cell is a bacterial cell selected from the group consisting of: *Clostridium beijerinckii*, *Clostridium sporogenes*, *Clostridium novyi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Salmonella choleraesuis*.

Embodiment 123: A composition comprising the isolated cell of any one of embodiments 111-122, and a pharmaceutically acceptable carrier.

Embodiment 124: 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 111-122 or the composition of embodiment 123.

Embodiment 125: A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells of any one of embodiments 111-122 or the composition of embodiment 123.

Embodiment 126: A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the isolated cells of any one of embodiments 111-122 or the composition of embodiment 123.

Embodiment 127: A method of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the isolated cells of any one of embodiments 111-122 or the composition of embodiment 123.

Embodiment 128: A method of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the isolated cells of any one of embodiments 111-122 or the composition of embodiment 123.

Embodiment 129: The method of any one of embodiments 124-128, wherein the administering comprises systemic administration.

Embodiment 130: The method of any one of embodiments 124-128, wherein the administering comprises intratumoral administration.

Embodiment 131: The method of any one of embodiments 124-130, wherein the isolated cell is derived from the subject.

Embodiment 132: The method of any one of embodiments 124-130, wherein the isolated cell is allogeneic with reference to the subject.

Embodiment 133: The method of any one of embodiments 124-132, wherein the method further comprises administering a checkpoint inhibitor.

Embodiment 134: The method of embodiment 133, 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-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.

Embodiment 135: The method of any one of embodiments 124-134, wherein the method further comprises administering an anti-CD40 antibody.

Embodiment 136: The method of any one of embodiments 125-135, wherein the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

Embodiment 137: A lipid-based structure comprising the engineered nucleic acid of any one of embodiments 1-108.

Embodiment 138: The lipid-based structure of embodiment 137, wherein the lipid-based structure comprises a extracellular vesicle.

Embodiment 139: The lipid-based structure of embodiment 138, wherein the extracellular vesicle is selected from the group consisting of: a nanovesicle and an exosome.

Embodiment 140: The lipid-based structure of embodiment 137, wherein the lipid-based structure comprises a lipid nanoparticle or a micelle.

Embodiment 141: The lipid-based structure of embodiment 137, wherein the lipid-based structure comprises a liposome.

Embodiment 142: A composition comprising the lipid-based structure of any one of embodiments 137-141, and a pharmaceutically acceptable carrier.

Embodiment 143: 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 any one of embodiments 137-141 or the composition of embodiment 142.

Embodiment 144: A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the lipid-based structures of any one of embodiments 137-141 or the composition of embodiment 142.

Embodiment 145: A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the lipid-based structures of any one of embodiments 137-141 or the composition of embodiment 142.

Embodiment 146: A method of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the lipid-based structures of any one of embodiments 137-141 or the composition of embodiment 142.

Embodiment 147: A method of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the lipid-based structures of any one of embodiments 137-141 or the composition of embodiment 142.

Embodiment 148: The method of any one of embodiments 143-147, wherein the administering comprises systemic administration.

Embodiment 149: The method of any one of embodiments 144-147, wherein the administering comprises intratumoral administration.

Embodiment 150: The method of any one of embodiments 143-149, the lipid-based structure is capable of engineering a cell in the subject.

Embodiment 151: The method of any one of embodiments 143-150, wherein the method further comprises administering a checkpoint inhibitor.

Embodiment 152: The method of embodiment 151, 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-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.

Embodiment 153: The method of any one of embodiments 143-152, wherein the method further comprises administering an anti-CD40 antibody.

Embodiment 154: The method of any one of embodiments 144-153, wherein the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

Embodiment 155: A nanoparticle comprising the engineered nucleic acid of any one of embodiments 1-108.

Embodiment 156: The nanoparticle of embodiment 155, wherein the nanoparticle comprises an inorganic material.

Embodiment 157: A composition comprising the nanoparticle of embodiment 155 or embodiment 156.

Embodiment 158: A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the nanoparticles of embodiment 155 or embodiment 156, or the composition of embodiment 157.

Embodiment 159: A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the nanoparticles of embodiment 155 or embodiment 156, or the composition of embodiment 157.

Embodiment 160: A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the nanoparticles of embodiment 155 or embodiment 156, or the composition of embodiment 157.

Embodiment 161: A method of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the nanoparticles of embodiment 155 or embodiment 156, or the composition of embodiment 157.

Embodiment 162: A method of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the nanoparticles of embodiment 155 or embodiment 156, or the composition of embodiment 157.

Embodiment 163: The method of any one of embodiments 158-162, wherein the administering comprises systemic administration.

Embodiment 164: The method of any one of embodiments 159-162, wherein the administering comprises intratumoral administration.

Embodiment 165: The method of any one of embodiments 158-164, the nanoparticle is capable of engineering a cell in the subject.

Embodiment 166: The method of any one of embodiments 158-165, wherein the method further comprises administering a checkpoint inhibitor.

Embodiment 167: The method of embodiment 166, 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-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.

Embodiment 168: The method of any one of embodiments 158-167, wherein the method further comprises administering an anti-CD40 antibody.

Embodiment 169: The method of any one of embodiments 159-168, wherein the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

Embodiment 170: A virus engineered to comprise the engineered nucleic acid of any one of embodiments 1-108.

Embodiment 171: The engineered virus of embodiment 170, 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).

Embodiment 172: The engineered virus of embodiment 170, wherein the virus is an oncolytic virus.

Embodiment 173: The engineered virus of embodiment 172, wherein the first expression cassette and the second expression cassette are capable of being expressed in a tumor cell.

Embodiment 174: The engineered virus of embodiment 173, wherein the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

Embodiment 175: The engineered virus of any one of embodiments 171-174, wherein the oncolytic virus is selected from the group consisting of: 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.

Embodiment 176: A composition comprising the engineered virus of any one of embodiments 170-175, and a pharmaceutically acceptable carrier.

Embodiment 177: A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered viruses of any one of embodiments 170-175 or the composition of embodiment 176.

Embodiment 178: A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the engineered viruses of any one of embodiments 170-175 or the composition of embodiment 176.

Embodiment 179: A method of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the engineered viruses of any one of embodiments 170-175 or the composition of embodiment 176.

Embodiment 180: A method of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the engineered viruses of any one of embodiments 170-175 or the composition of embodiment 176.

Embodiment 181: The method of any one of embodiments 177-180, wherein the administering comprises systemic administration.

Embodiment 182: The method of any one of embodiments 177-180, wherein the administering comprises intratumoral administration.

Embodiment 183: The method of any one of embodiments 177-182, the engineered virus infects a cell in the subject and expresses the first expression cassette and the second expression cassette.

Embodiment 184: The method of any one of embodiments 177-183, wherein the method further comprises administering a checkpoint inhibitor.

Embodiment 185: The method of embodiment 184, 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-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.

Embodiment 186: The method of any one of embodiments 177-185, wherein the method further comprises administering an anti-CD40 antibody.

Embodiment 187: The method of any one of embodiments 177-186 wherein the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

Embodiment 188: An engineered cell comprising:

a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide; and

b) a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula:

(L – E)x

wherein

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20,

wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and

wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

Embodiment 189: The engineered cell of embodiment 188, wherein the first expression cassette and the second expression cassette are encoded by separate polynucleotide sequences.

Embodiment 190: The engineered cell of embodiment 188, wherein the first expression cassette and the second expression cassette are encoded by a single polynucleotide sequence.

Embodiment 191: The engineered cell of any one of embodiments 188-190, wherein when the second expression cassette comprises two or more units of **$(L_1 – E)_x$**, each L_1 linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide.

Embodiment 192: The engineered cell of embodiment 190 or embodiment 191, wherein the engineered cell further comprises a second linker polynucleotide sequence, wherein the second linker polynucleotide links the first expression cassette to the second expression cassette.

Embodiment 193: The engineered cell of embodiment 192, wherein the second linker polynucleotide sequence is operably associated with the translation of each effector molecule and the ACP as separate polypeptides.

Embodiment 194: The engineered cell of any one of embodiments 188-193, wherein each linker polynucleotide sequence encodes a 2A ribosome skipping tag.

Embodiment 195: The engineered cell of embodiment 194, wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

Embodiment 196: The engineered cell of any one of embodiments 188-193, wherein each linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

Embodiment 197: The engineered cell of any one of embodiments 188-196, wherein the linker polynucleotide sequence encodes a cleavable polypeptide.

Embodiment 198: The engineered cell of embodiment 197, wherein the cleavable polypeptide comprises a furin polypeptide sequence.

Embodiment 199: The engineered cell of any one of embodiments 188-198, wherein the second expression cassette comprising one or more units of $\mathbf{(L_1 - E)_X}$ further comprises a polynucleotide sequence encoding a secretion signal peptide for each X.

Embodiment 200: The engineered cell of embodiment 199, wherein for each X the corresponding secretion signal peptide is operably associated with the effector molecule.

Embodiment 201: The engineered cell of embodiment 199 or embodiment 200, wherein each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule.

Embodiment 202: The engineered cell of any one of embodiments 199-201, wherein each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule.

Embodiment 203: The engineered cell of embodiment 202, wherein the non-native secretion signal peptide is a secretion signal peptide of a molecule selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

Embodiment 204: The engineered cell of any one of embodiments 188-203, wherein the ACP-responsive promoter comprises an ACP-binding domain and a promoter sequence.

Embodiment 205: The engineered cell embodiment 204, wherein the promoter sequence is derived from a promoter selected from the group consisting of: 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.

Embodiment 206: The engineered cell of any one of embodiments 188-205, wherein the ACP-responsive promoter is a synthetic promoter.

Embodiment 207: The engineered cell of any one of embodiments 188-206, wherein the ACP-responsive promoter comprises a minimal promoter.

Embodiment 208: The engineered cell of any one of embodiments 204-207, wherein the ACP-binding domain comprises one or more zinc finger binding sites.

Embodiment 209: The engineered cell of any one of embodiments 188-208, wherein the first promoter is a constitutive promoter, an inducible promoter, or a synthetic promoter.

Embodiment 210: The engineered cell of embodiment 209, wherein the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

Embodiment 211: The engineered cell of any one of embodiments 188-210, wherein each effector molecule is independently 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, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

Embodiment 212: The engineered cell of embodiment 211, wherein the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

Embodiment 213: The engineered cell of embodiment 212, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

Embodiment 214: The engineered cell of embodiment 211, wherein the homing molecule is selected from the group consisting of: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; and GPR15.

Embodiment 215: The engineered cell of embodiment 211, wherein the growth factor is selected from the group consisting of: FLT3L and GM-CSF.

Embodiment 216: The engineered cell of embodiment 211, wherein the co-activation molecule is selected from the group consisting of: c-Jun, 4-1BBL, and CD40L.

Embodiment 217: The engineered cell of embodiment 211, wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

Embodiment 218: The engineered cell of embodiment 217, wherein the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

Embodiment 219: The engineered cell of embodiment 217, wherein the immune checkpoint inhibitors are selected from the group consisting of: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

Embodiment 220: The engineered cell of embodiment 217, wherein the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

Embodiment 221: The engineered cell of any one of embodiments 188-217, wherein each effector molecule is a human-derived effector molecule.

Embodiment 222: The engineered cell of any one of embodiments 188-221, wherein the cell further comprises a third expression cassette comprising a third promoter and a third exogenous polynucleotide sequence encoding an antigen recognizing receptor, wherein the third promoter is operably linked to the third exogenous polynucleotide.

Embodiment 223: The engineered cell of any one of embodiments 188-221, wherein the first exogenous polynucleotide sequence further encodes an antigen recognizing receptor.

Embodiment 224: An engineered cell comprising:

a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) and an antigen recognizing receptor,

wherein the first promoter is operably linked to the first exogenous polynucleotide; and

b) a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula:

$(L - E)_X$

wherein

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20,

wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and

wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

Embodiment 225: An engineered cell comprising:

a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an antigen recognizing receptor,

wherein the first promoter is operably linked to the first exogenous polynucleotide; and

b) a second expression cassette comprising an activation-conditional control polypeptide-responsive (ACP-responsive) promoter and a second exogenous polynucleotide sequence having the formula:

(L – E)x

wherein

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20,

wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent.

Embodiment 226: The engineered cell of embodiment 225, wherein the cell further comprises a third expression cassette comprising a third promoter and a third exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the third promoter is operably linked to the third exogenous polynucleotide.

Embodiment 227: The engineered cell of embodiment 226, wherein the ACP is capable of inducing expression of the second expression cassette by binding to the ACP-responsive promoter.

Embodiment 228: The engineered cell of embodiment 225, wherein the ACP is the antigen recognizing receptor and the ACP is capable of inducing expression of the second expression cassette following binding of the ACP to a cognate antigen.

Embodiment 229: The engineered cell of embodiment 228, wherein the ACP-responsive promoter is an inducible promoter that is capable of being induced by the ACP binding to the cognate antigen.

Embodiment 230: The engineered cell of embodiment 229, wherein the ACP-responsive promoter is derived from a promoter region of a gene upregulated following binding of the ACP to the cognate antigen..

Embodiment 231: The engineered cell of any one of embodiments 224-227, wherein the ACP-responsive promoter is selected from the group consisting of a constitutive promoter, an inducible promoter, and a synthetic promoter.

Embodiment 232: The engineered cell of any one of embodiments 224-231, wherein the first expression cassette and the second expression cassette are encoded by separate polynucleotide sequences.

Embodiment 233: The engineered cell of any one of embodiments 224-232, wherein the ACP-responsive promoter comprises a minimal promoter.

Embodiment 234: The engineered cell of any one of embodiments 224-233, wherein the ACP-binding domain comprises one or more zinc finger binding sites.

Embodiment 235: The engineered cell of embodiment 224 or embodiment 225, wherein the first exogenous polynucleotide sequence further comprises a third linker polynucleotide sequence localized between the region of the first exogenous polynucleotide sequence encoding the ACP and the region of the first exogenous polynucleotide sequence encoding the antigen recognizing receptor.

Embodiment 236: The engineered cell of embodiment 235, wherein the third linker polynucleotide sequence is operably associated with the translation of the ACP and the antigen recognizing receptor as separate polypeptides.

Embodiment 237: The engineered cell of any one of embodiments 225-234, further comprising a third linker polynucleotide sequence localized between the first expression cassette and the second expression cassette.

Embodiment 238: The engineered cell of embodiment 237, wherein the third linker polynucleotide sequence is operably associated with the translation of the antigen receptor and each effector molecule as separate polypeptides.

Embodiment 239: The engineered cell of any one of embodiments 235-238, wherein the third linker polynucleotide sequence encodes a 2A ribosome skipping tag.

Embodiment 240: The engineered nucleic acid of embodiment 239, wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

Embodiment 241: The engineered cell of any one of embodiments 235-238, the third linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

Embodiment 242: The engineered nucleic acid of any one of embodiments 235-241, wherein the third linker polynucleotide sequence encodes a cleavable polypeptide.

Embodiment 243: The engineered nucleic acid of embodiment 242, wherein the cleavable polypeptide comprises a furin polypeptide sequence.

Embodiment 244: The engineered cell of any one of embodiments 222-243, wherein 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.

Embodiment 245: The engineered cell of any one of embodiments 222-244, wherein the antigen recognizing receptor recognizes GPC3.

Embodiment 246: The engineered cell of any one of embodiments 222-244, wherein the antigen recognizing receptor recognizes mesothelin.

Embodiment 247: The engineered cell of any one of embodiments 222-246, wherein the antigen recognizing receptor comprises an antigen-binding domain.

Embodiment 248: The engineered cell of embodiment 245 or embodiment 247, wherein the antigen-binding domain that binds to GPC3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region,

wherein the VH comprises:

a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (**SEQ ID NO: 119**),

a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (**SEQ ID NO: 120**), and

a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (**SEQ ID NO: 121**), and

wherein the VL comprises:

a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (**SEQ ID NO: 122**),

a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (**SEQ ID NO: 123**), and

a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (**SEQ ID NO: 124**).

Embodiment 249: The engineered cell of embodiment 248, wherein the VH region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of EVQLVETGGGMVQPEGSLKLSCAASGFTFNKNAMNWVRQAPGKGLEWVARIRNKT NNYATYYADSVKARFTISRDDSQSMLYLQMNNLKIEDTAMYYCVAGNSFA YWGQGTLVTVSA (**SEQ ID NO: 125**) or EVQLVESGGGLVQPGGSLRLSCAASGFTFNKNAMNWVRQAPGKGLEWVGRIRNKT NNYATYYADSVKARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQG TLVTVSA (**SEQ ID NO: 126**).

Embodiment 250: The engineered cell of embodiment 248 or embodiment 249, wherein the VL region comprises an amino acid sequence with at least 90 %, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the amino acid sequence of DIVMSQSPSSLVVSIGEKVTMTCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWA SSRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYNYPLTFGAGTKLELK (**SEQ ID NO: 127**), or DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKLLIYWA SSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFGQGTKLEIK (**SEQ ID NO: 128**).

Embodiment 251: The engineered cell of embodiment 246 or embodiment 247, wherein the antigen-binding domain that binds to MSLN comprises the three complementarity determining regions (CDRs) of a single-domain monoclonal antibody having the amino acid sequence of: QVQLVESGGGTVQAGGSLKLACAASGLPRTYNVMGWFRQAPGKEREGVAIIYTTTG ATYYRDSVKGRATISQDNAKKSVSLQMNSLRPEDTAIYYCVARQPNSGPWEYWGQ GTQVTVSS (**SEQ ID NO: 129**), or QVKLEESGGGSVQAGGSLRLSCTTSGYTNSYKWMGWFRQAPGQEREGVAVIYTGN DRTYYSDSVKGRFTISRDNAKNMIYLDMTRLRPEDSAVYECAIGHDGAWRYWGQG TQVTVSS (**SEQ ID NO: 130**).

Embodiment 252: The engineered cell of any one of embodiments 247-251, 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).

Embodiment 253: The engineered cell of any one of embodiments 247-251, wherein the antigen-binding domain comprises a single chain variable fragment (scFv).

Embodiment 254: The engineered cell of embodiment 253, wherein the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

Embodiment 255: The engineered cell of embodiment 254, wherein the VH and VL are separated by a peptide linker.

Embodiment 256: The engineered cell of embodiment 254 or embodiment 255, 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.

Embodiment 257: The engineered cell of any one of embodiments 222-256, wherein the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR).

Embodiment 258: The engineered cell of embodiment 257, wherein the antigen recognizing receptor is a CAR.

Embodiment 259: The engineered cell of embodiment 258, wherein the CAR comprises one or more intracellular signaling domains, and each of the one or more intracellular signaling domains is 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, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, a CD16a intracellular signaling domain, a DNAM-1 intracellular signaling domain, a KIR2DS1 intracellular signaling domain, a KIR3DS1 intracellular signaling domain, a NKp44 intracellular signaling domain, a NKp46 intracellular signaling domain, a FceRlg intracellular signaling domain, a NKG2D intracellular signaling domain, and an EAT-2 intracellular signaling domain.

Embodiment 260: The engineered cell of embodiment 258 or embodiment 259, 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, a BTLA transmembrane domain, an OX40 transmembrane domain, a DAP10 transmembrane domain, a DAP12 transmembrane domain, a CD16a transmembrane domain, a DNAM-1 transmembrane domain, a KIR2DS1 transmembrane domain, a KIR3DS1 transmembrane domain, an NKp44 transmembrane domain, an NKp46 transmembrane domain, an FceRlg transmembrane domain, and an NKG2D transmembrane domain.

Embodiment 261: The engineered cell of any one of embodiments 258-260, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

Embodiment 262: The engineered cell of any one of embodiments 188-261, wherein the ACP is a transcriptional modulator.

Embodiment 263: The engineered cell of any one of embodiments 188-261, wherein the ACP is a transcriptional repressor.

Embodiment 264: The engineered cell of any one of embodiments 188-261, wherein the ACP is a transcriptional activator.

Embodiment 265: The engineered cell of any one of embodiments 188-264, wherein the ACP further comprises a repressible protease and one or more cognate cleavage sites of the repressible protease.

Embodiment 266: The engineered cell of any one of embodiments 188-264, wherein the ACP further comprises a hormone-binding domain of estrogen receptor (ERT2 domain).

Embodiment 267: The engineered cell of any one of embodiments 264-266, wherein the ACP is a transcription factor.

Embodiment 268: The engineered cell of embodiment 237, wherein the ACP is a zinc-finger-containing transcription factor.

Embodiment 269: The engineered cell of embodiment 268, wherein the zinc finger-containing transcription factor comprises a DNA-binding zinc finger protein domain (ZF protein domain) and an effector domain.

Embodiment 270: The engineered cell of embodiment 269, wherein the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA).

Embodiment 271: The engineered cell of embodiment 270, wherein the ZF protein domain comprises one to ten ZFA.

Embodiment 272: The engineered cell of any one of embodiments 269-271, wherein 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 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 truncated Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif (SEQ ID NO: 162) of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain ("WRPW" disclosed as SEQ ID NO: 162); a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

Embodiment 273: The engineered cell of any one of embodiments 269-272, wherein the one or more cognate cleavage sites of the repressible protease are localized between the ZF protein domain and the effector domain.

Embodiment 274: The engineered cell of any one of embodiments 265-273, wherein the repressible protease is a hepatitis C virus (HCV) nonstructural protein 3 (NS3).

Embodiment 275: The engineered cell of embodiment 274, wherein the cognate cleavage site comprises an NS3 protease cleavage site.

Embodiment 276: The engineered cell of embodiment 275, wherein the NS3 protease cleavage site comprises a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B junction cleavage site.

Embodiment 277: The engineered cell of any one of embodiments 274-276, wherein the NS3 protease can be repressed by a protease inhibitor.

Embodiment 278: The engineered cell of embodiment 277, wherein the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir.

Embodiment 279: The engineered cell of embodiment 277, wherein the protease inhibitor is grazoprevir.

Embodiment 280: The engineered cell of embodiment 277, wherein the protease inhibitor is grazoprevir and elbasvir.

Embodiment 281: The engineered cell of embodiment 280, wherein the grazoprevir and the elbasvir is co-formulated in a pharmaceutical composition.

Embodiment 282: The engineered cell of embodiment 281, wherein the pharmaceutical composition is a tablet.

Embodiment 283: The engineered cell of embodiment 281 or 282, wherein the grazoprevir and the elbasvir are at a 2 to 1 weight ratio.

Embodiment 284: The engineered cell of embodiment 283, wherein the grazoprevir is 100 mg per unit dose and the elbasvir is 50 mg per unit dose.

Embodiment 285: The engineered cell of any one of embodiments 266-284, wherein the ACP is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof.

Embodiment 286: The engineered cell of embodiment 285, wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

Embodiment 287: The engineered cell of any one of embodiments 188-286, wherein the ACP further comprises a degron, and wherein the degron is operably linked to the ACP.

Embodiment 288: The engineered cell of embodiment 287, wherein the degron is selected from the group consisting of HCV NS4 degron, PEST (two copies of residues 277-307 of human IκBα), 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 (SEQ ID NO: 163), an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box.

Embodiment 289: The engineered cell of embodiment 287, wherein the degron 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.

Embodiment 290: The engineered cell of embodiment 289, wherein the CRBN polypeptide substrate domain is selected from the group consisting of: IKZF1, IKZF3, CKla, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN.

Embodiment 291: The engineered cell of embodiment 289, wherein the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences.

Embodiment 292: The engineered cell of embodiment 289, wherein the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRR DAL (**SEQ ID NO: 131**).

Embodiment 293: The engineered cell of any one of embodiments 289-292, wherein the IMiD is an FDA-approved drug.

Embodiment 294: The engineered cell of any one of embodiments 289-293, wherein the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

Embodiment 295: The engineered cell of any one of embodiments 285-294, wherein the degron is localized 5' of the repressible protease, 3' of the repressible protease, 5' of the ZF protein domain, 3' of the ZF protein domain, 5' of the effector domain, or 3' of the effector domain.

Embodiment 296: The engineered cell of any one of embodiments 190-295, wherein the engineered nucleic acid further comprises an insulator.

Embodiment 297: The engineered cell of embodiment 296, wherein the insulator is localized between the first expression cassette and the second expression cassette.

Embodiment 298: The engineered cell of any one of embodiments 190-297, wherein the first expression cassette is localized in the same orientation relative to the second expression cassette.

Embodiment 299: The engineered cell of any one of embodiments 190-297, wherein the first expression cassette is localized in the opposite orientation relative to the second expression cassette.

Embodiment 300: The engineered cell of any one of embodiments 188-299, wherein the cell further comprises an additional expression cassette comprising an additional promoter and an additional exogenous polynucleotide sequence having the formula:

(L – E)x

wherein

E comprises a polynucleotide sequence encoding an effector molecule,

L comprises a linker polynucleotide sequence,

X = 1 to 20,

wherein the additional promoter is operably linked to the additional exogenous polynucleotide, and wherein for the first iteration of the (L – E) unit, L is absent.

Embodiment 301: The engineered cell of embodiment 300, wherein when the additional expression cassette comprises two or more units of **(L – E)x**, each L linker polynucleotide sequence is operably associated with the translation of each effector molecule as a separate polypeptide.

Embodiment 302: The engineered cell of embodiment 300 or embodiment 301, wherein each linker polynucleotide sequence encodes a 2A ribosome skipping tag.

Embodiment 303: The engineered cell of embodiment 302, wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

Embodiment 304: The engineered cell of embodiment 300 or embodiment 301, wherein each linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES).

Embodiment 305: The engineered cell of any one of embodiments 300-304, wherein the linker polynucleotide sequence encodes a cleavable polypeptide.

Embodiment 306: The engineered cell of embodiment 305, wherein the cleavable polypeptide comprises a furin polypeptide sequence.

Embodiment 307: The engineered cell of any one of embodiments 300-306, wherein the additional expression cassette comprising one or more units of **(L – E)x** further comprises a polynucleotide sequence encoding a secretion signal peptide for each X.

Embodiment 308: The engineered cell of embodiment 307, wherein for each X the corresponding secretion signal peptide is operably associated with the effector molecule.

Embodiment 309: The engineered cell of embodiment 307 or embodiment 308, wherein each secretion signal peptide comprises a native secretion signal peptide native to the corresponding effector molecule.

Embodiment 310: The engineered cell of any one of embodiments 307-309, wherein each secretion signal peptide comprises a non-native secretion signal peptide that is non-native to the corresponding effector molecule.

Embodiment 311: The engineered cell of embodiment 310 wherein the non-native secretion signal peptide is a secretion signal peptide of a molecule selected from the group consisting of: IL12, IL2, optimized IL2, trypsiongen-2, Gaussia luciferase, CD5, CD8, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL6, IL8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, GM-CSFR, GM-CSF, and CXCL12.

Embodiment 312: The engineered cell of any one of embodiments 300-311, wherein the additional promoter is a constitutive promoter, an inducible promoter, or a synthetic promoter.

Embodiment 313: The engineered cell of any one of embodiments 300-311, wherein the additional promoter is a constitutive promoter selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

Embodiment 314: The engineered cell of any one of embodiments 300-313, wherein each effector molecule is independently 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, a receptor, a ligand, an antibody, a polynucleotide, a peptide, and an enzyme.

Embodiment 315: The engineered cell of embodiment 314, wherein the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha.

Embodiment 316: The engineered cell of embodiment 314, wherein the chemokine is selected from the group consisting of: CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, and XCL1.

Embodiment 317: The engineered cell of embodiment 314, wherein the homing molecule is selected from the group consisting of: anti-integrin alpha4,beta7; anti-MAdCAM; CCR9; CXCR4; SDFl; MMP-2; CXCR1; CXCR7; CCR2; and GPR15.

Embodiment 318: The engineered cell of embodiment 314, wherein the growth factor is selected from the group consisting of: FLT3L and GM-CSF.

Embodiment 319: The engineered cell of embodiment 314, wherein the co-activation molecule is selected from the group consisting of: c-Jun, 4-1BBL, and CD40L.

Embodiment 320: The engineered cell of embodiment 314, wherein the tumor microenvironment modifier is selected from the group consisting of: adenosine deaminase, TGFbeta inhibitors, immune checkpoint inhibitors, VEGF inhibitors, and HPGE2.

Embodiment 321: The engineered cell of embodiment 320, wherein the TGFbeta inhibitors are selected from the group consisting of: an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, and combinations thereof.

Embodiment 322: The engineered cell of embodiment 320, wherein the immune checkpoint inhibitors are selected from the group consisting of: anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNFa antibodies, anti-TREMl antibodies, and anti-TREM2 antibodies.

Embodiment 323: The engineered cell of embodiment 320, wherein the VEGF inhibitors comprise anti-VEGF antibodies, anti-VEGF peptides, or combinations thereof.

Embodiment 324: The engineered cell of any one of embodiments 300-320, wherein each effector molecule is a human-derived effector molecule.

Embodiment 325: The engineered cell of any one of embodiments 188-324, 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 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.

Embodiment 326: The engineered cell of any one of embodiments 188-325, wherein the cell is a Natural Killer (NK) cell.

Embodiment 327: The engineered cell of any one of embodiments 188-326, wherein the cell is autologous.

Embodiment 328: The engineered cell of any one of embodiments 188-326 wherein the cell is allogeneic.

Embodiment 329: The engineered cell of any one of embodiments 188-324, wherein the cell is a tumor cell selected from the group consisting of: an adenocarcinoma cell, 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, a mesothelioma cell, an ovarian tumor cell, a pancreatic tumor cell, a gastric tumor cell, a testicular yolk sac tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

Embodiment 330: The engineered cell of embodiment 329, wherein the cell was engineered via transduction with an oncolytic virus.

Embodiment 331: The engineered cell of embodiment 330, wherein the oncolytic virus is selected from the group consisting of: 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.

Embodiment 332: The engineered cell of embodiment 330 or embodiment 331, wherein the oncolytic virus is a recombinant oncolytic virus comprising the first expression cassette and the second expression cassette.

Embodiment 333: The engineered cell of any one of embodiments 188-324, wherein the cell is a bacterial cell selected from the group consisting of: *Clostridium beijerinckii*, *Clostridium sporogenes, Clostridium novyi, Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Salmonella choleraesuis*.

Embodiment 334: A composition comprising the engineered cell of any one of embodiments 188-333, and a pharmaceutically acceptable carrier.

Embodiment 335: A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the engineered cells of any one of embodiments 188-333 or the composition of embodiment 334.

Embodiment 336: A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the engineered cells of any one of embodiments 188-333 or the composition of embodiment 334.

Embodiment 337: A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the engineered cells of any one of embodiments 188-333 or the composition of embodiment 334.

Embodiment 338: A method of treating a subject having cancer, the method comprising administering a therapeutically effective dose of any of the engineered cells of any one of embodiments 188-333 or the composition of embodiment 334.

Embodiment 339: A method of reducing tumor volume in a subject, the method comprising administering to a subject having a tumor a composition comprising any of the engineered cells of any one of embodiments 188-333 or the composition of embodiment 334.

Embodiment 340: The method of any one of embodiments 335-339, wherein the administering comprises systemic administration.

Embodiment 341: The method of any one of embodiments 336-339, wherein the administering comprises intratumoral administration.

Embodiment 342: The method of any one of embodiments 335-341, wherein the engineered cell is derived from the subject.

Embodiment 343: The method of any one of embodiments 335-341, wherein the engineered cell is allogeneic with reference to the subject.

Embodiment 344: The method of any one of embodiments 335-343, wherein the method further comprises administering a checkpoint inhibitor.

Embodiment 345: The method of embodiment 344, 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-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.

Embodiment 346: The method of any one of embodiments 335-345, wherein the method further comprises administering an anti-CD40 antibody.

Embodiment 347: The method of any one of embodiments 336-346, wherein the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor.

Embodiment 348: The method of any one of embodiments 335-347, wherein the method further comprises administering a protease inhibitor.

Embodiment 349: The method of embodiment 348, wherein the protease inhibitor is administered in a sufficient amount to repress a repressible protease.

Embodiment 350: The method of embodiment 348 or 349, wherein the protease inhibitor is administered prior to, concurrently with, subsequent to administration of the engineered cells or the composition comprising the engineered cells.

Embodiment 351: The method of embodiment 350, wherein the protease inhibitor is selected from the group consisting of: simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovaprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir

Embodiment 352: The method of embodiment 350, wherein the protease inhibitor is grazoprevir.

Embodiment 353: The method of embodiment 350, wherein the protease inhibitor comprises grazoprevir and elbasvir.

Embodiment 354: The method of embodiment 353, wherein the grazoprevir and the elbasvir is co-formulated in a pharmaceutical composition.

Embodiment 355: The method of embodiment 354, wherein the pharmaceutical composition is a tablet.

Embodiment 356: The method of embodiment 354 or 355, wherein the grazoprevir and the elbasvir are at a 2 to 1 weight ratio.

Embodiment 357: The engineered nucleic acid of embodiment 356, wherein the grazoprevir is 100 mg per unit dose and the elbasvir is 50 mg per unit dose.

Embodiment 358: The method of any one of embodiments 335-347, wherein the method further comprises administering tamoxifen or a metabolite thereof.

Embodiment 359: The method of embodiment 358, wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

EXAMPLES

[00620] Below are examples of specific embodiments for carrying out the present disclosure. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present disclosure in any way. 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.

[00621] The practice of the present disclosure will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Example 1: Regulation of effector gene expression via drug-inducible regulatable transcription factors

[00622] An exemplary zinc finger transcription factor with a repressible protease and protease cleavage site (regulatable TF) to drive effector gene expression was constructed. The regulatable TF is a zinc-finger (ZF) DNA binding domain linked to an NS3 protease, NS3 cleavage site, and the activation domain of a transcription factor **(FIG. 1A**, the ZF domain is the circle shown on the left of the diagram in the top panel, the NS3 protease is the partial circle shown in the middle of the diagram in the top panel, and the activation domain is the square shown on the right of the diagram in the top panel). The full construct described in FIG. 1A is an example of activation-conditional control polypeptide (ACP). Expression of the regulatable TF is driven via the constitutive SFFV promoter. In untreated cells (e.g., in the absence of a protease inhibitor) the regulatable TF protein is made, but the NS3 protease self cleaves the activation domain from the ZF DNA binding domain of the regulatable TF protein, which inhibits the TF protein from inducing expression of an effector gene. However, in the presence of a protease inhibitor, such as asunaprevir (ASV), the NS3 protease is inhibited and the regulatable TF protein is stabilized. The regulatable TF then binds via the ZF DNA binding domain to a ZF-specific promoter region and drives

expression of the effector gene. The ZF-specific DNA sequence is titled the BD (binding domain) in **FIGs. 1, 2, 3, 4**, and **5**.

[00623] In this example, a regulatable TF dual vector system was synthesized. In the dual vector system, the regulatable TF gene is in one expression vector, while the ZF-BD and effector gene are on a separate expression vector. In this example, two different ZF-BD and effector gene expression cassettes and vectors were made, one with a minCMV promoter (**FIG. 1A**) and one with a minYB_TATA promoter (**FIG. 1C**). FIG. 1, panel A shows an exemplary schematic of a dual vector regulatable TF system with a minCMV promoter.

[00624] **Materials and Methods**

[00625] T cells were thawed and rested in X-Vivo 10^{TM} (X Lonza) media with 10 U/mL hrIL-2 (Day 0). On Day 1, T cells were activated with CD3/CD28 Dynabeads (Dynabeads T-activator) at a 3:1 ratio and supplemented with 100 U/mL hrIL-2. On Day 2, T cells were co-transduced with the ACP TF vector and the ACP TF-inducible mCherry vector using lentiviral vectors at 1x10^5 GVs (GoStix value) units for each lentivirus. Dynabeads were removed on Day 3 and T cells were diluted to 1x10^6 cell/ml in fresh media 100 U/mL hrIL-2. On Day 6, T cells were passaged into fresh media X-vivo 10 with 100 U/mL hrIL-2 in the presence or absence of 2.5 μM asunaprevir (ASV). T cells were incubated for a further two days and then harvested on Day 8 for flow cytometry to assess mCherry expression.

Results

[00626] Both dual vector systems showed induction of mCherry expression in T cells upon asunaprevir addition (**FIG. 1B** and **1D**). The cells expressing high levels of mCherry in each case likely represent the co-transduced cells that received both the regulatable TF and the mCherry vector. The minCMV dual vector system likely showed leaky expression of mCherry as the transduced but non-asunaprevir treated cells showed mCherry expression above the untransduced cells (**FIG. 1B**). In contrast, the minYB_TATA dual vector system shows minimal leaky expression as the transduced but non-asunaprevir treated cells showed little mCherry expression above the untransduced cells (**FIG. 1D**). mCherry was expressed in the transduced cells once asunaprevir was added to inhibit degradation of the regulatable TF, indicating that the mCherry expression was largely due to regulatable TF-induced transcription.

[00627] The minCMV dual vector system showed a higher mCherry expression in the induced state (asunaprevir treated cells) than the minYB_TATA system. Thus, altering the

minimal promoter allowed for control over the dynamic range and baseline expression in cells expressing payloads regulated by the regulatable TF.

Example 2: Single vector regulatable transcription factor regulation of IL-10 and IL-12 expression

[00628] A single vector TF and effector gene expression system was assessed.

[00629] **Materials and Methods**

[00630] Two regulatable TF and effector gene expression cassettes were constructed as shown in **FIG. 2A** and **FIG. 3A**. In **FIG. 2A**, an IL-10 effector gene with a minTK promoter and a 4x ZF binding sequence (ZF-BD) was arranged in a 3' to 5' orientation upstream of the 5' end of a drug inducible promoter and the regulatable TF. In **FIG. 3A**, an IL-12 effector gene with a minTK promoter and a 4x ZF binding sequence (ZF-BD) was arranged in a 3' to 5' orientation upstream of the 5' end of a drug inducible promoter and the regulatable TF.

[00631] T cells were thawed and rested in X-Vivo 10^{TM} media with 10 U/mL hrIL-2 (Day 0). On Day 1, T cells were activated with a 3:1 incubation of CD3/CD28 Dynabeads and supplemented with 100 U/mL hr IL-2. On Day 2, T cells were transduced with 1x10^5 GV units of a lentiviral vector containing the synTF (also referred to as Pro-Dial)-inducible IL-10 or IL-12 vector. A GFP lentiviral vector was used as a negative control. CD3/CD28 Dynabeads were removed on Day 4 and T cells were passaged. On Day 6, T cells were passaged into wells in the presence or absence of 2.5 μM asunaprevir in X-Vivo 10 media with 100 U/mL hrIL-2. Transduced T cells were incubated for a further two days and the supernatant collected via centrifugation for IL-10 or IL-12 quantification.

[00632] For IL-10, supernatants were diluted 1/100 in PBS/BSA and analyzed using the human IL-10 Quantikine ELISA kit (R&D Systems, cat # D1000B).

[00633] For IL-12, supernatants were diluted 1/100 in PBS/BSA and analyzed using the human IL-12 p70 Quantikine ELISA kit (R&D Systems, cat # D1200B).

Results

[00634] T cells transduced with the regulatable TF inducible IL-10 single vector showed a 6-fold increase in secreted IL-10 levels after addition of asunaprevir to inhibit the NS3 protease activity as compared to transduced T cells in the absence of asunaprevir (**FIG. 2B**). The control cells transduced with GFP vector produced no IL-10. Thus, the single vector regulatable TF system is capable of drug-regulated cytokine production.

[00635] In addition, T cells transduced with the regulatable TF inducible IL-12 single vector showed a 7.5 fold increase in secreted IL-12 levels after addition of asunaprevir to

inhibit the NS3 protease activity as compared to transduced T cells in the absence of asunaprevir (**FIG. 3B**). The control cells transduced with GFP vector produced no IL-12. Thus, the single vector regulatable TF system is capable of drug-regulated cytokine production of different cytokines.

Example 3: Co-expression of single vector regulatable transcription factor and additional proteins

[00636] A single vector regulatable TF and effector gene expression system with co-expression of an additional protein was assessed.

[00637] Materials and Methods

[00638] A regulatable TF and reporter gene expression cassette was constructed as shown in **FIG. 4A**. The regulatable TF gene was linked to a myc-tagged chimeric antigen receptor (CAR) via a 2A linker at the 3' end of the regulatable TF gene. An mCherry reporter gene with a minYB_TATA promoter and a 4x ZF binding sequence (ZF-BD) was arranged in a 3' to 5' orientation upstream of the 5' end of a drug inducible promoter and the linked regulatable TF-CAR genes.

[00639] On Day 0, Jurkat cells were plated in RPMI (RPMI media) at $1x10^6$ cells/ml in a 24 well plate (1ml/well). The Jurkat cells were transduced with $3x10^5$ GVs units of virus/well. On Day 2, Jurkat cells were passaged into wells in the presence or absence of 2 μM asunaprevir. On Day 6, Jurkats were harvested for flow cytometry. Cells were stained with a-Myc-APC antibody (R&D Systems, cat # IC3696A), to assess the expression of the CAR. mCherry expression was also assessed.

Results

[00640] As shown in **FIG. 4B**, the myc-tagged CAR was expressed at similar levels in both the asunaprevir-treated cells and untreated cells, indicating that the regulatable TF can be co-expressed with a CAR in Jurkat cells. The presence and activity of the protease in the regulatable TF did not affect the expression or stability of the CAR protein linked to the regulatable TF. This indicates that the regulatable TF system can function in CAR-T cells.

[00641] As shown in **FIG. 4C**, the addition of asunaprevir to inhibit the protease resulted in regulatable TF-induced expression of the mCherry reporter gene. Thus, the regulatable TF expression system can function correctly in the presence of an additional co-expressed protein to induce expression of an effector protein.

Example 4: CAR and Payload Expression

[00642] CAR and payload/cytokine expression was assessed in cells co-transduced with a CAR construct and effector gene expression systems for "armoring" cells. Payload/cytokine expression was assessed in cells transduced with an effector gene expression system with or without a CAR construct.

Materials and Methods

[00643] Effector gene (*e.g.*, cytokine payload of interest) expression cassettes were constructed and cloned into a viral vector then used to produce payload virus, see **Table 10**. A CAR construct targeting GPC3, a protein highly and specifically expressed in liver cancer [Sun et al, Medical Science Monitor, 2017], was constructed as shown in **FIG. 6A** (construct "1106") and cloned into a viral vector and used to produce GPC3-CAR virus. The GPC3-CAR was fused to a YFP reporter to monitor expression.

[00644] On Day 0, CD4/CD8 T cells (FL00711) were thawed and activated with Dynabeads (3:1) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + IL-2 (100 Units/ml). On Day 1, cells were transduced with $1x10^5$ GV of payload virus +/- $1x10^5$ GV of GPC3-CAR (construct 1106) virus. On Day 4, CAR transduction efficiency was assessed by flow cytometry (Cytoflex). Florescent median intensity (MFI) and percentage of cells expressing GPC3-CAR was analyzed by FlowJo. The cells were then transferred to Grex 24 (Wilson Wolf) in full T cell media + IL2 for further expansion.

[00645] On day 8, cells were harvested from Grex plate and counted then $1x10^6$ cells were spin down and resuspended in 1mL of Full T cells media without IL-2. Cells were seeded in a 24-well plate for 48 hours. On day 10, supernatant was harvested and cells were counted from the 24-well plate to determine their viability. Cells were spun down and supernatant transferred to -80C for further processing by Luminex using Luminex 3plex (IL-12/21/15) kit (LXSAHM-03; R&D) for payload expression.

[00646] For co-culturing experiments with target cells, $2.5x10^4$ HepG2 cells/well were seeded for 5hrs in full EMEM. Following which, EMEM was removed and $1x10^6$ cells total transduced T cells were added in T cell media without IL-2 (4:1, E:T). Supernatant was harvested 48 hours later. Payload expression and T cell activation was assessed using Luminex-6plex kit (LXSAHM-06; R&D) (IL-12/21/15/2/TNFa/IFNg).

Table 10 – Effector gene constructs

SB #	Promoter	Insert
SB00171	SSFV	IL12
SB00862	SSFV	ss12-IL12
SB00880	SSFV	ssIL12-T2A-ssIL21
SB00868	SSFV	IL21ss-IL21(ATUM)
SB01335	SSFV	ssIL-15
SB01961	SSFV	ss.IL21—Furin T2a —ss.mIgGk-IL15
SB01965	SSFV	IL12-Furin E2A-T2A- IL15

Results

[00647] CAR expression was assessed in cells also engineered to express an effector molecule (*e.g.*, cytokine) payload. As shown in **FIG. 6B** and **FIG. 7** (bottom panel), the GPC3-CAR was expressed in 25.0-58.2% of transduced cells 4 days after transduction. As shown in **FIG. 7** (top panel), GPC3-CAR expression level as assessed by YFP MFI was generally comparable between transduction with GPC3-CAR virus only ("1106") and the different payload constructs examined. As shown in **Table 11**, viability was also generally comparable between transduction with GPC3-CAR virus only ("1106") and the different payload constructs examined. Accordingly, CAR expression and viability was not significantly altered by engineering with an armoring effector molecule payload.

Table 11- Cell line cytokine payloads and viability

Cell line	**Viability (%)**	**Cytokine**
NV	85	
1106	83	
171+1106	85	IL-12
862+1106	83	IL-21
868+1106	83	IL-12
1335+1106	75	IL-15
880+1106	76	IL-12/21
1961+1106	82	IL-21/15
1965+1106	81	IL-12/15

[00648] Payload expression of cytokines was assessed in cells transduced with an armoring effector molecule expression system with or without cells also engineered to

express a CAR construct. As shown in **FIG. 8**, payload cytokine expression was detected in all constructs encoding the cytokine payload of interest only (left panels: **FIG. 8A** – IL-12; **FIG. 8B** – IL-15; **FIG. 8C** – IL-21). Expression of cytokines was also generally better in constructs engineered to encode a single cytokine (left panels vs right panels). Notably, when co-transduced with the GPC3-CAR, cytokine levels were reduced generally by a log-fold or undetected (right panels: **FIG. 8A** – IL-12; **FIG. 8B** – IL-15; **FIG. 8C** – IL-21).

[00649] Payload expression of cytokines was next assessed when CAR T cells were co-cultured with target cells. As shown in **FIG. 9**, no significant change of payload cytokine expression for IL-12 or IL-21 was observed in the presence of HepG2 cells, while expression of IL-15 was reduced by approximately 50% (top panels vs bottom panels: **FIG. 9A** – IL-12; **FIG. 9B** – IL-15; **FIG. 9C** – IL-21). In addition to payload cytokine expression, production of additional T cell activation cytokines was assessed. As shown in **FIG. 10**, no significant change of TNFα, IFNγ, or IL-2 expression was observed in the presence of HepG2 cells target cells (top panels vs bottom panels: **FIG. 10A** – TNFα; **FIG. 10B** – IFNγ; **FIG. 10C** – IL-2).

Example 5: In vivo CAR Activity in Co-Expression Systems

[00650] CAR expression, payload/cytokine expression, and anti-tumor activity was assessed in cells co-transduced with a CAR construct and effector gene expression systems for "armoring" cells.

Materials and Methods

[00651] Effector gene (*e.g.*, cytokine payload of interest) expression cassettes were constructed and cloned into a viral vector then used to produce payload virus, see **Table 12**. A CAR construct targeting GPC3, a protein highly and specifically expressed in liver cancer (Sun et al, Medical Science Monitor, 2017), was constructed as shown in **FIG. 11A** (construct "1108") and cloned into a viral vector and used to produce GPC3-CAR virus. The GPC3-CAR was fused to a YFP reporter to monitor expression.

[00652] On day 0, $6x10^6$ HepG2 fLuc cells were implanted (IP cavity) in NGS female mice. On day 7, body weight (BW) was measured and mice were randomized. On day 11, $5x10^6$ CAR-T cells were injected IV. Mice were observed for overall health condition, BLI measurement, and body weight twice a week. Mice were also bled once a week into EDTA tubes and spun down to separate cells from plasma. Plasma frozen at -80C until Luminex analysis using a Luminex-6plex kit (LXSAHM-06; R&D) (IL-12/21/15/2/TNFa/IFNg) and the cell pellet was resuspended and processed for flow cytometry. Mice were sacrificed when

body weight drops more than 15% of original weight. On day 45, all mice were sacrificed with tumors, lung, liver, and spleen were collected and fixed as well as tumors weighed.

[00653] On Day 0, CD4/CD8 T cells (FL00711) were thawed and activated with Dynabeads (3:1) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + IL-2 (100 Units/ml). On Day 1, cells were transduced with $1x10^5$ GV of payload virus +/- $1x10^5$ GV of GPC3-CAR virus (construct "1108"). On Day 4, CAR transduction efficiency was assessed by flow cytometry (Cytoflex). Florescent median intensity (MFI) and percentage of cells expressing GPC3-CAR was analyzed by FlowJo. The cells were then transferred to Grex 24 (Wilson Wolf) in full T cell media + IL2 for further expansion.

[00654] On day 9, cells were harvested from Grex plate and counted then $1x10^6$ cells were spin down and resuspended in 1mL of Full T cells media without IL-2. Cells were seeded in a 24-well plate for 48 hours. On day 11, supernatant was harvested and cells were counted from the 24-well plate to determine their viability. Cells were spun down and supernatant transferred to -80C for further processing by Luminex using Luminex 3plex (IL-12/21/15) kit (LXSAHM-03; R&D) for payload expression.

[00655] For *ex vivo* cell analysis, 1 ml PBS was added to blood samples, mixed and transferred to 15 ml conical tubes then 2 ml/tube lysis buffer added. Tubes were inverted 2x to mix the incubate at RT for 5 min. Next, ~14 ml of FACS buffer wash was added and repeated 2x. At last spin, cells were resuspended in ~250-400ul and transferred to v-bottom plate, then washed with 400 ul/well PBS. Next, 100 ul/well of viability dye (zombieUV; Biolegend) was added and incubated at RT dark for 15 min. Wells then received 200ul FACS buffer, spun at 400g 5 min, decanted, then 200 ul/well antibody mix added and incubated at RT for 45 min. Cells were washed with 200ul FACS buffer, spun at 400g 5 min, decanted, and was repeated 2x. Cells were resuspended in 150 ul/well FACS buffer, transferred to u-bottom plate and read. Antibodies were purchased from Biolegend and included: mCD45 clone 30-F11; hCD45 clone H130; hCD3 clone OKT3; hCD8 clone SK1.

Table 12 – Effector gene constructs

SB #	Promoter	Insert
SB00880	SSFV	ssIL12-T2A-ssIL21
SB00862	SSFV	ss12-IL12
SB00868	SSFV	IL21ss-IL21(ATUM)
SB01335	SSFV	ssIL-15
SB01478	SSFV	ssIL21-T2A-IL15_ss+propeptideIL15_mature

Results

[00656] CAR expression was assessed in T cells also engineered to express an armoring cytokine payload. As shown in **FIG. 11B**, the GPC3-CAR was expressed in greater than 54% of all constructs examined, greater than 99% of 5 out of 6 constructs, 4 days after transduction. Payload expression of cytokines was next assessed. As shown in **Table 13**, the engineered CAR T cells expressed their respective payload cytokine.

Table 13 – Cytokine expression (pg/ml)

	IL12	**IL21**	**IL15**
NV	56.54	19.34	6.382
1108 (CAR)	48.77	17.66	4.55
CAR+ 862	48.77	**152.5**	6.945
CAR+ 868	**3348**	70.14	6.94
CAR+ 880	**163.5**	**46.27**	6.946
CAR+1335	84.85	32.30	**3165**
CAR+1874	n/a	n/a	n/a
1478	-	**456.1**	**70.64**

*Bold denotes constructs engineered to express the indicated cytokine

[00657] Efficacy of the CAR T cells engineered with a armoring cytokine payload was then assessed. Mice treated with CAR-T cells + IL-12 (868) were sacrificed at day 21 and mice treated with CAR-T cells + IL-15/IL-21 (1478) were sacrificed at day 24 due to poor health conditions. As shown and summarized in **FIG. 12**, tumor sizes were assessed by BLI measurement on days 11, 14, 21, and 24 for mice treated with T cells transduced without virus (**FIG. 13A** – left panel), GPC3-CAR T alone without a cytokine (**FIG. 13A** – right panel), or GPC3-CAR T engineered with an armoring cytokine (**FIG. 13B** – IL-12/IL-21 co-expression; **FIG. 13C** – IL-15; **FIG. 13D** – IL-12; **FIG. 13E** – IL-21). At low dose (5e6 cell/ml), T cells transduced without virus or with the GPC3-CAR alone demonstrated increased tumor burden. In contrast, T cells engineered to co-express the CAR and IL-12/IL-21 (“880” FIG. 12; FIG. 13B) or IL-15 (“1335” FIG. 12; FIG. 13C) demonstrated tumor burden reduction by day 24. T cells engineered to co-express the CAR and IL-12 demonstrated initial tumor burden reduction (“868” FIG. 12; FIG. 13D), but the combination demonstrated high toxicity as mice were sacrificed due to poor health. T cells engineered to co-express the CAR and IL-21 demonstrated overall tumor size maintenance (“862” FIG. 12) with tumor burden reduction detected in individual mice (FIG. 13E), potentially due to the low levels of the cytokine. Accordingly, the results indicate CAR T cells co-expressing select

cytokines and cytokine combinations demonstrated efficacy in reducing tumor burden, but potentially present high toxicity suggesting that armoring cytokine expression may benefit from regulation.

[00658] T cell activation cytokines was assessed in plasma of treated mice. As shown in **FIG. 14**, no significant detection of TNFα (**FIG. 14A**) or IL-2 (**FIG. 14C**) was observed on Day 14, 3 days after treatment (top panel). IFNγ (**FIG. 14B**) was observed in treatment groups expressing IL-12 alone ("880") or co-expressing IL-12/21 ("880") on Day 14 (**FIG. 14B** - top panel). In contrast, TNFα (**FIG. 14A** - bottom panel) was observed in treatment groups that correlated with tumor reduction ("868"/"880"/"1335"; see FIG. 12 and FIG. 13) on Day 21, 10 days after treatment (bottom panel). IFNγ (**FIG. 14B** - bottom panel) was also observed in treatment groups that correlated with tumor reduction at Day 21, though notably higher detection (~10x) in IL-12 and IL-12/21 systems. IL-2 (**FIG. 14C** - bottom panel) was also observed was in IL-12 and IL-12/21 systems at Day 21, with notably higher detection in the IL-12 alone system. Accordingly, the results indicate CAR T cells engineered to express select armoring cytokines and armoring cytokine combinations demonstrated production of T cell activation cytokines.

[00659] Payload expression of cytokines was also assessed in the plasma of treated mice. As shown in **FIG. 15A**, IL-12 was observed on Day 14, 3 days after treatment (top panel) and on Day 21, 10 days after treatment (bottom panel) in IL-12 and IL-12/21 systems, though notably higher detection (~10x) in IL-12 alone. IL-12 expression also increased as the percentage of human CD45+ cells increased in mice (*see* below). As shown in **FIG. 15B**, IL-15 was observed on Day 14, 3 days after treatment (top panel) and on Day 21, 10 days after treatment (bottom panel) in the IL-15 system. IL-15 expression also increased as the percentage of human CD45+ cells increased in mice (*see* below), though more moderately than IL-12. Unexpectedly, IL-21 was only detected on Day 21, 10 days after treatment (bottom panel) in the IL-12 system ("868") and not in IL-21 alone or IL-12/IL-21 co-expression systems (**FIG. 15C**). Accordingly, the results indicate CAR T cells engineered to express armoring cytokines demonstrated production of payload cytokines in select systems.

[00660] Ex vivo analysis of T cell persistence was assessed. As shown in **FIG. 16A**, no or minimal human CD45+ CAR T cells were detected on Day 14, 3 days after treatment (right panel) correlating with no observed reduction in tumor size (left panel). In contrast, as shown in **FIG. 16B**, CD45+ CAR T cells were detected in the armored CAR systems ("868"/"880"/"1335"; right panel) that demonstrated tumor reduction (left panel), though notably all armored groups present high degree of toxicity, potentially due to GvHD.

Example 6: Tamoxifen Regulatable Transcription Factor Systems

[00661] Payload expression was assessed using a regulatable TF expression system.

Materials and Methods

[00662] Various regulatable TF (also referred to as an activation-conditional control polypeptide (ACP) for drug-inducible formats or "synTF") and expression cassettes were constructed as shown in **FIG. 17A**. Regulation of transcription factor activity was constructed through fusion of the zinc-finger with P65 activation motif and an ERT2 sequence that allows tamoxifen-controlled nuclear localization of the regulatable TF ("565"). A reporter payload sequence was constructed under the inducible control (mCherry driven by YB-TATA promoter and a 4x ZF binding sequence "ZFBD") on a separate construct from the regulatable TF, with ("2235") or without ("1066") a GPC3-CAR-YFP construct expressed by an SFFV promoter in an opposite orientation. The cassettes were cloned into a viral vector and used to produce virus.

[00663] On Day 0, CD4/CD8 T cells (FL00711) were thawed and activated with Dynabeads (3:1) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + IL-2 (100 Units/ml). On Day 2, cells were transduced with $1x10^5$ GV of virus. On Day 7, 4-hydroxytamoxifen (4-OHT), N-desmethyltamoxifen, or endoxifen were added to each well at 1uM, 0.25uM, 0.1uM, or with no drug. Florescent median intensity (MFI) was assessed of Day 9.

Results

[00664] ERT2 regulatable TF systems were assessed using 4-hydroxytamoxifen (4-OHT), N-desmethyltamoxifen, or endoxifen. As shown in **FIG. 17B**, 4-OHT treatment resulted in regulatable and titratable expression of the reporter either expressed alone (left panel) or expression from a construct also encoding a CAR construct (right panel). As shown in **FIG. 17C**, N-desmethyltamoxifen treatment did not result expression of the reporter relative to the no-drug control, both expressed alone (left panel) and from a construct also encoding a CAR construct (right panel). As shown in **FIG. 17D**, endoxifen treatment resulted in regulatable and titratable expression of the reporter either expressed alone (left panel) or expression from a construct also encoding a CAR construct (right panel). A summary of the results is presented in **FIG. 17E**. As shown in **FIG. 18** and **FIG. 19**, expression of the CAR was

confirmed and comparable across the untreated and highest concentration drug treatments. Accordingly, the results indicate T cells can be engineered with an ERT2 regulatable TF system to produce regulatable and titratable expression of a payload using 4-OHT or endoxifen, including in a CAR T system.

Example 7: Regulatable Transcription Factor Systems in Combination with CAR Expression

[00665] Various strategies for the dual-engineering of CAR and regulatable TF expression systems was assessed.

Materials and Methods

[00666] Various strategies for dual-engineering of CAR and regulatable TF expression systems are shown in **FIG. 20** and **FIG. 21** describing the various orientations, components, and two vector constructions examined.

[00667] On Day 0, CD4/CD8 T cells (FL00711) were thawed and activated with Dynabeads (3:1) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + IL-2 (100 Units/ml). On Day 2, cells were transduced with $1x10^5$ GV of virus. On Day 7, 2uM Grazoprevir or no drug was added. Florescent median intensity (MFI) was assessed of Day 9.

Results

[00668] Various orientations, components, and two vector constructions were examined to assess expression of a CAR, and in particular if CAR expression is improved when encoded on the payload vector, regulated TF vector, or alone. As shown in **FIG. 22**, assessment of the various strategies described in **FIG. 21** demonstrated CAR expression was only detected when encoded on the vector with the reporter payload, and not when on vector encoding the regulatable TF (ACP). As shown in **FIG. 23A** and **FIG. 23B**, a strategy encoding the payload and CAR on one vector and the regulatable TF on a separate vector resulted in about 50% of transduced cells expressing the CAR and reporter, with a large proportion co-expressing both. As shown in **FIG. 24A** and **FIG. 24B**, a strategy encoding the payload and regulatable TF on one vector and the CAR on a separate vector resulted in about 65% of transduced cells that express the CAR co-expressing the reporter, and about 35% of transduced cells expressing the reporter overall after induction. Accordingly, the results indicate (1) encoding CAR on the same vector as the payload and the regulatable TF on a separate vector produced more

cells in an induced (payload/reporter positive) state; and (2) encoding the payload and regulatable TF on one vector and the CAR on a separate vector produced more CAR-expressing cells.

Example 8: Regulatable Transcription Factor Systems Demonstrate Regulated Expression *in vitro* and *in vivo*

[00669] Regulatable TF expression systems were assessed both *in vitro* and *in vivo*.

Materials and Methods

[00670] Schematics of the ACP for drug-inducible formats (also referred to as "synTF") using an NS3/NS4 protease cleavage site and a VPR transcriptional effector domain (Construct "1845") as well as the expression cassette using a 4x BS minYB-TATA ACP-responsive promoter driving hIL-12 effector molecule payload are shown in **FIG. 25**. A construct with hIL-12 driven by a constitutive SFFV promoter (Construct "171") was also assessed. Relevant sequences of the constructs are provided in **Table 14**.

Table 14 – ACP Sequences

SB #	Domain	Sequence
SB01845	Zinc Finger	SRPGERPFQCRICMRNFSRRHGLDRHTRTHTGEKPFQCRICMR NFSDHSSLKRHLRTHTGSQKPFQCRICMRNFSVRHNLTRHLRT HTGEKPFQCRICMRNFSDHSNLSRHLKTHTGSQKPFQCRICMR NFSQRSSLVRHLRTHTGEKPFQCRICMRNFSESGHLKRHLRTH LRGS (SEQ ID NO: 88)
SB01845	Spacer-*Cleavage Site*-linker-**Protease**-linker-*Cleavage Site*-spacer	TCRDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPG GLEGGGGSGGT*EDVVCCHSIY*GKKKGDIDTYRYIGSSGTGCVVI VGRIVLSGSGTS**APITAYAQQTRGLLGCIITSLTGRDKNQVE GEVQIVSTATQTFLATCINGVCWAVYHGAGTRTIASPKGPV IQMYTNVDQDLVGWPAPQGSRSLTPCTCGSSDLYLVTRHA DVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLLCPAGHAVGL FRAAVCTRGVAKAVDFIPVENLETTMRSPVFTD**NSSPPAVTL THPITKIDREV*LYQEFDEMEECSQH*YPYDVPDYAGGGGSGGT (SEQ ID NO: 89)
SB01845	VPR	*See* SEQ ID NO: 90
SB01845	Full Construct (DNA)	atgTCTAGACCCGGAGAGCGCCCATTCCAGTGTCGGATTTGC ATGCGGAACTTTTCGAGAAGACACGGCCTGGACAGACATAC CCGTACTCATACAGGTGAAAAACCCTTTCAGTGTCGGATCT GTATGCGAAATTTCTCCGACCACAGCAGCCTGAAGAGACAT CTACGTACCCACACCGGCAGCCAGAAGCCATTTCAGTGTCG GATCTGTATGCGGAACTTCTCCGTGAGACACAACCTGACCA GACATCTACGTACGCACACCGGAGAGAAGCCATTCCAATGC CGAATATGCATGCGCAACTTCAGTGACCACAGCAACCTGAG CAGACACCTAAAAACCCACACCGGTTCCCAGAAGCCATTTC AGTGTCGGATCTGTATGCGGAACTTCTCCCAGCGCAGCAGC

		CTGGTGAGACATCTACGTACGCACACCGGAGAGAAGCCATT CCAATGCCGAATATGCATGCGCAACTTCAGTGAGAGCGGCC ACCTGAAGAGACACCTGCGTACGCACCTGAGGGGATCCaCC TGCAGGgactacaaagaccatgacggtgattataaagatcatgacatcgattacaaggatgac gatgacaagatggcccccaagaaaaagaggaaggtgggcattcacggggtgccgggtggactc gagggaggcggtggaagcggcggtaccGAGGACGTGGTGTGCTGCCACTC AATCTACGGCAAGAAGAAGGGTGATATCGACACCTACCGAT ACATAGGCTCTTCCGGGACAGGCTGCGTGGTCATAGTGGGC AGGATCGTCTTGTCCGGATCCGGCACTAGTGCGCCCATCAC GGCGTACGCCCAGCAGACGAGAGGCCTCCTAGGGTGTATAA TCACCAGCCTGACTGGCCGGGACAAAAACCAAGTGGAGGG TGAGGTCCAGATCGTGTCAACTGCTACCCAAACCTTCCTGG CAACGTGCATCAATGGGGTATGCTGGGCAGTCTACCACGGG GCCGGAACGAGGACCATCGCATCACCCAAGGGTCCTGTCAT CCAGATGTATACCAATGTGGACCAAGACCTTGTGGGCTGGC CCGCTCCTCAAGGTTCCCGCTCATTGACACCCTGTACCTGCG GCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTC ATTCCCGTGCGCCGGCGAGGTGATAGTAGAGGCTCTCTGCT GAGCCCCAGACCTATCAGCTACCTGAAGGGCTCTAGCGGCG GACCTCTGCTTTGTCCTGCTGGACATGCCGTGGGCCTGTTTA GAGCCGCCGTGTGTACAAGAGGCGTGGCCAAAGCCGTGGA CTTCATCCCCGTGGAAAACCTGGAAACCACCATGCGGAGCC CCGTGTTCACCGACAATTCTAGCCCTCCAGCCGTGACACTG ACACACCCCATCACCAAGATCGACAGAGAGGTGCTGTACCA AGAGTTCGACGAGATGGAAGAGTGCAGCCAGCACTACCCCT ACGACGTGCCAGATTATGCTGGCGGCGGAGGATCTGGCGGA ACAGAAGCCTCTGGAAGCGGCAGAGCTGACGCCCTGGATG ACTTCGACCTGGATATGCTGGGCAGCGACGCTCTGGACGAT TTTGACCTCGACATGCTGGGATCTGATGCACTCGACGATTTC GATTTGGACATGCTCGGCAGTGATGCCTTGGACGACTTTGA TCTTGATATGCTCATCAACAGCCGGTCCAGCGGCAGCCCCA AGAAAAAAAGAAAAGTGGGCTCCCAGTACCTGCCTGACAC CGACGACAGACACCGGATCGAGGAAAAGCGGAAGCGGACC TACGAGACATTCAAGAGCATCATGAAGAAGTCCCCATTCAG CGGCCCCACCGATCCTAGACCTCCACCTAGAAGAATCGCCG TGCCTAGCAGATCTAGCGCCTCCGTGCCTAAACCTGCTCCTC AGCCTTATCCTTTCACCAGCAGCCTGAGCACCATCAACTAC GACGAGTTCCCTACCATGGTGTTCCCCAGCGGCCAGATCTCT CAGGCTTCTGCTCTTGCTCCAGCTCCTCCTCAGGTTCTGCCT CAAGCTCCTGCACCAGCACCGGCTCCAGCTATGGTTTCTGCT TTGGCTCAGGCCCCTGCTCCTGTGCCTGTTCTTGCTCCTGGA CCACCTCAGGCTGTTGCTCCTCCTGCTCCAAAACCTACACAG GCCGGCGAAGGCACACTGTCTGAAGCTCTGCTGCAGCTCCA GTTCGATGACGAAGATCTGGGCGCCCTGCTGGGCAATTCTA CAGATCCTGCCGTGTTTACCGATCTGGCCAGCGTGGACAAC AGCGAGTTTCAGCAGCTCCTGAATCAGGGCATCCCTGTGGC TCCTCACACCACCGAACCTATGCTGATGGAATACCCCGAGG CCATCACCAGACTGGTCACCGGTGCTCAAAGACCACCTGAT CCAGCTCCAGCACCACTGGGAGCACCTGGACTGCCTAATGG ACTGCTGTCTGGCGACGAGGACTTCAGCTCTATCGCCGACA TGGATTTCTCTGCCCTGCTCGGCTCTGGCAGCGGCTCTAGAG ATAGCAGAGAAGGCATGTTCCTGCCTAAGCCTGAGGCCGGC TCTGCCATCTCCGATGTGTTCGAGGGAAGAGAAGTGTGCCA GCCTAAGCGGATCCGGCCTTTTCACCCTCCTGGAAGCCCTTG GGCCAACAGACCTCTGCCTGCTTCTCTGGCCCCTACACCAAC

		AGGACCTGTGCACGAACCTGTGGGCAGTCTGACCCCAGCTC CTGTTCCTCAACCTCTGGATCCCGCTCCTGCTGTGACACCTG AAGCCTCTCATCTGCTGGAAGATCCCGACGAAGAGACAAGC CAGGCCGTGAAGGCCCTGAGAGAAATGGCCGACACAGTGA TCCCTCAGAAAGAGGAAGCCGCCATCTGCGGACAGATGGAC CTGTCTCATCCTCCACCAAGAGGCCACCTGGACGAGCTGAC AACCACACTGGAATCCATGACCGAGGACCTGAACCTGGACA GCCCTCTGACACCCGAGCTGAACGAGATCCTGGACACCTTC CTGAACGACGAGTGTCTGCTGCACGCCATGCACATCTCTAC CGGCCTGAGCATCTTCGACACCAGCCTGTTTTGA (SEQ ID NO: 91)
SB02357	ACP-responsive promoter	cgggtttcgtaacaatcgcatgaggattcgcaacgccttcGGCGTAGCCGATGTCGC GctcccgtctcagtaaaggtcGGCGTAGCCGATGTCGCGcaatcggactgccttc gtacGGCGTAGCCGATGTCGCGcgtatcagtcgcctcggaacGGCGTAGC CGATGTCGCGcattcgtaagaggctcactctcccttacacggagtggataACTAGTT CTAGAGGGTATATAATGGGGGCCA (SEQ ID NO: 92)
SB02357	inducible promoter IL12 (4x ZF BS YB-TATA IL12)	cgggtttcgtaacaatcgcatgaggattcgcaacgccttcGGCGTAGCCGATGTCGC GctcccgtctcagtaaaggtcGGCGTAGCCGATGTCGCGcaatcggactgccttc gtacGGCGTAGCCGATGTCGCGcgtatcagtcgcctcggaacGGCGTAGC CGATGTCGCGcattcgtaagaggctcactctcccttacacggagtggataACTAGTT CTAGAGGGTATATAATGGGGGCCAACGCGTGCCGCCACCAT GTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTT CCTCGCTTCCCCTCTGGTCGCCATTTGGGAACTGAAGAAGG ACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCT GGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGG ACGGCATAACCTGGACCCTGGATCAGAGCTCCGAGGTGCTC GGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCG GCGACGCGGGCCAGTACACTTGCCACAAGGGTGGCGAAGT GCTGTCCCACTCCCTGCTGCTGCTGCACAAGAAAGAGGATG GAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCG AAGAACAAGACCTTCCTCCGCTGCGAAGCCAAGAACTACAG CGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTCCACCG ACCTGACTTTCTCCGTGAAGTCGTCACGGGGATCAAGCGAT CCTCAGGGCGTGACCTGTGGAGCCGCCACTCTGTCCGCCGA GAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTG GAATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGT CCCTGCCTATCGAGGTCATGGTCGATGCCGTGCATAAGCTG AAATACGAGAACTACACTTCCTCCTTCTTTATCCGCGACATC ATCAAGCCTGACCCCCCCAAGAACTTGCAGCTGAAGCCACT CAAGAACTCCCGCCAAGTGGAAGTGTCTTGGGAATATCCAG ACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCT GTGTGCAAGTGCAGGGAAAGTCCAAACGGGAGAAGAAAGA CCGGGTGTTCACCGACAAAACCTCCGCCACTGTGATTTGTC GGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATA CTACTCGTCCTCCTGGAGCGAATGGGCCAGCGTGCCTTGTTC CGGTGGCGGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGA GGAGGTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGG AATGTTCCCGTGCCTACACCACTCCCAAAACCTCCTGAGGG CTGTGTCGAACATGTTGCAGAAGGCCCGCCAGACCCTTGAG TTCTACCCCTGCACCTCGGAAGAAATTGATCACGAGGACAT CACCAAGGACAAGACCTCGACCGTGGAAGCCTGCCTGCCGC TGGAACTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAG ACAAGCTTTATCACTAACGGCAGCTGCCTGGCGTCGAGAAA GACCTCATTCATGATGGCGCTCTGTCTTTCCTCGATCTACGA AGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGAACG

		CCAAGCTGCTCATGGACCCGAAGCGGCAGATCTTCCTGGAC CAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCCCT GAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGG AAGAACCGGACTTCTACAAGACCAAGATCAAGCTGTGCATC CTGTTGCACGCTTTCCGCATTCGAGCCGTGACCATTGACCGC GTGATGTCCTACCTGAACGCCAGTtaa (SEQ ID NO: 161)
SB02357 and SB00171	hIL-12 protein	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAP GEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDA GQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCG AATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDA VHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEY PDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICR KNASISVRAQDRYYSSSWSEWASVPCSGGGSGGGSGGGSGGG SRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYP CTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNG SCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPK RQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIK LCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 93)
SB02357	hIL-12 DNA (codon optimized)	ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTG TTCCTCGCTTCCCCTCTGGTCGCCATTTGGGAACTGAAGAAG GACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCC TGGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGG ACGGCATAACCTGGACCCTGGATCAGAGCTCCGAGGTGCTC GGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTCG GCGACGCGGGCCAGTACACTTGCCACAAGGGTGGCGAAGT GCTGTCCCACTCCCTGCTGCTGCTGCACAAGAAAGAGGATG GAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCG AAGAACAAGACCTTCCTCCGCTGCGAAGCCAAGAACTACAG CGGTCGGTTCACCTGTTGGTGGCTGACGACAATCTCCACCG ACCTGACTTTCTCCGTGAAGTCGTCACGGGGATCAAGCGAT CCTCAGGGCGTGACCTGTGGAGCCGCCACTCTGTCCGCCGA GAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTG GAATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGT CCCTGCCTATCGAGGTCATGGTCGATGCCGTGCATAAGCTG AAATACGAGAACTACACTTCCTCCTTCTTTATCCGCGACATC ATCAAGCCTGACCCCCCCAAGAACTTGCAGCTGAAGCCACT CAAGAACTCCCGCCAAGTGGAAGTGTCTTGGGAATATCCAG ACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCT GTGTGCAAGTGCAGGGAAAGTCCAAACGGGAGAAGAAAGA CCGGGTGTTCACCGACAAAACCTCCGCCACTGTGATTTGTC GGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATA CTACTCGTCCTCCTGGAGCGAATGGGCCAGCGTGCCTTGTTC CGGTGGCGGATCAGGCGGAGGTTCAGGAGGAGGCTCCGGA GGAGGTTCCCGGAACCTCCCTGTGGCAACCCCCGACCCTGG AATGTTCCCGTGCCTACACCACTCCCAAAACCTCCTGAGGG CTGTGTCGAACATGTTGCAGAAGGCCCGCCAGACCCTTGAG TTCTACCCCTGCACCTCGGAAGAAATTGATCACGAGGACAT CACCAAGGACAAGACCTCGACCGTGGAAGCCTGCCTGCCGC TGGAACTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAG ACAAGCTTTATCACTAACGGCAGCTGCCTGGCGTCGAGAAA GACCTCATTCATGATGGCGCTCTGTCTTTCCTCGATCTACGA AGATCTGAAGATGTATCAGGTCGAGTTCAAGACCATGAACG CCAAGCTGCTCATGGACCCGAAGCGGCAGATCTTCCTGGAC CAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCCCT

		GAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGG AAGAACCGGACTTCTACAAGACCAAGATCAAGCTGTGCATC CTGTTGCACGCTTTCCGCATTCGAGCCGTGACCATTGACCGC GTGATGTCCTACCTGAACGCCAGTTAA (SEQ ID NO: 94)
SB00171	hIL-12 DNA	ATGTGCCATCAGCAGCTTGTCATATCTTGGTTTTCACTTGTA TTCCTGGCCAGCCCTTTGGTTGCGATCTGGGAGCTCAAGAA GGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTC CCGGTGAGATGGTCGTTTTGACATGTGACACTCCAGAAGAG GACGGTATTACGTGGACTCTGGACCAGTCCTCCGAAGTTCTT GGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGG GGATGCGGGACAATACACATGCCACAAGGGAGGCGAGGTG TTGTCTCATAGTTTGCTGCTTCTCCACAAGAAAGAGGATGG AATCTGGAGCACCGACATACTCAAGGATCAAAAGGAACCC AAAAATAAGACATTTCTGCGATGTGAGGCTAAGAACTATAG TGGCCGCTTCACTTGTTGGTGGCTGACTACCATCAGCACAG ATCTCACGTTTTCAGTAAAAAGTAGTAGAGGTTCAAGTGAT CCTCAAGGGGTAACGTGCGGTGCTGCAACACTGTCTGCTGA ACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCGTA GAATGCCAAGAGGACAGTGCTTGTCCTGCGGCCGAGGAGTC TCTCCCAATAGAAGTGATGGTGGACGCGGTGCATAAACTGA AATATGAGAACTACACAAGCAGTTTTTTTATAAGAGATATC ATCAAGCCCGATCCGCCGAAGAATTTGCAACTTAAACCGCT TAAAAACTCACGCCAGGTTGAAGTATCCTGGGAGTATCCGG ATACATGGTCAACACCACACAGCTATTTTTCCCTTACCTTCT GTGTGCAGGTCCAAGGGAAGAGCAAAAGGGAGAAGAAGGA CAGGGTATTCACTGATAAAACTTCCGCGACGGTCATCTGCC GAAAAAACGCTAGTATATCTGTACGGGCGCAGGATAGGTAC TATAGTTCTTCTTGGTCTGAGTGGGCCTCAGTTCCGTGCTCT GGGGGAGGAAGTGGAGGAGGGTCCGGCGGTGGAAGCGGGG GAGGGAGTCGCAACTTGCCAGTGGCTACACCAGATCCAGGC ATGTTTCCATGTCTGCATCATTCCCAGAATCTCCTGAGAGCG GTGTCAAATATGCTCCAAAAAGCGAGACAAACACTGGAATT TTACCCGTGTACCAGTGAGGAGATTGATCACGAGGACATAA CCAAGGACAAGACCTCAACTGTAGAAGCGTGTTTGCCGCTG GAGTTGACTAAGAATGAGTCCTGCCTCAATTCCAGAGAAAC TTCATTCATTACTAACGGCAGTTGTCTTGCATCCCGGAAAAC GTCCTTTATGATGGCCCTTTGCCTTAGTTCAATTTACGAGGA TCTTAAAATGTATCAAGTGGAGTTTAAAACCATGAATGCTA AACTTCTTATGGACCCCAAACGACAAATTTTTCTGGATCAG AATATGCTTGCCGTGATAGACGAACTCATGCAGGCGCTTAA TTTTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAG AACCTGATTTTTATAAAACGAAGATTAAACTGTGTATCCTGC TGCATGCCTTTCGCATCCGAGCTGTCACAATCGATAGGGTTA TGTCCTACCTTAACGCGAGCtaG (SEQ ID NO: 95)

[00671] For *in vitro* assessment, on Day 0, CD4/CD8 T cells (FL00711) were thawed and activated with Dynabeads (3:1) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + IL-2 (100 Units/ml). On Day 2, cells were transduced with $1x10^5$ GV of virus encoding the constructs described above. On Day 7, 0.1uM Grazoprevir (GRZ), 0.5uM Grazoprevir, or no drug was added. Transduced T cells were incubated for a further two days and the supernatant collected via centrifugation for IL-12 quantification. For IL-12,

supernatants were diluted 1/100 in PBS/BSA and analyzed using the human IL-12 p70 Quantikine ELISA kit (R&D Systems, cat # D1200B).

[0001] For *in vivo* assessment, T cells were transduced with SB01845 & SB02357, each at an estimated MOI of 5 based on viral titering in HEK cells. Positive control T cells were transduced with 5 MOI of SB00171, encoding constitutive hIL-12 (driven by SFFV). Negative controls were untransduced T cells. The number of lentiviral genomes integrated into the T cells was analyzed in bulk by PCR (copy # assay). NSG mice were randomized on day -2 and vehicle or Grazoprevir (Grz) dosing began in the afternoon on day 1 (Vehicle: 2.5% DMSO, 30% PEG400, 67.5% PBS). Grazoprevir potassium salt was dissolved sequentially in DMSO, PEG400, and PBS to reach 10mg/mL. Dosing: Drug treated mice received 25mg/kg Grz at each dosing time point; vehicle treated mice received equivalent volume of vehicle; all Grz dosing administered IP. Dosing continued BID on days 2-4. On day 2, 20e6 T cells per mouse were injected by tail vein injection after the AM drug dosing. Mice were bled on day 4 and sacrificed/bled on day 5. Luminex assay was run to assess levels of hIL-12 in mouse plasma. Presence of human T cells in mouse blood was analyzed by flow cytometry. The treatment groups are presented in **Table 15**.

Table 15 – Murine Regulated Armoring Treatment Groups

Treatment (IV)	Drug (IP)	Concentration	Frequency of Dose
non-engineered T cells	Vehicle	-	BID
non-engineered T cells	Grazoprevir	25 mg/kg	BID
constitutive IL12 T cells	Vehicle	-	BID
constitutive IL12 T cells	Grazoprevir	25 mg/kg	BID
inducible IL12 T cells	Vehicle	-	BID
inducible IL12 T cells	Grazoprevir	25 mg/kg	BID

Results

[00672] As shown in **FIG. 26**, a regulated expression system with an ACP for drug-inducible formats (also referred to as “synTF”) using an NS3/NS4 protease cleavage site and a VPR transcriptional effector domain (Construct “1845”) as well as the expression cassette using a 4x BS minYB-TATA ACP-responsive promoter driving hIL-12 effector molecule payload resulted in titratable hIL-12 secretion *in vitro* upon addition of increasing amounts of

GRZ, with minimal hIL-12 detected in the absence of drug and no detectable hIL-12 in the absence of virus.

[00673] Next, the constructs were assessed *in vivo*. The experimental design is shown in **FIG. 27**. T cells were assessed following transduction and injection. As shown in **Table 16**, T cells with the regulatable system demonstrated ~2.5 lower copy number viral copy number relative to the constitutive system, indicating lower transduction efficiency. As shown in **FIG. 28**, T cells at day 9 also demonstrated ~2-fold expansion in the regulatable system compared to the no virus control, potentially due to transduction with two viruses. As shown in **FIG. 29**, T cells from each group demonstrated similar glucose profiles, indicating T cell growth and metabolism was generally equivalent across all groups. Next, production of hIL-12 *in vivo* was assessed. As shown in **FIG. 30**, T cells were equally present for each group demonstrating that the regulatable armoring system did not alter T cell longevity *in vivo*. As shown in **FIG. 31**, administration of GRZ lead to detectable hIL-12 in the plasma of mice (~100-fold above vehicle) indicating the regulatable system provided a drug-inducible *in vivo* format for the regulated armoring of T cells.

Table 16 – Viral Copy Number

Description	Copy Number (copies/cell)
No virus (non-engineered)	UND
SB00171 - constitutive IL12 T cells	**8.8**
SB01845+2357 - inducible IL12 T cells	**3.4**

Example 9: Activation Inducible System

[00674] Enhancers that turn on transcription when CAR-T are activated by target cells are assessed.

Materials and Methods

[00675] **Library Generation:** A library of 15,000 enhancers linked to a minimal promoter (late Ade minimal promoter) was generated to screen for enhancers that turn on transcription when CAR-T are activated by target cells. Enhancers that were enriched in the ATAC-seq of activated T cells (Gate et al. Nat Genet. Author manuscript; available in PMC 2019 Jan 9; herein incorporated by reference for all purposes) were chosen for the library (all enhancers <400 bp were used). Length of library members is 199 bp, thus for enhancers longer than 199bp tiling was used to cover 2 regions of each enhancer with as much overlap as possible.

Top upregulated genes in single-cell RNA seq data (Xhangolli et al. Genomics Proteomics Bioinformatics. 2019 Apr;17(2):129-139. doi: 10.1016/j.gpb.2019.03.002; herein incorporated by reference for all purposes) were searched in the HACER database of human enhancers and enhancers from those genes were chosen. Synthetic enhancers were designed using pairs of transcription factors known from the literature to be upregulated in activated T cells. 4 binding sites for each TF were used to generate the synthetic enhancers (either aaaabbbb or abababab). All possible pairs from the following list of TFs were included: ATF2, ATF7, BACH1, BATF, Bcl-6, Blimp-1, BMI1, CBFB, CREB1, CREM, CTCF, E2F1, EBF1, EGR1, ETV6, FOS, FOXA1, FOXA2, GATA3, HIF1A, IKZF1, IKZF2, IRF4, JUN, JUNB, JUND, Lef1, NFAT, NFIA, NFIB, NFKB, NR2F1, Nur77, PU.1, RELA, RUNX3, SCRT1, SCRT2, SP1, STAT4, STAT5A, T-Bet, Tcf7, ZBED1, ZNF143, and ZNF217.

[00676] **Candidate Selection:** Using bioinformatic analysis of single-cell RNA-seq data and ATAC-seq data of activated and resting T cells, candidate enhancers were chosen to screen in parallel with the library. Using the single-cell RNA-seq data, transcription factors upregulated during CAR-T activation were identified. The ATAC-seq open enhancer regions were screened for binding sites of these upregulated transcription factors and a subset of these enhancers were chosen as candidates. Using the single-cell RNA-seq data, top genes upregulated in activated CAR-T relative to resting CAR-T were identified and the 2kb region upstream of those genes in the genome were chosen as candidate promoters.

[00677] **Screening**: The library and selected candidates are screened by flow sorting (min promoter drives expression of a fluorescent mKate protein). Activated or not activated (resting) T cells are analyzed by flow cytometry, sorted for high/low expression, and compared by NGS which promoters are identified in the high bin in activated but not resting T cells.

Results

[00678] A library of enhancer and selected candidates are screened for enhancing transcription when CAR-T are activated by target cells. Enhancers that turn on transcription when CAR-T are activated by target cells are identified.

Example 10: IL-12 and IL-15 Drug-inducible Expression Systems in T Cells

[00679] IL-12 and/or IL-15 payload expression was assessed in T cells for various regulatable TF expression system strategies. The strategies included co-expression of CAR constructs and CAR activity was assessed.

Materials and Methods

[00680] For *in vitro* assessment, on Day 0, CD4/CD8 T cells (donor derived) were thawed, seeded at 1e6 cells/mL/well in a 24 well-plate, and activated with anti-CD3/CD28 Dynabeads (3:1 bead to cell) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + rhIL-2 (100 Units/ml; Peprotech). On Day 1, 0.5ml media was removed and cells were transduced with 3e5 pg of virus for each the constructs indicated (relevant sequences of the constructs are provided in **Table 17**). On Day 2, 1.5mL of Optimizer media + 100U/mL rhIL-2 was added. Day 4 cells were counted and 1e6 cells/well were transferred to a 24 well Grex plate at a final volume of 8mL. On Day 8, 6mL of media was removed from the wells and cells counted then seeded at a concentration of 1e6 cells/mL in fresh media with grazoprevir (2, 1, 0.5, 0.1, 0.05, 0.01µM and no drug). Two parallel plates of cells were seeded for determining payload induction and T-cell killing. On Day 8, cells were also assessed for CAR expression by flow cytometry. Transduced T cells were incubated for a further two days and the supernatant collected via centrifugation for IL-12 and IL-15 quantification. For IL-12 and IL-15, supernatants analyzed by Luminex (R&D IL12/IL15).

[00681] For T cell killing assays, on Day 0 T cells were seeded at 1e6/mL +/- 2µM of GRZ for 48 hours to induce payload. On Day 2, target cells were counted and 35K were seeded per well in a 96 well plate for 4-6hrs in target cell media. Then target cell media was removed and T cells were counted and 35K CAR normalized T cells were seeded [E:T – 1:1] in 200ul of Optimizer media overnight. On Day 3, cells were transferred to a V-bottom plate, spun down, and supernatant harvested to determine killing activity using an LDH assay (CyQUANT LDH Cytotoxicity Assay; Life Technologies).

Table 17 – IL-12 and IL-15 Regulatable TF Expression Constructs

SB #	Domain	Sequence
SB01845		*See* Table 14 above for various constructs sequences
SB02110	3x Flag NLS ZF	MDYKDDDDKDYKDDDDKDYKDDDDKPKKKRKVSRPGERPFQCRICMRNFSRRHGLDRHTRTHTGEKPFQCRICMRNFSDHSSLKRHLRTHTGSQKPFQCRICMRNFSVRHNLTRHLRTHTGEKPFQCRICMRNFSDHSNLSRHLKTHTGSQKPFQCRICMRNFSQRSSLVRHLRTHTGEKPFQCRICMRNFSESGHLKRHLRTHLRGS (SEQ ID NO: 132)

SB02110	NS3 cutsite and linker	EDVVCCHSIYGKKKGDIDTYRYIGSSGTGCVVIVGRIVLSGSGTS (SEQ ID NO: 133)
SB02110	NS3 protease	APITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTATQTFLATCI NGVCWAVYHGAGTRTIASPKGPVIQMYTNVDQDLVGWPAPQGSR SLTPCTCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSG GPLLCPAGHAVGLFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTD (SEQ ID NO: 134)
SB02110	NS3 cutsite and linker	NSSPPAVTLTHPITKIDREVLYQEFDEMEECSQH (SEQ ID NO: 135)
SB02110	VPR	*See* SEQ ID NO: 90
SB02110	Full-length cassette (DNA)	ATGGACTACAAGGACGACGACGACAAGGATTACAAGGATGATG ATGATAAGGACTATAAGGACGATGATGACAAACCCAAGAAGAA GCGGAAGGTTTCCCGGCCTGGCGAGAGGCCTTTCCAGTGCAGAA TCTGCATGCGGAACTTCAGCAGACGGCACGGCCTGGACAGACAC ACCAGAACACACACAGGCGAGAAACCCTTCCAGTGCCGGATCT GTATGAGAAATTTCAGCGACCACAGCAGCCTGAAGCGGCACCTG AGAACCCATACCGGCAGCCAGAAACCATTTCAGTGTAGGATATG CATGCGCAATTTCTCCGTGCGGCACAACCTGACCAGACACCTGA GGACACACACCGGGGAGAAGCCTTTTCAATGTCGCATATGCATG AGAAACTTCTCTGACCACTCCAACCTGAGCCGCCACCTCAAAAC CCACACCGGCTCTCAAAAGCCCTTCCAATGTAGAATATGTATGA GGAACTTTAGCCAGCGGAGCAGCCTCGTGCGCCATCTGAGAACT CACACTGGCGAAAAGCCGTTTCAATGCCGTATCTGTATGCGCAA CTTTAGCGAGAGCGGCCACCTGAAGAGACATCTGCGCACACACC TGAGAGGCAGCGAGGATGTCGTGTGCTGCCACAGCATCTACGGA AAGAAGAAGGGCGACATCGACACCTATCGGTACATCGGCAGCA GCGGCACAGGCTGTGTTGTGATCGTGGGCAGAATCGTGCTGAGC GGCTCTGGAACAAGCGCCCCTATCACAGCCTACGCTCAGCAGAC AAGAGGCCTGCTGGGCTGCATCATCACAAGCCTGACCGGCAGA GACAAGAACCAGGTGGAAGGCGAGGTGCAGATCGTGTCTACAG CTACCCAGACCTTCCTGGCCACCTGTATCAATGGCGTGTGCTGG GCCGTGTATCACGGCGCTGGCACAAGAACAATCGCCTCTCCAAA GGGCCCCGTGATCCAGATGTACACCAACGTGGACCAGGACCTCG TTGGCTGGCCTGCTCCTCAAGGCAGCAGAAGCCTGACACCTTGC ACCTGTGGCTCCAGCGATCTGTACCTGGTCACCAGACACGCCGA CGTGATCCCTGTCAGAAGAAGAGGGGATTCCAGAGGCAGCCTG CTGAGCCCTAGACCTATCAGCTACCTGAAGGGCAGCTCTGGCGG ACCTCTGCTTTGTCCTGCTGGACATGCCGTGGGCCTGTTTAGAGC CGCCGTGTGTACAAGAGGCGTGGCCAAAGCCGTGGACTTCATCC CCGTGGAAAACCTGGAAACCACCATGCGGAGCCCCGTGTTCACC GACAATTCTAGCCCTCCAGCCGTGACACTGACACACCCCATCAC CAAGATCGACAGAGAGGTGCTGTACCAAGAGTTCGACGAGATG GAAGAGTGCAGCCAGCACGAGGCCTCTGGATCTGGTAGAGCCG ACGCTCTGGACGACTTCGACCTGGATATGCTGGGCTCTGACGCC CTGGATGATTTTGACCTCGACATGCTGGGAAGCGACGCCCTCGA CGATTTCGATTTGGACATGCTCGGCAGTGATGCACTCGATGACT TTGATCTTGATATGCTGATCAACAGCCGGTCCAGCGGCAGCCCT AAGAAAAAACGGAAAGTGGGCAGCCAGTATCTGCCCGACACCG ACGATCGGCACCGGATCGAGGAAAAGCGGAAGCGGACCTACGA GACATTCAAGAGCATCATGAAGAAGTCCCCATTCAGCGGCCCCA CCGATCCTAGACCTCCACCTAGAAGAATCGCCGTGCCTAGCAGA TCTAGCGCCTCCGTGCCTAAACCTGCTCCACAGCCTTATCCTTTC ACCTCTAGCCTGAGCACCATCAACTACGACGAGTTCCCTACCAT GGTGTTCCCCAGCGGCCAGATCTCTCAGGCATCTGCTCTTGCTCC

		AGCTCCACCTCAGGTTCTGCCTCAAGCTCCTGCTCCGGCTCCTGC ACCAGCTATGGTGTCTGCACTTGCTCAGGCACCAGCTCCAGTGC CTGTTCTTGCTCCTGGACCTCCTCAGGCTGTTGCTCCACCAGCAC CTAAACCTACACAGGCCGGCGAGGGAACACTGTCTGAAGCCCTG CTGCAACTCCAGTTCGATGACGAGGATCTGGGCGCCCTGCTGGG AAATTCTACAGACCCTGCCGTGTTTACCGATCTGGCCAGCGTGG ACAACAGCGAGTTTCAGCAGCTCCTGAACCAGGGCATCCCAGTG GCTCCTCACACCACAGAGCCCATGCTGATGGAATACCCCGAGGC CATCACCAGACTGGTCACCGGCGCACAAAGACCTCCAGATCCTG CTCCAGCACCGCTTGGAGCACCTGGACTGCCTAATGGACTGCTG TCTGGCGACGAGGACTTCAGCTCTATCGCCGACATGGATTTCTCT GCCCTGCTCGGCTCTGGCAGCGGCTCTAGAGATAGCAGAGAAGG CATGTTCCTGCCTAAGCCTGAGGCCGGCTCTGCCATCTCCGATGT GTTCGAGGGAAGAGAAGTGTGCCAGCCTAAGCGGATCCGGCCTT TTCACCCTCCTGGAAGCCCTTGGGCCAACAGACCTCTGCCTGCTT CTCTGGCCCCTACACCAACAGGACCTGTGCACGAACCTGTGGGC AGTCTTACCCCTGCTCCTGTTCCTCAGCCACTGGATCCCGCACCA GCTGTGACACCTGAAGCCAGCCATCTGCTGGAAGATCCCGACGA AGAAACCTCTCAGGCCGTGAAGGCCCTGAGAGAAATGGCCGAC ACAGTGATCCCTCAGAAAGAGGAAGCCGCCATTTGCGGACAGA TGGACCTGTCTCACCCTCCACCAAGAGGCCACCTGGACGAGCTG ACCACCACACTGGAATCCATGACCGAGGACCTGAACCTGGACA GCCCTCTGACACCCGAGCTGAACGAGATCCTGGACACCTTCCTG AACGACGAGTGTCTGCTGCACGCCATGCACATCTCTACCGGCCT GAGCATCTTCGACACCAGCCTGTTCTGA (SEQ ID NO: 136)
SB02661	hIL12	*See* SEQ ID NO: 93
SB02661	insulator + inducible YB-TATA promoter hIL12 on negative DNA strand	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccaccccccagaatag aatgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttc cagggtcaaggaaggcacgggggagggggcaaacaacagatggctggcaactagaaggcacagttaA CTGGCGTTCAGGTAGGACATCACGCGGTCAATGGTCACGGCTCG AATGCGGAAAGCGTGCAACAGGATGCACAGCTTGATCTTGGTCT TGTAGAAGTCCGGTTCTTCCAGGCTGGACTTTTGAGGCACAGTC TCGGAGTTGAAATTCAGGGCCTGCATCAGTTCATCAATCACGGC GAGCATATTCTGGTCCAGGAAGATCTGCCGCTTCGGGTCCATGA GCAGCTTGGCGTTCATGGTCTTGAACTCGACCTGATACATCTTCA GATCTTCGTAGATCGAGGAAAGACAGAGCGCCATCATGAATGA GGTCTTTCTCGACGCCAGGCAGCTGCCGTTAGTGATAAAGCTTG TCTCGCGGGAGTTCAGACACGATTCGTTCTTGGTCAGTTCCAGC GGCAGGCAGGCTTCCACGGTCGAGGTCTTGTCCTTGGTGATGTC CTCGTGATCAATTTCTTCCGAGGTGCAGGGGTAGAACTCAAGGG TCTGGCGGGCCTTCTGCAACATGTTCGACACAGCCCTCAGGAGG TTTTGGGAGTGGTGTAGGCACGGGAACATTCCAGGGTCGGGGGT TGCCACAGGGAGGTTCCGGGAACCTCCTCCGGAGCCTCCTCCTG AACCTCCGCCTGATCCGCCACCGGAACAAGGCACGCTGGCCCAT TCGCTCCAGGAGGACGAGTAGTATCTATCCTGCGCCCGGACGCT GATTGACGCGTTCTTCCGACAAATCACAGTGGCGGAGGTTTTGT CGGTGAACACCCGGTCTTTCTTCTCCCGTTTGGACTTTCCCTGCA CTTGCACACAGAAAGTGAGCGAGAAGTATGAGTGCGGGGTGCT CCAAGTGTCTGGATATTCCCAAGACACTTCCACTTGGCGGGAGT TCTTGAGTGGCTTCAGCTGCAAGTTCTTGGGGGGGTCAGGCTTG ATGATGTCGCGGATAAAGAAGGAGGAAGTGTAGTTCTCGTATTT CAGCTTATGCACGGCATCGACCATGACCTCGATAGGCAGGGACT CTTCCGCGGCAGGGCAGGCGCTGTCCTCCTGGCATTCCACGGAG TACTCATATTCCTTGTTGTCTCCCCTGACTCTCTCGGCGGACAGA

		GTGGCGGCTCCACAGGTCACGCCCTGAGGATCGCTTGATCCCCG TGACGACTTCACGGAGAAAGTCAGGTCGGTGGAGATTGTCGTCA GCCACCAACAGGTGAACCGACCGCTGTAGTTCTTGGCTTCGCAG CGGAGGAAGGTCTTGTTCTTCGGTTCTTTTTGGTCCTTGAGGATG TCAGTGGACCAGATTCCATCCTCTTTCTTGTGCAGCAGCAGCAG GGAGTGGGACAGCACTTCGCCACCCTTGTGGCAAGTGTACTGGC CCGCGTCGCCGAACTCCTTGACTTGAATGGTCAGGGTCTTTCCGC TTCCGAGCACCTCGGAGCTCTGATCCAGGGTCCAGGTTATGCCG TCCTCTTCTGGCGTATCGCAAGTCAGCACGACCATTTCTCCAGGG GCGTCCGGGTACCAATCCAGCTCGACCACGTAGACGTCCTTCTT CAGTTCCCAAATGGCGACCAGAGGGGAAGCGAGGAACACAAGG GAGAACCAGGAGATGACGAGTTGCTGATGGCACATGGTGGCGG CACCGGTACGCGTTGGCCCCCATTATATACCCTCTAGAACTAGTt atccactccgtgtaagggagagtgagcctcttacgaatgCGCGACATCGGCTACGCCgttc cgaggcgactgatacgCGCGACATCGGCTACGCCgtacgaaggcagtccgattgCGC GACATCGGCTACGCCgacctttactgagacgggagCGCGACATCGGCTACG CCgaaggcgttgcgaatcctcatgcgattgttacgaaacccgTTAATTAAAGAGCGAGA TTCCGTCTCAAAGAAAAAAAAAGTAATGAAATGAATAAAATGA GTCCTAGAGCCAGTAAATGTCGTAAATGTCTCAGCTAGTCAGGT AGTAAAAGGTCTCAACTAGGCAGTGGCAGAGCAGGATTCAAAT TCAGGGCTGTTGTGATGCCTCCGCAGACTCTGAGCGCCACCTGG TGGTAATTTGTCTGTGCCTCTTCTGACGTGGAAGAACAGCAACT AACACACTAACACGGCATTTACTATGGGCCAGCCATTGT (SEQ ID NO: 137)
SB02661	GPC3 CAR	MLLLVTSLLLCELPHPAFLLIPHMEVQLVESGGGLVQPGGSLRLSCA ASGFTFNKNAMNWVRQAPGKGLEWVGRIRNKTNNYATYYADSV KARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGNSFAYWGQGT LVTVSAGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKS SQSLLYSSNQKNYLAWYQQKPGQPPKLLIYWASSRESGVPDRFSGS GSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFGQGTKLEIKTTTP APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGMVSKGEELFT GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPV PWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFF KDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNY NSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDG PVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDEL YK (SEQ ID NO: 138)
SB02661	SFFV GPC3 CAR	gtaacgccattttgcaaggcatggaaaaataccaaaccaagaatagagaagttcagatcaagggcgggta catgaaaatagctaacgttgggccaaacaggatatctgcggtgagcagtttcggccccggcccggggcca agaacagatggtcaccgcagtttcggccccggcccgaggccaagaacagatggtccccagatatggccc aaccctcagcagtttcttaagacccatcagatgtttccaggctcccccaaggacctgaaatgaccctgcgcc ttatttgaattaaccaatcagcctgcttctcgcttctgttcgcgcgcttctgcttcccgagctctataaaagagct cacaacccctcactcggcgcgccagtcctccgacagactgagtcgcccgggGGATCcTACGTA GCCGCCACCATGCTGCTGCTGGTCACATCTCTGCTGCTGTGCGA GCTGCCCCATCCTGCCTTTCTGCTGATCCCTCACATGGAAGTGCA GCTGGTGGAATCTGGCGGAGGACTGGTTCAACCTGGCGGCTCTC TGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAACAAGAAC GCCATGAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTTGAATG GGTCGGACGGATCAGAAACAAGACCAACAACTACGCCACCTAC TACGCCGACAGCGTGAAGGCCAGGTTCACCATCTCCAGAGATGA CAGCAAGAACAGCCTGTACCTCCAGATGAACTCCCTGAAAACCG

		AGGACACCGCCGTGTACTATTGCGTGGCCGGCAATAGCTTTGCC TACTGGGGACAGGGCACCCTGGTTACAGTTTCTGCTGGTGGTGG AGGCTCAGGAGGGGGAGGTTCCGGAGGAGGCGGTTCGGACATC GTGATGACACAGAGCCCTGACAGCCTGGCCGTGTCTCTGGGAGA AAGAGCCACCATCAACTGCAAGAGCAGCCAGAGCCTGCTGTACT CCAGCAACCAGAAGAACTACCTGGCCTGGTATCAGCAAAAGCC CGGCCAGCCTCCTAAGCTGCTGATCTATTGGGCCAGCTCCAGAG AAAGCGGCGTGCCCGATAGATTTTCTGGCTCTGGCAGCGGCACC GACTTCACCCTGACAATTTCTAGCCTGCAAGCCGAGGACGTGGC CGTGTATTACTGCCAGCAGTACTACAACTACCCTCTGACCTTCGG CCAGGGCACCAAGCTGGAAATCAAGACCACCACACCAGCTCCTC GGCCTCCAACTCCTGCTCCTACAATTGCCAGCCAGCCTCTGTCTC TGAGGCCCGAAGCTTGTAGACCAGCTGCCGGCGGAGCTGTGCAT ACAAGAGGACTGGATTTCGCCTGCGACATCTACATATGGGCCCC CCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTAC GAAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTT TCATGCGGCCTGTCCAAACAACTCAAGAAGAGGACGGGTGTTCA TGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTCGGGT GAAGTTCTCACGATCTGCGGACGCCCCCGCATACCAACAGGGAC AGAACCAACTCTACAATGAACTGAACCTGGGGCGAAGAGAAGA ATATGACGTTCTGGATAAGCGCAGAGGCCGCGACCCCGAAATG GGAGGTAAGCCGAGAAGGAAAAATCCACAGGAAGGACTGTATA ATGAGTTGCAAAAGGATAAGATGGCGGAAGCATATTCAGAGAT TGGGATGAAAGGCGAAAGAAGAAGAGGCAAAGGACATGATGG GCTGTATCAGGGCCTTTCCACGGCCACAAAAGATACGTATGACG CGCTGCACATGCAAGCTCTTCCTCCCAGGGGAATGGTGTCCAAA GGAGAAGAACTGTTCACCGGAGTGGTGCCGATTCTGGTGGAACT GGACGGCGATGTCAACGGACACAAGTTCTCAGTCAGCGGAGAA GGGGAAGGGGATGCTACGTACGGGAAGCTCACCCTGAAGCTCA TCTGTACTACTGGGAAGCTGCCTGTGCCCTGGCCAACTCTGGTG ACAACCCTCGGTTACGGGTTGCAGTGCTTCGCACGCTACCCTGA CCACATGAAGCAGCACGATTTCTTCAAATCCGCCATGCCTGAGG GTTACGTGCAAGAGCGGACCATCTTTTTCAAGGACGATGGCAAC TACAAGACTCGGGCCGAAGTGAAATTCGAAGGGGACACTCTTGT GAACCGCATCGAGTTGAAGGGCATTGACTTCAAGGAAGATGGC AACATTTTGGGACACAAGCTGGAGTACAACTACAATTCCCATAA CGTGTACATCACCGCCGACAAGCAGAAAAACGGGATCAAGGCC AACTTCAAGATCCGCCACAATATCGAGGATGGCGGAGTGCAGCT GGCTGACCACTACCAGCAGAACACCCCCATCGGTGATGGCCCAG TTCTGCTCCCCGATAACCATTACCTGTCGTACCAATCGGCATTGA GCAAGGACCCCAACGAGAAGCGCGATCACATGGTGTTGCTGGA ATTTGTGACCGCTGCCGGCATTACCCTCGGGATGGACGAGCTCT ACAAGTAA (SEQ ID NO: 139)
SB02662	insulator + inducible YB-TATA promoter IL-12 _2A_ IL15 on negative DNA strand	tccccagcatgcctgctattctcttcccaatcctcccccttgctgtcctgccccaccccaccccccagaatag aatgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttc cagggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagTCA GCTGGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGCA GGAACTCTTTGATATTCTTTTCTTCCAGTTCCTCGCATTCTTTGCA GCCGGACTCGGTCACATTGCCGTTGGAGGACAGGCTGTTGTTGG CCAGGATGATCAGGTTTTCCACGGTATCGTGGATGGAGGCGTCG CCGCTTTCCAGGCTGATCACTTGCAGCTCGAGCAGAAAGCACTT CATGGCGGTCACTTTACAGCTAGGGTGCACGTCGCTCTCGGTGT ACAGTGTGGCGTCGATGTGCATGCTCTGGATCAGATCCTCGATC TTCTTCAGATCGCTGATCACGTTGACCCAGTTGCCTGTAGAGCCG GGCACCCAAAGAAGCAGCACCCACAGCAGCAGTGTGTCGGTTTC

		CATAGGTCCAGGGTTTTCCTCCACGTCTCCACACGTCAGTAGACT CCCGCGTCCCTCGCCAGATCCTCTCTTCCGTCTACTGGCGTTCAG GTAGGACATCACGCGGTCAATGGTCACGGCTCGAATGCGGAAA GCGTGCAACAGGATGCACAGCTTGATCTTGGTCTTGTAGAAGTC CGGTTCTTCCAGGCTGGACTTTTGAGGCACAGTCTCGGAGTTGA AATTCAGGGCCTGCATCAGTTCATCAATCACGGCGAGCATATTC TGGTCCAGGAAGATCTGCCGCTTCGGGTCCATGAGCAGCTTGGC GTTCATGGTCTTGAACTCGACCTGATACATCTTCAGATCTTCGTA GATCGAGGAAAGACAGAGCGCCATCATGAATGAGGTCTTTCTCG ACGCCAGGCAGCTGCCGTTAGTGATAAAGCTTGTCTCGCGGGAG TTCAGACACGATTCGTTCTTGGTCAGTTCCAGCGGCAGGCAGGC TTCCACGGTCGAGGTCTTGTCCTTGGTGATGTCCTCGTGATCAAT TTCTTCCGAGGTGCAGGGGTAGAACTCAAGGGTCTGGCGGGCCT TCTGCAACATGTTCGACACAGCCCTCAGGAGGTTTTGGGAGTGG TGTAGGCACGGGAACATTCCAGGGTCGGGGGTTGCCACAGGGA GGTTCCGGGAACCTCCTCCGGAGCCTCCTCCTGAACCTCCGCCT GATCCGCCACCGGAACAAGGCACGCTGGCCCATTCGCTCCAGGA GGACGAGTAGTATCTATCCTGCGCCCGGACGCTGATTGACGCGT TCTTCCGACAAATCACAGTGGCGGAGGTTTTGTCGGTGAACACC CGGTCTTTCTTCTCCCGTTTGGACTTTCCCTGCACTTGCACACAG AAAGTGAGCGAGAAGTATGAGTGCGGGGTGCTCCAAGTGTCTG GATATTCCCAAGACACTTCCACTTGGCGGGAGTTCTTGAGTGGC TTCAGCTGCAAGTTCTTGGGGGGGTCAGGCTTGATGATGTCGCG GATAAAGAAGGAGGAAGTGTAGTTCTCGTATTTCAGCTTATGCA CGGCATCGACCATGACCTCGATAGGCAGGGACTCTTCCGCGGCA GGGCAGGCGCTGTCCTCCTGGCATTCCACGGAGTACTCATATTC CTTGTTGTCTCCCCTGACTCTCTCGGCGGACAGAGTGGCGGCTCC ACAGGTCACGCCCTGAGGATCGCTTGATCCCCGTGACGACTTCA CGGAGAAAGTCAGGTCGGTGGAGATTGTCGTCAGCCACCAACA GGTGAACCGACCGCTGTAGTTCTTGGCTTCGCAGCGGAGGAAGG TCTTGTTCTTCGGTTCTTTTTGGTCCTTGAGGATGTCAGTGGACC AGATTCCATCCTCTTTCTTGTGCAGCAGCAGCAGGGAGTGGGAC AGCACTTCGCCACCCTTGTGGCAAGTGTACTGGCCCGCGTCGCC GAACTCCTTGACTTGAATGGTCAGGGTCTTTCCGCTTCCGAGCAC CTCGGAGCTCTGATCCAGGGTCCAGGTTATGCCGTCCTCTTCTGG CGTATCGCAAGTCAGCACGACCATTTCTCCAGGGGCGTCCGGGT ACCAATCCAGCTCGACCACGTAGACGTCCTTCTTCAGTTCCCAA ATGGCGACCAGAGGGGAAGCGAGGAACACAAGGGAGAACCAG GAGATGACGAGTTGCTGATGGCACATGGTGGCGGCACCGGTAC GCGTTGGCCCCCATTATATACCCTCTAGAACTAGTtatccactccgtgtaa gggagagtgagcctcttacgaatgCGCGACATCGGCTACGCCgttccgaggcgactgata cgCGCGACATCGGCTACGCCgtacgaaggcagtccgattgCGCGACATCGGC TACGCCgacctttactgagacgggagCGCGACATCGGCTACGCCgaaggcgttgcg aatcctcatgcgattgttacgaaacccgTTAATTAAAGAGCGAGATTCCGTCTCA AAGAAAAAAAAAGTAATGAAATGAATAAAATGAGTCCTAGAGC CAGTAAATGTCGTAAATGTCTCAGCTAGTCAGGTAGTAAAAGGT CTCAACTAGGCAGTGGCAGAGCAGGATTCAAATTCAGGGCTGTT GTGATGCCTCCGCAGACTCTGAGCGCCACCTGGTGGTAATTTGT CTGTGCCTCTTCTGACGTGGAAGAACAGCAACTAACACACTAAC ACGGCATTTACTATGGGCCAGCCATTGT (SEQ ID NO: 140)
SB02662	CAR	*See* SB02661 sequences
SB02662	IL-12	*See* SEQ ID NO: 93
SB02662	IL-15	METDTLLLWVLLLWVPGSTGNWVNVISDLKKIEDLIQSMHIDATLY TESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSL

		SSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 141)
SB02662	2A peptide	RRKRGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 142)
SB02664	insulator + inducible minTK promoter hIL12 on negative DNA strand	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccacccccagaatag aatgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttc cagggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagttaA CTGGCGTTCAGGTAGGACATCACGCGGTCAATGGTCACGGCTCG AATGCGGAAAGCGTGCAACAGGATGCACAGCTTGATCTTGGTCT TGTAGAAGTCCGGTTCTTCCAGGCTGGACTTTTGAGGCACAGTC TCGGAGTTGAAATTCAGGGCCTGCATCAGTTCATCAATCACGGC GAGCATATTCTGGTCCAGGAAGATCTGCCGCTTCGGGTCCATGA GCAGCTTGGCGTTCATGGTCTTGAACTCGACCTGATACATCTTCA GATCTTCGTAGATCGAGGAAAGACAGAGCGCCATCATGAATGA GGTCTTTCTCGACGCCAGGCAGCTGCCGTTAGTGATAAAGCTTG TCTCGCGGGAGTTCAGACACGATTCGTTCTTGGTCAGTTCCAGC GGCAGGCAGGCTTCCACGGTCGAGGTCTTGTCCTTGGTGATGTC CTCGTGATCAATTTCTTCCGAGGTGCAGGGGTAGAACTCAAGGG TCTGGCGGGCCTTCTGCAACATGTTCGACACAGCCCTCAGGAGG TTTTGGGAGTGGTGTAGGCACGGGAACATTCCAGGGTCGGGGGT TGCCACAGGGAGGTTCCGGGAACCTCCTCCGGAGCCTCCTCCTG AACCTCCGCCTGATCCGCCACCGGAACAAGGCACGCTGGCCCAT TCGCTCCAGGAGGACGAGTAGTATCTATCCTGCGCCCGGACGCT GATTGACGCGTTCTTCCGACAAATCACAGTGGCGGAGGTTTTGT CGGTGAACACCCGGTCTTTCTTCTCCCGTTTGGACTTTCCCTGCA CTTGCACACAGAAAGTGAGCGAGAAGTATGAGTGCGGGGTGCT CCAAGTGTCTGGATATTCCCAAGACACTTCCACTTGGCGGGAGT TCTTGAGTGGCTTCAGCTGCAAGTTCTTGGGGGGGTCAGGCTTG ATGATGTCGCGGATAAAGAAGGAGGAAGTGTAGTTCTCGTATTT CAGCTTATGCACGGCATCGACCATGACCTCGATAGGCAGGGACT CTTCCGCGGCAGGGCAGGCGCTGTCCTCCTGGCATTCCACGGAG TACTCATATTCCTTGTTGTCTCCCCTGACTCTCTCGGCGGACAGA GTGGCGGCTCCACAGGTCACGCCCTGAGGATCGCTTGATCCCCG TGACGACTTCACGGAGAAAGTCAGGTCGGTGGAGATTGTCGTCA GCCACCAACAGGTGAACCGACCGCTGTAGTTCTTGGCTTCGCAG CGGAGGAAGGTCTTGTTCTTCGGTTCTTTTTGGTCCTTGAGGATG TCAGTGGACCAGATTCCATCCTCTTTCTTGTGCAGCAGCAGCAG GGAGTGGGACAGCACTTCGCCACCCTTGTGGCAAGTGTACTGGC CCGCGTCGCCGAACTCCTTGACTTGAATGGTCAGGGTCTTTCCGC TTCCGAGCACCTCGGAGCTCTGATCCAGGGTCCAGGTTATGCCG TCCTCTTCTGGCGTATCGCAAGTCAGCACGACCATTTCTCCAGGG GCGTCCGGGTACCAATCCAGCTCGACCACGTAGACGTCCTTCTT CAGTTCCCAAATGGCGACCAGAGGGGAAGCGAGGAACACAAGG GAGAACCAGGAGATGACGAGTTGCTGATGGCACATGGTGGCGG CACCGGTttaagcgggtcgctgcagggtcgctcggtgttcgaggccacacgcgtcaccttaatatgc gaaACTAGTtatccactccgtgtaagggagagtgagcctcttacgaatgCGCGACATCGG CTACGCCgttccgaggcgactgatacgCGCGACATCGGCTACGCCgtacgaaggc agtccgattgCGCGACATCGGCTACGCCgacctttactgagacgggagCGCGACA TCGGCTACGCCgaaggcgttgcgaatcctcatgcgattgttacgaaacccgTTAATTAA AGAGCGAGATTCCGTCTCAAAGAAAAAAAAAAGTAATGAAATGA ATAAAATGAGTCCTAGAGCCAGTAAATGTCGTAAATGTCTCAGC TAGTCAGGTAGTAAAAGGTCTCAACTAGGCAGTGGCAGAGCAG GATTCAAATTCAGGGCTGTTGTGATGCCTCCGCAGACTCTGAGC GCCACCTGGTGGTAATTTGTCTGTGCCTCTTCTGACGTGGAAGA ACAGCAACTAACACACTAACACGGCATTTACTATGGGCCAGCCA

		TTGT (SEQ ID NO: 143)
SB02664	CAR	*See* SB02661 sequences
SB02664	IL-12	*See* SEQ ID NO: 93
SB02665	insulator + inducible minTK promoter IL-12 _2A_ IL15 on negative DNA strand	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccaccccccagaatag aatgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttc cagggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagTCA GCTGGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGCA GGAACTCTTTGATATTCTTTTCTTCCAGTTCCTCGCATTCTTTGCA GCCGGACTCGGTCACATTGCCGTTGGAGGACAGGCTGTTGTTGG CCAGGATGATCAGGTTTTCCACGGTATCGTGGATGGAGGCGTCG CCGCTTTCCAGGCTGATCACTTGCAGCTCGAGCAGAAAGCACTT CATGGCGGTCACTTTACAGCTAGGGTGCACGTCGCTCTCGGTGT ACAGTGTGGCGTCGATGTGCATGCTCTGGATCAGATCCTCGATC TTCTTCAGATCGCTGATCACGTTGACCCAGTTGCCTGTAGAGCCG GGCACCCAAAGAAGCAGCACCCACAGCAGCAGTGTGTCGGTTTC CATAGGTCCAGGGTTTTCCTCCACGTCTCCACACGTCAGTAGACT CCCGCGTCCCTCGCCAGATCCTCTCTTCCGTCTACTGGCGTTCAG GTAGGACATCACGCGGTCAATGGTCACGGCTCGAATGCGGAAA GCGTGCAACAGGATGCACAGCTTGATCTTGGTCTTGTAGAAGTC CGGTTCTTCCAGGCTGGACTTTTGAGGCACAGTCTCGGAGTTGA AATTCAGGGCCTGCATCAGTTCATCAATCACGGCGAGCATATTC TGGTCCAGGAAGATCTGCCGCTTCGGGTCCATGAGCAGCTTGGC GTTCATGGTCTTGAACTCGACCTGATACATCTTCAGATCTTCGTA GATCGAGGAAAGACAGAGCGCCATCATGAATGAGGTCTTTCTCG ACGCCAGGCAGCTGCCGTTAGTGATAAAGCTTGTCTCGCGGGAG TTCAGACACGATTCGTTCTTGGTCAGTTCCAGCGGCAGGCAGGC TTCCACGGTCGAGGTCTTGTCCTTGGTGATGTCCTCGTGATCAAT TTCTTCCGAGGTGCAGGGGTAGAACTCAAGGGTCTGGCGGGCCT TCTGCAACATGTTCGACACAGCCCTCAGGAGGTTTTGGGAGTGG TGTAGGCACGGGAACATTCCAGGGTCGGGGGTTGCCACAGGGA GGTTCCGGGAACCTCCTCCGGAGCCTCCTCCTGAACCTCCGCCT GATCCGCCACCGGAACAAGGCACGCTGGCCCATTCGCTCCAGGA GGACGAGTAGTATCTATCCTGCGCCCGGACGCTGATTGACGCGT TCTTCCGACAAATCACAGTGGCGGAGGTTTTGTCGGTGAACACC CGGTCTTTCTTCTCCCGTTTGGACTTTCCCTGCACTTGCACACAG AAAGTGAGCGAGAAGTATGAGTGCGGGGTGCTCCAAGTGTCTG GATATTCCCAAGACACTTCCACTTGGCGGGAGTTCTTGAGTGGC TTCAGCTGCAAGTTCTTGGGGGGGTCAGGCTTGATGATGTCGCG GATAAAGAAGGAGGAAGTGTAGTTCTCGTATTTCAGCTTATGCA CGGCATCGACCATGACCTCGATAGGCAGGGACTCTTCCGCGGCA GGGCAGGCGCTGTCCTCCTGGCATTCCACGGAGTACTCATATTC CTTGTTGTCTCCCCTGACTCTCTCGGCGGACAGAGTGGCGGCTCC ACAGGTCACGCCCTGAGGATCGCTTGATCCCCGTGACGACTTCA CGGAGAAAGTCAGGTCGGTGGAGATTGTCGTCAGCCACCAACA GGTGAACCGACCGCTGTAGTTCTTGGCTTCGCAGCGGAGGAAGG TCTTGTTCTTCGGTTCTTTTTGGTCCTTGAGGATGTCAGTGGACC AGATTCCATCCTCTTTCTTGTGCAGCAGCAGCAGGGAGTGGGAC AGCACTTCGCCACCCTTGTGGCAAGTGTACTGGCCCGCGTCGCC GAACTCCTTGACTTGAATGGTCAGGGTCTTTCCGCTTCCGAGCAC CTCGGAGCTCTGATCCAGGGTCCAGGTTATGCCGTCCTCTTCTGG CGTATCGCAAGTCAGCACGACCATTTCTCCAGGGGCGTCCGGGT ACCAATCCAGCTCGACCACGTAGACGTCCTTCTTCAGTTCCCAA ATGGCGACCAGAGGGGAAGCGAGGAACACAAGGGAGAACCAG GAGATGACGAGTTGCTGATGGCACATGGTGGCGGCACCGGTttaag

		cgggtcgctgcagggtcgctcggtgttcgaggccacacgcgtcaccttaatatgcgaaACTAGTtatc cactccgtgtaagggagagtgagcctcttacgaatgCGCGACATCGGCTACGCCgttccg aggcgactgatacgCGCGACATCGGCTACGCCgtacgaaggcagtccgattgCGCG ACATCGGCTACGCCgacctttactgagacgggagCGCGACATCGGCTACGC CgaaggcgttgcgaatcctcatgcgattgttacgaaacccgTTAATTAAAGAGCGAGAT TCCGTCTCAAAGAAAAAAAAAAGTAATGAAATGAATAAAATGAG TCCTAGAGCCAGTAAATGTCGTAAATGTCTCAGCTAGTCAGGTA GTAAAAGGTCTCAACTAGGCAGTGGCAGAGCAGGATTCAAATTC AGGGCTGTTGTGATGCCTCCGCAGACTCTGAGCGCCACCTGGTG GTAATTTGTCTGTGCCTCTTCTGACGTGGAAGAACAGCAACTAA CACACTAACACGGCATTTACTATGGGCCAGCCATTGT (SEQ ID NO: 144)
SB02665	CAR	*See* SB02661 sequences
SB02665	IL-12	*See* SEQ ID NO: 93
SB02665	IL-15	*See* SEQ ID NO: 141
SB02665	2A peptide	*See* SEQ ID NO: 142

Results

[00682] IL-12 and/or IL-15 payload expression was assessed in T cells for various regulatable TF expression system strategies and constructs. An drug-inducible format ACP (also referred to as "synTF") using an NS3/NS4 protease cleavage site and a VPR transcriptional effector domain (SB01845) or a modified version of the ACP with linkers shortened and the entire construct codon optimized (SB02110) were assessed. All payload constructs (SB02661, SB02662, SB02664, SB02665) encoded a GPC3-CAR driven by an SFFV promoter and all cytokine payloads included an A2 insulator and promoter driving expression in the opposite direction as the CAR cassette (*e.g.*, *see* orientation of reporter and CAR cassettes in construct "2235" in **FIG. 20**). The inducible synTF promoter was either derived from a YB-TATA promoter (SB02661, SB02662) or minTK (SB02664, SB02665). A summary of the systems assessed in shown in **FIG. 32**. SB00171 and SB01335 encoding constitutive hIL-12 and IL-15, respectively, each driven by SFFV were used as controls.

[00683] As shown in **FIG. 33** and quantified in **Table 18**, IL-12 production was observed in a concentration dependent manner following addition of the NS3 protease inhibitor grazoprevir. As shown in **FIG. 34** and quantified in **Table 19**, IL-15 production was observed for constructs encoding IL-15 using a ribosome skipping tag 2A multicistronic system in a concentration dependent manner for the NS3 protease inhibitor grazoprevir, with IL-15 expressed at lower levels relatively to IL-12. Production was notably greater when using ACP construct SB01845 compared to SB02110 for all combinations for both IL-12 and IL-15 (solid vs. dashed **FIG. 33** and **Table 18A** vs **Table 18B**, respectively). In addition, the YB-TATA based inducible promoter demonstrated greater expression than the minTK

promoter (SB02661 vs SB02664 and SB02662 vs SB02665) and less IL-12 was observed when expressed as part of a 2A/IL-15 peptide. Accordingly, regulated cytokine expression was observed in a drug-dependent in T cells for the various systems with ACP construct SB01845 together with SB02661 demonstrating the highest production of IL-12 alone and together with SB02662 IL-12 and IL-15 in a multicistronic system.

[00684] CAR profiles for the various constructs was also assessed. Expression was monitored by flow cytometry (YFP) for the various constructs, with a GPC3-CAR only construct ("1106") as a control. As shown in **FIG. 35**, all cytokine payload constructs expressed the CAR ranging from ~50-70%. As expected, the ACP construct choice did not noticeably affect expression (1845 vs 2110). CAR activity was then assessed through T cell killing. As shown in **FIG. 36** and quantified in **Table 20**, killing (LDH release) was observed and comparable for all constructs tested that expressed the CAR, including the CAR only control, regardless of ACP used (1845 vs 2110). Killing was also assessed in the presence or absence of grazoprevir. No noticeable differences in killing were observed in the presence or absence of grazoprevir, although the assay was not designed to directly test cytokine armoring influence as the assay was performed in the absence of additional immune system components. Accordingly, CAR expression and killing activity was demonstrated for each regulatable TF expression system examined using drug-inducible ACP formats.

Table 18A – IL-12 production with ACP 1845 in T Cells

[GRZ]uM	2357+1845	2661+1845	2662+1845	2664+1845	2665+1845	NV	SB00171
2	7001.8	94155.5	22296.0	48693.2	13746.7		
1	6335.4	98314.1	22220.4	56098.2	15942.5		
0.5	7085.0	119580.0	25885.7	58288.7	16808.8		
0.1	5651.3	91022.2	22464.0	42428.9	16092.4		
0.05	4194.9	72358.4	18026.4	45813.9	13788.3		
0.01	892.5	20383.8	5233.6	21137.9	6018.5		
No drug	32.0	51.2	32.0	2013.4	236.1	12.4	13165.0

Table 18B – IL-12 production with ACP 2110 in T Cells

[GRZ]uM	2357+2110	2661+2110	2662+2110	2664+2110	2665+2110	NV	SB00171
2	1240.1	10790.5	1773.9	7118.3	1567.9		
1	1101.5	12533.9	1893.7	8182.8	1516.3		
0.5	1101.5	11006.3	1910.9	7134.9	1567.9		
0.1	979.7	9785.9	1378.4	5668.0	1636.6		
0.05	892.5	7634.1	1292.0	6585.4	1705.3		
0.01	380.4	3134.1	344.5	3184.8	944.8		
No drug	32.0	32.0	12.4	1378.4	163.0	12.4	13165.0

Table 19 - IL-15 Production in T Cells

[GRZ]uM	2662+2110	2665+2110	2662+1845	2665+1845	NV	SB01335
2	9.03	7.59	78.72	44.40		
1	9.03	6.87	77.32	48.61		
0.5	9.03	6.87	89.91	48.61		
0.1	6.87	6.87	73.83	47.21		
0.05	6.87	7.59	58.43	38.08		
0.01	3.97	5.42	19.01	19.01		
No drug	1.77	1.77	2.51	2.51	12.4	9213

Table 20 – T cell Killing (LDH)

[GRZ]uM	1845 Rep 1	1845 Rep 2	1845 Rep 3	2110 Rep 1	2110 Rep 2	2110 Rep 3
NV	5.57	4.43	5.10			
1106	44.55	37.87	36.82			
2661	53.82	50.67	46.18	51.03	41.96	47.76
2661+Grz	47.90	49.04	38.92	48.13	45.50	46.59
2662	41.59	46.66	42.36	41.60	38.70	41.33
2662+Grz	47.99	48.85	46.85	40.06	38.34	40.15
2664	40.16	47.23	48.09	48.04	46.68	49.94
2664+Grz	45.41	45.99	54.59	47.04	51.84	51.66
2665	36.43	34.62	41.31	41.06	42.96	44.32
2665+Grz	36.72	43.60	49.24	39.88	42.60	44.50

Example 11: IL-12 Drug-inducible Expression Systems in NK Cells

[00685] IL-12 payload expression was assessed in NK cells for various regulatable TF expression system strategies. The strategies included co-expression of CAR constructs and CAR expression was assessed.

Materials and Methods

[00686] For *in vitro* assessment, starting on Day 0, NK cells (iCD3- CD56 cells isolated from health donor PBMCs) were expanded for 10 days with mitoC WT K562 cell, 500 U/ml rhIL-2, and 10 ng/ml rhIL-15. Day 10, cells were spun down in a large volume of PBS then resuspended in NK MACS medium (Miltenyi) without serum or supplements at 10e6/mL concentration. Cells were then seeded at 1e6 cells (100ul) cells + 1ul of 1:10 BX795 (Tocris Bioscience Cat #4318) for 30mins in a 48-well retronectin coated non TC treated plate then virus added at an MOI = 25 (IU) (MOI = 12.5 for each virus for each of the constructs indicated; relevant sequences of the constructs are provided in **Table 17**) followed by 200ul of NK MACS media (no serum/supplements). The cells and virus were then spinoculated (800g 2hrs at 32C, rest the plate for 2hrs at 37C incubator and transfer cells to a 24-well Grex

plate in complete NK MACS media + 500U/mL of rhIL-2). On Day 14, media was partially exchanged removing 5ml media and supplemented with fresh media + rhIL-2 (500U/mL). On Day 17, cells were counted and the required number of cells were spun down in fresh complete media + 500U/mL rhIL2. Cells were seeded at 0.2e6 cells/200ul/well in a U-bottom 96 well-plate in increasing concentration of Grazoprevir (1, 0.1, 0.01, 0.01µM GRZ and no drug). On Days 12, 19, and 21 (24, 48, 96 hours, respectively), cells were transferred to a V-bottom plate, spun down, and supernatant saved for analysis using Luminex (IL-12 Milliplex kit). Cells were also assessed for CAR expression by flow cytometry.

Results

[00687] IL-12 payload expression was assessed in NK cells for various regulatable TF expression system strategies and constructs. A drug-inducible format ACP (also referred to as "synTF") using an NS3/NS4 protease cleavage site and a VPR transcriptional effector domain (SB01845) or a modified version of the ACP with linkers shortened and the entire construct codon optimized (SB02110) were assessed. The payload construct SB02661 encoded a GPC3-CAR driven by an SFFV promoter, with the IL-12 cytokine payload encoded in a cassette including an A2 insulator and YB-TATA promoter driving expression in the opposite direction as the CAR cassette (*e.g., see* orientation of reporter and CAR cassettes in construct "2235" in **FIG. 20**).

[00688] As shown in **FIG. 37** and quantified in **Table 21**, IL-12 production was observed in a concentration dependent manner following addition of the NS3 protease inhibitor grazoprevir starting at 24 hours after treatment. IL-12 levels were similar between hours 48 and 96, suggesting production peaked at least 48 hours. Production was also notably greater when using ACP construct SB01845 compared to SB02110 for all combinations for both IL-12 and IL-15 (square vs. circle **FIG. 37**, respectively, and **Table 21**). CAR expression was also monitored by flow cytometry (YFP) with low overall YFP expression detected (data not shown). Accordingly, regulated cytokine expression was observed in a drug-dependent manner in NK cells for the various systems with ACP construct SB01845 together, with construct SB02661 demonstrating the highest production of IL-12.

Table 21 - IL-12 Production in NK Cells (24hr)

[GRZ]uM	2661+2110 (24hr)	2661+1845 (24hr)	2661+2110 (48hr)	2661+1845 (48hr)	2661+2110 (96hr)	2661+1845 (96hr)
1	94.72	209.46	206.33	419.42	269.83	384.29
0.1	61.30	89.49	220.91	365.22	129.36	332.50

0.01	4.88	4.88	18.42	26.82	26.57	24.90
0.001	4.88	4.88	0.00	0.00	4.18	4.53
No drug	4.88	4.88	0.00	0.00	0.79	8.36

Example 12: Additional IL-12 and IL-15 Drug-inducible Systems in T Cells

[00689] IL-12 and/or IL-15 payload expression was assessed in T cells for additional various regulatable TF expression system strategies, including assessment of different transcription activators and production of cytokines encoded separately or in multicistronic systems.

Materials and Methods

[00690] For *in vitro* assessment, on Day 0, CD4/CD8 T cells (donor derived) were thawed, seeded at 1e6 cells/mL/well in a 24 well-plate, and activated with anti-CD3/CD28 Dynabeads (3:1 bead to cell) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + rhIL-2 (100 Units/ml; Peprotech). On Day 1, 0.5ml media was removed and cells were transduced with 3e5 pg of virus for each the constructs indicated (relevant sequences of the constructs are provided in **Table 22**). On Day 2, 1.5mL of Optimizer media + 100U/mL rhIL-2 was added. Day 4 cells were counted and 1e6 cells/well were transferred to a 24 well Grex plate at a final volume of 8mL. On Day 8, 6mL of media was removed from the wells and cells counted then seeded at a concentration of 1e6 cells/mL in fresh media with grazoprevir (2, 1, 0.5, 0.1, 0.05, 0.01µM and no drug). Transduced T cells were incubated for a further two days and the supernatant collected via centrifugation for IL-12 and IL-15 quantification. For IL-12 and IL-15, supernatants analyzed by Luminex (R&D IL12/IL15 kit).

Table 22 – Additional IL-12 and IL-15 Regulatable TF Expression Constructs

SB #	Domain	Sequence
SB02667	p65-based synTF	MSRPGERPFQCRICMRNFSRRHGLDRHTRTHTGEKPFQCRICMRNFS DHSSLKRHLRTHTGSQKPFQCRICMRNFSVRHNLTRHLRTHTGEKPF QCRICMRNFSDHSNLSRHLKTHTGSQKPFQCRICMRNFSQRSSLVRH LRTHTGEKPFQCRICMRNFSESGHLKRHLRTHLRGSTCRDYKDHDG DYKDHDIDYKDDDDKMAPKKKRKVGIHGVPGGLEGGGGSGGTED VVCCHSIYGKKKGDIDTYRYIGSSGTGCVVIVGRIVLSGSGTSAPITA YAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTATQTFLATCINGVC WAVYHGAGTRTIASPKGPVIQMYTNVDQDLVGWPAPQGSRSLTPC TCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLLC PAGHAVGLFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPP AVTLTHPITKIDREVLYQEFDEMEECSQHYPYDVPDYAGGGGSGGT DEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAP

		APVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALL GNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAIT RLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLSQI SSQL (SEQ ID NO: 145)
SB02667	IL-12	*See* SEQ ID NO: 93
SB02667	insulator + inducible YB-TATA promoter IL12 inverted strand, forward strand SFFV synTF-p65	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccaccccccagaataga atgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttcca gggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagttaACT GGCGTTCAGGTAGGACATCACGCGGTCAATGGTCACGGCTCGAA TGCGGAAAGCGTGCAACAGGATGCACAGCTTGATCTTGGTCTTGT AGAAGTCCGGTTCTTCCAGGCTGGACTTTTGAGGCACAGTCTCGG AGTTGAAATTCAGGGCCTGCATCAGTTCATCAATCACGGCGAGC ATATTCTGGTCCAGGAAGATCTGCCGCTTCGGGTCCATGAGCAGC TTGGCGTTCATGGTCTTGAACTCGACCTGATACATCTTCAGATCTT CGTAGATCGAGGAAAGACAGAGCGCCATCATGAATGAGGTCTTT CTCGACGCCAGGCAGCTGCCGTTAGTGATAAAGCTTGTCTCGCGG GAGTTCAGACACGATTCGTTCTTGGTCAGTTCCAGCGGCAGGCAG GCTTCCACGGTCGAGGTCTTGTCCTTGGTGATGTCCTCGTGATCA ATTTCTTCCGAGGTGCAGGGGTAGAACTCAAGGGTCTGGCGGGC CTTCTGCAACATGTTCGACACAGCCCTCAGGAGGTTTTGGGAGTG GTGTAGGCACGGGAACATTCCAGGGTCGGGGGTTGCCACAGGGA GGTTCCGGGAACCTCCTCCGGAGCCTCCTCCTGAACCTCCGCCTG ATCCGCCACCGGAACAAGGCACGCTGGCCCATTCGCTCCAGGAG GACGAGTAGTATCTATCCTGCGCCCGGACGCTGATTGACGCGTTC TTCCGACAAATCACAGTGGCGGAGGTTTTGTCGGTGAACACCCG GTCTTTCTTCTCCCGTTTGGACTTTCCCTGCACTTGCACACAGAAA GTGAGCGAGAAGTATGAGTGCGGGGTGCTCCAAGTGTCTGGATA TTCCCAAGACACTTCCACTTGGCGGGAGTTCTTGAGTGGCTTCAG CTGCAAGTTCTTGGGGGGGTCAGGCTTGATGATGTCGCGGATAA AGAAGGAGGAAGTGTAGTTCTCGTATTTCAGCTTATGCACGGCAT CGACCATGACCTCGATAGGCAGGGACTCTTCCGCGGCAGGGCAG GCGCTGTCCTCCTGGCATTCCACGGAGTACTCATATTCCTTGTTGT CTCCCCTGACTCTCTCGGCGGACAGAGTGGCGGCTCCACAGGTCA CGCCCTGAGGATCGCTTGATCCCCGTGACGACTTCACGGAGAAA GTCAGGTCGGTGGAGATTGTCGTCAGCCACCAACAGGTGAACCG ACCGCTGTAGTTCTTGGCTTCGCAGCGGAGGAAGGTCTTGTTCTT CGGTTCTTTTTGGTCCTTGAGGATGTCAGTGGACCAGATTCCATC CTCTTTCTTGTGCAGCAGCAGCAGGGAGTGGGACAGCACTTCGCC ACCCTTGTGGCAAGTGTACTGGCCCGCGTCGCCGAACTCCTTGAC TTGAATGGTCAGGGTCTTTCCGCTTCCGAGCACCTCGGAGCTCTG ATCCAGGGTCCAGGTTATGCCGTCCTCTTCTGGCGTATCGCAAGT CAGCACGACCATTTCTCCAGGGGCGTCCGGGTACCAATCCAGCTC GACCACGTAGACGTCCTTCTTCAGTTCCCAAATGGCGACCAGAGG GGAAGCGAGGAACACAAGGGAGAACCAGGAGATGACGAGTTGC TGATGGCACATCATGGTGGCGACACCGGTACGCGTTGGCCCCCAT TATATACCCTCTAGAACTAGTtatccactccgtgtaagggagagtgagcctcttacgaat gCGCGACATCGGCTACGCCgttccgaggcgactgatacgCGCGACATCGGCT ACGCCgtacgaaggcagtccgattgCGCGACATCGGCTACGCCgacctttactgagac gggagCGCGACATCGGCTACGCCgaaggcgttgcgaatcctcatgcgattgttacgaaacc cgTTAATTAAAGAGCGAGATTCCGTCTCAAAGAAAAAAAAAGTAA TGAAATGAATAAAATGAGTCCTAGAGCCAGTAAATGTCGTAAAT GTCTCAGCTAGTCAGGTAGTAAAAGGTCTCAACTAGGCAGTGGC AGAGCAGGATTCAAATTCAGGGCTGTTGTGATGCCTCCGCAGACT CTGAGCGCCACCTGGTGGTAATTTGTCTGTGCCTCTTCTGACGTG

		GAAGAACAGCAACTAACACACTAACACGGCATTTACTATGGGCC AGCCATTGTCCATCTAGATGGccgataaaataaaagattttatttagtctccagaaaaagg ggggaatgaaagaccccacctgtaggtttggcaagctagctgcagtaacgccattttgcaaggcatggaaa aataccaaaccaagaatagagaagttcagatcaagggcgggtacatgaaaatagctaacgttgggccaaac aggatatctgcggtgagcagtttcggccccggcccggggccaagaacagatggtcaccgcagtttcggcc ccggcccgaggccaagaacagatggtccccagatatggcccaaccctcagcagtttcttaagacccatcag atgtttccaggctcccccaaggacctgaaatgaccctgcgccttatttgaattaaccaatcagcctgcttctcgc ttctgttcgcgcgcttctgcttcccgagctctataaaagagctcacaacccctcactcggcgcgccagtcctcc gacagactgagtcgcccgggGGATCCGCCACCATGAGTAGACCTGGCGAAA GACCATTTCAGTGCAGAATTTGTATGCGGAACTTCAGCAGAAGG CACGGCCTGGACAGACACACCAGAACACACACAGGCGAGAAGC CTTTCCAGTGTAGAATCTGTATGCGCAATTTCAGCGACCACAGCA GCCTGAAGCGGCACCTGAGAACCCATACCGGCAGCCAGAAACCA TTTCAATGCCGCATCTGTATGAGAAACTTCTCCGTGCGGCACAAC CTGACCAGACACCTGAGGACACACACCGGGGAGAAACCCTTCCA GTGCCGGATATGCATGAGGAATTTCTCCGACCACTCCAACCTGAG CCGCCACCTGAAAACTCACACCGGCTCTCAAAAGCCATTTCAGTG TCGTATATGTATGCGGAATTTTTCCCAGCGGAGCAGCCTCGTGCG CCATCTGAGGACTCATACTGGCGAAAAGCCCTTCCAATGTCGCAT ATGCATGCGCAACTTTAGCGAGTCCGGCCACCTGAAGAGACATC TGCGGACACACCTGAGAGGCAGCACCTGTAGAGACTACAAGGAC CACGACGGCGATTATAAGGATCACGACATCGACTACAAAGACGA CGATGACAAGATGGCCCCTAAGAAGAAGCGGAAAGTCGGCATCC ATGGCGTGCCAGGTGGACTTGAAGGTGGCGGAGGATCTGGCGGC ACAGAGGATGTTGTGTGCTGCCACAGCATCTACGGCAAGAAGAA GGGCGACATCGATACCTATCGGTACATCGGCAGCAGCGGCACAG GCTGCGTTGTGATCGTGGGAAGAATCGTGCTGAGCGGCTCTGGC ACAAGCGCCCCTATTACAGCCTACGCTCAGCAGACAAGAGGCCT GCTGGGCTGCATCATCACAAGCCTGACCGGCAGAGACAAGAACC AGGTGGAAGGCGAGGTGCAGATCGTGTCTACAGCTACCCAGACC TTCCTGGCCACCTGTATCAATGGCGTGTGCTGGGCCGTGTATCAC GGCGCTGGAACCAGAACAATCGCCTCTCCTAAGGGACCCGTGAT CCAGATGTACACCAACGTGGACCAGGACCTCGTTGGCTGGCCTG CTCCTCAGGGAAGTAGAAGCCTGACACCTTGCACCTGTGGCTCCA GCGATCTGTACCTGGTCACCAGACACGCCGACGTGATCCCTGTCA GAAGAAGAGGAGATTCCAGAGGCAGCCTGCTGAGCCCTAGACCT ATCAGCTACCTGAAGGGCAGCTCTGGCGGACCTCTGCTTTGTCCT GCTGGACATGCCGTGGGCCTGTTTAGAGCCGCCGTGTGTACAAG AGGCGTGGCAAAGGCCGTGGACTTCATCCCCGTGGAAAACCTGG AAACCACCATGCGGAGCCCCGTGTTCACCGACAATTCTAGCCCTC CAGCCGTGACACTGACACACCCCATCACCAAGATCGACAGAGAG GTGCTGTACCAAGAGTTCGACGAGATGGAAGAGTGCAGCCAGCA CTACCCCTACGACGTGCCAGATTATGCTGGCGGAGGTGGCAGCG GAGGCACCGATGAATTTCCAACCATGGTGTTCCCCAGCGGCCAG ATCTCTCAGGCATCTGCTCTTGCTCCAGCTCCACCTCAGGTTCTGC CTCAAGCTCCTGCTCCGGCTCCTGCACCAGCTATGGTGTCTGCAC TTGCTCAGGCACCAGCTCCAGTGCCTGTTCTTGCTCCTGGACCTC CTCAGGCTGTTGCTCCACCAGCACCTAAACCTACACAGGCCGGCG AGGGAACACTGTCTGAAGCCCTGCTGCAACTCCAGTTCGATGAC GAAGATCTGGGCGCCCTGCTGGGAAACTCTACAGATCCTGCCGT GTTTACCGATCTGGCCAGCGTGGACAACAGCGAGTTTCAGCAGCT CCTGAACCAGGGCATCCCAGTGGCTCCTCACACCACAGAGCCTAT GCTGATGGAATACCCCGAGGCCATCACCAGACTGGTCACCGGCG CACAAAGACCACCTGATCCTGCTCCAGCACCGCTTGGAGCACCTG GACTGCCTAATGGACTGCTGTCTGGCGACGAGGACTTCAGTTCTA

		TCGCTGATATGGATTTCTCTGCCCTTCTGTCTCAGATTAGTAGCCA GCTGTAA (SEQ ID NO: 146)
SB02668	p65-based synTF	*See* SEQ ID NO: 145
SB02668	IL-12	*See* SEQ ID NO: 93
SB02668	IL-15	*See* SEQ ID NO: 141
SB02668	2A peptide	*See* SEQ ID NO: 142
SB02668	insulator + inducible YB-TATA promoter IL12_2A_IL-15 inverted strand, forward strand SFFV synTF-p65	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccaccccccagaataga atgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttcca gggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagttaGCT GGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGCAGGA ACTCTTTGATATTCTTTTCTTCCAGTTCCTCGCATTCTTTGCAGCC GGACTCGGTCACATTGCCGTTGGAGGACAGGCTGTTGTTGGCCAG GATGATCAGGTTTTCCACGGTATCGTGGATGGAGGCGTCGCCGCT TTCCAGGCTGATCACTTGCAGCTCGAGCAGAAAGCACTTCATGGC GGTCACTTTACAGCTAGGGTGCACGTCGCTCTCGGTGTACAGTGT GGCGTCGATGTGCATGCTCTGGATCAGATCCTCGATCTTCTTCAG ATCGCTGATCACGTTGACCCAGTTGCCTGTAGAGCCGGGCACCCA AAGAAGCAGCACCCACAGCAGCAGTGTGTCGGTTTCCATAGGTC CAGGGTTTTCCTCCACGTCTCCACACGTCAGTAGACTCCCGCGTC CCTCGCCAGATCCTCTCTTCCGTCTACTGGCGTTCAGGTAGGACA TCACGCGGTCAATGGTCACGGCTCGAATGCGGAAAGCGTGCAAC AGGATGCACAGCTTGATCTTGGTCTTGTAGAAGTCCGGTTCTTCC AGGCTGGACTTTTGAGGCACAGTCTCGGAGTTGAAATTCAGGGC CTGCATCAGTTCATCAATCACGGCGAGCATATTCTGGTCCAGGAA GATCTGCCGCTTCGGGTCCATGAGCAGCTTGGCGTTCATGGTCTT GAACTCGACCTGATACATCTTCAGATCTTCGTAGATCGAGGAAAG ACAGAGCGCCATCATGAATGAGGTCTTTCTCGACGCCAGGCAGC TGCCGTTAGTGATAAAGCTTGTCTCGCGGGAGTTCAGACACGATT CGTTCTTGGTCAGTTCCAGCGGCAGGCAGGCTTCCACGGTCGAGG TCTTGTCCTTGGTGATGTCCTCGTGATCAATTTCTTCCGAGGTGCA GGGGTAGAACTCAAGGGTCTGGCGGGCCTTCTGCAACATGTTCG ACACAGCCCTCAGGAGGTTTTGGGAGTGGTGTAGGCACGGGAAC ATTCCAGGGTCGGGGGTTGCCACAGGGAGGTTCCGGGAACCTCC TCCGGAGCCTCCTCCTGAACCTCCGCCTGATCCGCCACCGGAACA AGGCACGCTGGCCCATTCGCTCCAGGAGGACGAGTAGTATCTAT CCTGCGCCCGGACGCTGATTGACGCGTTCTTCCGACAAATCACAG TGGCGGAGGTTTTGTCGGTGAACACCCGGTCTTTCTTCTCCCGTTT GGACTTTCCCTGCACTTGCACACAGAAAGTGAGCGAGAAGTATG AGTGCGGGGTGCTCCAAGTGTCTGGATATTCCCAAGACACTTCCA CTTGGCGGGAGTTCTTGAGTGGCTTCAGCTGCAAGTTCTTGGGGG GGTCAGGCTTGATGATGTCGCGGATAAAGAAGGAGGAAGTGTAG TTCTCGTATTTCAGCTTATGCACGGCATCGACCATGACCTCGATA GGCAGGGACTCTTCCGCGGCAGGGCAGGCGCTGTCCTCCTGGCA TTCCACGGAGTACTCATATTCCTTGTTGTCTCCCCTGACTCTCTCG GCGGACAGAGTGGCGGCTCCACAGGTCACGCCCTGAGGATCGCT TGATCCCCGTGACGACTTCACGGAGAAAGTCAGGTCGGTGGAGA TTGTCGTCAGCCACCAACAGGTGAACCGACCGCTGTAGTTCTTGG CTTCGCAGCGGAGGAAGGTCTTGTTCTTCGGTTCTTTTTGGTCCTT GAGGATGTCAGTGGACCAGATTCCATCCTCTTTCTTGTGCAGCAG CAGCAGGGAGTGGGACAGCACTTCGCCACCCTTGTGGCAAGTGT ACTGGCCCGCGTCGCCGAACTCCTTGACTTGAATGGTCAGGGTCT TTCCGCTTCCGAGCACCTCGGAGCTCTGATCCAGGGTCCAGGTTA TGCCGTCCTCTTCTGGCGTATCGCAAGTCAGCACGACCATTTCTC

		CAGGGGCGTCCGGGTACCAATCCAGCTCGACCACGTAGACGTCC TTCTTCAGTTCCCAAATGGCGACCAGAGGGGAAGCGAGGAACAC AAGGGAGAACCAGGAGATGACGAGTTGCTGATGGCACATCATGG TGGCGACACCGGTACGCGTTGGCCCCCATTATATACCCTCTAGAA CTAGTtatccactccgtgtaagggagagtgagcctcttacgaatgCGCGACATCGGCTAC GCCgttccgaggcgactgatacgCGCGACATCGGCTACGCCgtacgaaggcagtccga ttgCGCGACATCGGCTACGCCgacctttactgagacgggagCGCGACATCGGC TACGCCgaaggcgttgcgaatcctcatgcgattgttacgaaacccgTTAATTAAAGAGCG AGATTCCGTCTCAAAGAAAAAAAAAGTAATGAAATGAATAAAAT GAGTCCTAGAGCCAGTAAATGTCGTAAATGTCTCAGCTAGTCAG GTAGTAAAAGGTCTCAACTAGGCAGTGGCAGAGCAGGATTCAAA TTCAGGGCTGTTGTGATGCCTCCGCAGACTCTGAGCGCCACCTGG TGGTAATTTGTCTGTGCCTCTTCTGACGTGGAAGAACAGCAACTA ACACACTAACACGGCATTTACTATGGGCCAGCCATTGTCCATCTA GATGGccgataaaataaaagattttatttagtctccagaaaaaggggggaatgaaagaccccacctgta ggtttggcaagctagctgcagtaacgccattttgcaaggcatggaaaaataccaaaccaagaatagagaagt tcagatcaagggcgggtacatgaaaatagctaacgttgggccaaacaggatatctgcggtgagcagtttcg gccccggcccggggccaagaacagatggtcaccgcagtttcggccccggcccgaggccaagaacagat ggtccccagatatggcccaaccctcagcagtttcttaagacccatcagatgtttccaggctcccccaaggacc tgaaatgaccctgcgccttatttgaattaaccaatcagcctgcttctcgcttctgttcgcgcgcttctgcttcccg agctctataaaagagctcacaacccctcactcggcgcgccagtcctccgacagactgagtcgcccgggG GATCCGCCACCATGAGTAGACCTGGCGAAAGACCATTTCAGTGC AGAATTTGTATGCGGAACTTCAGCAGAAGGCACGGCCTGGACAG ACACACCAGAACACACACAGGCGAGAAGCCTTTCCAGTGTAGAA TCTGTATGCGCAATTTCAGCGACCACAGCAGCCTGAAGCGGCAC CTGAGAACCCATACCGGCAGCCAGAAACCATTTCAATGCCGCAT CTGTATGAGAAACTTCTCCGTGCGGCACAACCTGACCAGACACCT GAGGACACACACCGGGGAGAAACCCTTCCAGTGCCGGATATGCA TGAGGAATTTCTCCGACCACTCCAACCTGAGCCGCCACCTGAAAA CTCACACCGGCTCTCAAAAGCCATTTCAGTGTCGTATATGTATGC GGAATTTTTCCCAGCGGAGCAGCCTCGTGCGCCATCTGAGGACTC ATACTGGCGAAAAGCCCTTCCAATGTCGCATATGCATGCGCAACT TTAGCGAGTCCGGCCACCTGAAGAGACATCTGCGGACACACCTG AGAGGCAGCACCTGTAGAGACTACAAGGACCACGACGGCGATTA TAAGGATCACGACATCGACTACAAAGACGACGATGACAAGATGG CCCCTAAGAAGAAGCGGAAAGTCGGCATCCATGGCGTGCCAGGT GGACTTGAAGGTGGCGGAGGATCTGGCGGCACAGAGGATGTTGT GTGCTGCCACAGCATCTACGGCAAGAAGAAGGGCGACATCGATA CCTATCGGTACATCGGCAGCAGCGGCACAGGCTGCGTTGTGATC GTGGGAAGAATCGTGCTGAGCGGCTCTGGCACAAGCGCCCCTAT TACAGCCTACGCTCAGCAGACAAGAGGCCTGCTGGGCTGCATCA TCACAAGCCTGACCGGCAGAGACAAGAACCAGGTGGAAGGCGA GGTGCAGATCGTGTCTACAGCTACCCAGACCTTCCTGGCCACCTG TATCAATGGCGTGTGCTGGGCCGTGTATCACGGCGCTGGAACCA GAACAATCGCCTCTCCTAAGGGACCCGTGATCCAGATGTACACC AACGTGGACCAGGACCTCGTTGGCTGGCCTGCTCCTCAGGGAAG TAGAAGCCTGACACCTTGCACCTGTGGCTCCAGCGATCTGTACCT GGTCACCAGACACGCCGACGTGATCCCTGTCAGAAGAAGAGGAG ATTCCAGAGGCAGCCTGCTGAGCCCTAGACCTATCAGCTACCTGA AGGGCAGCTCTGGCGGACCTCTGCTTTGTCCTGCTGGACATGCCG TGGGCCTGTTTAGAGCCGCCGTGTGTACAAGAGGCGTGGCAAAG GCCGTGGACTTCATCCCCGTGGAAAACCTGGAAACCACCATGCG GAGCCCCGTGTTCACCGACAATTCTAGCCCTCCAGCCGTGACACT GACACACCCCATCACCAAGATCGACAGAGAGGTGCTGTACCAAG AGTTCGACGAGATGGAAGAGTGCAGCCAGCACTACCCCTACGAC

		GTGCCAGATTATGCTGGCGGAGGTGGCAGCGGAGGCACCGATGA ATTTCCAACCATGGTGTTCCCCAGCGGCCAGATCTCTCAGGCATC TGCTCTTGCTCCAGCTCCACCTCAGGTTCTGCCTCAAGCTCCTGCT CCGGCTCCTGCACCAGCTATGGTGTCTGCACTTGCTCAGGCACCA GCTCCAGTGCCTGTTCTTGCTCCTGGACCTCCTCAGGCTGTTGCTC CACCAGCACCTAAACCTACACAGGCCGGCGAGGGAACACTGTCT GAAGCCCTGCTGCAACTCCAGTTCGATGACGAAGATCTGGGCGC CCTGCTGGGAAACTCTACAGATCCTGCCGTGTTTACCGATCTGGC CAGCGTGGACAACAGCGAGTTTCAGCAGCTCCTGAACCAGGGCA TCCCAGTGGCTCCTCACACCACAGAGCCTATGCTGATGGAATACC CCGAGGCCATCACCAGACTGGTCACCGGCGCACAAAGACCACCT GATCCTGCTCCAGCACCGCTTGGAGCACCTGGACTGCCTAATGGA CTGCTGTCTGGCGACGAGGACTTCAGTTCTATCGCTGATATGGAT TTCTCTGCCCTTCTGTCTCAGATTAGTAGCCAGCTGTAA (SEQ ID NO: 177)
SB02670	VPR-based synTF	MSRPGERPFQCRICMRNFSRRHGLDRHTRTHTGEKPFQCRICMRNFS DHSSLKRHLRTHTGSQKPFQCRICMRNFSVRHNLTRHLRTHTGEKPF QCRICMRNFSDHSNLSRHLKTHTGSQKPFQCRICMRNFSQRSSLVRH LRTHTGEKPFQCRICMRNFSESGHLKRHLRTHLRGSTCRDYKDHDG DYKDHDIDYKDDDDKMAPKKKRKVGIHGVPGGLEGGGGSGGTED VVCCHSIYGKKKGDIDTYRYIGSSGTGCVVIVGRIVLSGSGTSAPITA YAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTATQTFLATCINGVC WAVYHGAGTRTIASPKGPVIQMYTNVDQDLVGWPAPQGSRSLTPC TCGSSDLYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLLC PAGHAVGLFRAAVCTRGVAKAVDFIPVENLETTMRSPVFTDNSSPP AVTLTHPITKIDREVLYQEFDEMEECSQHYPYDVPDYAGGGGSGGT EASGSGRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDML GSDALDDFDLDMLINSRSSGSPKKKRKVGSQYLPDTDDRHRIEEKR KRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPF TSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAM VSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFD DEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPML MEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM DFSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEGREVCQPKRIRPF HPPGSPWANRPLPASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTP EASHLLEDPDEETSQAVKALREMADTVIPQKEEAAICGQMDLSHPPP RGHLDELTTTLESMTEDLNLDSPLTPELNEILDTFLNDECLLHAMHIS TGLSIFDTSLF (SEQ ID NO: 147)
SB02670	IL-12	*See* SEQ ID NO: 93
SB02670	insulator + inducible YB-TATA promoter IL12 inverted strand, forward strand SFFV synTF-VPR	tccccagcatgcctgctattctcttcccaatcctccccccttgctgtcctgccccacccccacccccccagaatagaa atgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttcca gggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagttaACT GGCGTTCAGGTAGGACATCACGCGGTCAATGGTCACGGCTCGAA TGCGGAAAGCGTGCAACAGGATGCACAGCTTGATCTTGGTCTTGT AGAAGTCCGGTTCTTCCAGGCTGGACTTTTGAGGCACAGTCTCGG AGTTGAAATTCAGGGCCTGCATCAGTTCATCAATCACGGCGAGC ATATTCTGGTCCAGGAAGATCTGCCGCTTCGGGTCCATGAGCAGC TTGGCGTTCATGGTCTTGAACTCGACCTGATACATCTTCAGATCTT CGTAGATCGAGGAAAGACAGAGCGCCATCATGAATGAGGTCTTT CTCGACGCCAGGCAGCTGCCGTTAGTGATAAAGCTTGTCTCGCGG GAGTTCAGACACGATTCGTTCTTGGTCAGTTCCAGCGGCAGGCAG GCTTCCACGGTCGAGGTCTTGTCCTTGGTGATGTCCTCGTGATCA ATTTCTTCCGAGGTGCAGGGGTAGAACTCAAGGGTCTGGCGGGC CTTCTGCAACATGTTCGACACAGCCCTCAGGAGGTTTTGGGAGTG

		GTGTAGGCACGGGAACATTCCAGGGTCGGGGGTTGCCACAGGGA GGTTCCGGGAACCTCCTCCGGAGCCTCCTCCTGAACCTCCGCCTG ATCCGCCACCGGAACAAGGCACGCTGGCCCATTCGCTCCAGGAG GACGAGTAGTATCTATCCTGCGCCCGGACGCTGATTGACGCGTTC TTCCGACAAATCACAGTGGCGGAGGTTTTGTCGGTGAACACCCG GTCTTTCTTCTCCCGTTTGGACTTTCCCTGCACTTGCACACAGAAA GTGAGCGAGAAGTATGAGTGCGGGGTGCTCCAAGTGTCTGGATA TTCCCAAGACACTTCCACTTGGCGGGAGTTCTTGAGTGGCTTCAG CTGCAAGTTCTTGGGGGGGTCAGGCTTGATGATGTCGCGGATAA AGAAGGAGGAAGTGTAGTTCTCGTATTTCAGCTTATGCACGGCAT CGACCATGACCTCGATAGGCAGGGACTCTTCCGCGGCAGGGCAG GCGCTGTCCTCCTGGCATTCCACGGAGTACTCATATTCCTTGTTGT CTCCCCTGACTCTCTCGGCGGACAGAGTGGCGGCTCCACAGGTCA CGCCCTGAGGATCGCTTGATCCCCGTGACGACTTCACGGAGAAA GTCAGGTCGGTGGAGATTGTCGTCAGCCACCAACAGGTGAACCG ACCGCTGTAGTTCTTGGCTTCGCAGCGGAGGAAGGTCTTGTTCTT CGGTTCTTTTTGGTCCTTGAGGATGTCAGTGGACCAGATTCCATC CTCTTTCTTGTGCAGCAGCAGCAGGGAGTGGGACAGCACTTCGCC ACCCTTGTGGCAAGTGTACTGGCCCGCGTCGCCGAACTCCTTGAC TTGAATGGTCAGGGTCTTTCCGCTTCCGAGCACCTCGGAGCTCTG ATCCAGGGTCCAGGTTATGCCGTCCTCTTCTGGCGTATCGCAAGT CAGCACGACCATTTCTCCAGGGGCGTCCGGGTACCAATCCAGCTC GACCACGTAGACGTCCTTCTTCAGTTCCCAAATGGCGACCAGAGG GGAAGCGAGGAACACAAGGGAGAACCAGGAGATGACGAGTTGC TGATGGCACATCATGGTGGCGACACCGGTACGCGTTGGCCCCCAT TATATACCCTCTAGAACTAGTtatccactccgtgtaagggagagtgagcctcttacgaat gCGCGACATCGGCTACGCCgttccgaggcgactgatacgCGCGACATCGGCT ACGCCgtacgaaggcagtccgattgCGCGACATCGGCTACGCCgacctttactgagac gggagCGCGACATCGGCTACGCCgaaggcgttgcgaatcctcatgcgattgttacgaaacc cgTTAATTAAAGAGCGAGATTCCGTCTCAAAGAAAAAAAAAGTAA TGAAATGAATAAAATGAGTCCTAGAGCCAGTAAATGTCGTAAAT GTCTCAGCTAGTCAGGTAGTAAAAGGTCTCAACTAGGCAGTGGC AGAGCAGGATTCAAATTCAGGGCTGTTGTGATGCCTCCGCAGACT CTGAGCGCCACCTGGTGGTAATTTGTCTGTGCCTCTTCTGACGTG GAAGAACAGCAACTAACACACTAACACGGCATTTACTATGGGCC AGCCATTGTCCATCTAGATGGccgataaaataaaagattttatttagtctccagaaaaagg ggggaatgaaagaccccacctgtaggtttggcaagctagctgcagtaacgccattttgcaaggcatggaaa aataccaaaccaagaatagagaagttcagatcaagggcgggtacatgaaaatagctaacgttgggccaaac aggatatctgcggtgagcagtttcggccccggcccggggccaagaacagatggtcaccgcagtttcggcc ccggcccgaggccaagaacagatggtccccagatatggcccaaccctcagcagtttcttaagacccatcag atgtttccaggctcccccaaggacctgaaatgaccctgcgccttatttgaattaaccaatcagcctgcttctcgc ttctgttcgcgcgcttctgcttcccgagctctataaaagagctcacaacccctcactcggcgcgccagtcctcc gacagactgagtcgcccgggGGATCCGCCACCATGAGTAGACCTGGCGAAA GACCATTTCAGTGCAGAATTTGTATGCGGAACTTCAGCAGAAGG CACGGCCTGGACAGACACACCAGAACACACACAGGCGAGAAGC CTTTCCAGTGTAGAATCTGTATGCGCAATTTCAGCGACCACAGCA GCCTGAAGCGGCACCTGAGAACCCATACCGGCAGCCAGAAACCA TTTCAATGCCGCATCTGTATGAGAAACTTCTCCGTGCGGCACAAC CTGACCAGACACCTGAGGACACACACCGGGGAGAAACCCTTCCA GTGCCGGATATGCATGAGGAATTTCTCCGACCACTCCAACCTGAG CCGCCACCTGAAAACTCACACCGGCTCTCAAAAGCCATTTCAGTG TCGTATATGTATGCGGAATTTTTCCCAGCGGAGCAGCCTCGTGCG CCATCTGAGGACTCATACTGGCGAAAAGCCCTTCCAATGTCGCAT ATGCATGCGCAACTTTAGCGAGTCCGGCCACCTGAAGAGACATC TGCGGACACACCTGAGAGGCAGCACCTGTAGAGACTACAAGGAC

		CACGACGGCGATTATAAGGATCACGACATCGACTACAAAGACGA CGATGACAAGATGGCCCCTAAGAAGAAGCGGAAAGTCGGCATCC ATGGCGTGCCAGGTGGACTTGAAGGTGGCGGAGGATCTGGCGGC ACAGAGGATGTTGTGTGCTGCCACAGCATCTACGGCAAGAAGAA GGGCGACATCGATACCTATCGGTACATCGGCAGCAGCGGCACAG GCTGCGTTGTGATCGTGGGAAGAATCGTGCTGAGCGGCTCTGGC ACAAGCGCCCCTATTACAGCCTACGCTCAGCAGACAAGAGGCCT GCTGGGCTGCATCATCACAAGCCTGACCGGCAGAGACAAGAACC AGGTGGAAGGCGAGGTGCAGATCGTGTCTACAGCTACCCAGACC TTCCTGGCCACCTGTATCAATGGCGTGTGCTGGGCCGTGTATCAC GGCGCTGGAACCAGAACAATCGCCTCTCCTAAGGGACCCGTGAT CCAGATGTACACCAACGTGGACCAGGACCTCGTTGGCTGGCCTG CTCCTCAGGGAAGTAGAAGCCTGACACCTTGCACCTGTGGCTCCA GCGATCTGTACCTGGTCACCAGACACGCCGACGTGATCCCTGTCA GAAGAAGAGGAGATTCCAGAGGCAGCCTGCTGAGCCCTAGACCT ATCAGCTACCTGAAGGGCAGCTCTGGCGGACCTCTGCTTTGTCCT GCTGGACATGCCGTGGGCCTGTTTAGAGCCGCCGTGTGTACAAG AGGCGTGGCAAAGGCCGTGGACTTCATCCCCGTGGAAAACCTGG AAACCACCATGCGGAGCCCCGTGTTCACCGACAATTCTAGCCCTC CAGCCGTGACACTGACACACCCCATCACCAAGATCGACAGAGAG GTGCTGTACCAAGAGTTCGACGAGATGGAAGAGTGCAGCCAGCA CTACCCCTACGACGTGCCAGATTATGCTGGCGGAGGTGGCAGCG GAGGCACCGAAGCCTCTGGAAGCGGCAGAGCTGACGCCCTGGAT GACTTCGACCTGGATATGCTGGGCAGCGACGCTCTGGACGATTTT GACCTCGACATGCTGGGATCTGATGCACTCGACGATTTCGATTTG GACATGCTCGGCAGTGATGCCTTGGACGACTTTGATCTTGATATG CTCATCAACAGCCGGTCCAGCGGCAGCCCCAAGAAAAAAAGAAA AGTGGGCTCCCAGTACCTGCCTGACACCGACGACAGACACCGGA TCGAGGAAAAGCGGAAGCGGACCTACGAGACATTCAAGAGCATC ATGAAGAAGTCCCCATTCAGCGGCCCCACCGATCCTAGACCTCCA CCTAGAAGAATCGCCGTGCCTAGCAGATCTAGCGCCTCCGTGCCT AAACCTGCTCCTCAGCCTTATCCTTTCACCAGCAGCCTGAGCACC ATCAACTACGACGAGTTCCCTACCATGGTGTTCCCCAGCGGCCAG ATCTCTCAGGCTTCTGCTCTTGCTCCAGCTCCTCCTCAGGTTCTGC CTCAAGCTCCTGCACCAGCACCGGCTCCAGCTATGGTTTCTGCTT TGGCTCAGGCCCCTGCTCCTGTGCCTGTTCTTGCTCCTGGACCACC TCAGGCTGTTGCTCCTCCTGCTCCAAAACCTACACAGGCCGGCGA AGGCACACTGTCTGAAGCTCTGCTGCAGCTCCAGTTCGATGACGA AGATCTGGGCGCCCTGCTGGGCAATTCTACAGATCCTGCCGTGTT TACCGATCTGGCCAGCGTGGACAACAGCGAGTTTCAGCAGCTCCT GAATCAGGGCATCCCTGTGGCTCCTCACACCACCGAACCTATGCT GATGGAATACCCCGAGGCCATCACCAGACTGGTCACCGGTGCTC AAAGACCACCTGATCCAGCTCCAGCACCACTGGGAGCACCTGGA CTGCCTAATGGACTGCTGTCTGGCGACGAGGACTTCAGCTCTATC GCCGACATGGATTTCTCTGCCCTGCTCGGCTCTGGCAGCGGCTCT AGAGATAGCAGAGAAGGCATGTTCCTGCCTAAGCCTGAGGCCGG CTCTGCCATCTCCGATGTGTTCGAGGGAAGAGAAGTGTGCCAGCC TAAGCGGATCCGGCCTTTTCACCCTCCTGGAAGCCCTTGGGCCAA CAGACCTCTGCCTGCTTCTCTGGCCCCTACACCAACAGGACCTGT GCACGAACCTGTGGGCAGTCTGACCCCAGCTCCTGTTCCTCAACC TCTGGATCCCGCTCCTGCTGTGACACCTGAAGCCTCTCATCTGCT GGAAGATCCCGACGAAGAGACAAGCCAGGCCGTGAAGGCCCTG AGAGAAATGGCCGACACAGTGATCCCTCAGAAAGAGGAAGCCGC CATCTGCGGACAGATGGACCTGTCTCATCCTCCACCAAGAGGCCA CCTGGACGAGCTGACAACCACACTGGAATCCATGACCGAGGACC

		TGAACCTGGACAGCCCTCTGACACCCGAGCTGAACGAGATCCTG GACACCTTCCTGAACGACGAGTGTCTGCTGCACGCCATGCACATC TCTACCGGCCTGAGCATCTTCGACACCAGCCTGTTTTGA (SEQ ID NO: 148)
SB02671	IL-12	*See* SEQ ID NO: 93
SB02671	IL-15	*See* SEQ ID NO: 141
SB02671	2A peptide	*See* SEQ ID NO: 142
SB02671	VPR-based synTF	*See* SEQ ID NO: 147
SB02671	insulator + inducible YB-TATA promoter IL12_2A_IL-15 inverted strand, forward strand SFFV synTF-VPR	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccaccccccagaataga atgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttcca gggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagttaGCT GGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGCAGGA ACTCTTTGATATTCTTTTCTTCCAGTTCCTCGCATTCTTTGCAGCC GGACTCGGTCACATTGCCGTTGGAGGACAGGCTGTTGTTGGCCAG GATGATCAGGTTTTCCACGGTATCGTGGATGGAGGCGTCGCCGCT TTCCAGGCTGATCACTTGCAGCTCGAGCAGAAAGCACTTCATGGC GGTCACTTTACAGCTAGGGTGCACGTCGCTCTCGGTGTACAGTGT GGCGTCGATGTGCATGCTCTGGATCAGATCCTCGATCTTCTTCAG ATCGCTGATCACGTTGACCCAGTTGCCTGTAGAGCCGGGCACCCA AAGAAGCAGCACCCACAGCAGCAGTGTGTCGGTTTCCATAGGTC CAGGGTTTTCCTCCACGTCTCCACACGTCAGTAGACTCCCGCGTC CCTCGCCAGATCCTCTCTTCCGTCTACTGGCGTTCAGGTAGGACA TCACGCGGTCAATGGTCACGGCTCGAATGCGGAAAGCGTGCAAC AGGATGCACAGCTTGATCTTGGTCTTGTAGAAGTCCGGTTCTTCC AGGCTGGACTTTTGAGGCACAGTCTCGGAGTTGAAATTCAGGGC CTGCATCAGTTCATCAATCACGGCGAGCATATTCTGGTCCAGGAA GATCTGCCGCTTCGGGTCCATGAGCAGCTTGGCGTTCATGGTCTT GAACTCGACCTGATACATCTTCAGATCTTCGTAGATCGAGGAAAG ACAGAGCGCCATCATGAATGAGGTCTTTCTCGACGCCAGGCAGC TGCCGTTAGTGATAAAGCTTGTCTCGCGGGAGTTCAGACACGATT CGTTCTTGGTCAGTTCCAGCGGCAGGCAGGCTTCCACGGTCGAGG TCTTGTCCTTGGTGATGTCCTCGTGATCAATTTCTTCCGAGGTGCA GGGGTAGAACTCAAGGGTCTGGCGGGCCTTCTGCAACATGTTCG ACACAGCCCTCAGGAGGTTTTGGGAGTGGTGTAGGCACGGGAAC ATTCCAGGGTCGGGGGTTGCCACAGGGAGGTTCCGGGAACCTCC TCCGGAGCCTCCTCCTGAACCTCCGCCTGATCCGCCACCGGAACA AGGCACGCTGGCCCATTCGCTCCAGGAGGACGAGTAGTATCTAT CCTGCGCCCGGACGCTGATTGACGCGTTCTTCCGACAAATCACAG TGGCGGAGGTTTTGTCGGTGAACACCCGGTCTTTCTTCTCCCGTTT GGACTTTCCCTGCACTTGCACACAGAAAGTGAGCGAGAAGTATG AGTGCGGGGTGCTCCAAGTGTCTGGATATTCCCAAGACACTTCCA CTTGGCGGGAGTTCTTGAGTGGCTTCAGCTGCAAGTTCTTGGGGG GGTCAGGCTTGATGATGTCGCGGATAAAGAAGGAGGAAGTGTAG TTCTCGTATTTCAGCTTATGCACGGCATCGACCATGACCTCGATA GGCAGGGACTCTTCCGCGGCAGGGCAGGCGCTGTCCTCCTGGCA TTCCACGGAGTACTCATATTCCTTGTTGTCTCCCCTGACTCTCTCG GCGGACAGAGTGGCGGCTCCACAGGTCACGCCCTGAGGATCGCT TGATCCCCGTGACGACTTCACGGAGAAAGTCAGGTCGGTGGAGA TTGTCGTCAGCCACCAACAGGTGAACCGACCGCTGTAGTTCTTGG CTTCGCAGCGGAGGAAGGTCTTGTTCTTCGGTTCTTTTTGGTCCTT GAGGATGTCAGTGGACCAGATTCCATCCTCTTTCTTGTGCAGCAG CAGCAGGGAGTGGGACAGCACTTCGCCACCCTTGTGGCAAGTGT ACTGGCCCGCGTCGCCGAACTCCTTGACTTGAATGGTCAGGGTCT

		TTCCGCTTCCGAGCACCTCGGAGCTCTGATCCAGGGTCCAGGTTA TGCCGTCCTCTTCTGGCGTATCGCAAGTCAGCACGACCATTTCTC CAGGGGCGTCCGGGTACCAATCCAGCTCGACCACGTAGACGTCC TTCTTCAGTTCCCAAATGGCGACCAGAGGGGAAGCGAGGAACAC AAGGGAGAACCAGGAGATGACGAGTTGCTGATGGCACATCATGG TGGCGACACCGGTACGCGTTGGCCCCCATTATATACCCTCTAGAA CTAGTtatccactccgtgtaagggagagtgagcctcttacgaatgCGCGACATCGGCTAC GCCgttccgaggcgactgatacgCGCGACATCGGCTACGCCgtacgaaggcagtccga ttgCGCGACATCGGCTACGCCgacctttactgagacgggagCGCGACATCGGC TACGCCgaaggcgttgcgaatcctcatgcgattgttacgaaacccgTTAATTAAAGAGCG AGATTCCGTCTCAAAGAAAAAAAAAGTAATGAAATGAATAAAAT GAGTCCTAGAGCCAGTAAATGTCGTAAATGTCTCAGCTAGTCAG GTAGTAAAAGGTCTCAACTAGGCAGTGGCAGAGCAGGATTCAAA TTCAGGGCTGTTGTGATGCCTCCGCAGACTCTGAGCGCCACCTGG TGGTAATTTGTCTGTGCCTCTTCTGACGTGGAAGAACAGCAACTA ACACACTAACACGGCATTTACTATGGGCCAGCCATTGTCCATCTA GATGGccgataaaataaaagattttatttagtctccagaaaaaggggggaatgaaagaccccacctgta ggtttggcaagctagctgcagtaacgccattttgcaaggcatggaaaaataccaaaccaagaatagagaagt tcagatcaagggcgggtacatgaaaatagctaacgttgggccaaacaggatatctgcggtgagcagtttcg gccccggcccggggccaagaacagatggtcaccgcagtttcggccccggcccgaggccaagaacagat ggtccccagatatggcccaaccctcagcagtttcttaagacccatcagatgtttccaggctcccccaaggacc tgaaatgaccctgcgccttatttgaattaaccaatcagcctgcttctcgcttctgttcgcgcgcttctgcttcccg agctctataaaagagctcacaacccctcactcggcgcgccagtcctccgacagactgagtcgcccgggG GATCCGCCACCATGAGTAGACCTGGCGAAAGACCATTTCAGTGC AGAATTTGTATGCGGAACTTCAGCAGAAGGCACGGCCTGGACAG ACACACCAGAACACACACAGGCGAGAAGCCTTTCCAGTGTAGAA TCTGTATGCGCAATTTCAGCGACCACAGCAGCCTGAAGCGGCAC CTGAGAACCCATACCGGCAGCCAGAAACCATTTCAATGCCGCAT CTGTATGAGAAACTTCTCCGTGCGGCACAACCTGACCAGACACCT GAGGACACACACCGGGGAGAAACCCTTCCAGTGCCGGATATGCA TGAGGAATTTCTCCGACCACTCCAACCTGAGCCGCCACCTGAAAA CTCACACCGGCTCTCAAAAGCCATTTCAGTGTCGTATATGTATGC GGAATTTTTCCCAGCGGAGCAGCCTCGTGCGCCATCTGAGGACTC ATACTGGCGAAAAGCCCTTCCAATGTCGCATATGCATGCGCAACT TTAGCGAGTCCGGCCACCTGAAGAGACATCTGCGGACACACCTG AGAGGCAGCACCTGTAGAGACTACAAGGACCACGACGGCGATTA TAAGGATCACGACATCGACTACAAAGACGACGATGACAAGATGG CCCCTAAGAAGAAGCGGAAAGTCGGCATCCATGGCGTGCCAGGT GGACTTGAAGGTGGCGGAGGATCTGGCGGCACAGAGGATGTTGT GTGCTGCCACAGCATCTACGGCAAGAAGAAGGGCGACATCGATA CCTATCGGTACATCGGCAGCAGCGGCACAGGCTGCGTTGTGATC GTGGGAAGAATCGTGCTGAGCGGCTCTGGCACAAGCGCCCCTAT TACAGCCTACGCTCAGCAGACAAGAGGCCTGCTGGGCTGCATCA TCACAAGCCTGACCGGCAGAGACAAGAACCAGGTGGAAGGCGA GGTGCAGATCGTGTCTACAGCTACCCAGACCTTCCTGGCCACCTG TATCAATGGCGTGTGCTGGGCCGTGTATCACGGCGCTGGAACCA GAACAATCGCCTCTCCTAAGGGACCCGTGATCCAGATGTACACC AACGTGGACCAGGACCTCGTTGGCTGGCCTGCTCCTCAGGGAAG TAGAAGCCTGACACCTTGCACCTGTGGCTCCAGCGATCTGTACCT GGTCACCAGACACGCCGACGTGATCCCTGTCAGAAGAAGAGGAG ATTCCAGAGGCAGCCTGCTGAGCCCTAGACCTATCAGCTACCTGA AGGGCAGCTCTGGCGGACCTCTGCTTTGTCCTGCTGGACATGCCG TGGGCCTGTTTAGAGCCGCCGTGTGTACAAGAGGCGTGGCAAAG GCCGTGGACTTCATCCCCGTGGAAAACCTGGAAACCACCATGCG GAGCCCCGTGTTCACCGACAATTCTAGCCCTCCAGCCGTGACACT

		GACACACCCCATCACCAAGATCGACAGAGAGGTGCTGTACCAAG AGTTCGACGAGATGGAAGAGTGCAGCCAGCACTACCCCTACGAC GTGCCAGATTATGCTGGCGGAGGTGGCAGCGGAGGCACCGAAGC CTCTGGAAGCGGCAGAGCTGACGCCCTGGATGACTTCGACCTGG ATATGCTGGGCAGCGACGCTCTGGACGATTTTGACCTCGACATGC TGGGATCTGATGCACTCGACGATTTCGATTTGGACATGCTCGGCA GTGATGCCTTGGACGACTTTGATCTTGATATGCTCATCAACAGCC GGTCCAGCGGCAGCCCCAAGAAAAAAAGAAAAGTGGGCTCCCA GTACCTGCCTGACACCGACGACAGACACCGGATCGAGGAAAAGC GGAAGCGGACCTACGAGACATTCAAGAGCATCATGAAGAAGTCC CCATTCAGCGGCCCCACCGATCCTAGACCTCCACCTAGAAGAATC GCCGTGCCTAGCAGATCTAGCGCCTCCGTGCCTAAACCTGCTCCT CAGCCTTATCCTTTCACCAGCAGCCTGAGCACCATCAACTACGAC GAGTTCCCTACCATGGTGTTCCCCAGCGGCCAGATCTCTCAGGCT TCTGCTCTTGCTCCAGCTCCTCCTCAGGTTCTGCCTCAAGCTCCTG CACCAGCACCGGCTCCAGCTATGGTTTCTGCTTTGGCTCAGGCCC CTGCTCCTGTGCCTGTTCTTGCTCCTGGACCACCTCAGGCTGTTGC TCCTCCTGCTCCAAAACCTACACAGGCCGGCGAAGGCACACTGTC TGAAGCTCTGCTGCAGCTCCAGTTCGATGACGAAGATCTGGGCGC CCTGCTGGGCAATTCTACAGATCCTGCCGTGTTTACCGATCTGGC CAGCGTGGACAACAGCGAGTTTCAGCAGCTCCTGAATCAGGGCA TCCCTGTGGCTCCTCACACCACCGAACCTATGCTGATGGAATACC CCGAGGCCATCACCAGACTGGTCACCGGTGCTCAAAGACCACCT GATCCAGCTCCAGCACCACTGGGAGCACCTGGACTGCCTAATGG ACTGCTGTCTGGCGACGAGGACTTCAGCTCTATCGCCGACATGGA TTTCTCTGCCCTGCTCGGCTCTGGCAGCGGCTCTAGAGATAGCAG AGAAGGCATGTTCCTGCCTAAGCCTGAGGCCGGCTCTGCCATCTC CGATGTGTTCGAGGGAAGAGAAGTGTGCCAGCCTAAGCGGATCC GGCCTTTTCACCCTCCTGGAAGCCCTTGGGCCAACAGACCTCTGC CTGCTTCTCTGGCCCCTACACCAACAGGACCTGTGCACGAACCTG TGGGCAGTCTGACCCCAGCTCCTGTTCCTCAACCTCTGGATCCCG CTCCTGCTGTGACACCTGAAGCCTCTCATCTGCTGGAAGATCCCG ACGAAGAGACAAGCCAGGCCGTGAAGGCCCTGAGAGAAATGGC CGACACAGTGATCCCTCAGAAAGAGGAAGCCGCCATCTGCGGAC AGATGGACCTGTCTCATCCTCCACCAAGAGGCCACCTGGACGAG CTGACAACCACACTGGAATCCATGACCGAGGACCTGAACCTGGA CAGCCCTCTGACACCCGAGCTGAACGAGATCCTGGACACCTTCCT GAACGACGAGTGTCTGCTGCACGCCATGCACATCTCTACCGGCCT GAGCATCTTCGACACCAGCCTGTTTTGA (SEQ ID NO: 149)
SB02675	IL-15	*See* SEQ ID NO: 141
SB02675	p65-based synTF	*See* SEQ ID NO: 145
SB02675	insulator + inducible YB-TATA promoter IL-15 inverted strand, forward strand SFFV synTF-p65	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccacccccagaataga atgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttcca gggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagttaGCT GGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGCAGGA ACTCTTTGATATTCTTTTCTTCCAGTTCCTCGCATTCTTTGCAGCC GGACTCGGTCACATTGCCGTTGGAGGACAGGCTGTTGTTGGCCAG GATGATCAGGTTTTCCACGGTATCGTGGATGGAGGCGTCGCCGCT TTCCAGGCTGATCACTTGCAGCTCGAGCAGAAAGCACTTCATGGC GGTCACTTTACAGCTAGGGTGCACGTCGCTCTCGGTGTACAGTGT GGCGTCGATGTGCATGCTCTGGATCAGATCCTCGATCTTCTTCAG ATCGCTGATCACGTTGACCCAGTTGCCTGTAGAGCCGGGCACCCA AAGAAGCAGCACCCACAGCAGCAGTGTGTCGGTTTCCATCATGG

		TGGCGACACCGGTACGCGTTGGCCCCCATTATATACCCTCTAGAA CTAGTtatccactccgtgtaagggagagtgagcctcttacgaatgCGCGACATCGGCTAC GCCgttccgaggcgactgatacgCGCGACATCGGCTACGCCgtacgaaggcagtccga ttgCGCGACATCGGCTACGCCgacctttactgagacgggagCGCGACATCGGC TACGCCgaaggcgttgcgaatcctcatgcgattgttacgaaacccgTTAATTAAAGAGCG AGATTCCGTCTCAAAGAAAAAAAAAGTAATGAAATGAATAAAAT GAGTCCTAGAGCCAGTAAATGTCGTAAATGTCTCAGCTAGTCAG GTAGTAAAAGGTCTCAACTAGGCAGTGGCAGAGCAGGATTCAAA TTCAGGGCTGTTGTGATGCCTCCGCAGACTCTGAGCGCCACCTGG TGGTAATTTGTCTGTGCCTCTTCTGACGTGGAAGAACAGCAACTA ACACACTAACACGGCATTTACTATGGGCCAGCCATTGTCCATCTA GATGGccgataaaataaaagattttatttagtctccagaaaaaggggggaatgaaagaccccacctgta ggtttggcaagctagctgcagtaacgccattttgcaaggcatggaaaaataccaaaccaagaatagagaagt tcagatcaagggcgggtacatgaaaatagctaacgttgggccaaacaggatatctgcggtgagcagtttcg gccccggcccggggccaagaacagatggtcaccgcagtttcggccccggcccgaggccaagaacagat ggtccccagatatggcccaaccctcagcagtttcttaagacccatcagatgtttccaggctcccccaaggacc tgaaatgaccctgcgccttatttgaattaaccaatcagcctgcttctcgcttctgttcgcgcgcttctgcttcccg agctctataaaagagctcacaacccctcactcggcgcgccagtcctccgacagactgagtcgcccgggG GATCCGCCACCATGAGTAGACCTGGCGAAAGACCATTTCAGTGC AGAATTTGTATGCGGAACTTCAGCAGAAGGCACGGCCTGGACAG ACACACCAGAACACACACAGGCGAGAAGCCTTTCCAGTGTAGAA TCTGTATGCGCAATTTCAGCGACCACAGCAGCCTGAAGCGGCAC CTGAGAACCCATACCGGCAGCCAGAAACCATTTCAATGCCGCAT CTGTATGAGAAACTTCTCCGTGCGGCACAACCTGACCAGACACCT GAGGACACACACCGGGGAGAAACCCTTCCAGTGCCGGATATGCA TGAGGAATTTCTCCGACCACTCCAACCTGAGCCGCCACCTGAAAA CTCACACCGGCTCTCAAAAGCCATTTCAGTGTCGTATATGTATGC GGAATTTTTCCCAGCGGAGCAGCCTCGTGCGCCATCTGAGGACTC ATACTGGCGAAAAGCCCTTCCAATGTCGCATATGCATGCGCAACT TTAGCGAGTCCGGCCACCTGAAGAGACATCTGCGGACACACCTG AGAGGCAGCACCTGTAGAGACTACAAGGACCACGACGGCGATTA TAAGGATCACGACATCGACTACAAAGACGACGATGACAAGATGG CCCCTAAGAAGAAGCGGAAAGTCGGCATCCATGGCGTGCCAGGT GGACTTGAAGGTGGCGGAGGATCTGGCGGCACAGAGGATGTTGT GTGCTGCCACAGCATCTACGGCAAGAAGAAGGGCGACATCGATA CCTATCGGTACATCGGCAGCAGCGGCACAGGCTGCGTTGTGATC GTGGGAAGAATCGTGCTGAGCGGCTCTGGCACAAGCGCCCCTAT TACAGCCTACGCTCAGCAGACAAGAGGCCTGCTGGGCTGCATCA TCACAAGCCTGACCGGCAGAGACAAGAACCAGGTGGAAGGCGA GGTGCAGATCGTGTCTACAGCTACCCAGACCTTCCTGGCCACCTG TATCAATGGCGTGTGCTGGGCCGTGTATCACGGCGCTGGAACCA GAACAATCGCCTCTCCTAAGGGACCCGTGATCCAGATGTACACC AACGTGGACCAGGACCTCGTTGGCTGGCCTGCTCCTCAGGGAAG TAGAAGCCTGACACCTTGCACCTGTGGCTCCAGCGATCTGTACCT GGTCACCAGACACGCCGACGTGATCCCTGTCAGAAGAAGAGGAG ATTCCAGAGGCAGCCTGCTGAGCCCTAGACCTATCAGCTACCTGA AGGGCAGCTCTGGCGGACCTCTGCTTTGTCCTGCTGGACATGCCG TGGGCCTGTTTAGAGCCGCCGTGTGTACAAGAGGCGTGGCAAAG GCCGTGGACTTCATCCCCGTGGAAAACCTGGAAACCACCATGCG GAGCCCCGTGTTCACCGACAATTCTAGCCCTCCAGCCGTGACACT GACACACCCCATCACCAAGATCGACAGAGAGGTGCTGTACCAAG AGTTCGACGAGATGGAAGAGTGCAGCCAGCACTACCCCTACGAC GTGCCAGATTATGCTGGCGGAGGTGGCAGCGGAGGCACCGATGA ATTTCCAACCATGGTGTTCCCCAGCGGCCAGATCTCTCAGGCATC TGCTCTTGCTCCAGCTCCACCTCAGGTTCTGCCTCAAGCTCCTGCT

		CCGGCTCCTGCACCAGCTATGGTGTCTGCACTTGCTCAGGCACCA GCTCCAGTGCCTGTTCTTGCTCCTGGACCTCCTCAGGCTGTTGCTC CACCAGCACCTAAACCTACACAGGCCGGCGAGGGAACACTGTCT GAAGCCCTGCTGCAACTCCAGTTCGATGACGAAGATCTGGGCGC CCTGCTGGGAAACTCTACAGATCCTGCCGTGTTTACCGATCTGGC CAGCGTGGACAACAGCGAGTTTCAGCAGCTCCTGAACCAGGGCA TCCCAGTGGCTCCTCACACCACAGAGCCTATGCTGATGGAATACC CCGAGGCCATCACCAGACTGGTCACCGGCGCACAAAGACCACCT GATCCTGCTCCAGCACCGCTTGGAGCACCTGGACTGCCTAATGGA CTGCTGTCTGGCGACGAGGACTTCAGTTCTATCGCTGATATGGAT TTCTCTGCCCTTCTGTCTCAGATTAGTAGCCAGCTGTAA (SEQ ID NO: 150)
SB02676	VPR-based synTF	*See* SEQ ID NO: 147
SB02676	IL-15	*See* SEQ ID NO: 141
SB02676	insulator + inducible YB-TATA promoter IL-15 inverted strand, forward strand SFFV synTF-VPR	tccccagcatgcctgctattctcttcccaatcctccccttgctgtcctgccccaccccaccccccagaatagaatgacacctactcagacaatgcgatgcaatttcctcattttattaggaaaggacagtgggagtggcaccttccagggtcaaggaaggcacgggggaggggcaaacaacagatggctggcaactagaaggcacagttaGCTGGTGTTGATGAACATCTGCACGATGTGCACGAAGCTCTGCAGGAACTCTTTGATATTCTTTTCTTCCAGTTCCTCGCATTCTTTGCAGCCGGACTCGGTCACATTGCCGTTGGAGGACAGGCTGTTGTTGGCCAGGATGATCAGGTTTTCCACGGTATCGTGGATGGAGGCGTCGCCGCTTTCCAGGCTGATCACTTGCAGCTCGAGCAGAAAGCACTTCATGGCGGTCACTTTACAGCTAGGGTGCACGTCGCTCTCGGTGTACAGTGTGGCGTCGATGTGCATGCTCTGGATCAGATCCTCGATCTTCTTCAGATCGCTGATCACGTTGACCCAGTTGCCTGTAGAGCCGGGCACCCAAAGAAGCAGCACCCACAGCAGCAGTGTGTCGGTTTCCATCATGGTGGCGACACCGGTACGCGTTGGCCCCCATTATATACCCTCTAGAACTAGTtatccactccgtgtaagggagagtgagcctcttacgaatgCGCGACATCGGCTACGCCgttccgaggcgactgatacgCGCGACATCGGCTACGCCgtacgaaggcagtccgattgCGCGACATCGGCTACGCCgacctttactgagacgggagCGCGACATCGGCTACGCCgaaggcgttgcgaatcctcatgcgattgttacgaaacccgTTAATTAAAGAGCGAGATTCCGTCTCAAAGAAAAAAAAAGTAATGAAATGAATAAAATGAGTCCTAGAGCCAGTAAATGTCGTAAATGTCTCAGCTAGTCAGGTAGTAAAAGGTCTCAACTAGGCAGTGGCAGAGCAGGATTCAAATTCAGGGCTGTTGTGATGCCTCCGCAGACTCTGAGCGCCACCTGGTGGTAATTTGTCTGTGCCTCTTCTGACGTGGAAGAACAGCAACTAACACACTAACACGGCATTTACTATGGGCCAGCCATTGTCCATCTAGATGGccgataaaataaaagattttatttagtctccagaaaaaggggggaatgaaagaccccacctgtaggtttggcaagctagctgcagtaacgccattttgcaaggcatggaaaaataccaaaccaagaatagagaagttcagatcaagggcgggtacatgaaaatagctaacgttgggccaaacaggatatctgcggtgagcagtttcggccccggcccggggccaagaacagatggtcaccgcagtttcggccccggcccgaggccaagaacagatggtccccagatatggcccaaccctcagcagtttcttaagacccatcagatgtttccaggctcccccaaggacctgaaatgaccctgcgccttatttgaattaaccaatcagcctgcttctcgcttctgttcgcgcgcttctgcttcccgagctctataaaagagctcacaacccctcactcggcgcgccagtcctccgacagactgagtcgcccgggGGATCCGCCACCATGAGTAGACCTGGCGAAAGACCATTTCAGTGCAGAATTTGTATGCGGAACTTCAGCAGAAGGCACGGCCTGGACAGACACACCAGAACACACACAGGCGAGAAGCCTTTCCAGTGTAGAATCTGTATGCGCAATTTCAGCGACCACAGCAGCCTGAAGCGGCACCTGAGAACCCATACCGGCAGCCAGAAACCATTTCAATGCCGCATCTGTATGAGAAACTTCTCCGTGCGGCACAACCTGACCAGACACCTGAGGACACACACCGGGGAGAAACCCTTCCAGTGCCGGATATGCATGAGGAATTTCTCCGACCACTCCAACCTGAGCCGCCACCTGAAAACTCACACCGGCTCTCAAAAGCCATTTCAGTGTCGTATATGTATGC

		GGAATTTTTCCCAGCGGAGCAGCCTCGTGCGCCATCTGAGGACTC ATACTGGCGAAAAGCCCTTCCAATGTCGCATATGCATGCGCAACT TTAGCGAGTCCGGCCACCTGAAGAGACATCTGCGGACACACCTG AGAGGCAGCACCTGTAGAGACTACAAGGACCACGACGGCGATTA TAAGGATCACGACATCGACTACAAAGACGACGATGACAAGATGG CCCCTAAGAAGAAGCGGAAAGTCGGCATCCATGGCGTGCCAGGT GGACTTGAAGGTGGCGGAGGATCTGGCGGCACAGAGGATGTTGT GTGCTGCCACAGCATCTACGGCAAGAAGAAGGGCGACATCGATA CCTATCGGTACATCGGCAGCAGCGGCACAGGCTGCGTTGTGATC GTGGGAAGAATCGTGCTGAGCGGCTCTGGCACAAGCGCCCCTAT TACAGCCTACGCTCAGCAGACAAGAGGCCTGCTGGGCTGCATCA TCACAAGCCTGACCGGCAGAGACAAGAACCAGGTGGAAGGCGA GGTGCAGATCGTGTCTACAGCTACCCAGACCTTCCTGGCCACCTG TATCAATGGCGTGTGCTGGGCCGTGTATCACGGCGCTGGAACCA GAACAATCGCCTCTCCTAAGGGACCCGTGATCCAGATGTACACC AACGTGGACCAGGACCTCGTTGGCTGGCCTGCTCCTCAGGGAAG TAGAAGCCTGACACCTTGCACCTGTGGCTCCAGCGATCTGTACCT GGTCACCAGACACGCCGACGTGATCCCTGTCAGAAGAAGAGGAG ATTCCAGAGGCAGCCTGCTGAGCCCTAGACCTATCAGCTACCTGA AGGGCAGCTCTGGCGGACCTCTGCTTTGTCCTGCTGGACATGCCG TGGGCCTGTTTAGAGCCGCCGTGTGTACAAGAGGCGTGGCAAAG GCCGTGGACTTCATCCCCGTGGAAAACCTGGAAACCACCATGCG GAGCCCCGTGTTCACCGACAATTCTAGCCCTCCAGCCGTGACACT GACACACCCCATCACCAAGATCGACAGAGAGGTGCTGTACCAAG AGTTCGACGAGATGGAAGAGTGCAGCCAGCACTACCCCTACGAC GTGCCAGATTATGCTGGCGGAGGTGGCAGCGGAGGCACCGAAGC CTCTGGAAGCGGCAGAGCTGACGCCCTGGATGACTTCGACCTGG ATATGCTGGGCAGCGACGCTCTGGACGATTTTGACCTCGACATGC TGGGATCTGATGCACTCGACGATTTCGATTTGGACATGCTCGGCA GTGATGCCTTGGACGACTTTGATCTTGATATGCTCATCAACAGCC GGTCCAGCGGCAGCCCCAAGAAAAAAAGAAAAGTGGGCTCCCA GTACCTGCCTGACACCGACGACAGACACCGGATCGAGGAAAAGC GGAAGCGGACCTACGAGACATTCAAGAGCATCATGAAGAAGTCC CCATTCAGCGGCCCCACCGATCCTAGACCTCCACCTAGAAGAATC GCCGTGCCTAGCAGATCTAGCGCCTCCGTGCCTAAACCTGCTCCT CAGCCTTATCCTTTCACCAGCAGCCTGAGCACCATCAACTACGAC GAGTTCCCTACCATGGTGTTCCCCAGCGGCCAGATCTCTCAGGCT TCTGCTCTTGCTCCAGCTCCTCCTCAGGTTCTGCCTCAAGCTCCTG CACCAGCACCGGCTCCAGCTATGGTTTCTGCTTTGGCTCAGGCCC CTGCTCCTGTGCCTGTTCTTGCTCCTGGACCACCTCAGGCTGTTGC TCCTCCTGCTCCAAAACCTACACAGGCCGGCGAAGGCACACTGTC TGAAGCTCTGCTGCAGCTCCAGTTCGATGACGAAGATCTGGGCGC CCTGCTGGGCAATTCTACAGATCCTGCCGTGTTTACCGATCTGGC CAGCGTGGACAACAGCGAGTTTCAGCAGCTCCTGAATCAGGGCA TCCCTGTGGCTCCTCACACCACCGAACCTATGCTGATGGAATACC CCGAGGCCATCACCAGACTGGTCACCGGTGCTCAAAGACCACCT GATCCAGCTCCAGCACCACTGGGAGCACCTGGACTGCCTAATGG ACTGCTGTCTGGCGACGAGGACTTCAGCTCTATCGCCGACATGGA TTTCTCTGCCCTGCTCGGCTCTGGCAGCGGCTCTAGAGATAGCAG AGAAGGCATGTTCCTGCCTAAGCCTGAGGCCGGCTCTGCCATCTC CGATGTGTTCGAGGGAAGAGAAGTGTGCCAGCCTAAGCGGATCC GGCCTTTTCACCCTCCTGGAAGCCCTTGGGCCAACAGACCTCTGC CTGCTTCTCTGGCCCCTACACCAACAGGACCTGTGCACGAACCTG TGGGCAGTCTGACCCCAGCTCCTGTTCCTCAACCTCTGGATCCCG CTCCTGCTGTGACACCTGAAGCCTCTCATCTGCTGGAAGATCCCG

		ACGAAGAGACAAGCCAGGCCGTGAAGGCCCTGAGAGAAATGGC CGACACAGTGATCCCTCAGAAAGAGGAAGCCGCCATCTGCGGAC AGATGGACCTGTCTCATCCTCCACCAAGAGGCCACCTGGACGAG CTGACAACCACACTGGAATCCATGACCGAGGACCTGAACCTGGA CAGCCCTCTGACACCCGAGCTGAACGAGATCCTGGACACCTTCCT GAACGACGAGTGTCTGCTGCACGCCATGCACATCTCTACCGGCCT GAGCATCTTCGACACCAGCCTGTTTTGA (SEQ ID NO: 151)

Results

[00691] IL-12 and/or IL-15 payload expression was assessed in T cells for various regulatable TF expression system strategies and constructs. A drug-inducible format ACP (also referred to as "synTF") using an NS3/NS4 protease cleavage site and either a VPR transcriptional effector domain (constructs SB02667 and SB02668) or a p65 transcriptional effector domain (constructs SB02670 and SB02671) were assessed. All constructs encoded cytokine payloads included an A2 insulator and YB-TATA promoter driving expression in the opposite direction as the ACP cassette (*e.g.*, *see* orientation of reporter and ACP/synTF cassettes in construct "1560" in **FIG. 20**). A summary of the systems assessed in shown in **FIG. 38**.

[00692] As shown in **FIG. 39** and quantified in **Table 23**, IL-12 production was observed in a concentration dependent manner following addition of the NS3 protease inhibitor grazoprevir when using a p65 transcriptional effector domain, with greater production (~8-fold) observed when expressed alone (SB02667) as compared to in a ribosome skipping tag 2A multicistronic system (SB02668), *see* circle vs. square in **FIG. 39**, respectively, and **Table 23**. As shown in **FIG. 40** and **FIG. 41** and quantified in **Table 24**, IL-15 production was also observed for constructs encoding IL-15 in a concentration dependent manner for the NS3 protease inhibitor grazoprevir when using a p65 transcriptional effector domain in both a ribosome skipping tag 2A multicistronic system (SB02668; **FIG. 40** diamond) and expressed as a single payload (SB02675; **FIG. 41** circle). IL-15 expressed at greater levels when encoded as a single payload, though at notably low levels in the 2A multicistronic system. Notably, there was no observable cytokine expression for constructs using a VPR transcriptional effector domain other than for IL-15 encoded as a single payload (SB02676; **FIG. 41** triangle). Accordingly, regulated cytokine expression was observed in a drug-dependent in T cells for the various systems.

Table 23 – IL-12 (pg/mL) Production in T cells for Additional Constructs

[GRZ]uM	2667 #1	2667 #2	2668 #1	2668 #2	2671 #1	2671 #2	2670 #1	2670 #2
2	2088.21	1672.93	251.45	239.53	2.96	2.96	2.96	2.96
1	2304.49	1593.19	251.45	239.53	2.96	2.96	2.96	2.96
0.5	1833.06	1315.96	145.52	145.52	2.96	2.96	2.96	2.96
0.1	192.19	133.97	22.96	22.96	2.96	2.96	2.96	2.96
0.05	12.70	2.96	0	0	2.96	2.96	2.96	2.96
0.01	0	0	0	0	0	0	0	0
No drug	0	0	0	0	0	0	0	0

Table 24 – IL-15 (pg/mL) Production in T cells for Additional Constructs

[GRZ]uM	2668 #1	2668 #2	2671 #1	2671 #2	2675 #1	2675 #2	2676 #1	2676 #2
2	1.102	0.694	0	0	9.91	8.93	7.00	6.05
1	1.102	0.694	0	0	11.39	7.97	7.00	5.58
0.5	0.305	0.694	0	0	9.42	8.93	7.00	5.58
0.1	0.305	0.305	0	0	3.28	2.83	7.48	6.05
0.05	0.305	0.305	0	0	0.69	0.69	5.11	5.11
0.01	0.305	0.305	0	0	0.31	0.31	0.69	0.69
No drug	0	0	0	0	0	0	0	0

Example 13: *in vivo* Assement of Various Grazoprevir Dosing Regimens

[00693] Regulatable TF expression systems, including various Grz dosing and T cell injection regimens, were assessed both *in vitro* and *in vivo*.

Materials and Methods

[00694] For *in vivo* assessment, T cells were transduced with SB01845 & SB02357 ("inducible IL-12"), each at an estimated MOI of 5 based on viral titering in HEK cells. Control T cells ("constitutive IL-12") were transduced with 5 MOI of SB00171, encoding constitutive hIL-12 driven by SFFV. Negative controls were untransduced T cells. The number of lentiviral genomes integrated into the T cells was analyzed in bulk by PCR (copy # assay). NSG mice were randomized on day -2 and vehicle or Grazoprevir (Grz) dosing began in the afternoon on day 1 (Vehicle: 2.5% DMSO, 30% PEG400, 67.5% PBS). Grazoprevir potassium salt was dissolved sequentially in DMSO, PEG400, and PBS to reach 10mg/mL. All Grz dosing was administered IP. 20e6 T cells per mouse were injected by tail vein injection, as indicated. Specific Grz dosing and T cell injection regimens are indicated in the tables below. Luminex assay was run to assess levels of hIL-12 in mouse plasma. Presence of human T cells in mouse blood was analyzed by flow cytometry.

Results

[00695] A first series of Grazoprevir (Grz) dosing regimens were assessed, as described in **Table 25**.

Table 25 - Grazoprevir (Grz) dosing regimens

Dosing schedule	**Day -2**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 8/12**
Previous – 25 BID	Randomize mice	25mg/kg Grz, PM	25 mg/kg Grz BID	25 mg/kg GRZ BID	25 mg/kg GRZ BID Harvest plasma	Harvest plasma
1 x day – 50 mg/kg	Randomize mice	25mg/kg Grz, PM	50 mg/kg Grz AM, Inject T cells	50 mg/kg Grz AM	50 mg/kg Grz AM Harvest plasma	Harvest plasma
2x day – 25/50	Randomize mice	25mg/kg Grz, PM	25mg/kg Grz AM, Inject T cells, 50mg/kg Grz PM	50mg/kg Grz AM, 25 mg/kg Grz PM	25 mg/kg GRZ AM & PM Harvest plasma	Harvest plasma
1x day – 75mg/kg	Randomize mice	25mg/kg Grz, PM	75 mg/kg Grz AM, Inject T cells	75 mg/kg Grz AM	50 mg/kg Grz AM Harvest plasma	Harvest plasma

[00696] Persistence of the injected T cells was assessed. As shown in **FIG. 42**, and quantitated in **Table 26A** (Day 4), **Table 26B** (Day 8), and **Table 26C** (Day 12), populations of injected human T cells were observed in the blood through Day 12 (note 50mg/kg and 75mg/kg groups of cells from Day 12 were not included in the analysis due to loss of the samples). IL-12 levels were assessed in plasma. As shown in **FIG. 43**, significant Grazoprevir dose dependent induction of IL-12 was observed on Day 4 (2 days post Grz dosing), ranging from ~100-fold to ~700-fold above vehicle. As shown in **FIG. 44**, serum IL-12 levels returned to baseline by Day 8 (left panel) and remained stable at Day 12 (right panel). Notably, mice receiving 75mg/kg of GRZ experienced toxicity with moderate to severe weight loss following the 75mg/kg daily dose on days 2-3 (mice recovered mildly 24 hours after each dose). In addition, 2 of 8 mice in the 75mg/kg group were found dead on the final day (Day 12) with no death seen in the other groups, with necropsy revealing abnormalities in the liver and ovarian ducts, including one mouse having fused organs. All mice in the 75mg/kg group presented with enlarged spleens, many with inflamed oviducts, at Day 12. Accordingly, the Grazoprevir (Grz) dosing regimens assessed demonstrated drug-dependent IL-12 production, with toxicity observed in certain dosing schemes.

Table 26A – Human T cells in the blood (hCD3+:hCD45+ shown as % of live cells); Day 4

Treatment	Mouse #1	Mouse #2	Mouse #3	Mouse #4	Mouse #5	Mouse #6	Mouse #7	Mouse #8
Constitutive IL-12 + Vehicle	3.0	4.5	5.77	3.86	5.56			
Inducible IL-12 + Vehicle	3.93	2.38	6.12	5.63	6.18			
25mg/kg BID	5.42	0.08	4.07	3.61	3.88	5.91	4.2	3.14
25+50 mg/kg	2.18	6.98	6.53	13	2.24	4.72	5.64	7.89
50mg/kg	4.09	2.96	3.7	1.62	4.16	2.57	4.55	2.58
75mg/kg	2.95	3.14	2.6	*0**	2.41	*0**	1.43	2.87

Table 26B – Human T cells in the blood (hCD3+:hCD45+ shown as % of live cells); Day 8

Treatment	Mouse #1	Mouse #2	Mouse #3	Mouse #4	Mouse #5	Mouse #6	Mouse #7	Mouse #8
Constitutive IL-12 + Vehicle	7.2	9.34	6.2	8.89	8.19			
Inducible IL-12 + Vehicle	3.05	8.17	6.04	5.45	3.71			
25mg/kg BID	6.78	0.077	3.63	2.46	2.07	3.45	2.27	4.09
25+50 mg/kg	7.09	7.85	9	12	6.34	7.02	8.65	5.04
50mg/kg	7.36	10.9	7.31	8.1	9.23	5.88	6.63	7.34
75mg/kg	8.19	9.67	6.55	0.028	11.6	4.7e-003	11	3.79

Table 26C – Human T cells in the blood (hCD3+:hCD45+ shown as % of live cells); Day 12

Treatment	Mouse #1	Mouse #2	Mouse #3	Mouse #4	Mouse #5	Mouse #6	Mouse #7	Mouse #8
Constitutive IL-12 + Vehicle	23	33.5	27.5	28.2	45.7			
Inducible IL-12 + Vehicle	4.52	10.8	18.1	14.9	13.3			
25mg/kg BID	13.5	0.21	13.3	9.36	9.33	14	16.1	13.8
25+50 mg/kg	10.9	12.2	11.1	16.3	2.61	9.13		
50mg/kg	**							
75mg/kg	**							

** Lost to technical error

[00697] An “on/off/on” Grazoprevir (Grz) dosing regimen was next assessed, with the calendar illustrating the particular dosing regimen in **FIG. 45**. Following the results of the experiments above, animals were dosed (IP) 60mg/kg for 3 days once a day. Recombinant (hIL-2 1x10^4 IU) was also administered as indicated.

[00698] Persistence of the injected T cells was assessed. As shown in **FIG. 46**, and quantitated in **Table 27A** (without drug) and **Table 27B** (with drug), populations of injected human T cells were significantly less at Day 4 when Grz was administered, with no difference seen in the inducible system groups at Days 11, 18, and 25. As previously observed, the constitutive IL-12 group demonstrated increased human T cell numbers in blood, though notably mice in this group demonstrated toxicity and required sacrificing by Day 29.

[00699] IL-12 cytokine levels were assessed in plasma for the various groups. As shown in **FIG. 47**, significant Grazoprevir dose dependent induction of IL-12 was observed on Day 4 in the inducible system, with levels between the groups equivalent during the “off” week (Day 11), and further significant Grazoprevir dose dependent induction of IL-12 during the second “on” week (Day 18). During the first week of dosing, 3 of 10 mice in Grz treatment group lost ~15% weight and received only 30mg/kg on day 3. IL-12 serum levels in the no-drug did also demonstrate increased serum IL-12 levels over time, suggesting leaky expression of the inducible cassette. Toxicity was also monitored in the different groups. As shown in **FIG. 48**, body weights remained generally stable for the inducible IL-12 group not administered drug, with a single enlarged spleen and no other abnormalities observed in this group at end time point. Body weights for the constitutive IL-12 group were also generally stable (or slightly increasing) until Day 22 with a decline in body weights afterwards and eventual euthanasia of the group by Day 29, 2 with mice found dead at Day 24, 3 mice euthanized at Day 26 due to weight loss, and spleens for all the mice significantly enlarged compared to the normal NSG mouse spleen (data not shown). In contrast, body weights for the inducible IL-12 group administered Grazoprevir demonstrated transient weight loss for most of the group followed by general weight stabilization. The Grazoprevir also showed signs of toxicity at the end time point, including pale kidneys, thick diaphragm, enlarged spleen, thick liver lobe, white/yellow lump growing inside the liver, and organs (liver, stomach, spleen) sticking with peritoneal wall and each other. The results indicate the Grazoprevir regimen demonstrated drug-dependent IL-12 production with acute toxicity in contrast to the long term lethality of constitutive IL-12 expression in the systems examined.

Table 27A – Human T cells in the blood (hCD3+:hCD45+ shown as % of live cells); no drug

	Constitutive IL-12			Inducible IL-12 (no drug)				
Mouse	#1	#2	#3	#1	#2	#3	#4	#5
Day 4	6.68	27.9	16.6	17.5	31.5	13.9	9.17	14.2
Day 11	8.84	12.4	13.9	1.54	3.83	4.84	2.89	2.84
Day 18	12.2	31	33.9	4.98	8.01	8.08	4.35	7.17
Day 25	25	46.3	43	22.5	22.5	23.6	10	9
Day 29	nd	nd	nd	25.4	41.1	44.1	32.1	31.6

Table 27B – Human T cells in the blood (hCD3+:hCD45+ shown as % of live cells); w/ Grz

	Inducible IL-12 (Grz administered)									
Mouse	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Day 4	10.2	0.82	5.8	2.11	5.39	4.92	4.03	4.04	3.76	3.6
Day 11	2	0.95	2.55	1.53	1.59	1.07	1.51	1.98	2.27	2.71
Day 18	6.36	3.74	4.73	11.8	16.7	9.5	5.18	4.87	7.61	4.26
Day 25	17.6	8	14.2	nd	11.7	14.4	11.7	12.9	9.8	26.2
Day 29	43.7	24.2	21	nd	45.9	18.2	36.4	17.7	38.2	54.6

Example 14: Activation Inducible System

[00700] Promoters that turn on transcription when CAR cells are activated by target cells are assessed.

Materials and Methods

[00701] **Candidate Selection:** Using single-cell RNA-seq data for CAR-T cells cultured with or without cognate target cells (Xhangolli et al. Genomics Proteomics Bioinformatics. 2019 Apr;17(2):129-139. doi: 10.1016/j.gpb.2019.03.002; herein incorporated by reference for all purposes), top genes upregulated in activated CAR-T cells relative to resting CAR-T cells were identified and regions from 2kb upstream to -100bp for those genes identified were chosen as candidate promoters.

[00702] **Screening**: Pan T cells were virally transduced as described with a GPC3-CAR only construct ("1106") and viral vector encoding The candidate promoters were either (1) paired with YBTATA (SEQ ID NO: 155; "YBTATA" constructs; or (2) trimmed to the translational start site of the respective gene ("trimmed") constructs to direct transcription initiation upstream of an mKate reporter. Candidate constructs were screened by flow sorting for mKate expression (gated by YFP CAR+ cells) following either 24 or 48 hours of culturing alone or activated through co-culturing with HepG2 target cells. In addition, candidates based on NFAT transcription factor binding sites was also assessed. The screen workflow is shown in **FIG. 49**. Constructs assessed are shown in **FIG. 50**.

Results

[00703] Candidates were screened for enhancing transcription when CAR-T were co-cultured with target cells, with flow-cytometry results for culturing with target cells for 24 hours shown in **FIG. 51** and 48 hours shown in **FIG. 52**. The 5x NFAT BS min AdePro construct ("2096"; SEQ ID NO: 154) demonstrated increased reporter expression when cultured with target cells at both 24 and 48 hours, though notably the construct had a high basal level of reporter expression in the absence of target cells. The 24 hour and 48 hours timepoints identified trimmed CCL3 ("2935") as demonstrating increased reporter expression when cultured with target cells, notably with reporter expression generally equivalent to background in the absence of target cells. The 24 hour timepoint also identified trimmed CCL4 ("2936") as demonstrating increased reporter expression when cultured with target cells, notably with reporter expression generally equivalent to background in the absence of target cells. The 48 hour timepoint also identified trimmed MT2a ("2942") as demonstrating increased reporter expression when cultured with target cells, with reporter expression ~3-fold above background in the absence of target cells. Histograms of the identified promoters demonstrating reporter expression and their controls (CAR only cultured without HepG2 targets, CAR only cultured with HepG2 targets, CAR + promoter cultured without HepG2 targets) are shown in **FIG. 53** and fold-changes as assessed by MFI in **Table 28**. Sequences for the identified promoters are provided in **Table 29**. The data demonstrated promoters that turn on transcription when CAR cells are activated by target cells were identified.

Table 28 - Fold change in mKate gMFI target cells/no treatment

Constructs	24h Fold change	48h Fold change
GPC3 CAR only	1.77	1.89
GPC3 CAR + SFFV mKate	0.97	0.43
GPC3 CAR + 5x NFAT BS min AdePro mKate	8.81	9.51
GPC3 CAR + CCL3 Pro mKate	12.12	9.03
GPC3 CAR + CCL4 Pro mKate	5.52	1.85
GPC3 CAR + MT2A_1 Pro mKate	2.63	3.91

Table 29 - Activation Inducible Promoter Sequences

SB #	Gene	Sequence

SB02935	CCL3	GGACAGAATTCCAAAGGCATGGTCGCACTTGGCTTCTGTCCTCTGTTATTCTCC AGCATCAAATGTATCAACTCTAACCCCTTTGGGGGGAATACAAGGCCTGTCCT GGTTTGGTCCCAATTTAGCTTTATCATCCATATTCACCCCCACTGCTCTGCAGCT CCACTGAAGCACCCCCTCTTTCCTCTGAACCCACAATGTCACACTCAGGACTCT GCCTCAGCTGGGCACTCATCTATAGATGCCTAAATCCCGGGCAGTTATCCAGA CACAACTAAAGTTCCATCCCTTCCATGAAGCCTTCCCCAACCCTCTGGTGGAAG GTCACTTCTTCCCCTCGTGGGATTCTGAGCTTTCATTTCTTTTTCTACTAGGAGT CCTAGCACTTTCGGCTAAATGCTACAATTACCTGTTCATACACTCTACCTGCCC CCACGAGATCAGGGGCATCTCAGAAACAAAGATCATTAAAACCAACTAAATCT ATTTCTCATTATAAAATGAGGTATGCTGATTGATTGTGAAAGAATAAAATAAC AAAGTATGGAAAAGAAAAAAAAGCATATAATCTGGCTGAGAAGGTAGAGACC CTTCCACACCACTGAAATTATGTATTGAAAAGAATAAGTAAAAAACTGCTTCA ATTTGGCATGATTTATGTAAGTATAGTATAGGATCCTTAAAATGGTTCAAAGA AATGGGAAATCAAGACTTCATTTTGGCCAAAACCATTGAACAGAAACTTCAGC ATATTTATCAATAATTTCTTTCAGATTAAACAACTGACAACAACCTATTTTTCA ACCAGTGATGTTGGAAATGTTTTTTTAAAAATTAGTTTATAAATTTGTGGGCTG ACCAAGAAGGTAATAAAGTCTAACTAAGTAAAATGAGAAAAATTCAGAAAAA GAAAAAAATAAGAAAATAAATCACCCAGGGACCTATCACACAAATATAAGAA CTATTCATTCTTTAAGGCATGTATTTCCAAGCCTTTGTATTTTTTTCCATGCTTA GGGTTGGCAAGGAATATATATATATTTGTACAAATATATATGTGTATATGTACA AATACATGTATATATAGTACAAATATATATATATATTTGTACAATTCTTCAGAC TTTGTAGAATTTGTATAATGTCGTATCTTGCTTTTTTTAACCACTGATGTTATAA GCATATTTATGCCACTTCATTCATTTTAGAGACTTAATAATAAATGATCTAGTG GATAATTTATCATTCCCTGATGGAGAAAAATTTAGCTTTGTTTATTTTAGAGTT ATAAACGATGCTGGGTCAGGTATCTTTATGTTTGAAGATGGCTCCATATTTGGG TTGTTTCCACAGAACTCTTTCCTAGAAATGCTTTTTCTAGGTTAATGGCTACAG ATATTTCTAGGCACCTGACATATTGACACCCACCTCTAAAGTATTTTTATGATC CACAACTAGCGTTTAACACAGCGCCCTAGTCACTACATGACTAATAAATAGAC AAATGACTGAAACATGACCTCATGCTTTCTATTCCTCCAGCTTTCATTCAGTTC TTTGCCTCTGGGAGGAGGAAGGGTTGTGCAGCCCTCCACAGCATCAGCCCATC AACCCTATCCCTGTGGTTATAGCAGCTGAGGAAGCAGAATTGCAGCTCTGTGG GAAGGAATGGGGCTGGAGAGTTCATGCACAGACCAGTTCTTATGAGAAGGGA CTGACTAAGAATAGCCTTGGGTTGACATATACCCCTCTTCACACTCACAGGAG AAACCATTTCCCTATGAAACTATAACAAGTCATGAGTTGAGAGCTGAGAGTTA GAGAATAGCTCAAAGATGCTATTCTTGGATATCCTGAGCCCCTGTGGTCACCA GGGACCCTGAGTTGTGCAACTTAGCATGACAGCATCACTACGCTTAAAAATTT CCCTCCTCACCCCCAGATTCCATTTCCCCATCCGCCAGGGCTGCCTATAAAGAG GAGAGCTGGTTTCAGACTTCAGAAGGACACGGGCAGCAGACAGTGGTCAGTCC TTTCTTGGCTCTGCTGACACTCGAGCCCACATTCCGTCACCTGCTCAGAATC (SEQ ID NO: 156)
SB02936	CCL4	TTAAGTCTTCAACCCATTGTGAGTTGATTTTTACAGATGGTGTAAGGAAGGGGT CCAGTTTCAATCTTTTGCATACAGCTAGCCAGTTATCCCAGTACCATTTATTGA CTGGGAAGTCCTTTCCCTGTTGCTTGTTTTTGTTAACTTTGTCAAAGTTCGGATG GTTGCAGGTATGCAGCATTATTTCTGAGCTCTCTATTTTGTTCTATTGGTCTATG AGTCTGTTTTTGTACCAAAGCCATGCTGTTTTGGTTACTGCAGCCCTGTGGTAT AGTTTGAAGTCATGGCTCCCTTTTTTTACTCTTCTTTTTTCTTTCTCAGAGAAGA GCAGGTAACAATGTGGGGTTAAAGGTGAGCAGGTGGGTTAGAGTAGTGGAGC TAAAGGCAGATAGAGGTCTTGATCCAATTATCCTCATTCCCTTCATTTGTCCAA CCTTGCACATTCCTGTATGCAGGGCCTTTGTAAGCTTCACCTTCTGTACAGGTG CCCAGCAACTGTCAAACCAGCCAGGGCTTAACATGAGCATGGGCATTCCCTAT GGACTGGCTGGGACACACCCACATCCTTAGATTACACATTTTGCCTGTAACATA GATAAACTAATTACTAGGTATATAATTCTGTTTCGTTTTGTTTTGCTTTGTTTTG TTTTTGGGACAGGGTCTCATTCTGTCAAACAAGCGGGAGTGCAGTGGCACAAT CACCACTCACTGCAGCCTGGACTTTTCTGGGCTCAGGTGATCTTCCCACCTCAG CCTACTGAGCAGCTGGGACTACAGGTGTGTATCACCACTCCCAGCCAAAATAT TTTTTTATAGAGACAGTGTTTCACCATGTTGCCCAGTCTGGTATCGAACTTCTG GGCTCAAGCTATCTGCCTGCCTTGACCTCCCAAAGTGCTGGGATTACAGGTGTG AGGCCCTGTACCCAGCTCAATCCTGTTTTTATACTAGAAGAACATTCTCTATCT GGGTCTTCCAAACTTGATTCTTACTGGTTAATTTGTCCTGTTCTTTTGCTGGTCC AAGAAAATATCCTGAAATCTTTATTTCTTCTCCGTTTCCTCCTTGTCCTAGGACA

		GACTAGCCCTATCCCCTTCCTGAATTAAGTCCGAATATAGTCAGTCTTTGAGTG TGGAATAGCTCCTAGCAGTCTATCAGTCAACGGGTTCTCTTTGTGGTCACATTC TATGTTTATTCAGGAGACTACAGCATGGAAAGAAAATGGACTTTGGAGTCAGG TGAATCTTGATTCAAATCTTGGCAGTGCCATTCATCAGCTCTGTGGCATTGAGC GCATCAGTGGACCACTCTCTGATCCCCAATTGCCTTGTCTGTAAAATGAGATTA GACACCCCTGCTCAAGATTATGGTAGGATTATATATAATGTGTGTAACACAGC TTTTCCAGTGCCTGGTACAAGGTAAGTGTCCAATAAGAAGTTACAAATGTTCCT GAGCCACCCTACTGGGTCCCTCCTCACATCCAATGCTGGACTCTTCATGCCCAA GGAAACATATAAGGCACAAAAGTCAGTGGCACTCAGCTCAGCAGAAGAAGGC ACAGGAAGGGGATGGGGGAGTCCTGGCTCCCACTTTGTTCTTGGGGTCAGGCT TGTGGCCAGCCTGGAAAGACTGACATGAACCCTCCAGCATCTGAGGGTGCCAA CCACCAAGAGCAGCACAGTTCTTGTCTACAAGCCACTTGTAGCAGGTGTGAAC ATTTCATCTTTTCCTCTGGGGTTTGCAACTCTCTCCCCAGTCTCGGTCACTGCCT TTGGCTGTACCACTTCCCTTTTCTTCTCGCCTGCACCTCCCTCATCTTTCCTCTAT GATGACATCGCCCTGGGGAAGAGAAGCTGAGAGGAACTCCTCACTCAGCTAGC TTCAGGAGCATGACGTCATCTCTACCATGGAAATTCCACTCACTCTCCTGTGCC CCCACATTTGTCCTAGGCCTCAGAGTCCCTATAAAGAGAGATTCCCAAGTCAG TATCAGCACAGGACACAGCTGGGTTCTGAAGCTTCTGAGTTCTGCAGCCTCAC CTCTGAGAAAACCTCTTTTCCACCAATACC (SEQ ID NO: 157)
SB02942	MT2A	GGGATTTGGTATATAATACTGTGCATACATAAATAACAACAACAAAATTGGTC CACTGCCCAGAGAGCTCTCGATCCACAGAGCTGCCAGACATCATGAGATTTCA AAAGATCTTGGCTCTAAAATCAGAGGATGTTTGGATATAAATTCTAGTACAAC CCAGTCATTTGACAGATGAGTAAACCAGGGATCAGAAGAGTCCAGGCATGTAA TTGCCCCAAGGTCACTGAGTAAGTGAGTGGCCGAGTGACCACTGAAATTTAGT CCATCTGAGAGCTCGCCCCCCTGCCCTGTGCGGTGTTTTTACAAGAGTGAGCAC GCAGGTTCCAGGGTGGTCAAGAGGTGTTTACTTTCATTTTGTAGTCGGCCTTCA TACAAGGTGGGGGCTGGGAAGGGAAGAGCAGTCCAGGCCATTACCCCCATGA ATTCATTTGACAAATATTTATTAAGCGTCTACTGCAAACTTGGCCCGGTGCTGG AAGCTGAAGACACAATTCCAGAGGCAGACAATGACTGCCCTTGGCATTTCCAG TGGTCAGGGGTTGGTGAGGAGGGGCAGAGACACCAATCCAAGAGTCCCCGCA GTTTATACATACCACAGGCACGGAAAGCGCCAGGAGGGGAAAGAACAGGATG TTTACCAGCACCTTCCACAGGCAGCCACGGTCATGGGGGTCAGGACAGGGTCC CCAGAGGAAGTGACGATTCGGCTGAGCTAGAAAGACATTTCCACAGGAACTTA CAGTAAGGGCTGCAAGGACAGCCTGTCCTCCCCCGCCCACCACCTCACTAAAC TTTCACTGTGGCAATCGGCATTCCCTAAGCCTGCCAGGAAGCTTCCAGTAACCA CTTCCTGACTCCTAGCATGACACACTTCGGGCCTTCCAAGGTTGACGATCTAAG GCCCTTACACAGCGCCAGACACGCGCAGGCAGGGAGGCAGGATGTGCCTCTCC AGACAGGAATGTGGACGCCACCTGGGCTCTTCCCCTCAGCCTCCACAGGGGAA GAATATTCTTGTGGGGTTTTTCCCTTCCAAATGTCTCAGGGCGATTCCAGTGTT CCCGCTAGTTCCTCCCTCCCAGGCTAGAACACAAATCCTTCCCACTCCCTGCCT GGCAAACACCTTCTGACCCTCAGGCCCAAGGCAATGGCCCACCTCCTCCCAGG CTGGATGGGGTCTCCTCCTCTCTGTTCCCCCAGCCCCTGAGCTTCCTGAGGACC AAGCTTGTGGCTTCTTCTCCTTACTCTTCCTCCTTGGTGTCTCTATGTTAGAGGG CCGTTAGCATCTGCTGGGGCCTGGTCGCATTCACCCTGCTCTGCCACTCACTGG CTGTGTGACTCTGGACAAATTAACTTCTCTGGACCTGCAGTTTCTCCTCTCTAC AATGAGAATACTGGAGAGTCCTTATCTTATGGGTTGCTACAGAATTAAGTGAC ATCTCACACACAACACACTTCCTACAGTCCCTGTTACACGCTAAAAGTACTCAA CATGCAACGGATACGTCATCAGTAACCACCCCACGGGTTTACTGTGATGCTGC ACAATTATTAAGCCTTGGCTGCTACAGAGTTGTAACCTGTCTGCACTTCCAACC GGTTTGGGAATGCAAGCAGCATTCCCAAGTCCCGCTTTCACCCGCGCGCTAAC GGCTCAGGTTCGAGTACAGGACAGGAGGGAGGGGAGCTGTGCACACGGCGGA GGCGCACGGCGTGGGCACCCAGCACCCGGTACACTGTGTCCTCCCGCTGCACC CAGCCCCTTCCGCGCCGAGGCGTCCCCGAGGCGCAAGTGGGCCGCCTTCAGGG AACTGACCGCCCGCGGCCCGTGTGCAGAGCCGGGTGCGCCCGGCCCAGTGCGC GCGGCCGGGTGTTTCGCCTGGAGCCGCAAGTGACTCAGCGCGGGGCGTGTGCA GGCAGCGCCCGGCCGGGGCGGGGCTTTTGCACTCGTCCCGGCTCTTTCTAGCTA TAAACACTGCTTGCCGCGCTGCACTCCACCACGCCTCCTCCAAGTCCCAGCGA ACCCGCGTGCAACCTGTCCCGACTCTAGCCGCCTCTTCAGCTCGCC (SEQ ID NO: 158)

Example 15: IL-12 Drug-inducible Systems in T Cells with Grazoprevir/Elbasvir

[00704] IL-12 payload expression was assessed in T cells for various regulatable TF expression system strategies using either grazoprevir or the combination grazoprevir/elbasvir.

Materials and Methods

[00705] For *in vitro* assessment, on Day 0, CD4/CD8 T cells (donor derived) were thawed, seeded at 1e6 cells/mL/well in a 24 well-plate, and activated with anti-CD3/CD28 Dynabeads (3:1 bead to cell) in complete T cell media (Optimizer+ Supplements–Gibco +5% human serum) + rhIL-2 (100 Units/ml; Peprotech). On Day 1, 0.5ml media was removed and cells were transduced with 3e5 pg of virus for each the constructs indicated (relevant sequences of the constructs are provided in **Table 17**). On Day 2, 1.5mL of Optimizer media + 100U/mL rhIL-2 was added. Day 7 cells were treated with grazoprevir (2, 1, 0.5, 0.1, 0.05, 0.01, .005µM and no drug) with or without elbasvir at a ratio of 2:1, respectively, in line with the ratio of compounds in Zepatier®. Transduced T cells were incubated for a further two days and the supernatant collected via centrifugation for IL-12 quantification and assessed by Luminex (R&D IL12).

Results

[00706] IL-12 payload expression was assessed in T cells for various regulatable TF expression system strategies and constructs. An drug-inducible format ACP (also referred to as "synTF") using an NS3/NS4 protease cleavage site and a VPR transcriptional effector domain (SB01845) or a modified version of the ACP with linkers shortened and the entire construct codon optimized (SB02110) were assessed. The payload construct SB02661 encoded a GPC3-CAR driven by an SFFV promoter, with the IL-12 cytokine payload encoded in a cassette including an A2 insulator and YB-TATA promoter driving expression in the opposite direction as the CAR cassette (*e.g., see* orientation of reporter and CAR cassettes in construct "2235" in **FIG. 20**). Control T cells were transduced with constitutive IL-12 construct SB00171. As shown in **FIG. 54** and quantified in **Table 30**, IL-12 production was observed in a concentration dependent manner following addition of the NS3 protease inhibitor grazoprevir, in line with those seen above. The kinetics of IL-12 production were generally equivalent regardless of elbasvir addition. Accordingly, elbasvir did not impact grazoprevir potency in the induction of IL-12 in a drug-inducible format ACP TF expression system.

Table 30 - IL-12 Production with Grazoprevir (Grz) With or Without Elbasvir

GRZ conc (uM)	171 GRZ	1845+2661 GRZ	2110+2661 GRZ	NV	171 GRZ + Elbasvir	1845+2661 GRZ + Elbasvir	2110+2661 GRZ + Elbasvir
2	65992	78330	12647	1314	57054	70010	11823
1	58677	83739	14837	1133	66533	84048	16897
0.5	61896	83559	14193	1288	63545	91776	16228
0.1	73436	88685	15146	1159	71788	90926	17825
0.05	86701	77119	15249	1468	84332	88556	17026
0.01	91312	15558	5847	1133	90488	23156	7779
0.005	86701	7779	2924	567	84332	11578	3889
0	86701	1005	902	953	84332	1133	927

[00707] While the present disclosure has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the present disclosure and appended claims.

[00708] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

CLAIMS

What is claimed is:

1. An engineered expression system comprising:

(a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding a transcriptional modulator,
wherein the first promoter is operably linked to the first exogenous polynucleotide, wherein the transcriptional modulator comprises (i) a DNA-binding domain, (ii) a transcriptional effector domain, and (iii) a hormone-binding domain of estrogen receptor (ERT2 domain); and wherein the ERT2 domain is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof; and
(b) a second expression cassette comprising a transcriptional modulator-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)**x
wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, and X = 1 to 20, and wherein the effector molecule is a cytokine,

wherein the transcriptional modulator-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and

wherein the transcriptional modulator is capable of inducing expression of the second expression cassette by binding to the transcriptional modulator-responsive promoter, wherein (i) the first expression cassette is comprised within a first nucleic acid and the second expression cassette is comprised within a second nucleic acid, or (ii) wherein the first expression cassette and the second expression cassette are comprised within a single nucleic acid; and
(c) an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor comprising an antigen-binding domain, wherein the first expression cassette and/or the second expression cassette further comprises the additional exogenous polynucleotide sequence, or an additional expression cassette comprising an additional promoter and an additional exogenous polynucleotide sequence encoding

an antigen recognizing receptor comprising an antigen-binding domain, wherein the engineered expression system further comprises the additional expression cassette.

2. The engineered expression system of claim 1, further comprising a linker polynucleotide sequence localized between the first expression cassette and the second expression cassette,
wherein the linker polynucleotide sequence is operably associated with the translation of the transcriptional modulator and each effector molecule as separate polypeptides, and/or
wherein the linker polynucleotide sequence encodes a 2A ribosome skipping tag and wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A, and/or
wherein the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES), and/or
wherein the linker polynucleotide sequence encodes a cleavable polypeptide and/or wherein the cleavable polypeptide comprises a furin polypeptide sequence.

3. The engineered expression system of claim 1 or 2, wherein:

(a) the transcriptional modulator-responsive promoter comprises an transcriptional modulator-binding domain sequence and a promoter sequence,
wherein the promoter sequence is derived from a promoter selected from the group consisting of: 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,
wherein the transcriptional modulator-responsive promoter comprises a synthetic promoter,
wherein the transcriptional modulator-responsive promoter comprises a minimal promoter, and/or
wherein the transcriptional modulator-binding domain comprises one or more zinc finger binding sites;

(b) the first promoter comprises a constitutive promoter, an inducible promoter, or a synthetic promoter,
wherein the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb; and/or

(c) wherein the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha,
wherein each cytokine is a human-derived cytokine.

4. The engineered expression system of any one of claims 1-3,
wherein the additional promoter is operably linked to the additional exogenous polynucleotide, wherein the additional exogenous polynucleotide sequence are encoded by the same polynucleotide as the first expression cassette or the second expression cassette, and
wherein the antigen recognizing receptor recognizes GPC3,
wherein the antigen recognizing receptor comprises an antigen-binding domain,
wherein the antigen-binding domain that binds to GPC3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region,

wherein the VH comprises:
a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (SEQ ID NO: 119),
a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (SEQ ID NO: 120), and
a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (SEQ ID NO: 121), and
wherein the VL comprises:
a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (SEQ ID NO: 122),

a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (SEQ ID NO: 123), and

a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (SEQ ID NO: 124),

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),
wherein the VH and VL are separated by a peptide linker,
wherein when the antigen-binding domain comprises an scFv, 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,
wherein the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR),
wherein when the antigen recognizing receptor is a CAR, the CAR comprises one or more intracellular signaling domains, and each of the one or more intracellular signaling domains is 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, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, a CD16a intracellular signaling domain, a DNAM-1 intracellular signaling domain, a KIR2DS1 intracellular signaling domain, a KIR3DS1 intracellular signaling domain, a NKp44 intracellular signaling domain, a NKp46 intracellular signaling domain, a FceRlg intracellular signaling domain, a NKG2D intracellular signaling domain, and an EAT-2 intracellular signaling domain,
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, a BTLA transmembrane domain, an OX40 transmembrane domain, a DAP10 transmembrane domain, a DAP12 transmembrane domain, a CD16a transmembrane domain, a DNAM-1 transmembrane domain, a KIR2DS1 transmembrane domain, a KIR3DS1 transmembrane domain, an NKp44 transmembrane domain, an NKp46 transmembrane domain, an FceRlg transmembrane domain, and an NKG2D transmembrane domain, and/or
wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

5. The engineered expression system of any one of claims 1-4,
wherein the transcriptional modulator is a transcriptional repressor or the transcriptional modulator is a transcriptional activator,
wherein the transcriptional modulator further comprises a repressible protease and one or more cognate cleavage sites of the repressible protease,
wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

6. The engineered expression system of any one of claims 1-5, wherein:
(a) the engineered expression system further comprises an insulator, wherein the insulator is localized between the first expression cassette, the second expression cassette, and/or the additional expression cassette if present;
(b) the first expression cassette is localized in the same orientation relative to the second expression cassette or the first expression cassette is localized in the opposite orientation relative to the second expression cassette; and/or
(c) the engineered expression system is a nucleic acid selected from the group consisting of: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid.

7. One or more expression vectors comprising the first expression cassette, the second expression cassette, and/or the additional expression cassette if present of any one of the engineered expression systems of any one of claims 1-6, and

wherein (a) a first vector comprises the first expression cassette and the additional expression cassette if present, and a second vector comprises the second expression cassette, (b) a first vector comprises the first expression cassette, and a second vector

comprises the second expression cassette and the the additional expression cassette if present, (c) a first vector comprises the first expression cassette and the second expression cassette, and a second vector comprises the additional expression cassette if present, or (d) a vector comprises the first expression cassette, the second expression cassette, and the additional expression cassette if present.

8. An isolated cell comprising the engineered expression system of any one of claims 1-6 or the one or more expression vectors of claim 7,
wherein the engineered expression system is recombinantly expressed,
wherein the engineered expression system is expressed from a one or more vectors or one or more selected loci from the genome of the cell,
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, and/or
wherein the cell is autologous or the cell is allogeneic.

9. A pharmaceutical composition comprising the engineered expression system of any one of claims 1-6, the one or more expression vectors of claim 7, or the isolated cell of claim 8, and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

10. The isolated cell of claim 8 or the composition of claim 9 for use in therapy.

11. Use of the isolated cell of claim 8 or the composition of claim 9 in the manufacture of a medicament for treating cancer in a subject.

12. The use of claim 11, wherein:

(a) the medicament is to be systemically administered or intratumorally administered;

(b) the isolated cell is derived from the subject or the isolated cell is allogeneic with reference to the subject;

(c) the use further comprises that a checkpoint inhibitor is to be administered;

(d) the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor; and

(e) the use further comprises that tamoxifen or a metabolite thereof is to be administered.

13. The use of claim 12, wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

14. A kit comprising the isolated cell of claim 8 or the pharmaceutical composition of claim 9, wherein the kit further comprises written instructions for using the cell or composition for treating and/or preventing cancer in a subject.

15. The engineered expression system of any one of claims 1-4, wherein the VH region comprises an amino acid sequence comprising
EVQLVETGGGMVQPEGSLKLSCAASGFTFNKNAMNWVRQAPGKGLEWVAR
IRNKTNNYATYYADSVKARFTISRDDSQSMLYLQMNNLKIEDTAMYYCVAG
NSFA YWGQGTLVTVSA (SEQ ID NO: 125) or
EVQLVESGGGLVQPGGSLRLSCAASGFTFNKNAMNWVRQAPGKGLEWVGRI
RNKTNNYATYYADSVKARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGN
SFAYWGQGTLVTVSA (SEQ ID NO: 126), and
wherein the VL region comprises an amino acid sequence comprising
DIVMSQSPSSLVVSIGEKVTMTCKSSQSLLYSSNQKNYLAWYQQKPGQSPKL
LIYWASSRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYNYPLTFG
AGTKLELK (SEQ ID NO: 127), or
DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKL
LIYWASSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFG
QGTKLEIK (SEQ ID NO: 128).