CLAIMS

1. A system for regulating expression of a target gene in a cell, comprising:

a first transmembrane receptor comprising a first ligand binding domain and a first signaling domain, wherein the first signaling domain activates a first signaling pathway of the cell upon binding of a first ligand to the first ligand binding domain; and

a first expression cassette comprising a nucleic acid sequence encoding at least a portion of a gene modulating polypeptide (GMP) placed under control of a first promoter, wherein the GMP comprises an actuator moiety, and wherein the first promoter is activated to drive expression of the at least the portion of the GMP upon binding of the first ligand to the first ligand binding domain,

wherein, upon expression, the at least the portion of the GMP regulates expression of the target gene.

2. The system of claim 1, wherein:

(a) the system further comprises a second transmembrane receptor comprising a second ligand binding domain and a second signaling domain, wherein the second signaling domain activates a second signaling pathway of the cell upon binding of a second ligand to the second ligand binding domain, optionally wherein:

(1) the first promoter is activated to drive expression of the GMP upon binding of the second ligand to the second ligand binding domain; or

(2) the system further comprises a second expression cassette comprising a nucleic acid sequence encoding an additional GMP placed under control of a second promoter, wherein the additional GMP comprises a second actuator moiety, and wherein the second promoter is activated to drive expression of the second actuator moiety upon binding of the second ligand to the second ligand binding domain,

optionally wherein the additional GMP regulates expression of an additional target gene in the cell;

(b) the nucleic acid sequence of the first expression cassette encodes a first portion of the GMP placed under control of the first promoter, wherein the first portion of the GMP comprises a first portion of the actuator moiety, and wherein the first promoter is activated to drive expression of the first portion of the GMP upon binding of the first ligand to the first ligand binding domain;

the system further comprises a second transmembrane receptor comprising a second ligand binding domain and a second signaling domain, wherein the second signaling domain activates a second signaling pathway of the cell upon binding of a second ligand to the second ligand binding domain; and

the system further comprises a second expression cassette comprising a nucleic acid sequence encoding a second portion of the GMP placed under control of a second promoter, wherein the second portion of the GMP comprises a second portion of the actuator moiety, and wherein the second promoter is activated to drive expression of the second portion of the GMP upon binding of the second ligand to the second ligand binding domain,

wherein the first portion of the actuator moiety and the second portion of the actuator moiety complex to form an actuator moiety that is functional, wherein the functional actuator moiety regulates expression of the target gene;

(c) the nucleic acid sequence of the first expression cassette encodes a first portion of the GMP placed under control of the first promoter, wherein the first portion of the GMP comprises a first portion of the actuator moiety, and wherein the first promoter is activated to drive expression of the first portion of the GMP upon binding of the first ligand to the first ligand binding domain; and

the system further comprises a second expression cassette comprising a nucleic acid sequence encoding a second portion of the GMP placed under control of a second promoter, wherein the second portion of the GMP comprises a second portion of the actuator moiety, and wherein the second promoter is activated to drive expression of the second portion of the GMP upon binding of the first ligand to the first ligand binding domain,

wherein the first portion of the actuator moiety and the second portion of the actuator moiety complex to form an actuator moiety that is functional, wherein the functional actuator moiety regulates expression of the target gene;

(d) (i) the first transmembrane receptor further comprises a cleavage moiety, and (ii) the nucleic acid sequence of the first expression cassette encodes a fusion protein comprising the GMP linked to a nuclear export signal peptide,

wherein the cleavage moiety cleaves the cleavage recognition site of the fusion protein when the fusion protein is in proximity to the cleavage moiety to release the actuator moiety, and wherein the released actuator moiety regulates expression of the target gene,

optionally wherein the cleavage moiety is linked to an intracellular region of the transmembrane receptor;

(e) the nucleic acid sequence of the first expression cassette encodes a fusion protein comprising the GMP linked to a nuclear export signal peptide, wherein the GMP comprises the actuator moiety linked to a cleavage recognition site,

wherein upon release of the actuator moiety via cleavage by a cleavage moiety at the cleavage recognition site, the released actuator moiety regulates expression of the target gene,

optionally wherein:

(1) the system further comprises the cleavage moiety; or

(2) the cleavage moiety cleaves the cleavage recognition site of the expressed fusion protein when in proximity to the cleavage recognition site; or

(f) the nucleic acid sequence of the first expression cassette encodes a fusion protein comprising the GMP linked to a nuclear export signal peptide, wherein the GMP comprises the actuator moiety linked to a cleavage recognition site; and

the system further comprises a second expression cassette comprising a nucleic acid sequence encoding a cleavage moiety, wherein the nucleic acid sequence of the second expression cassette is placed under the control of a second promoter activated by the first signaling pathway to drive expression of the cleavage moiety upon binding of the first ligand to the first ligand binding domain,

wherein the expressed cleavage moiety cleaves the cleavage recognition site of the expressed fusion protein when in proximity to the cleavage recognition site to release the actuator moiety, and wherein the released actuator moiety regulates expression of the target gene.

3. A system for regulating expression of a target gene in a cell, comprising:

a first transmembrane receptor comprising a first ligand binding domain and a first signaling domain, wherein the first signaling domain activates a first signaling pathway of the cell upon binding of a first ligand to the first ligand binding domain;

a gene modulating polypeptide (GMP), the GMP comprising an actuator moiety linked to a cleavage recognition site; and

a first expression cassette comprising a nucleic acid sequence encoding at least a portion of a cleavage moiety, wherein the nucleic acid sequence is placed under the control of

a first promoter activated by the first signaling pathway to drive expression of the cleavage moiety upon binding of the first ligand to the first ligand binding domain,

wherein the expressed cleavage moiety cleaves the cleavage recognition site when in proximity to the cleavage recognition site to release the actuator moiety from the GMP, and wherein the released actuator moiety regulates expression of the target gene, and

wherein:

(a) the first transmembrane receptor comprises the GMP, wherein the expressed cleavage moiety cleaves the cleavage recognition site to release the actuator moiety from the first transmembrane receptor;

(b) the system further comprises a fusion protein, wherein the fusion protein comprises the GMP linked to a nuclear export signal peptide, wherein the expressed cleavage moiety cleaves the cleavage recognition site to release the actuator moiety from the fusion protein; or

(c) the nucleic acid sequence of the first expression cassette further encodes a fusion protein comprising the GMP linked to a nuclear export signal peptide, wherein the expressed cleavage moiety cleaves the cleavage recognition site to release the actuator moiety from the fusion protein.

4. The system of claim 3, wherein, in (b):

(1) the nucleic acid sequence of the first expression cassette encodes a first portion of the actuator moiety; and

(2) the system further comprises a second expression cassette comprising a nucleic acid sequence encoding a second portion of the cleavage moiety, wherein the nucleic acid sequence of the second expression cassette is placed under control of a second promoter activated by the first signaling pathway to drive expression of the second portion of the cleavage moiety upon binding of the first ligand to the first ligand binding domain,

wherein (i) the first portion and the second portion of the cleavage moiety complex to form a cleavage moiety that is functional, and (ii) upon cleavage of the cleavage recognition site by the functional cleavage moiety, the actuator moiety is released from the nuclear export signal peptide of the fusion protein to regulate expression of the target gene.

5. The system of any one of claims 1-4, wherein:

(a) (i) the first promoter comprises a first endogenous promoter that is activated upon

binding of the first ligand to the first ligand binding domain, and/or (ii) the second promoter comprises a second endogenous promoter that is activated upon binding of the second ligand to the second ligand binding domain, optionally wherein:

(1) (i) the nucleic acid sequence encoding the GMP is operably linked to the first endogenous promoter, and/or (ii) the nucleic acid sequence encoding the additional GMP is operably linked to the second endogenous promoter; or

(2) (i) the first expression cassette comprises a first gene encoding a first endogenous protein, wherein the first gene is located upstream of the nucleic acid sequence encoding the GMP, and wherein expression of the first endogenous protein is driven by the first endogenous promoter, and/or (ii) the second expression cassette comprises a second gene encoding an second endogenous protein, wherein the second gene is located upstream of the nucleic acid sequence encoding the additional GMP, and wherein expression of the second endogenous protein is driven by the second endogenous promoter, optionally wherein:

(A) (i) the first gene and the nucleic acid sequence encoding the GMP are joined by a nucleic acid sequence encoding a first peptide linker, and/or (ii) the second gene and the nucleic acid sequence encoding the additional GMP are joined by a nucleic acid sequence encoding a second peptide linker, optionally wherein:

(A1) the first peptide linker and/or the second peptide linker comprises a protease recognition sequence; or

(A2) the first peptide linker and/or the second peptide linker comprises a self-cleaving segment, optionally wherein the self-cleaving segment comprises a 2A peptide, optionally wherein the 2A peptide is T2A, P2A, E2A, or F2A; or

(B) (i) the first gene and the nucleic acid sequence encoding the GMP are joined by a nucleic acid sequence comprising a first internal ribosome entry site (IRES), and/or (ii) the second gene and the nucleic acid sequence encoding the additional GMP are joined by a nucleic acid sequence comprising a second IRES; or

(b) (i) the first promoter comprises a first exogenous promoter that is activated upon binding of the first ligand to the first ligand binding domain, and/or (ii) the second promoter comprises a second exogenous promoter that is activated upon binding of the second ligand to the second ligand binding domain, optionally wherein:

(1) (i) the first exogenous promoter comprises a synthetic promoter sequence, and/or (ii) the second exogenous promoter comprises a synthetic promoter sequence; or

(2) (i) the nucleic acid sequencing encoding the GMP is operably linked to the

first exogenous promoter, and/or (ii) the nucleic acid sequencing encoding the additional GMP is operably linked to the second exogenous promoter.

6. The system of any one of claims 1-5, wherein:

(a) (i) the first transmembrane receptor comprises an endogenous receptor, and/or (ii) the second transmembrane receptor comprises an endogenous receptor;

(b) (i) the first transmembrane receptor comprises a synthetic receptor, and/or (ii) the second transmembrane receptor comprises a synthetic receptor;

(c) (i) the first transmembrane receptor comprises a chimeric antigen receptor (CAR), a T cell receptor (TCR), G-protein coupled receptor (GPCR), integrin receptor, or a Notch receptor, and/or (ii) the second transmembrane receptor comprises a chimeric antigen receptor (CAR), a T cell receptor (TCR), G-protein coupled receptor (GPCR), integrin receptor, or a Notch receptor, optionally wherein:

(1) (i) the first transmembrane receptor comprises a GPCR, and/or (ii) the second transmembrane receptor comprises a GPCR; or

(2) (i) the first transmembrane receptor comprises a chimeric antigen receptor (CAR), and/or (ii) the second transmembrane receptor comprises a chimeric antigen receptor (CAR), optionally wherein:

(A) the ligand binding domain of the CAR comprises at least one of a Fab, a single-chain Fv (scFv), an extracellular receptor domain, and an Fc binding domain;

(B) the signaling domain of the CAR comprises an immunoreceptor tyrosine-based activation motif (ITAM);

(C) the signaling domain of the CAR comprises an immunoreceptor tyrosine-based inhibition motif (ITIM); or

(D) the signaling domain of the CAR comprises a co-stimulatory domain;

(d) the actuator moiety of the GMP and/or the actuator of the additional GMP is an RNA-guided actuator moiety, and wherein the system further comprises a guide-RNA that complexes with the RNA-guided actuator moiety, optionally wherein:

(1) the RNA-guided actuator moiety is Cas9, optionally wherein:

(A) Cas9 is an S. pyogenes Cas9;

(B) Cas9 is an S. aureus Cas9; or

(C) Cas9 substantially lacks nuclease activity; or

(2) the RNA-guided actuator moiety is Cpf1, optionally wherein Cpf1 substantially lacks nuclease activity;

(e) the GMP and/or the additional GMP comprises a nuclear localization sequence (NLS);

(f) the GMP and/or the additional GMP comprises a transcription activator or transcription repressor;

(g) the first promoter and/or the second promoter is selected from an IL-2, IFN-γ, IRF4, NR4A1, PRDM1, TBX21, CD69, CD25, and GZMB promoter;

(h) the target gene and/or the additional target gene encodes for a cytokine, a T cell receptor (TCR), or an immune checkpoint inhibitor,

optionally wherein the target gene and/or the additional target gene encodes for an immune checkpoint inhibitor, optionally wherein the immune checkpoint inhibitor is PD-1, CTLA-4, LAG3, TIM-3, A2AR, B7-H3, B7-H4, BTLA, IDO, KIR, or VISTA; or

(i) the cell is an immune cell, a hematopoietic progenitor cell, or a hematopoietic stem cell,

optionally wherein the cell is an immune cell, optionally wherein the immune cell is a lymphocyte, optionally wherein:

(1) the lymphocyte is a T cell; or

(2) the lymphocyte is a natural killer (NK) cell.

7. An expression cassette comprising a promoter operably linked to a nucleic acid sequence encoding a gene modulating polypeptide (GMP) that comprises an actuator moiety, wherein the expression cassette is characterized in that the promoter is activatable to drive expression of the GMP from the expression cassette upon activation of a transmembrane receptor of a cell by binding of a ligand to the transmembrane receptor.

8. The expression cassette of claim 7, wherein:

(a) the transmembrane receptor comprises a signaling domain, and wherein the signaling domain activates a signaling pathway of the cell when the transmembrane receptor is activated;

(b) the signaling domain of the transmembrane receptor activates an immune cell signaling pathway;

(c) a transcription factor of the activated signaling pathway of the cell binds the

promoter, thereby activating the promoter to drive expression of the GMP from the expression cassette;

(d) the promoter comprises an endogenous promoter sequence;

(e) the promoter comprises a synthetic promoter sequence;

(f) the actuator moiety is an RNA-guided actuator moiety, optionally wherein:

(1) the RNA-guided actuator moiety is Cas9, optionally wherein:

(A) Cas9 is an S. pyogenes Cas9;

(B) Cas9 is an S. aureus Cas9; or

(C) Cas9 substantially lacks nuclease activity; or

(2) the RNA-guided actuator moiety is Cpf1, optionally wherein Cpf1 substantially lacks nuclease activity;

(g) the promoter is an IL-2, IFN-γ, IRF4, NR4A1, PRDM1, TBX21, CD69, CD25, or GZMB promoter;

(h) the GMP comprises a nuclear localization sequence (NLS);

(i) the GMP comprises a transcription activator or a transcription repressor; or

(j) the expression cassette is integrated into the cell genome, optionally wherein:

(1) the expression cassette is integrated into the cell genome via lentivirus;

(2) the expression cassette is integrated into the cell genome at a region comprising a safe harbor site;

(3) the expression cassette is integrated into the AAVS1 site of chromosome 19;

(4) the expression cassette is integrated into the CCR5 site of chromosome 3; or

(5) the expression cassette is integrated into the cell genome via a programmable nuclease,

optionally wherein the programmable nuclease is a RNA-guided nuclease, a zinc finger nuclease (ZNF), or a transcription activator-like effector nuclease (TALEN).

9. The expression cassette of claim 7, further comprising at least one integration sequence which facilitates integration of the expression cassette into a cell genome,

optionally wherein:

(a) the at least one integration sequence facilitates integration of the expression

cassette into a region of the cell genome such that the nucleic acid sequence encoding the GMP is operably linked to an endogenous promoter,

optionally wherein the at least one integration sequence facilitates integration of the expression cassette into a region of the cell genome such that the nucleic acid sequence encoding the GMP is (i) operably linked to an endogenous promoter and (ii) located downstream of a gene encoding an endogenous protein, wherein expression of the endogenous protein in the cell is driven by the endogenous promoter, optionally wherein:

(1) the nucleic acid sequence encoding the GMP is joined to the gene by a nucleic acid sequence encoding a peptide linker, optionally wherein:

(A) the nucleic acid sequence encoding the GMP is joined in-frame to the gene;

(B) the peptide linker comprises a protease recognition sequence; or

(C) the peptide linker comprises a self-cleaving segment,

optionally wherein the self-cleaving segment comprises a 2A peptide, optionally wherein the 2A peptide is T2A, P2A, E2A, or F2A; or

(2) the nucleic acid sequence encoding the GMP is joined to the gene by a nucleic acid sequence comprising an internal ribosome entry site (IRES);

(b) the at least one integration sequence comprises a homology sequence, and wherein the expression cassette is integrated into the cell genome via homology-directed repair (HDR), optionally wherein:

(1) two integration sequences flank the nucleic acid sequence encoding the GMP, each integration sequence of the two comprising a homology sequence; or

(2) the homology sequence facilitates integration of the expression cassette into a targeted region of the cell genome; or

(c) the nucleic acid sequence encoding the GMP is located downstream of the promoter after integration of the expression cassette.

10. A cell comprising the system of any one of claims 1-6 or the expression cassette of any one of claims 7-9.

11. The cell of claim 10, wherein:

(a) the cell is a hematopoietic cell, a hematopoietic progenitor cell, or a hematopoietic stem cell,

optionally wherein the cell is a hematopoietic cell, and wherein the hematopoietic cell is a lymphocyte, natural killer (NK) cell, monocyte, macrophage, or dendritic cell (DC);

(b) the first expression cassette of the system is present in the cell as part of a plasmid; or

(c) the first expression cassette of the system is integrated into the cell genome, optionally wherein:

(1) the first expression cassette of the system is integrated into the cell genome at a region comprising a genomic safe harbor site;

(2) the first expression cassette of the system is integrated into the AAVS1 site of chromosome 19;

(3) the first expression cassette of the system is integrated into the CCR5 site of chromosome 3; or

(4) the first expression cassette is integrated into the cell genome via a programmable nuclease,

optionally wherein the programmable nuclease is a RNA-guided nuclease, a zinc finger nuclease (ZNF), or a transcription activator-like effector nuclease (TALEN).

12. A cell comprising the system of any one of claims 1-6 or the expression cassette of any one of claims 7-9 for use in therapy.

13. Use of a cell comprising the system of any one of claims 1-6 or the expression cassette of any one of claims 7-9 in the manufacture of a medicament for the treatment of a disease selected from the group consisting of a cancer, an infectious disease, and an autoimmune disease.