INDUCIBLE CELL DEATH SYSTEMS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/116,433 filed November 20, 2020, 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 Month XX, 20XX, is named XXXXXUS_sequencelisting.txt, and is X,XXX,XXX bytes in size.

BACKGROUND

[0003] Currently available cell and gene therapy products can lack control, which can lead to safety concerns such as toxicity in subjects that receive the therapies. Thus, additional methods of controlling and regulating these therapies are needed.

SUMMARY

[0003a] In an aspect of the present disclosure, there is provided an engineered cell comprising an inducible cell death system, or an engineered nucleic acid comprising the inducible cell death system, for use in therapy,

wherein the inducible cell death system comprises two or more polypeptide monomers, wherein each polypeptide monomer comprises one or more ligand binding domains and a cell death-inducing domain, wherein the polypeptide monomers are configured to oligomerize upon contacting the polypeptide monomers with a cognate ligand of the one or more ligand binding domains and generate a cell-death inducing signal in a cell in which the polypeptide monomers are expressed, and wherein:

i) each polypeptide monomer of the two or more polypeptide monomers comprises the same ligand binding domain, wherein the same ligand binding domain comprises a domain or functional fragment thereof selected from the group consisting of: a hormone-binding domain of estrogen receptor (ER) domain, optionally comprising the amino acid sequence of SEQ ID NO: 42; an ABI domain, optionally comprising the amino sequence of SEQ ID NO: 31; a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53; a caffeine-binding single-domain antibody, optionally comprising the amino acid sequence of SEQ ID NO: 33; a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38; a heavy chain variable region (VH) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 50, and/or the light chain variable region (VL) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 51; optionally comprising the amino acid sequence of

SEQ ID NO: 43; a progesterone receptor domain, optionally comprising the amino acid sequence of SEQ ID NO: 52; and combinations thereof; or

ii) a first polypeptide monomer of the two or more polypeptide monomers comprises a first ligand binding domain, a second polypeptide monomer of the two or more polypeptide monomers comprises a second ligand binding domain, and wherein the second ligand binding domain is different from the first ligand binding domain.

[0003b] In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising:

a) an inducible cell death system, an engineered nucleic acid encoding the inducible cell death system, or an engineered cell comprising the inducible cell death system, wherein the inducible cell death system comprises two or more polypeptide monomers, wherein each polypeptide monomer comprises one or more ligand binding domains and a cell death-inducing domain, wherein the polypeptide monomers are configured to oligomerize upon contacting the polypeptide monomers with a cognate ligand of the one or more ligand binding domains and generate a cell-death inducing signal in a cell in which the polypeptide monomers are expressed, and wherein:

i) each polypeptide monomer of the two or more polypeptide monomers comprises the same ligand binding domain, wherein the same ligand binding domain comprises a domain or functional fragment thereof selected from the group consisting of: a hormone-binding domain of estrogen receptor (ER) domain, optionally comprising the amino acid sequence of SEQ ID NO: 42; an ABI domain, optionally comprising the amino sequence of SEQ ID NO: 31; a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53; a caffeine-binding single-domain antibody, optionally comprising the amino acid sequence of SEQ ID NO: 33; a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38; a heavy chain variable region (VH) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 50, and/or the light chain variable region (VL) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 51; optionally comprising the amino acid sequence of SEQ ID NO: 43; a progesterone receptor domain, optionally comprising the amino acid sequence of SEQ ID NO: 52; and combinations thereof; or

ii) a first polypeptide monomer of the two or more polypeptide monomers comprises a first ligand binding domain, a second polypeptide monomer of the two or more polypeptide monomers comprises a second ligand binding domain, and wherein the second ligand binding domain is different from the first ligand binding domain;

and

b) a pharmaceutically acceptable carrier.

[0003c] In a further aspect of the present disclosure, there is provided an inducible cell death system comprising an activation-conditional control polypeptide (ACP),

wherein the ACP comprises a ligand binding domain and a transcriptional effector domain, and

wherein upon binding of the ligand binding domain to a cognate ligand, the ACP is capable of modulating transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter,

wherein the gene of interest comprises a cell death inducing polypeptide.

[0003d] In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising:

a) the inducible cell death system comprising an activation-conditional control polypeptide (ACP) as disclosed herein; or an isolated cell comprising the inducible cell death system comprising an activation-conditional control polypeptide (ACP) as disclosed herein; or an engineered nucleic acid encoding the inducible cell death system comprising an activation-conditional control polypeptide (ACP) as disclosed herein; and

b) a pharmaceutically acceptable carrier.

[0003e] In a further aspect of the present disclosure, there is provided a multimeric activation-conditional control polypeptide (ACP) comprising:

a) a first chimeric polypeptide, wherein the first chimeric polypeptide comprises a first ligand binding domain and a transcriptional activation domain; and

b) a second chimeric polypeptide, wherein the second chimeric polypeptide comprises a second ligand binding domain and a nucleic acid-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide oligomerize to form the multimeric ACP via a cognate ligand that binds to each ligand binding domain, and

wherein the multimeric ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter,

wherein the gene of interest is a cell death-inducing polypeptide, optionally wherein the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated

modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related cell death-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase, optionally wherein the caspase 9 or a functional truncation thereof, comprises the amino acid sequence of SEQ ID NO: 39, optionally wherein the DTA comprises the amino acid sequence of SEQ ID NO: 41, optionally wherein the granzyme B comprises the amino acid sequence of SEQ ID NO: 47, optionally wherein the Bax comprises the amino acid sequence of SEQ ID NO: 32, and wherein the transcriptional activation 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); and a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain).

[0003f] In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising:

a) the multimeric activation-conditional control polypeptide (ACP) as disclosed herein; or an isolated cell comprising the multimeric activation-conditional control polypeptide (ACP) as disclosed herein; or an engineered nucleic acid encoding the multimeric activation-conditional control polypeptide (ACP) as disclosed herein; and

b) a pharmaceutically acceptable carrier.

[0003g] In a further aspect of the present disclosure, there is provided a pharmaceutical composition as disclosed herein for use in therapy.

[0004] Disclosed herein are inducible cell death systems that can be used for inducing cell death in a regulated manner, for example in a safety switch system.

[0005] In some examples, the present disclosure provides an inducible cell death system comprising two or more polypeptide monomers, wherein each polypeptide monomer comprises one or more ligand binding domains and a cell death-inducing domain, wherein the polypeptide monomers are configured to oligomerize upon contacting the polypeptide monomers with a cognate ligand of the one or more ligand binding domains and generate a cell-death inducing signal in a cell in which the polypeptide monomers are expressed , and wherein:

the one or more ligand binding domains of each of the two or more polypeptide monomers comprise a domain or functional fragment thereof selected from the group consisting of: an ABI domain, optionally comprising the amino sequence of SEQ ID NO: 31; a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53; a caffeine-binding single-domain antibody, optionally comprising the amino acid sequence of SEQ ID NO: 33; a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38; a hormone-binding domain of estrogen receptor (ER) domain, optionally comprising the amino acid sequence of SEQ ID NO: 42; a heavy chain variable region (VH) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 50, and/or the light chain variable region (VL) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 51; an FKBP domain , optionally comprising the amino acid sequence of SEQ ID NO: 43; and a progesterone receptor domain, optionally comprising the amino acid sequence of SEQ ID NO: 52; or

the one or more ligand binding domains of a first of the two or more polypeptide monomers comprise an FKBP domain, optionally comprising the amino acid sequence of SEQ ID NO: 43, and the one or more ligand binding domains of a second of the two or more polypeptide monomers comprise an FRB domain, optionally comprising the amino acid sequence of SEQ ID NO: 44; or

the one or more ligand binding domains of a first of the two or more polypeptide monomers comprise a cereblon domain, optionally comprising the amino acid sequence set forth in one of SEQ ID NOs: 127 and 129, and the one or more ligand binding domains of a second of the two or more polypeptide monomers comprise a degron, optionally comprising the amino acid sequence set forth in one of SEQ ID NOs: 131 and 133, and

optionally wherein the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related cell death-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase, optionally wherein the caspase 9 or a functional truncation thereof, comprises the amino acid sequence of SEQ ID NO: 39, optionally wherein the DTA comprises the amino acid sequence of SEQ ID NO: 41, optionally wherein the granzyme B comprises the amino acid sequence of SEQ ID NO: 47, optionally wherein the Bax comprises the amino acid sequence of SEQ ID NO: 32.

[0006] In some examples, each polypeptide monomer comprises the same ligand binding domain, optionally wherein each polypeptide monomer comprises:

an FKBP domain, optionally wherein the cognate ligand is FK1012, a derivative thereof, or an analog thereof; or

an ABI domain and a PYL domain, optionally wherein the cognate ligand is abscisic acid; or

a first cannabidiol binding domain comprising the amino acid sequence of SEQ ID NO: 34, and a second cannabidiol binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 35, 36, 37, and 38, optionally wherein the cognate ligand is a phytocannabinoid, optionally the phytocannabinoid is cannabidiol; or

a hormone-binding domain of estrogen receptor (ER) domain and an FKBP domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof or an analog thereof and/or tamoxifen or a metabolite thereof, optionally wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen;

two caffeine-binding single-domain antibodies, optionally wherein each caffeine-binding single-domain antibody comprises the amino acid sequence of SEQ ID NO: 33, optionally wherein the cognate ligand is caffeine or a derivative thereof; or

a first progesterone receptor domain comprising the amino acid sequence of SEQ ID NO: 52, and a second first progesterone receptor domain comprising the amino acid sequence of SEQ ID NO: 52, optionally wherein the cognate ligand is mifepristone or a derivative thereof,

optionally wherein the polypeptide monomers are configured to form homooligomers upon contact with the cognate ligand, and optionally wherein the homooligomers comprise homodimers.

[0007] In some examples, a first polypeptide monomer comprises a first ligand binding domain and a second polypeptide monomer comprises a second ligand binding domain, optionally wherein:

the first monomer comprises an FKBP domain and the second monomer comprises an FRB domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof; or

[0008] the first polypeptide monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and the second polypeptide monomer comprises an FKBP domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof and/or tamoxifen or a metabolite thereof; or

the first polypeptide monomer comprises an FRB domain and the second polypeptide monomer comprises a hormone-binding domain of estrogen receptor (ER) domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof and/or tamoxifen or a metabolite thereof; or

wherein the first polypeptide monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and an FKBP domain, and the second polypeptide monomer comprises an FRB domain and a hormone-binding domain of estrogen receptor (ER) domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof and/or tamoxifen or a metabolite thereof; or

the first polypeptide monomer comprises an ABI domain and the second polypeptide monomer comprises a PYL domain, optionally wherein the cognate ligand comprises abscisic acid; or

the first polypeptide monomer comprises a heavy chain variable region (VH) of an anti-nicotine antibody and the second polypeptide monomer comprises a light chain variable region (VL) of

an anti-nicotine antibody, optionally wherein the anti-nicotine antibody is a Nic12 antibody, optionally wherein the VH comprises the amino acid sequence of SEQ ID NO: 50, and optionally wherein the VL comprises the amino acid sequence of SEQ ID NO: 51, and optionally wherein the cognate ligand is nicotine or a derivative thereof; or
the first polypeptide monomer comprises a cannabidiol binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 35, 36, 37, and 38 and the second polypeptide monomer comprises a cannabidiol binding domain comprising the amino acid sequence of SEQ ID NO: 34. optionally wherein the cognate ligand is a phytocannabinoid, optionally wherein the phytocannabinoid is cannabidiol; or
the first polypeptide monomer comprises a cereblon domain comprising the amino acid sequence set forth in one of SEQ ID NOs: 127 and 129 and the second polypeptide monomer comprises a degron domain comprising the amino acid sequence set forth in one of SEQ ID NOs: 131 and 133, optionally wherein the cognate ligand is an IMiD, optionally wherein the IMiD is an FDA-approved drug, and optionally wherein the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide,
optionally wherein the polypeptide monomers are configured to form heterooligomers upon contact with the cognate ligand, and optionally wherein the heterooligomers comprise heterodimers.

[0009] In some examples, the at least one of the two or more polypeptide monomers further comprises a linker localized between each ligand binding domain and cell death-inducing domain, optionally wherein the linker comprises an amino acid sequence selected from the group consisting of: GGGGSGGGGSGGGGSVDGF (SEQ ID NO: 101) and ASGGGGSAS (SEQ ID NO: 102), optionally wherein each polypeptide monomer further comprises a linker localized between each ligand binding domain and cell death-inducing domain.

[0010] In some examples, the present disclosure provides an inducible cell death system comprising an activation-conditional control polypeptide (ACP),
wherein the ACP comprises a ligand binding domain and a transcriptional effector domain, and

[0011] wherein upon binding of the ligand binding domain to a cognate ligand, the ACP is capable of modulating transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter,
wherein the gene of interest comprises a cell death inducing polypeptide, and
optionally wherein the ligand binding domain comprises a degron, optionally wherein the degron is capable of inducing degradation of the ACP, and
optionally 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, an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box, or 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 regulatable polypeptide, optionally 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, optionally wherein the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences, optionally wherein the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRD AL (SEQ ID NO: 103).

[0012] In some examples, the present disclosure provides an inducible cell death system comprising an activation-conditional control polypeptide (ACP), wherein the ACP comprises one or more ligand binding domains and a transcription factor comprising a nucleic acid-binding domain and a transcriptional effector domain,

wherein the ACP undergoes nuclear localization upon binding of the ligand binding domain to a cognate ligand, and

wherein when localized to a cell nucleus, the ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter,

wherein the gene of interest comprises a cell death-inducing domain,

optionally wherein the transcriptional 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

wherein upon binding of the ligand binding domain to a cognate ligand, the cognate ligand inhibits the pro-survival polypeptide,
optionally wherein the pro-survival polypeptide is selected from the group consisting of: XIAP, a modified XIAP, Bcl-2, Bcl-xL, Bcl-w, Bcl-2-related protein A1 (BCL2A1), Mcl-1, FLICE-like inhibitory protein (c-FLIP), and an adenoviral E1B-19K protein,
optionally wherein the ligand binding domain is localized at the N-terminal region of the pro-survival polypeptide or at the C-terminal region of the pro-survival polypeptide.

[0015] In some examples the present disclosure provides an inducible cell death system comprising a regulatable cell survival polypeptide and a cell death-inducing polypeptide, wherein the cell-survival polypeptide comprises a pro-survival polypeptide and a heterologous ligand binding domain,
wherein when expressed the cell survival polypeptide is capable of inhibiting the cell death-inducing polypeptide, and
wherein upon binding to a cognate ligand, the cognate ligand inhibits the pro-survival polypeptide, optionally wherein the cell survival polypeptide is selected from the group consisting of: XIAP, a modified XIAP, Bcl-2, Bcl-xL, Bcl-w, Bcl-2-related protein A1 (BCL2A1), Mcl-1, FLICE-like inhibitory protein (c-FLIP), and an adenoviral E1B-19K protein, optionally wherein the ligand binding domain is localized at the N-terminal region of the pro-survival polypeptide or at the C-terminal region of the pro-survival polypeptide.

[0016] In some examples, the XIAP comprises the amino acid sequence of SEQ ID NO: 107, or the modified XIAP comprises one or more amino acid substitutions within positions 305-325 of SEQ ID NO: 107, optionally wherein the one or more amino acid substitutions are at one or more positions of SEQ ID NO: 107 selected from the group consisting of: 305, 306, 308, and 325, optionally wherein the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, optionally wherein the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, optionally wherein the amino acid substitution at position 308 of SEQ ID NO: 107 is selected from the group consisting of T308S and T308D, optionally wherein the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[0017] In some examples the ligand binding domain comprises a domain, or functional fragment thereof, selected from the group consisting of:
an ABI domain, optionally comprising the amino acid sequence of SEQ ID NO: 31, and optionally wherein the cognate ligand is abscisic acid;
a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53, and optionally wherein the cognate ligand is abscisic acid;

a caffeine-binding single-domain antibody optionally comprising the amino acid sequence of SEQ ID NO: 33, and optionally wherein the cognate ligand is caffeine or a derivative thereof;
a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38, optionally wherein the cognate ligand is a phytocannabinoid, optionally wherein the phytocannabinoid is cannabidiol;
a hormone-binding domain of estrogen receptor (ER) domain optionally comprising the amino acid sequence of SEQ ID NO: 42, optionally wherein the cognate ligand is tamoxifen or a metabolite thereof, and optionally wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen;
a heavy chain variable region (VH) of an anti-nicotine antibody optionally comprising the amino acid sequence of SEQ ID NO: 50, and optionally wherein the cognate ligand is nicotine or a derivative thereof;
a light chain variable region (VL) of an anti-nicotine antibody optionally comprising the amino acid sequence of SEQ ID NO: 51, and optionally wherein the cognate ligand is nicotine or a derivative thereof;
a progesterone receptor domain optionally comprising the amino acid sequence of SEQ ID NO: 52, and optionally wherein the cognate ligand is mifepristone or a derivative thereof;

[0018] an FKBP domain optionally comprising the amino acid sequence of SEQ ID NO: 43, and optionally wherein the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof; and
an FRB domain optionally comprising the amino acid sequence of SEQ ID NO: 44, and optionally wherein the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

[0019] In some examples, the ligand binding domain comprises a degron, optionally wherein the degron is capable of inducing degradation of the regulatable cell survival polypeptide, and optionally 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, an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box, optionally 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 regulatable polypeptide, optionally 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, optionally wherein the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences, optionally wherein the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRD AL (SEQ ID NO: 103), optionally wherein the cognate ligand is an IMiD, optionally wherein the IMiD is an FDA-approved drug, and optionally wherein the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

[0020] In some examples, the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related cell death-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic chytochrom P450-2B1, and Purine nucleoside phosphorylase, optionally wherein the caspase 9 or a functional truncation thereof, comprises the amino acid sequence of SEQ ID NO: 39, optionally wherein the DTA comprises the amino acid sequence of SEQ ID NO: 41, optionally wherein the Bax comprises the amino acid sequence of SEQ ID NO: 32.

[0021] In some examples, the present disclosure provides an activation-conditional control polypeptide (ACP) comprising:

a light chain variable region (VL) of an anti-nicotine antibody optionally comprising the amino acid sequence of SEQ ID NO: 51, and optionally wherein the cognate ligand is nicotine or a derivative thereof;
a progesterone receptor domain optionally comprising the amino acid sequence of SEQ ID NO: 52, and optionally wherein the cognate ligand is mifepristone or a derivative thereof;
an FKBP domain optionally comprising the amino acid sequence of SEQ ID NO: 43, and optionally wherein the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof; and
an FRB domain optionally comprising the amino acid sequence of SEQ ID NO: 44, and optionally wherein the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof,
optionally wherein the gene of interest is a cell death-inducing polypeptide, optionally wherein the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related cell death-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase, optionally wherein the caspase 9 or a functional truncation thereof, comprises the amino acid sequence of SEQ ID NO: 39, optionally wherein the DTA comprises the amino acid sequence of SEQ ID NO: 41, optionally wherein the granzyme B comprises the amino acid sequence of SEQ ID NO: 47, optionally wherein the Bax comprises the amino acid sequence of SEQ ID NO: 32.

[0022] The ACP as disclosed herein, wherein the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain), optionally wherein the ZF protein domain is modular in design and is composed of an array of zinc finger motifs, optionally wherein the ZF-protein domain comprises one to ten zinc finger motifs.

[0023] In some examples, the chimeric polypeptide further comprises a linker localized between the nucleic acid-binding domain and the transcriptional effector domain, optionally wherein the linker comprises one or more 2A ribosome skipping tags, optionally wherein each 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

[0024] In some examples, the chimeric polypeptide comprises a first ligand binding domain operably linked to the nucleic acid-binding domain and a second ligand binding domain operably linked to the transcriptional effector domain; optionally wherein:
each of the first and second ligand binding domains comprises a hormone-binding domain of estrogen receptor (ER) domain, optionally wherein the cognate ligand is tamoxifen or a metabolite thereof, optionally wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen; or
each of the first and second ligand binding domains comprises a progesterone receptor domain., optionally wherein the cognate ligand is mifepristone or a derivative thereof, and optionally wherein when the ligand binding domain comprises an ABI domain or a PYL domain, the cognate ligand is abscisic acid; or
each of the first and second ligand binding domains comprises a caffeine-binding single-domain antibody, optionally wherein the cognate ligand is caffeine or a derivative thereof; or
each of the first and the second ligand binding domains comprises a cannabidiol binding domain, optionally wherein the cognate ligand is a cannabidiol or a phytocannabinoid, optionally wherein the cannabidiol binding domain comprises a single-domain antibody or a nanobody, and optionally wherein the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38.

[0025] In some examples, the nucleic acid-binding domain binds to the ACP-responsive promoter, optionally wherein the ACP-responsive promoter comprises an ACP-binding domain sequence and a promoter sequence, optionally wherein the promoter sequence comprises a minimal promoter, optionally wherein the promoter sequence is an inducible promoter and further comprises a responsive element selected from the group consisting of: NFκB 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, inducer molecule-responsive promoters, and tandem repeats thereof, and optionally wherein the ACP-responsive promoter comprises a synthetic promoter, and optionally wherein the ACP-binding domain comprises one or more zinc finger binding sites.

[0026] In some examples, the ligand binding domain is localized N-terminal to the transcriptional effector domain or C-terminal to the transcriptional effector domain.

[0027] In some examples, the present disclosure provides an isolated cell comprising an inducible cell death system as previously described or an ACP as previously described.

[0028] In some examples, the present disclosure provides an engineered nucleic acid encoding an inducible cell death system as previously described or an ACP as previously described.

[0029] In some examples, provided herein is an isolated cell comprising an inducible cell death polypeptide comprising two or more monomers, wherein each monomer comprises one or more ligand binding domains and a cell death-inducing domain, wherein each of the one or more ligand binding domains comprises a domain, or functional fragment thereof, selected from the group consisting of: an ABI domain, a PYL domain, a caffeine-binding single-domain antibody, a cannabidiol binding domain, a hormone-binding domain of estrogen receptor (ER) domain, heavy chain variable region (VH) of an anti-nicotine antibody, light chain variable region (VL) of an anti-nicotine antibody, a progesterone receptor domain, an FKBP domain, and an FRB domain, wherein each monomer is oligomerizable via a cognate ligand that binds to the ligand binding domain, and wherein when the ligand oligomerizes each monomer, a cell death-inducing signal is generated in the cell.

[0030] In some examples, the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related apoptosis-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase.

[0031] In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D.

[0032] In some examples, the cell death-inducing domain comprises Bid, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the Bid amino acid sequence of Table D.

[0033] In some examples, the ABI domain comprises the amino acid sequence of Table D. In some examples, the PYL domain comprises the amino acid sequence of Table D.

[0034] In some examples, the caffeine-binding single-domain antibody comprises the amino acid sequence of Table D.

[0035] In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequences for CA14, DB6, DB11, DB18, and DB21 as shown in Table D.

[0036] In some examples, the hormone-binding domain of estrogen receptor (ER) domain comprises the amino acid sequence of Table D.

[0037] In some examples, the heavy chain variable region (VH) of an anti-nicotine antibody comprises the VH amino acid sequence of Table D. In some examples, the light chain variable region (VL) of an anti-nicotine antibody comprises the VL amino acid sequence of Table D.

[0038] In some examples, the progesterone receptor domain comprises the amino acid sequence of Table D.

[0039] In some examples, the FKBP domain comprises the amino acid sequence of Table D.

[0040] In some examples, the FRB domain comprises the amino acid sequence of Table D.

[0041] In some examples, each monomer comprises the same ligand binding domain. In some examples, the inducible cell death polypeptide comprises homooligomers. In some examples, the homooligomers comprise homodimers. In some examples, each monomer comprises an FKBP domain. In some examples, the ligand is FK1012, a derivative thereof, or an analog thereof.

[0042] In some examples, the cell death-inducing domain comprises Bid, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the Bid amino acid sequence of Table D.

[0043] In some examples, each monomer comprises an ABI domain and a PYL domain. In some examples, the ligand is abscisic acid. In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing comprises the caspase 9 amino acid sequence of Table D.

[0044] In some examples, each monomer comprises a cannabidiol binding domain comprising the CA14 amino acid sequence of Table D and a cannabidiol binding domain comprising an amino acid sequence selected from the group consisting of the sequences of DB6, DB11, DB18, and DB21 of Table D.

[0045] In some examples, each monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and an FKBP domain. In some examples, each monomer comprises an FRB domain and a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D. In some examples, the ligand is rapamycin, a derivative thereof, or an analog thereof. In some examples, the ligand is 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.

[0046] In some examples, each monomer comprises two caffeine-binding single-domain antibodies. In some examples, each caffeine-binding single-domain antibody comprises the amino acid sequence of Table D. In some examples, the ligand is caffeine or a derivative thereof.

[0047] In some examples, a first monomer comprises a first ligand binding domain and a second monomer comprises a second ligand binding domain. In some examples, the inducible cell death polypeptide comprises heterooligomers. In some examples, the heterooligomers comprise heterodimers. In some examples, the first monomer comprises an FKBP domain and the second monomer comprises an FRB domain. In some examples, the cell death-inducing domain comprises Bid, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the Bid amino acid sequence of Table D.

[0048] In some examples, the first monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and the second monomer comprises an FKBP domain. In some examples, the first monomer comprises an FRB domain and the second monomer comprises a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the first monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and an FKBP domain, and the second monomer comprises an FRB domain and the second monomer comprises a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D. In some examples, the ligand is rapamycin, a derivative thereof, or an analog thereof. In some examples, the ligand is 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.

[0049] In some examples, the first monomer comprises an ABI domain and the second monomer comprises a PYL domain. In some examples, the ligand is abscisic acid.

[0050] In some examples, the first monomer comprises a heavy chain variable region (VH) of an anti-nicotine antibody and the second monomer comprises a light chain variable region (VL) of an anti-nicotine antibody. In some examples, the anti-nicotine antibody is a Nic12 antibody. In some examples, the VH comprises the VH amino acid sequence of Table D. In some examples, the VL comprises the VL amino acid sequence of Table D. In some examples, the ligand is nicotine or a derivative thereof.

[0051] In some examples, the first monomer comprises a cannabidiol binding domain comprising an amino acid sequence selected from the group consisting of the sequences of DB6, DB11,

DB18, and DB21 of Table D and the second monomer comprises a cannabidiol binding domain comprising the amino acid sequence of CA14 of Table D. In some examples, the ligand is a cannabidiol or a phytocannabinoid.

[0052] In some examples, each monomer further comprises a linker localized between each ligand binding domain and cell death-inducing domain. In some examples, the linker comprises an amino acid sequence selected from the group consisting of: GGGGSGGGGSGGGGSVDGF (SEQ ID NO: 85) and ASGGGGSAS (SEQ ID NO: 86).

[0053] Also disclosed herein is an isolated cell comprising an activation-conditional control polypeptide (ACP), wherein the ACP comprises one or more ligand binding domains and a transcription factor comprising a nucleic acid-binding domain and a transcriptional effector domain, wherein the ACP undergoes nuclear localization upon binding of the ligand binding domain to a cognate ligand, and wherein when localized to a cell nucleus, the ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter.

[0054] Also disclosed herein is an isolated cell comprising a multimeric activation-conditional control polypeptide (ACP), wherein the multimeric ACP comprises: (a) a first chimeric polypeptide, wherein the first chimeric polypeptide comprises a first ligand binding domain and a transcriptional activation domain; and (b) a second chimeric polypeptide, wherein the second chimeric polypeptide comprises a second ligand binding domain and a nucleic acid-binding domain, wherein the first chimeric polypeptide and the second chimeric polypeptide multimerize to form the multimeric ACP via a cognate ligand that binds to each ligand binding domain, and wherein the multimeric ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter.

[0055] In some examples, each ligand binding domain comprises a domain, or functional fragment thereof, selected from the group consisting of: an ABI domain, a PYL domain, a caffeine-binding single-domain antibody, a cannabidiol binding domain, a hormone-binding domain of estrogen receptor (ER) domain, heavy chain variable region (VH) of an anti-nicotine antibody, light chain variable region (VL) of an anti-nicotine antibody, a progesterone receptor domain, an FKBP domain, and an FRB domain.

[0056] In some examples, the ABI domain comprises the ABI amino acid sequence of Table D. In some examples, the PYL domain comprises the PYL amino acid sequence of Table D. In some examples, the caffeine-binding single-domain antibody comprises the amino acid sequence of Table D. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequences of CA14, DB6, DB11, DB18, and DB21 of Table D. In some examples, the hormone-binding domain of estrogen receptor (ER) domain

comprises the amino acid sequence of Table D. In some examples, the heavy chain variable region (VH) of an anti-nicotine antibody comprises the VH amino acid sequence of Table D. In some examples, the light chain variable region (VL) of an anti-nicotine antibody comprises the VL amino acid sequence of Table D. In some examples, the progesterone receptor domain comprises the amino acid sequence of Table D. In some examples, the FKBP domain comprises the amino acid sequence of Table D. In some examples, the FRB domain comprises the amino acid sequence of Table D.

[0057] In some examples, the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain). In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). In some examples, the transcriptional 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain); a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

[0058] In some examples, the chimeric polypeptide further comprises a linker localized between the nucleic acid-binding domain and the transcriptional effector domain. In some examples, the linker comprises one or more 2A ribosome skipping tags. In some examples, each 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

[0059] In some examples, the chimeric polypeptide comprises a first ligand binding domain operably linked to the nucleic acid-binding domain and a second ligand binding domain operably linked to the transcriptional effector domain.

[0060] In some examples, each of the first and second ligand binding domains comprises a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the cognate ligand is 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.

[0061] In some examples, each of the first and second ligand binding domains comprises a progesterone receptor domain. In some examples, the cognate ligand is mifepristone or a derivative thereof.

[0062] In some examples, when the ligand binding domain comprises an ABI domain or a PYL domain, the cognate ligand is abscisic acid.

[0063] In some examples, when the ligand binding domain comprises a caffeine-binding single-domain antibody, the cognate ligand is caffeine or a derivative thereof.

[0064] In some examples, when the ligand binding domain comprises a cannabidiol binding domain, the cognate ligand is a cannabidiol or a phytocannabinoid. In some examples, the cannabidiol binding domain comprises a single-domain antibody or a nanobody. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequence of CA14, DB6, DB11, DB18, and DB21 of Table D.

[0065] In some examples, when the ligand binding domain comprises a hormone-binding domain of estrogen receptor (ER) domain, the cognate ligand is 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.

[0066] In some examples, when the ligand binding domain comprises a heavy chain variable region (VH) of an anti-nicotine antibody or a light chain variable region (VL) of an anti-nicotine antibody, the cognate ligand is nicotine or a derivative thereof.

[0067] In some examples, when the ligand binding domain is a progesterone receptor domain, the cognate ligand is mifepristone or a derivative thereof.

[0068] In some examples, when the ligand binding domain comprises an FKBP domain or an FRB domain, the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

[0069] In some examples, the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain).

[0070] In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). In some examples, the ZF protein domain comprises one to ten ZFA.

[0071] In some examples, the nucleic acid-binding domain binds to the ACP-responsive promoter. In some examples, the ACP-responsive promoter comprises an ACP-binding domain sequence and a promoter sequence. 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, minTATA, minTK, inducer

molecule-responsive promoters, and tandem repeats thereof. In some examples, the ACP-responsive promoter comprises a synthetic promoter. In some examples, the ACP-responsive promoter comprises a minimal promoter.

[0072] In some examples, the ACP-binding domain comprises one or more zinc finger binding sites.

[0073] In some examples, the transcriptional 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); and a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain).

[0074] Also disclosed herein is an isolated cell comprising an activation-conditional control polypeptide (ACP), wherein the ACP comprises a ligand binding domain and a transcriptional effector domain, and wherein upon binding of the ligand binding domain to a cognate ligand, the ACP is capable of modulating transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter.

[0075] In some examples, ligand binding domain is localized 5’ of the transcriptional effector domain or 3’ of the transcriptional effector domain. In some examples, the transcriptional effector domain comprises a transcriptional repressor domain. In some examples, the transcriptional repressor domain is selected from the group consisting of: a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

[0076] In some examples, the transcriptional effector domain comprises a transcriptional activator domain. In some examples, the transcriptional activator 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); and a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain).

[0077] In some examples, the ACP is a transcription factor. In some examples, the ACP is a zinc-finger-containing transcription factor. In some examples, the zinc finger-containing transcription factor comprises a DNA-binding zinc finger protein domain (ZF protein domain) and the transcriptional repressor domain or the transcriptional activator domain. In some examples, the ZF protein domain is modular in design and is composed of a zinc finger array (ZFA). In some examples, the ZFA comprises one to ten ZF motifs.

[0078] In some examples, the DNA-binding zinc finger protein domain binds to the ACP-responsive promoter. In some examples, the ACP-responsive promoter comprises an ACP-binding domain and a promoter sequence. 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, minTATA, minTK, inducer molecule-responsive promoters, and tandem repeats thereof. In some examples, the ACP-responsive promoter is a synthetic promoter. In some examples, the ACP-responsive promoter comprises a minimal promoter. In some examples, the ACP-binding domain comprises one or more zinc finger binding sites.

[0079] In some examples, the gene of interest is a cell death-inducing polypeptide.

[0080] In some examples, the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related apoptosis-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase.

[0081] In some examples, the cell death-inducing polypeptide is caspase 9 or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D. In some examples, the cell death-inducing polypeptide is Diphtheria toxin fragment A (DTA). In some examples, the cell death-inducing domain comprises the DTA amino acid sequence of Table D. In some examples, the cell death-inducing polypeptide

is granzyme B. In some examples, the cell death-inducing domain comprises the granzyme B amino acid sequence of Table D. In some examples, the cell death-inducing polypeptide is Bax. In some examples, the cell death-inducing domain comprises the Bax amino acid sequence of Table D.

[0082] Also disclosed herein is an isolated cell comprising a regulatable cell survival polypeptide and a cell death-inducing polypeptide, wherein the cell-survival polypeptide comprises a ligand binding domain, wherein when expressed the cell survival polypeptide is capable of inhibiting the cell death-inducing polypeptide, and wherein upon binding to a cognate ligand, the cognate ligand inhibits the pro-survival polypeptide.

[0083] In some examples, the cell survival polypeptide is selected from the group consisting of: XIAP, Bcl-2, Bcl-xL, Bcl-w, Bcl-2-related protein A1 (BCL2A1), Mcl-1, FLICE-like inhibitory protein (c-FLIP), and an adenoviral E1B-19K protein**.** In some examples, the cell survival polypeptide is XIAP.

[0084] In some examples, the ligand binding domain is localized at the N-terminal region of the pro-survival polypeptide or at the C-terminal region of the pro-survival polypeptide.

[0085] In some examples, the ligand binding domain comprises a domain, or functional fragment thereof, selected from the group consisting of: an ABI domain, a PYL domain, a caffeine-binding single-domain antibody, a cannabidiol binding domain, a hormone-binding domain of estrogen receptor (ER domain), heavy chain variable region (VH) of an anti-nicotine antibody, light chain variable region (VL) of an anti-nicotine antibody, a progesterone receptor domain, an FKBP domain, and an FRB domain.

[0086] In some examples, the ABI domain comprises the ABI amino acid sequence of Table D. In some examples, the PYL domain comprises the PYL amino acid sequence of Table D. In some examples, the caffeine-binding single-domain antibody comprises the amino acid sequence of Table D. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of sequences of CA14, DB6, DB11, DB18, and DB21 of Table D. In some examples, the hormone-binding domain of estrogen receptor (ER) domain comprises the amino acid sequence of Table D. In some examples, the heavy chain variable region (VH) of an anti-nicotine antibody comprises the VH amino acid sequence of Table D. In some examples, the light chain variable region (VL) of an anti-nicotine antibody comprises the VL amino acid sequence of Table D. In some examples, the progesterone receptor domain comprises the amino acid sequence of Table D. In some examples, the FKBP domain comprises the amino acid sequence of Table D. In some examples, the FRB domain comprises the amino acid sequence of Table D.

[0087] In some examples, when the ligand binding domain comprises an ABI domain or a PYL domain, the cognate ligand is abscisic acid.

[0088] In some examples, when the ligand binding domain comprises a caffeine-binding single-domain antibody, the cognate ligand is caffeine or a derivative thereof.

[0089] In some examples, when the ligand binding domain comprises a cannabidiol binding domain, the cognate ligand is a cannabidiol or a phytocannabinoid. In some examples, when the ligand binding domain comprises a hormone-binding domain of estrogen receptor (ER) domain, the cognate ligand is 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.

[0090] In some examples, when the ligand binding domain comprises a heavy chain variable region (VH) of an anti-nicotine antibody or a light chain variable region (VL) of an anti-nicotine antibody, the cognate ligand is nicotine or a derivative thereof.

[0091] In some examples, when the ligand binding domain is a progesterone receptor domain, the cognate ligand is mifepristone or a derivative thereof.

[0092] In some examples, when the ligand binding domain comprises an FKBP domain, or an FRB domain, the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

[0093] In some examples, the ligand binding domain comprises a degron. In some examples, the degron is capable of inducing degradation of the regulatable cell survival polypeptide. 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, an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box. 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 regulatable polypeptide. 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. In some examples, the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences. In some examples, the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRD AL (SEQ ID NO: 87).

[0094] In some examples, the ligand is an IMiD. In some examples, the IMiD is an FDA-approved drug. In some examples, the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

[0095] In some examples, the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related apoptosis-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic chytochrom P450-2B1, and Purine nucleoside phosphorylase.

[0096] In some examples, the cell death-inducing polypeptide is caspase 9 or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D.

[0097] In some examples, the cell death-inducing polypeptide is Diphtheria toxin fragment A (DTA). In some examples, the cell death-inducing domain comprises the DTA amino acid sequence of Table D.

[0098] In some examples, the cell death-inducing polypeptide is Bax. In some examples, the cell death-inducing domain comprises the Bax amino acid sequence of Table D.

[0099] Also disclosed herein is an engineered nucleic acid comprising: an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding an inducible cell

death polypeptide monomer, wherein the promoter is operably linked to the exogenous polynucleotide, wherein the inducible cell death polypeptide monomer comprises one or more ligand binding domains and a cell death-inducing domain, wherein each of the one or more ligand binding domains comprises a domain, or functional fragment thereof, selected from the group consisting of: an ABI domain, a PYL domain, a caffeine-binding single-domain antibody, a cannabidiol binding domain, a hormone-binding domain of estrogen receptor (ER) domain, heavy chain variable region (VH) of an anti-nicotine antibody, light chain variable region (VL) of an anti-nicotine antibody, a progesterone receptor domain, an FKBP domain, and an FRB domain, wherein when expressed, the cell death polypeptide monomer is oligomerizable via a cognate ligand that binds to the ligand binding domain, and wherein when the ligand oligomerizes two or more of the cell death polypeptide monomers, a cell death-inducing signal is generated in a cell.

[00100] In some examples, the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related apoptosis-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase.

[00101] In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D.

[00102] In some examples, the cell death-inducing domain comprises Bid, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the Bid amino acid sequence of Table D.

[00103] In some examples, the ABI domain comprises the amino acid sequence of Table D. In some examples, the PYL domain comprises the amino acid sequence of Table D.

[00104] In some examples, the caffeine-binding single-domain antibody comprises the amino acid sequence of Table D.

[00105] In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequences for CA14, DB6, DB11, DB18, and DB21 as shown in Table D.

[00106] In some examples, the hormone-binding domain of estrogen receptor (ER) domain comprises the amino acid sequence of Table D.

[00107] In some examples, the heavy chain variable region (VH) of an anti-nicotine antibody comprises the VH amino acid sequence of Table D. In some examples, the light chain variable region (VL) of an anti-nicotine antibody comprises the VL amino acid sequence of Table D.

[00108] In some examples, the progesterone receptor domain comprises the amino acid sequence of Table D.

[00109] In some examples, the FKBP domain comprises the amino acid sequence of Table D.

[00110] In some examples, the FRB domain comprises the amino acid sequence of Table D.

[00111] In some examples, each monomer comprises the same ligand binding domain. In some examples, the inducible cell death polypeptide comprises homooligomers. In some examples, the homooligomers comprise homodimers. In some examples, each monomer comprises an FKBP domain. In some examples, the ligand is FK1012, a derivative thereof, or an analog thereof.

[00112] In some examples, the cell death-inducing domain comprises Bid, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the Bid amino acid sequence of Table D.

[00113] In some examples, each monomer comprises an ABI domain and a PYL domain. In some examples, the ligand is abscisic acid. In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D.

[00114] In some examples, each monomer comprises a cannabidiol binding domain comprising the CA14 amino acid sequence of Table D and a cannabidiol binding domain comprising an amino acid sequence selected from the group consisting of the sequences of DB6, DB11, DB18, and DB21 of Table D.

[00115] In some examples, each monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and an FKBP domain. In some examples, each monomer comprises an FRB domain and a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D. In some examples, the ligand is rapamycin, a derivative thereof, or an analog thereof. In some examples, the ligand is 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.

[00116] In some examples, each monomer comprises two caffeine-binding single-domain antibodies. In some examples, each caffeine-binding single-domain antibody comprises the amino acid sequence of Table D. In some examples, the ligand is caffeine or a derivative thereof.

[00117] In some examples, a first monomer comprises a first ligand binding domain and a second monomer comprises a second ligand binding domain. In some examples, the inducible cell death polypeptide comprises heterooligomers. In some examples, the heterooligomers comprise heterodimers. In some examples, the first monomer comprises an FKBP domain and the second monomer comprises an FRB domain. In some examples, the cell death-inducing domain comprises Bid, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the Bid amino acid sequence of Table D.

[00118] In some examples, the first monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and the second monomer comprises an FKBP domain. In some examples, the first monomer comprises an FRB domain and the second monomer comprises a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the first monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and an FKBP domain, and the second monomer comprises an FRB domain and the second monomer comprises a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the cell death-inducing domain comprises caspase 9, or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D. In some examples, the ligand is rapamycin, a derivative thereof, or an analog thereof. In some examples, the ligand is 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.

[00119] In some examples, the first monomer comprises an ABI domain and the second monomer comprises a PYL domain. In some examples, the ligand is abscisic acid.

[00120] In some examples, the first monomer comprises a heavy chain variable region (VH) of an anti-nicotine antibody and the second monomer comprises a light chain variable region (VL) of an anti-nicotine antibody. In some examples, the anti-nicotine antibody is a Nic12 antibody. In some examples, the VH comprises the VH amino acid sequence of Table D. In some examples, the VL comprises the VL amino acid sequence of Table D. In some examples, the ligand is nicotine or a derivative thereof.

[00121] In some examples, the first monomer comprises a cannabidiol binding domain comprising an amino acid sequence selected from the group consisting of the sequences of DB6,

DB11, DB18, and DB21 of Table D and the second monomer comprises a cannabidiol binding domain comprising the amino acid sequence of CA14 of Table D. In some examples, the ligand is a cannabidiol or a phytocannabinoid.

[00122] In some examples, each monomer further comprises a linker localized between each ligand binding domain and cell death-inducing domain. In some examples, the linker comprises an amino acid sequence selected from the group consisting of: GGGGSGGGGSGGGGSVDGF (SEQ ID NO: 88) and ASGGGGSAS (SEQ ID NO: 89).

[00123] Also disclosed herein is an engineered nucleic acid comprising: an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the promoter is operably linked to the exogenous polynucleotide, wherein the ACP comprises one or more ligand binding domains and a transcription factor comprising a nucleic acid-binding domain and a transcriptional effector domain, wherein when expressed, the ACP undergoes nuclear localization upon binding of the ligand binding domain to a cognate ligand, and wherein when localized to a cell nucleus, the ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter.

[00124] In some examples, each ligand binding domain comprises a domain, or functional fragment thereof, selected from the group consisting of: an ABI domain, a PYL domain, a caffeine-binding single-domain antibody, a cannabidiol binding domain, a hormone-binding domain of estrogen receptor (ER) domain, heavy chain variable region (VH) of an anti-nicotine antibody, light chain variable region (VL) of an anti-nicotine antibody, a progesterone receptor domain, an FKBP domain, and an FRB domain.

[00125] In some examples, the ABI domain comprises the ABI amino acid sequence of Table D. In some examples, the PYL domain comprises the PYL amino acid sequence of Table D. In some examples, the caffeine-binding single-domain antibody comprises the amino acid sequence of Table D. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequences of CA14, DB6, DB11, DB18, and DB21 of Table D. In some examples, the hormone-binding domain of estrogen receptor (ER) domain comprises the amino acid sequence of Table D. In some examples, the heavy chain variable region (VH) of an anti-nicotine antibody comprises the VH amino acid sequence of Table D. In some examples, the light chain variable region (VL) of an anti-nicotine antibody comprises the VL amino acid sequence of Table D. In some examples, the progesterone receptor domain comprises the amino acid sequence of Table D. In some examples, the FKBP domain comprises the amino acid sequence of Table D. In some examples, the FRB domain comprises the amino acid sequence of Table D.

[00126] In some examples, the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain). In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). In some examples, the transcriptional 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain); a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

[00127] In some examples, the chimeric polypeptide further comprises a linker localized between the nucleic acid-binding domain and the transcriptional effector domain. In some examples, the linker comprises one or more 2A ribosome skipping tags. In some examples, each 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

[00128] In some examples, the chimeric polypeptide comprises a first ligand binding domain operably linked to the nucleic acid-binding domain and a second ligand binding domain operably linked to the transcriptional effector domain.

[00129] In some examples, each of the first and second ligand binding domains comprises a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the cognate ligand is 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.

[00130] In some examples, each of the first and second ligand binding domains comprises a progesterone receptor domain. In some examples, the cognate ligand is mifepristone or a derivative thereof.

[00131] In some examples, when the ligand binding domain comprises an ABI domain or a PYL domain, the cognate ligand is abscisic acid.

[00132] In some examples, when the ligand binding domain comprises a caffeine-binding single-domain antibody, the cognate ligand is caffeine or a derivative thereof.

[00133] In some examples, when the ligand binding domain comprises a cannabidiol binding domain, the cognate ligand is a cannabidiol or a phytocannabinoid. In some examples, the cannabidiol binding domain comprises a single-domain antibody or a nanobody. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequence of CA14, DB6, DB11, DB18, and DB21 of Table D.

[00134] In some examples, when the ligand binding domain comprises a hormone-binding domain of estrogen receptor (ER) domain, the cognate ligand is 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.

[00135] In some examples, when the ligand binding domain comprises a heavy chain variable region (VH) of an anti-nicotine antibody or a light chain variable region (VL) of an anti-nicotine antibody, the cognate ligand is nicotine or a derivative thereof.

[00136] In some examples, when the ligand binding domain is a progesterone receptor domain, the cognate ligand is mifepristone or a derivative thereof.

[00137] In some examples, when the ligand binding domain comprises an FKBP domain or an FRB domain, the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

[00138] In some examples, the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain).

[00139] In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). In some examples, the ZF protein domain comprises one to ten ZFA.

[00140] In some examples, the nucleic acid-binding domain binds to the ACP-responsive promoter. In some examples, the ACP-responsive promoter comprises an ACP-binding domain sequence and a promoter sequence. 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, minTATA, minTK, inducer molecule-responsive promoters, and tandem repeats thereof. In some examples, the ACP-responsive promoter comprises a synthetic promoter. In some examples, the ACP-responsive promoter comprises a minimal promoter.

[00141] In some examples, the ACP-binding domain comprises one or more zinc finger binding sites.

[00142] In some examples, the transcriptional activator 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); and a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain).

[00143] An engineered nucleic acid comprising: an expression cassette comprising a promoter and an exogenous polynucleotide sequence having the formula: $\mathbf{C_1 – L –C_2}$ wherein C_1 comprises a polynucleotide sequence encoding a first chimeric polypeptide comprising a first ligand binding domain and a transcriptional activation domain, L comprises a linker polynucleotide sequence, C_2 comprises a polynucleotide sequence encoding a second chimeric polypeptide comprising a second ligand binding domain and a nucleic acid-binding domain; wherein the promoter is operably linked to the exogenous polynucleotide; wherein when expressed, the first chimeric polypeptide and the second chimeric polypeptide multimerize to form an activation-conditional control polypeptide (ACP) via a cognate ligand that binds to each ligand binding domain; and wherein the multimeric ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter.

[00144] In some examples, each ligand binding domain comprises a domain, or functional fragment thereof, selected from the group consisting of: an ABI domain, a PYL domain, a caffeine-binding single-domain antibody, a cannabidiol binding domain, a hormone-binding domain of estrogen receptor (ER) domain, heavy chain variable region (VH) of an anti-nicotine antibody, light chain variable region (VL) of an anti-nicotine antibody, a progesterone receptor domain, an FKBP domain, and an FRB domain.

[00145] In some examples, the ABI domain comprises the ABI amino acid sequence of Table D. In some examples, the PYL domain comprises the PYL amino acid sequence of Table D. In some examples, the caffeine-binding single-domain antibody comprises the amino acid sequence of Table D. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequences of CA14, DB6, DB11, DB18, and DB21 of Table D. In some examples, the hormone-binding domain of estrogen receptor (ER) domain comprises the amino acid sequence of Table D. In some examples, the heavy chain variable region (VH) of an anti-nicotine antibody comprises the VH amino acid sequence of Table D. In some examples, the light chain variable region (VL) of an anti-nicotine antibody comprises the VL amino acid sequence of Table D. In some examples, the progesterone receptor domain comprises the amino acid sequence of Table D. In some examples, the FKBP domain comprises the FKBP

amino acid sequence of Table D. In some examples, the FRB domain comprises the FRB amino acid sequence of Table D.

[00146] In some examples, the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain). In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). In some examples, the transcriptional 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain); a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

[00147] In some examples, the chimeric polypeptide further comprises a linker localized between the nucleic acid-binding domain and the transcriptional effector domain. In some examples, the linker comprises one or more 2A ribosome skipping tags. In some examples, each 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A.

[00148] In some examples, the chimeric polypeptide comprises a first ligand binding domain operably linked to the nucleic acid-binding domain and a second ligand binding domain operably linked to the transcriptional effector domain.

[00149] In some examples, each of the first and second ligand binding domains comprises a hormone-binding domain of estrogen receptor (ER) domain. In some examples, the cognate ligand is 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.

[00150] In some examples, each of the first and second ligand binding domains comprises a progesterone receptor domain.

[00151] In some examples, the cognate ligand is mifepristone or a derivative thereof.

[00152] In some examples, when the ligand binding domain comprises an ABI domain or a PYL domain, the cognate ligand is abscisic acid.

[00153] In some examples, when the ligand binding domain comprises a caffeine-binding single-domain antibody, the cognate ligand is caffeine or a derivative thereof.

[00154] In some examples, when the ligand binding domain comprises a cannabidiol binding domain, the cognate ligand is a cannabidiol or a phytocannabinoid. In some examples, the cannabidiol binding domain comprises a single-domain antibody or a nanobody. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of the sequences of CA14, DB6, DB11, DB18, and DB21 of Table D.

[00155] In some examples, when the ligand binding domain comprises a hormone-binding domain of estrogen receptor (ER) domain, the cognate ligand is 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.

[00156] In some examples, when the ligand binding domain comprises a heavy chain variable region (VH) of an anti-nicotine antibody or a light chain variable region (VL) of an anti-nicotine antibody, the cognate ligand is nicotine or a derivative thereof.

[00157] In some examples, when the ligand binding domain is a progesterone receptor domain, the cognate ligand is mifepristone or a derivative thereof.

[00158] In some examples, when the ligand binding domain comprises an FKBP domain or an FRB domain, the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

[00159] In some examples, the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain). In some examples, the ZF protein domain is modular in design and is composed of zinc finger arrays (ZFA). In some examples, the ZF protein domain comprises one to ten ZFA.

[00160] In some examples, the nucleic acid-binding domain binds to the ACP-responsive promoter. In some examples, the ACP-responsive promoter comprises an ACP-binding domain sequence and a promoter sequence. 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, minTATA, minTK, inducer molecule-responsive promoters, and tandem repeats thereof. In some examples, the ACP-responsive promoter comprises a synthetic promoter. In some examples, the ACP-responsive promoter comprises a minimal promoter. In some examples, the ACP-binding domain comprises one or more zinc finger binding sites. In some examples, the transcriptional activator 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); and a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain).

[00161] In some examples, the linker polynucleotide sequence is operably associated with the translation of each chimeric polypeptide as a separate polypeptide. 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.

[00162] Also disclosed herein is an engineered nucleic acid comprising: an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP) comprising a ligand binding domain and a transcriptional effector domain, wherein the promoter is operably linked to the exogenous polynucleotide, and wherein when expressed and upon binding of the ligand binding domain to a cognate ligand, the ACP is capable of modulating transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter.

[00163] Also disclosed herein is an engineered nucleic acid comprising: an expression cassette comprising a promoter and an exogenous polynucleotide sequence encoding a regulatable cell survival polypeptide comprising a ligand binding domain, wherein the promoter is operably linked to the exogenous polynucleotide, wherein when expressed, the cell survival polypeptide is capable of inhibiting a cell death-inducing polypeptide, and wherein upon binding to a cognate ligand, the cognate ligand inhibits the pro-survival polypeptide.

[00164] In some examples, the cell survival polypeptide is selected from the group consisting of: XIAP, Bcl-2, Bcl-xL, Bcl-w, Bcl-2-related protein A1 (BCL2A1), Mcl-1, FLICE-like inhibitory protein (c-FLIP), and an adenoviral E1B-19K protein**.** In some examples, the cell survival polypeptide is XIAP.

[00165] In some examples, the ligand binding domain is localized at the N-terminal region of the pro-survival polypeptide or at the C-terminal region of the pro-survival polypeptide.

[00166] In some examples, the ligand binding domain comprises a domain, or functional fragment thereof, selected from the group consisting of: an ABI domain, a PYL domain, a caffeine-binding single-domain antibody, a cannabidiol binding domain, a hormone-binding

domain of estrogen receptor (ER domain), heavy chain variable region (VH) of an anti-nicotine antibody, light chain variable region (VL) of an anti-nicotine antibody, a progesterone receptor domain, an FKBP domain, and an FRB domain.

[00167] In some examples, the ABI domain comprises the ABI amino acid sequence of Table D. In some examples, the PYL domain comprises the PYL amino acid sequence of Table D. In some examples, the caffeine-binding single-domain antibody comprises the amino acid sequence of Table D. In some examples, the cannabidiol binding domain comprises an amino acid sequence selected from the group consisting of sequences of CA14, DB6, DB11, DB18, and DB21 of Table D. In some examples, the hormone-binding domain of estrogen receptor (ER) domain comprises the amino acid sequence of Table D. In some examples, the heavy chain variable region (VH) of an anti-nicotine antibody comprises the VH amino acid sequence of Table D. In some examples, the light chain variable region (VL) of an anti-nicotine antibody comprises the VL amino acid sequence of Table D. In some examples, the progesterone receptor domain comprises the amino acid sequence of Table D. In some examples, the FKBP domain comprises the amino acid sequence of Table D. In some examples, the FRB domain comprises the amino acid sequence of Table D.

[00168] In some examples, when the ligand binding domain comprises an ABI domain or a PYL domain, the cognate ligand is abscisic acid.

[00169] In some examples, when the ligand binding domain comprises a caffeine-binding single-domain antibody, the cognate ligand is caffeine or a derivative thereof.

[00170] In some examples, when the ligand binding domain comprises a cannabidiol binding domain, the cognate ligand is a cannabidiol or a phytocannabinoid. In some examples, when the ligand binding domain comprises a hormone-binding domain of estrogen receptor (ER) domain, the cognate ligand is 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.

[00171] In some examples, when the ligand binding domain comprises a heavy chain variable region (VH) of an anti-nicotine antibody or a light chain variable region (VL) of an anti-nicotine antibody, the cognate ligand is nicotine or a derivative thereof.

[00172] In some examples, when the ligand binding domain is a progesterone receptor domain, the cognate ligand is mifepristone or a derivative thereof.

[00173] In some examples, when the ligand binding domain comprises an FKBP domain, or an FRB domain, the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

[00174] In some examples, the ligand binding domain comprises a degron. In some examples, the degron is capable of inducing degradation of the regulatable cell survival polypeptide. 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, an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box. 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 regulatable polypeptide. 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. In some examples, the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences. In some examples, the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRD AL (SEQ ID NO: 90).

[00175] In some examples, the ligand is an IMiD. In some examples, the IMiD is an FDA-approved drug. In some examples, the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

[00176] In some examples, the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme

B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related apoptosis-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic chytochrom P450-2B1, and Purine nucleoside phosphorylase.

[00177] In some examples, the cell death-inducing polypeptide is caspase 9 or a functional truncation thereof. In some examples, the cell death-inducing domain comprises the caspase 9 amino acid sequence of Table D.

[00178] In some examples, the cell death-inducing polypeptide is Diphtheria toxin fragment A (DTA). In some examples, the cell death-inducing domain comprises the DTA amino acid sequence of Table D.

[00179] In some examples, the cell death-inducing polypeptide is Bax. In some examples, the cell death-inducing domain comprises the Bax amino acid sequence of Table D.

[00180] An engineered nucleic acid comprising: (a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding a first chimeric polypeptide, wherein the first chimeric polypeptide comprises a first ligand binding domain and a transcriptional activation domain, wherein the first promoter is operably linked to the first exogenous polynucleotide; and (b) a second expression cassette comprising a second promoter and a second exogenous polynucleotide sequence encoding a second chimeric polypeptide, wherein the second chimeric polypeptide comprises a second ligand binding domain and a nucleic acid-binding domain, wherein the second promoter is operably linked to the second exogenous polynucleotide, wherein when expressed, the first chimeric polypeptide and the second chimeric polypeptide multimerize to form an activation-conditional control polypeptide (ACP) via a cognate ligand that binds to each ligand binding domain, and wherein the multimeric ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter.

[00181] In some examples, the first promoter, the second promoter, or both the first promoter and the second promoter comprise(s) a constitutive promoter, an inducible promoter, or a synthetic promoter.

[00182] In some examples, the constitutive promoter is selected from the group consisting of: CAG, HLP, CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb.

[00183] In some examples, the inducible promoter is 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.

[00184] In some examples, an inducible cell death polypeptide or system as described herein comprises a modified XIAP, wherein the modified XIAP comprises one or more amino acid substitutions within to positions 306-325 of SEQ ID NO: 107.

[00185] In some examples, the one or more amino acid substitutions are at one or more positions of SEQ ID NO: 107 selected from the group consisting of: 305, 306, 308, and 325.

[00186] In some examples, the one or more amino acid substitutions are at position 305 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M.

[00187] In some examples, the one or more amino acid substitutions are at position 306 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S.

[00188] In some examples, the one or more amino acid substitutions are at position 308 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 308 of SEQ ID NO: 107 is selected from the group consisting of T308S and T308D. In some examples, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S. In some examples, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308D.

[00189] In some examples, the one or more amino acid substitutions are at position 325 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00190] In some examples, the one or more amino acid substitutions are two amino acid substitutions.

[00191] In some examples, each of the two amino acid substitutions are at a position of SEQ ID NO: 107 selected from the group consisting of: 305, 306, 308, and 325.

[00192] In some examples, the two amino acid substitutions are at positions 305 and 306 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M and the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S.

[00193] In some examples, the two amino acid substitutions are at positions 305 and 308 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M and the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S.

[00194] In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M and the amino acid substitution at position 308 of SEQ ID NO: 107 is T308D.

[00195] In some examples, the two amino acid substitutions are at positions 305 and 325 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00196] In some examples, the two amino acid substitutions are at positions 306 and 308 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S and the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S.

[00197] In some examples, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S and the amino acid substitution at position 308 of SEQ ID NO: 107 is T308D.

[00198] In some examples, the two amino acid substitutions are at positions 306 and 325 of SEQ ID NO: 107.

[00199] In some examples, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00200] In some examples, the two amino acid substitutions are at positions 308 and 325 of SEQ ID NO: 107.

[00201] In some examples, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00202] In some examples, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308D and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00203] In some examples, the one or more additional amino acid substitutions are three amino acid substitutions. In some examples, each of the three amino acid substitutions are at a position of SEQ ID NO: 107 selected from the group consisting of: 305, 306, 308, and 325. In some examples, the three amino acid substitutions are at positions 305, 306, and 308 of SEQ ID NO: 107.

[00204] In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, and the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S.

[00205] In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, and the amino acid substitution at position 308 of SEQ ID NO: 107 is T308D.

[00206] In some examples, the three amino acid substitutions are at positions 305, 306, and 325 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00207] In some examples, the three amino acid substitutions are at positions 305, 308, and 325 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S, and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308D, and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00208] In some examples, the three amino acid substitutions are at positions 306, 308, and 325 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S, and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S. In some examples, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, the amino acid substitution at position 308 of SEQ ID NO: 107 is T3084, and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S.

[00209] In some examples, the one or more additional amino acid substitutions are four amino acid substitutions.

[00210] In some examples, the four amino acid substitutions are at positions 305, 306, 308, and 325 of SEQ ID NO: 107. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308S, and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S. In some examples, the amino acid substitution at position 305 of SEQ ID NO: 107 is G305M, the amino acid substitution at position 306 of SEQ ID NO: 107 is G306S, the amino acid substitution at position 308 of SEQ ID NO: 107 is T308D, and the amino acid substitution at position 325 of SEQ ID NO: 107 is P325S

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[00211] These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, and accompanying drawings.

[00212] **FIG. 1A, FIG. 1B, FIG. 1C, and FIG. 1D** provide examples of inducible cell death systems.

[00213] **FIG. 2A** shows the domains and organization of the indicated constructs for an inducible cell-death system.

[00214] **FIG. 2B** shows mCherry expression in cells expressing the indicated constructs following the addition of an IMiD.

CLAIMS

What is claimed is:

1. An engineered cell comprising an inducible cell death system, or an engineered nucleic acid comprising the inducible cell death system, for use in therapy,

wherein the inducible cell death system comprises two or more polypeptide monomers, wherein each polypeptide monomer comprises one or more ligand binding domains and a cell death-inducing domain, wherein the polypeptide monomers are configured to oligomerize upon contacting the polypeptide monomers with a cognate ligand of the one or more ligand binding domains and generate a cell-death inducing signal in a cell in which the polypeptide monomers are expressed, and wherein:

i) each polypeptide monomer of the two or more polypeptide monomers comprises the same ligand binding domain, wherein the same ligand binding domain comprises a domain or functional fragment thereof selected from the group consisting of: a hormone-binding domain of estrogen receptor (ER) domain, optionally comprising the amino acid sequence of SEQ ID NO: 42; an ABI domain, optionally comprising the amino sequence of SEQ ID NO: 31; a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53; a caffeine-binding single-domain antibody, optionally comprising the amino acid sequence of SEQ ID NO: 33; a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38; a heavy chain variable region (VH) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 50, and/or the light chain variable region (VL) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 51; optionally comprising the amino acid sequence of SEQ ID NO: 43; a progesterone receptor domain, optionally comprising the amino acid sequence of SEQ ID NO: 52; and combinations thereof; or

ii) a first polypeptide monomer of the two or more polypeptide monomers comprises a first ligand binding domain, a second polypeptide monomer of the two or more polypeptide monomers comprises a second ligand binding domain, and wherein the second ligand binding domain is different from the first ligand binding domain.

2. The engineered cell or engineered nucleic acid for use of claim 1, wherein:

the first ligand binding domain of (ii) comprises a cereblon domain, optionally comprising the amino acid sequence set forth in one of SEQ ID NOs: 127 and 129, and wherein the second ligand binding domain of (ii) comprises a degron, optionally comprising the amino acid sequence set forth in one of SEQ ID NOs: 131 and 133, optionally wherein the cognate ligand is an IMiD, optionally wherein the IMiD is an FDA-approved drug, and optionally

wherein the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide; or

the first polypeptide monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and the second polypeptide monomer comprises an FKBP domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof and/or tamoxifen or a metabolite thereof; or

the first polypeptide monomer comprises an FRB domain and the second polypeptide monomer comprises a hormone-binding domain of estrogen receptor (ER) domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof and/or tamoxifen or a metabolite thereof; or

wherein the first polypeptide monomer comprises a hormone-binding domain of estrogen receptor (ER) domain and an FKBP domain, and the second polypeptide monomer comprises an FRB domain and a hormone-binding domain of estrogen receptor (ER) domain, optionally wherein the cognate ligand is rapamycin or a derivative thereof and/or tamoxifen or a metabolite thereof; or

the first polypeptide monomer comprises an ABI domain and the second polypeptide monomer comprises a PYL domain, optionally wherein the cognate ligand comprises abscisic acid; or

the first polypeptide monomer comprises a heavy chain variable region (VH) of an anti-nicotine antibody and the second polypeptide monomer comprises a light chain variable region (VL) of an anti-nicotine antibody, optionally wherein the anti-nicotine antibody is a Nic12 antibody, optionally wherein the VH comprises the amino acid sequence of SEQ ID NO: 50, and optionally wherein the VL comprises the amino acid sequence of SEQ ID NO: 51, and optionally wherein the cognate ligand is nicotine or a derivative thereof; or

the first polypeptide monomer comprises a cannabidiol binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 35, 36, 37, and 38 and the second polypeptide monomer comprises a cannabidiol binding domain comprising the amino acid sequence of SEQ ID NO: 34. optionally wherein the cognate ligand is a phytocannabinoid, optionally wherein the phytocannabinoid is cannabidiol.

3. The engineered cell or engineered nucleic acid for use of claim 1, wherein the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein

(TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related cell death-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase, optionally wherein the caspase 9 or a functional truncation thereof, comprises the amino acid sequence of SEQ ID NO: 39, optionally wherein the DTA comprises the amino acid sequence of SEQ ID NO: 41, optionally wherein the granzyme B comprises the amino acid sequence of SEQ ID NO: 47, optionally wherein the Bax comprises the amino acid sequence of SEQ ID NO: 32.

4. A pharmaceutical composition comprising:

a) an inducible cell death system, an engineered nucleic acid encoding the inducible cell death system, or an engineered cell comprising the inducible cell death system, wherein the inducible cell death system comprises two or more polypeptide monomers, wherein each polypeptide monomer comprises one or more ligand binding domains and a cell death-inducing domain, wherein the polypeptide monomers are configured to oligomerize upon contacting the polypeptide monomers with a cognate ligand of the one or more ligand binding domains and generate a cell-death inducing signal in a cell in which the polypeptide monomers are expressed, and wherein:

i) each polypeptide monomer of the two or more polypeptide monomers comprises the same ligand binding domain, wherein the same ligand binding domain comprises a domain or functional fragment thereof selected from the group consisting of: a hormone-binding domain of estrogen receptor (ER) domain, optionally comprising the amino acid sequence of SEQ ID NO: 42; an ABI domain, optionally comprising the amino sequence of SEQ ID NO: 31; a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53; a caffeine-binding single-domain antibody, optionally comprising the amino acid sequence of SEQ ID NO: 33; a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38; a heavy chain variable region (VH) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 50, and/or the light chain variable region (VL) of an anti-nicotine antibody, optionally comprising the amino acid sequence of SEQ ID NO: 51; optionally comprising the amino acid sequence

of SEQ ID NO: 43; a progesterone receptor domain, optionally comprising the amino acid sequence of SEQ ID NO: 52; and combinations thereof; or

ii) a first polypeptide monomer of the two or more polypeptide monomers comprises a first ligand binding domain, a second polypeptide monomer of the two or more polypeptide monomers comprises a second ligand binding domain, and wherein the second ligand binding domain is different from the first ligand binding domain;

and

b) a pharmaceutically acceptable carrier.

5. An inducible cell death system comprising an activation-conditional control polypeptide (ACP),

wherein the ACP comprises a ligand binding domain and a transcriptional effector domain, and

wherein upon binding of the ligand binding domain to a cognate ligand, the ACP is capable of modulating transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter,

wherein the gene of interest comprises a cell death inducing polypeptide.

6. The inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5, wherein the ligand binding domain comprises a degron, optionally wherein the degron is capable of inducing degradation of the ACP,

optionally 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, an Siah binding motif, an SPOP SBC docking motif, and a PCNA binding PIP box, or 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 regulatable polypeptide, optionally 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, optionally wherein the CRBN polypeptide substrate domain is a chimeric fusion product of native CRBN polypeptide sequences, optionally wherein the CRBN polypeptide substrate domain is a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRD AL (SEQ ID NO: 103).

7. An inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5, wherein the transcriptional effector domain is comprised in a transcription factor that further comprises a nucleic acid-binding domain.

8. The inducible cell death system comprising an activation -conditional control polypeptide (ACP) of claim 5, wherein the transcriptional 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain); a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

9. The inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5, wherein:

the ligand binding domain comprises: a hormone-binding domain of estrogen receptor (ER) domain optionally comprising the amino acid sequence of SEQ ID NO: 42, optionally wherein the cognate ligand is tamoxifen or a metabolite thereof, and optionally wherein the

tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen; or

the ligand binding domain comprises: a progesterone receptor domain optionally comprising the amino acid sequence of SEQ ID NO: 52, and optionally wherein the cognate ligand is mifepristone or a derivative thereof, or

the ligand binding domain comprises: a domain or functional fragment thereof selected from the group consisting of: an ABI domain, optionally comprising the amino acid sequence of SEQ ID NO: 31, and optionally wherein the cognate ligand is abscisic acid; a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53, and optionally wherein the cognate ligand is abscisic acid; a caffeine-binding single-domain antibody optionally comprising the amino acid sequence of SEQ ID NO: 33, and optionally wherein the cognate ligand is caffeine or a derivative thereof; a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38, optionally wherein the cognate ligand is a phytocannabinoid, optionally wherein the phytocannabinoid is cannabidiol; a hormone-binding domain of estrogen receptor (ER) domain optionally comprising the amino acid sequence of SEQ ID NO: 42, optionally wherein the cognate ligand is tamoxifen or a metabolite thereof, and optionally wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen; a heavy chain variable region (VH) of an anti-nicotine antibody optionally comprising the amino acid sequence of SEQ ID NO: 50, and optionally wherein the cognate ligand is nicotine or a derivative thereof; a light chain variable region (VL) of an anti-nicotine antibody optionally comprising the amino acid sequence of SEQ ID NO: 51, and optionally wherein the cognate ligand is nicotine or a derivative thereof; a progesterone receptor domain optionally comprising the amino acid sequence of SEQ ID NO: 52, and optionally wherein the cognate ligand is mifepristone or a derivative thereof; an FRB domain optionally comprising the amino acid sequence of SEQ ID NO: 44, and optionally wherein the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

10. The inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5, wherein:

the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain), optionally wherein the ZF protein domain is modular in design and is composed of an array of zinc finger motifs, optionally wherein the ZF-protein domain comprises one to ten zinc finger motifs.

11. The inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5, wherein: the gene of interest is a cell death-inducing polypeptide, optionally wherein the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related cell death-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase, optionally wherein the caspase 9 or a functional truncation thereof, comprises the amino acid sequence of SEQ ID NO: 39, optionally wherein the DTA comprises the amino acid sequence of SEQ ID NO: 41, optionally wherein the granzyme B comprises the amino acid sequence of SEQ ID NO: 47, optionally wherein the Bax comprises the amino acid sequence of SEQ ID NO: 32.

12. A pharmaceutical composition comprising:

a) the inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5; or an isolated cell comprising the inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5; or an engineered nucleic acid encoding the inducible cell death system comprising an activation-conditional control polypeptide (ACP) of claim 5; and

b) a pharmaceutically acceptable carrier.

13. A pharmaceutical composition of claim 12 for use in therapy.

14. A multimeric activation-conditional control polypeptide (ACP) comprising:

a) a first chimeric polypeptide, wherein the first chimeric polypeptide comprises a first ligand binding domain and a transcriptional activation domain; and

b) a second chimeric polypeptide, wherein the second chimeric polypeptide comprises a second ligand binding domain and a nucleic acid-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide oligomerize to form the multimeric ACP via a cognate ligand that binds to each ligand binding domain, and wherein the multimeric ACP is capable of inducing transcriptional expression of a gene of interest operably linked to an ACP-responsive promoter,

wherein the gene of interest is a cell death-inducing polypeptide, optionally wherein the cell death-inducing domain is derived from a protein selected from the group consisting of: caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, Diphtheria toxin fragment A (DTA), Bax, Bak, Bok, Bad, Bcl-xS, Bak, Bik, Bcl-2-interacting protein 3 (BNIP3), Fas, Fas-associated protein with death domain (FADD), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), a TNF receptor (TNF-R), APAF-1, granzyme B, second mitochondria-derived activator of caspases (SMAC), Omi, Bmf, Bid, Bim, p53-upregulated modulator of apoptosis (PUMA), Noxa, Blk, Hrk, Cytochrome c, Arts, TNF-related cell death-inducing ligand (TRAIL), Herpes Simplex Virus thymidine kinase (HSV-TK), Varicella Zoster Virus thymidine kinase (VZV-TK), viral Spike protein, Carboxyl esterase, cytosine deaminase, nitroreductase Fksb, Carboxypeptidase G2, Carboxypeptidase A, Horseradish peroxidase, Linamarase, Hepatic cytochrome P450-2B1, and Purine nucleoside phosphorylase, optionally wherein the caspase 9 or a functional truncation thereof, comprises the amino acid sequence of SEQ ID NO: 39, optionally wherein the DTA comprises the amino acid sequence of SEQ ID NO: 41, optionally wherein the granzyme B comprises the amino acid sequence of SEQ ID NO: 47, optionally wherein the Bax comprises the amino acid sequence of SEQ ID NO: 32, and wherein the transcriptional activation 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 tripartite activator comprising the VP64, the p65, and the HSF1 activation domains (VPH activation domain); and a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300 (p300 HAT core activation domain).

15. The multimeric activation-conditional control polypeptide (ACP) of claim 14, wherein the one or more ligand binding domains of a first of the two or more polypeptide monomers comprise a cereblon domain, optionally comprising the amino acid sequence set forth in one of SEQ ID NOs: 127 and 129, and the one or more ligand binding domains of a second of the two or more polypeptide monomers comprise a degron, optionally comprising the amino acid sequence set forth in one of SEQ ID NOs: 131 and 133, optionally wherein the cognate ligand is

an IMiD, optionally wherein the IMiD is an FDA-approved drug, and optionally wherein the IMiD is selected from the group consisting of: thalidomide, lenalidomide, and pomalidomide.

16. The multimeric activation-conditional control polypeptide (ACP) of claim 14, wherein the nucleic acid-binding domain comprises a DNA-binding zinc finger protein domain (ZF protein domain), optionally wherein the ZF protein domain is modular in design and is composed of an array of zinc finger motifs, optionally wherein the ZF-protein domain comprises one to ten zinc finger motifs.

17. The multimeric activation-conditional control polypeptide (ACP) of claim 14, wherein the nucleic acid-binding domain binds to the ACP-responsive promoter, optionally wherein the ACP-responsive promoter comprises an ACP-binding domain sequence and a promoter sequence, optionally wherein the promoter sequence comprises a minimal promoter, optionally wherein the promoter sequence is an inducible promoter and further comprises a responsive element selected from the group consisting of: NFκB 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, inducer molecule-responsive promoters, and tandem repeats thereof, and optionally wherein the ACP-responsive promoter comprises a synthetic promoter, and optionally wherein the ACP-binding domain comprises one or more zinc finger binding sites.

18. The multimeric activation-conditional control polypeptide (ACP) of claim 14, wherein the one or more ligand binding domains of each of the two or more polypeptide monomers comprise a domain or functional fragment thereof selected from the group consisting of: an ABI domain, optionally comprising the amino acid sequence of SEQ ID NO: 31, and optionally wherein the cognate ligand is abscisic acid; a PYL domain, optionally comprising the amino acid sequence of SEQ ID NO: 53, and optionally wherein the cognate ligand is abscisic acid; a caffeine-binding single-domain antibody optionally comprising the amino acid sequence of SEQ ID NO: 33, and optionally wherein the cognate ligand is caffeine or a derivative thereof; a cannabidiol binding domain, optionally comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 34, 35, 36, 37, and 38, optionally wherein the cognate ligand is a phytocannabinoid, optionally wherein the phytocannabinoid is cannabidiol; a hormone-binding domain of estrogen receptor (ER) domain optionally comprising the amino acid sequence of SEQ ID NO: 42, optionally wherein the cognate ligand is tamoxifen or a metabolite thereof, and optionally wherein the tamoxifen metabolite is selected from the group consisting of: 4-

hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen; a heavy chain variable region (VH) of an anti-nicotine antibody optionally comprising the amino acid sequence of SEQ ID NO: 50, and optionally wherein the cognate ligand is nicotine or a derivative thereof; a light chain variable region (VL) of an anti-nicotine antibody optionally comprising the amino acid sequence of SEQ ID NO: 51, and optionally wherein the cognate ligand is nicotine or a derivative thereof; a progesterone receptor domain optionally comprising the amino acid sequence of SEQ ID NO: 52, and optionally wherein the cognate ligand is mifepristone or a derivative thereof; an FRB domain optionally comprising the amino acid sequence of SEQ ID NO: 44, and optionally wherein the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof; or

the one or more ligand binding domains of a first of the two or more polypeptide monomers comprise an FKBP domain, optionally comprising the amino acid sequence of SEQ ID NO: 43, and the one or more ligand binding domains of a second of the two or more polypeptide monomers comprise an FRB domain, optionally comprising the amino acid sequence of SEQ ID NO: 44, optionally wherein the cognate ligand is rapamycin, AP1903, AP20187, FK1012, derivatives thereof, or analogs thereof.

19. A pharmaceutical composition comprising:

a) the multimeric activation-conditional control polypeptide (ACP) of claim 14; or an isolated cell comprising the multimeric activation-conditional control polypeptide (ACP) of claim 14; or an engineered nucleic acid encoding the multimeric activation-conditional control polypeptide (ACP) of claim 14; and

b) a pharmaceutically acceptable carrier.

20. A pharmaceutical composition of claim 19 for use in therapy.