CONDITIONALLY ACTIVE HETERODIMERIC POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/276,725, filed January 8, 2016, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant Nos. R01 CA196277, P50 GM081879, and R01 GM055040 awarded by the National Institutes of Health. The government has certain rights in the invention.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

[0003] A Sequence Listing is provided herewith as a text file, "UCSF-524WO_seqlist_ST25.txt" created on January 6, 2017 and having a size of 2,005 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

[0004] In cell-based adoptive immunotherapy, immune cells isolated from a patient can be modified to express synthetic proteins that enable the cells to perform new therapeutic functions after they are subsequently transferred back into the patient. Examples of such synthetic proteins are chimeric antigen receptors (CARs) and engineered T cell Receptors (TCR). An example of a currently used CAR is a fusion of an extracellular recognition domain (e.g., an antigen-binding domain), a transmembrane domain, and one or more intracellular signaling domains. Upon antigen engagement, the intracellular signaling portion of the CAR can initiate an activation-related response in an immune cell, such as release of cytolytic molecules to induce tumor cell death, etc. However, such CARs are not capable of being pharmacologically controlled. Safe human testing and clinical use of such CARs and engineered TCR requires fine control of the powerful stimulating activity of these highly engineered proteins and protein complexes. In some cases, control is required in order to inhibit, halt or otherwise modulate immune cell activation when activation from the designer stimulating receptor is unwanted, becomes undesirable or is no longer necessary.

SUMMARY

[0005] The present disclosure provides conditionally active, heterodimeric polypeptides. The conditionally active, heterodimeric polypeptides are active in the presence of a dimerizing agent that induces dimerization of the polypeptides of the heterodimer. A conditionally active, heterodimeric polypeptide of the present disclosure is useful in a variety of research and treatment methods, which are also provided.

[0006] The present disclosure provides a heterodimeric, conditionally active polypeptide comprising: a) a first chimeric polypeptide comprising a first member of a dimerization pair and a first heterologous polypeptide; and b) a second chimeric polypeptide comprising a second member of a dimerization pair and a second heterologous polypeptide, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the first chimeric polypeptide and the second chimeric polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator. In some cases, a) the first heterologous polypeptide is a T-cell receptor (TCR) alpha chain; and b) the second heterologous polypeptide is a TCR beta chain. In some cases: a) the first heterologous polypeptide is a first polypeptide of a chimeric antigen receptor (CAR) heterodimer; and b) the second heterologous polypeptide is a second polypeptide of a CAR heterodimer. In some cases: a) the first heterologous polypeptide is an N-terminal portion of an RNA-guided endonuclease; and b) the second heterologous polypeptide is a C-terminal portion of the RNA-guided endonuclease, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide, mediated by the dimerization agent that induces binding of the LBD to the co-regulator, restores enzymatic function of the RNA-guided endonuclease. In some cases, the RNA-guided endonuclease is a class 2 CRISPR/Cas endonuclease. In some cases, the class 2 CRISPR/Cas endonuclease is a type II CRISPR/Cas protein, a type V CRISPR/Cas protein, or a type VI CRISPR/Cas protein. In some cases: a) the first heterologous polypeptide is an N-terminal portion of an enzyme; and b) the second heterologous polypeptide is a C-terminal portion of the enzyme, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide restore enzymatic activity of the enzyme. In some cases, the enzyme is a kinase, a protease, a phosphatase, or a caspase. In some cases, the first polypeptide and the second polypeptide exhibit an activity when brought into proximity upon dimerization mediated by the dimerization agent, but do not exhibit the activity individually. In some cases: a) the first heterologous polypeptide is an N-terminal portion of an antigen receptor; and b) the second heterologous polypeptide is a C-terminal portion of the antigen receptor, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide restore signaling activity of the antigen receptor. In some cases: a) the first heterologous polypeptide is an N-terminal portion of a

receptor; and b) the second heterologous polypeptide is a C-terminal portion of the antigen receptor, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide mediated by the dimerization agent restores signaling activity of the receptor. In some cases, the LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from an estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor. In some cases, the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285. In some cases, the co-regulator of the nuclear hormone receptor is selected from: a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1); b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2); c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3); d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4); e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5); f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6); g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7); h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8); i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9); j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10); k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11); l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12); m) αβV: SPGSREWFKDMLS (SEQ ID NO:13); n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14); o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15); p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16); q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17); r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18); s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19); t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20); u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21); v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22); w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23); x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24); y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25); z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26); aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27); bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28); cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29); dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30); ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31); ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32); gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33); hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34); ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35); jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36); kk) ASC2–2:

AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37); ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38); mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39); nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40); oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41); pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42); qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43); rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44); ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45); tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46); uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47); vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48); ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49); xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50); yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51); zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52); aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53); bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54); ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55); ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56); eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57); fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58); ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59); hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60); iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61); jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62); kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63); lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64); mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65); nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66); ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67); ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[0007] The present disclosure provides a heterodimeric, conditionally active receptor comprising: a) a first chimeric polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second chimeric polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain; or comprising: a) a first chimeric polypeptide comprising: i) a first member of a specific binding pair; ii) a modulatory domain; iii) a first member of a dimerization pair; iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and b) a second chimeric polypeptide comprising: i) a second member of the dimerization pair; and ii) an intracellular signaling domain, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the

nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor, wherein the first chimeric polypeptide and the second chimeric polypeptide are dimerized in the presence of a dimerizer that induces binding of the LBD to the co-regulator. In some cases, the first polypeptide comprises a hinge region interposed between the first member of the specific binding pair and the transmembrane domain. In some cases, the first member of the specific binding pair is an antibody or antibody fragment, a ligand, a receptor, or a non-antibody-based recognition scaffold. In some cases, the hinge region is an immunoglobulin IgG hinge region or a hinge derived from CD8. In some cases, the first and second modulatory domains are selected from 4-1BB (CD137), CD28, ICOS, BTLA, OX-40, CD27, CD30, GITR, HVEM, DAP10, DAP12, and CD28. In some cases, the intracellular signaling domain is selected from ZAP70 and CD3-zeta. In some cases, the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM). In some cases, the LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor. In some cases, the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285. In some cases, the co-regulator of the nuclear hormone receptor is a peptide selected from a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1); b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2); c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3); d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4); e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5); f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6); g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7); h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8); i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9); j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10); k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11); l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12); m) αβV: SPGSREWFKDMLS (SEQ ID NO:13); n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14); o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15); p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16); q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17); r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18); s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19); t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20); u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21); v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ

ID NO:22); w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23); x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24); y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25); z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26); aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27); bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28); cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29); dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30); ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31); ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32); gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33); hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34); ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35); jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36); kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37); ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38); mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39); nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40); oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41); pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42); qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43); rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44); ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45); tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46); uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47); vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48); ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49); xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50); yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51); zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52); aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53); bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54); ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55); ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56); eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57); fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58); ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59); hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60); iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61); jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62); kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63); lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64); mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65); nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66); ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67); ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69). In some cases: i) the first and second modulatory domains are derived from 4-1BB; ii) the first and second members of the dimerization pair are PPARγ and SRC3; and ii) the signaling domain comprises an

ITAM. In some cases, the first member of the specific binding pair is a single-chain Fv. In some cases, the first member of the specific binding pair binds an epitope present on a cell, on a solid surface, or a lipid bilayer. In some cases, the cell is a cancer cell. In some cases, the intracellular signaling domain is an intracellular inhibitory domain. In some cases, the intracellular inhibitory domain is derived from a protein selected from the group consisting of: PD-1, CTLA4, HPK1, SHP1, SHP2, Sts1, and Csk.

[0008] The present disclosure provides a heterodimeric, conditionally repressible synthetic immune cell receptor (ICR) comprising: a synthetic stimulatory ICR comprising a first member of a dimerization pair linked to the synthetic stimulatory ICR; and a synthetic ICR repressor comprising a second member of the dimerization pair linked to an intracellular inhibitory domain, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor, wherein the synthetic stimulatory ICR and the synthetic ICR repressor are dimerized in the presence of a dimerizer that induces binding of the LBD to the co-regulator. In some cases, the synthetic stimulatory ICR comprises an intracellular co-stimulatory domain. In some cases, the intracellular co-stimulatory domain is selected from the group consisting of: 4-1BB (CD137), CD28, ICOS, OX-40, BTLA, CD27, CD30, GITR, and HVEM. In some cases, the first member of a dimerization pair is linked intracellularly to the synthetic stimulatory ICR and the second member of the dimerization pair is linked intracellularly to the intracellular inhibitory domain. In some cases, the synthetic ICR repressor further comprises a transmembrane domain. In some cases, the second member of the dimerization pair is linked intracellularly to the transmembrane domain. In some cases, the second member of the dimerization pair is extracellular and linked to the intracellular inhibitory domain by way of the transmembrane domain. In some cases, the stimulatory ICR binds a soluble antigen. In some cases, the stimulatory ICR binds a cell surface antigen. In some cases, the intracellular inhibitory domain is an inhibitory domain derived from a protein selected from the group consisting of: PD-1, CTLA4, HPK1, SHP1, SHP2, Sts1 and Csk. In some cases, the synthetic stimulatory ICR comprises an intracellular signaling domain selected from the group consisting of: a CD3-zeta signaling domain, a ZAP70 signaling domain and an immunoreceptor tyrosine-based activation motif (ITAM). In some cases, the LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor. In some cases, the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC,

TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285. In some cases, the co-regulator of the nuclear hormone receptor is selected from: a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1); b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2); c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3); d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4); e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5); f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6); g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7); h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8); i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9); j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10); k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11); l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12); m) αβV: SPGSREWFKDMLS (SEQ ID NO:13); n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14); o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15); p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16); q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17); r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18); s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19); t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20); u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21); v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22); w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23); x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24); y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25); z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26); aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27); bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28); cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29); dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30); ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31); ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32); gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33); hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34); ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35); jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36); kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37); ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38); mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39); nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40); oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41); pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42); qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43); rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44); ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45); tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46); uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47); vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48); ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49); xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50); yy) CoRNR1 (N-CoR):

MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51); zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52); aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53); bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54); ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55); ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56); eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57); fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58); ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59); hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60); iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61); jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62); kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63); lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64); mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65); nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66); ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67); ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69). In some cases, the synthetic stimulatory ICR is a synthetic chimeric antigen receptor (CAR) or portion thereof. In some cases, the synthetic stimulatory ICR is a synthetic T cell receptor (TCR) or portion thereof.

[0009] The present disclosure provides a heterodimeric, conditionally repressible synthetic chimeric antigen receptor (CAR) comprising: a) a synthetic stimulatory CAR comprising: i) a extracellular recognition domain; ii) a transmembrane domain linked to the extracellular recognition domain; iii) a first member of a dimerization pair linked to the transmembrane domain; and iv) an intracellular stimulation domain; and b) a synthetic CAR repressor comprising: i) a second member of the dimerization pair; and ii) an intracellular inhibitory domain linked to the second member of the dimerization pair, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the synthetic stimulatory CAR and the synthetic CAR repressor are dimerized in the presence of a ligand that induces binding of the LBD to the co-regulator. In some cases, the synthetic CAR repressor further comprises a transmembrane domain linked to the second member of the dimerization pair, the intracellular inhibitory domain or both. In some cases, the LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor. In some

cases, the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285. In some cases, the co-regulator of the nuclear hormone receptor is selected from: a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1); b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2); c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3); d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4); e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5); f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6); g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7); h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8); i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9); j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10); k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11); l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12); m) αβV: SPGSREWFKDMLS (SEQ ID NO:13); n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14); o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15); p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16); q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17); r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18); s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19); t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20); u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21); v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22); w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23); x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24); y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25); z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26); aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27); bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28); cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29); dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30); ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31); ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32); gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33); hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34); ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35); jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36); kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37); ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38); mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39); nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40); oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41); pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42); qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43); rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44); ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45); tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46); uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47); vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48); ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ

ID NO:49); xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50); yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51); zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52); aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53); bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54); ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55); ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56); eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57); fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58); ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59); hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60); iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61); jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62); kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63); lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64); mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65); nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66); ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67); ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[0010] The present disclosure provides a heterodimeric, conditionally repressible synthetic T cell receptor (TCR) comprising: a) a synthetic stimulatory TCR comprising: i) a transmembrane domain; ii) a first member of a dimerization pair linked to the transmembrane domain; iii) an engineered TCR polypeptide comprising at least one TCR alpha or beta chain, wherein the at least one TCR alpha or beta chain is linked to the transmembrane domain or the first member of a dimerization pair; and b) a synthetic TCR repressor comprising: i) a second member of the dimerization pair; and ii) an intracellular inhibitory domain linked to the second member of the dimerization pair, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the synthetic stimulatory TCR and the synthetic TCR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator. In some cases, the synthetic TCR repressor further comprises a transmembrane domain linked to the second member of the dimerization pair, the intracellular inhibitory domain or both. In some cases, the engineered TCR polypeptide further comprises a TCR gamma chain. In some cases, the LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a

retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor. In some cases, the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285. In some cases, the co-regulator of the nuclear hormone receptor is selected from: a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1); b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2); c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3); d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4); e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5); f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6); g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7); h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8); i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9); j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10); k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11); l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12); m) αβV: SPGSREWFKDMLS (SEQ ID NO:13); n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14); o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15); p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16); q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17); r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18); s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19); t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20); u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21); v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22); w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23); x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24); y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25); z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26); aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27); bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28); cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29); dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30); ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31); ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32); gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33); hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34); ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35); jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36); kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37); ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38); mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39); nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40); oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41); pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42); qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43); rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44); ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45); tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46); uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47); vv) ERAP140:

HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48); ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49); xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50); yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51); zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52); aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53); bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54); ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55); ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56); eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57); fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58); ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59); hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60); iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61); jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62); kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63); lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64); mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65); nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66); ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67); ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[0011] The present disclosure provides a heterodimeric, conditionally active chimeric antigen receptor (CAR) comprising: a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain; or comprising: a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a modulatory domain; iii) a first member of a dimerization pair; iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and b) a second polypeptide comprising: i) a second member of the dimerization pair; and ii) an intracellular signaling domain, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the first polypeptide and the second polypeptide are dimerized in the presence of a dimerizing agent that induces binding of the LBD to the co-regulator.In some cases, the first polypeptide comprises a hinge region interposed between the first member of the specific binding pair and the transmembrane domain. In some cases, the first member of the specific binding pair is an antibody or antibody fragment, a ligand, or a receptor. In some cases, the hinge region is an immunoglobulin IgG hinge region or a hinge

derived from CD8. In some cases, the first and second modulatory domains are selected from 4-1BB (CD137), CD28, ICOS, BTLA, OX-40, CD27, CD30, GITR, HVEM, DAP10, DAP12, and CD28. In some cases, the intracellular signaling domain is selected from ZAP70 and CD3-zeta. In some cases, the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM). In some cases, the LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor. In some cases, the LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor. In some cases, the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285. In some cases, the co-regulator of the nuclear hormone receptor is selected from: a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1); b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2); c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3); d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4); e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5); f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6); g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7); h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8); i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9); j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10); k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11); l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12); m) αβV: SPGSREWFKDMLS (SEQ ID NO:13); n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14); o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15); p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16); q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17); r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18); s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19); t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20); u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21); v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22); w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23); x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24); y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25); z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26); aa) GRIP1–3:

PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27); bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28); cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29); dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30); ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31); ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32); gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33); hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34); ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35); jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36); kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37); ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38); mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39); nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40); oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41); pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42); qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43); rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44); ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45); tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46); uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47); vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48); ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49); xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50); yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51); zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52); aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53); bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54); ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55); ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56); eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57); fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58); ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59); hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60); iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61); jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62); kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63); lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64); mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65); nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66); ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67); ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69). In some cases, the first member of the specific binding pair is a single-chain Fv. In some cases, the first member of the specific binding pair binds an epitope present on a cell, on a solid surface, or a lipid bilayer. In some cases, the cell is a cancer cell.

[0012] The present disclosure provides a mammalian cell genetically modified to produce the heterodimeric, conditionally active polypeptide or receptor as described above or elsewhere herein. In some cases, the

cell is a stem cell, a progenitor cell, or a cell derived from a stem cell or a progenitor cell. In some cases, the cell is a T lymphocyte or an NK cell.

[0013] The present disclosure provides a nucleic acid comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide as described above or elsewhere herein. In some cases, the nucleotide sequences are operably linked to a promoter. In some cases, the promoter is an inducible promoter. In some cases, the promoter is a cell type-specific or tissue-specific promoter. In some cases, the promoter is a T lymphocyte-specific promoter or an NK cell-specific promoter. In some cases, the nucleic acid is *in vitro* transcribed RNA. The present disclosure provides a recombinant expression vector comprising the nucleic acid. In some cases, the recombinant expression vector is a viral vector, e.g., a lentiviral vector, a retroviral vector, or an adeno-associated viral vector.

[0014] The present disclosure provides a method of modulating an activity of a eukaryotic cell, the method comprising: a) expressing the heterodimeric, conditionally active polypeptide or receptor as described above, or elsewhere herein, in the eukaryotic cell; and b) contacting the cell with the ligand.

[0015] The present disclosure provides a method of modulating an activity of a T lymphocyte, the method comprising contacting the T lymphocyte with a dimerizing agent and a second member of a specific binding pair, wherein the T lymphocyte is genetically modified to produce a heterodimeric, conditionally active receptor as described above, or elsewhere herein, and wherein, in the presence of the dimerizing agent and the second member of a specific binding pair, the heterodimeric, conditionally active receptor dimerizes and modulates an activity of the T lymphocyte, thereby producing a modulated T lymphocyte. In some cases, the second member of a specific binding pair is an antigen. In some cases, the contacting occurs *in vivo*. In some cases, the T lymphocyte is activated, thereby producing an activated T lymphocyte. In some cases, the activated T lymphocyte mediates killing of a target cell. In some cases, the activated T lymphocyte produces IL-2 and/or IFN-γ. In some cases, the target cell is a cancer cell.

[0016] The present disclosure provides a method of making the cell of any one of claims 66-68, the method comprising genetically modifying a mammalian cell with an expression vector comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide as described above or elsewhere herein, or genetically modifying a mammalian cell with an RNA comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor as described above or elsewhere herein. In some cases, the genetic modification is carried out *ex vivo*. In some cases, the cell is a T lymphocyte, a stem cell, an NK cell, a progenitor cell, a cell derived from a stem cell, or a cell derived from a progenitor cell.

[0017] The present disclosure provides a method of treating a cancer in an individual, the method comprising: i) genetically modifying T lymphocytes obtained from the individual with an expression vector comprising nucleotide sequences encoding the heterodimeric, conditionally active chimeric

antigen receptor (CAR) as described above or elsewhere herein, wherein the antigen-binding domain of the heterodimeric, conditionally active CAR is specific for an epitope on a cancer cell in the individual, and wherein said genetic modification is carried out *ex vivo*; ii) introducing the genetically modified T lymphocytes into the individual; and iii) administering to the individual an effective amount of a dimerizing agent, wherein the dimerizing agent induces dimerization of the heterodimeric, conditionally active receptor, wherein said dimerization provides for activation of the genetically modified T lymphocytes and killing of the cancer cell, thereby treating the cancer. In some cases, the dimerizing agent is a nuclear hormone that binds the LBD of the nuclear hormone receptor and the co-regulator.

[0018] The present disclosure provides a method of modulating the activity of a host cell, the method comprising contacting the host cell with a dimerizing agent and a second member of a specific binding pair, wherein the T lymphocyte is genetically modified to produce a heterodimeric, conditionally active receptor as described above or elsewhere herein, and wherein, in the presence of the dimerizing agent and the second member of a specific binding pair, the heterodimeric, conditionally active receptor dimerizes and modulates at least one activity of the host cell. In some cases, the activity is proliferation, cell survival, apoptosis, gene expression, or immune activation. In some cases, the second member of a specific binding pair is an antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A-1F depict amino acid sequences of mineralocorticoid receptors (MR). FIG. 1A: full-length MR amino acid sequence; FIG. 1B-1E: amino acid sequences of ligand-binding domain (LBD) of MR; FIG. 1F: multiple sequence alignment of LBD of MR of various species. (SEQ ID NOs:70-84).

FIG. 2A-2I depict amino acid sequences of androgen receptors (AR). FIG. 2A: full-length AR amino acid sequence; FIG. 2B-2I: amino acid sequences of LBD of AR. (SEQ ID NOs:85-93).

FIG. 3A-3D depict amino acid sequences of progesterone receptors (PR). FIG. 3A: full-length PR amino acid sequence; FIG. 3B-3C: amino acid sequences of LBD of PR; FIG. 3D: multiple sequence alignment of LBD of PR of various species. (SEQ ID NOs:94-103).

FIG. 4A-4D depict amino acid sequences of thyroid hormone receptor-beta (TRβ). FIG. 3A: full-length PR amino acid sequence; FIG. 3B-3C: amino acid sequences of LBD of TRβ; FIG. 3D: multiple sequence alignment of LBD of TRβ of various species. (SEQ ID NO:104-118).

FIG. 5A-5H depict amino acid sequences of estrogen receptor-alpha (ERα). FIG. 5A: full-length ERα amino acid sequence; FIG. 5B-5G: amino acid sequences of LBD of ERα; FIG. 5H; multiple sequence alignment of LBD of ERα of various species. (SEQ ID NO:119-133).

FIG. 6A-6C depict amino acid sequences of estrogen receptor-beta (ERβ). FIG. 6A: full-length ERβ amino acid sequence; FIG. 6B: amino acid sequence of LBD of ERβ; FIG. 6C; multiple sequence alignment of LBD of ERβ of various species. (SEQ ID NO:134-143).

FIG. 7A-7E depict amino acid sequences of peroxisome proliferator-activated receptor-gamma (PPAR-γ). FIG. 7A: full-length PPAR-γ amino acid sequence; FIG. 7B-7D: amino acid sequences of LBD of PPAR-γ; FIG. 7E: multiple sequence alignment of LBD of PPAR-γ of various species. (SEQ ID NOs:144-157).

FIG. 8A-8C depict amino acid sequences of glucocorticoid receptor (GR). FIG. 8A: full-length GR amino acid sequence; FIG. 8B: amino acid sequence of LBD of GR; FIG. 8C; multiple sequence alignment of LBD of GR of various species. (SEQ ID NOs:158-166).

FIG. 9A-9C depict amino acid sequences of vitamin D receptor (VDR). FIG. 9A: full-length VDR amino acid sequence; FIG. 9B: amino acid sequence of LBD of VDR; FIG. 9C; multiple sequence alignment of LBD of VDR of various species. (SEQ ID NOs:167-172).

FIG. 10A-10C depict amino acid sequences of thyroid hormone receptor-alpha (TRα). FIG. 10A: full-length TRα amino acid sequence; FIG. 10B: amino acid sequence of LBD of TRα; FIG. 10C; multiple sequence alignment of LBD of TRα of various species. (SEQ ID NOs:173-185).

FIG. 11A-11C depict amino acid sequences of retinoic acid receptor-beta (RARβ). FIG. 11A: full-length RARβ amino acid sequence; FIG. 11B: amino acid sequence of LBD of RARβ; FIG. 11C; multiple sequence alignment of LBD of RARβ of various species. (SEQ ID NOs:186-196).

FIG. 12 is a schematic diagram of an ON-switch CAR featuring the Ligand Binding Domain (LBD) of a Nuclear Hormone Receptor, a co-regulator peptide, and a small molecule as the ternary hetero-dimerizing module.

FIG. 13 is a schematic diagram of a PPARγ-based ON-switch CAR construct.

FIG. 14 depicts IL-2 cytokine production by ON-switch CAR+ Jurkat cells (transduced with the indicated lentiviral constructs) after 18 hours of co-culturing with K562 target cells (+/- CD19 Ag, as indicated), in the presence or absence of rosiglitazone dimerizer (10 micromolar).

FIG. 15 is a schematic diagram of an estrogen receptor alpha-based ON-switch CAR construct.

FIGs. 16A-16B depict CD69 upregulation by ER CAR+ Jurkat cells after 24 hours of co-culturing with K562 target cells expressing CD19 or an irrelevant antigen ("meso"), in the presence or absence of a small-molecule dimerizer (4-hydroxytamoxifen or rapalog AP21967). CD69 expression in ER CAR+ Jurkat cells is strongly induced by 4- hydroxytamoxifen (EC50 ~100 nM) only in the presence of K562 cells expressing CD19. Rapamycin CAR and dead rapamycin CAR were tested as positive and negative controls, respectively. Dead rapamycin CAR comprises a mutation in the ITAM domain such that it does not signal. The data series presented in FIG. 16B from back to front are CD19 antigen + 4-hydroxytamoxifen 10 μM, 1 μM, 100 nM, 10 nM, 1 nM, 0 nM, meso antigen + 10 μM rapalog, and meso antigen + 0 μM rapalog.

FIG. 17 provides a schematic diagram of an estrogen receptor beta-based ON-switch CAR construct.

FIGs. 18A-18B depict CD69 upregulation by primary human T cells expressing ER-beta/CoRNR-based on-switch CAR measured after 24 hours of co-culturing with K562 target cells expressing CD19 or an irrelevant antigen ("meso"), in the presence or absence of a small-molecule dimerizer (4-hydroxytamoxifen or rapalog). CD69 expression is induced in a dimerizer dose dependent manner. The data series presented in FIG. 18B from back to front are CD19 antigen + 4-hydroxytamoxifen 10 μM, 1 μM, 100 nM, 10 nM, 1 nM, 0 nM, meso antigen + 10 μM rapalog, and meso antigen + 0 μM rapalog.

FIG. 19 provides a table showing various possible combinations of LBD, co-regulator, and dimerization agent.

FIG. 20A-20C depict amino acid sequences of Lyn kinase (FIG. 20A), an N-terminal portion of a Lyn kinase (FIG. 20B), and a C-terminal portion of a Lyn kinase (FIG. 20C). (SEQ ID NOs:197-199)

FIG. 21 provides an amino acid sequence of a Fak kinase. (SEQ ID NO:200)

FIG. 22A-22B depict amino acid sequences of an FXR (FIG. 22A) and a LBD of an FXR (FIG. 22B). (SEQ ID NOs:201-202)

FIG. 23A-23B depict amino acid sequences of an LXRα (FIG. 23A) and a LBD of an LXRα (FIG. 23B). (SEQ ID NOs:203-204)

FIG. 24A-24B depict amino acid sequences of an RORγ (FIG. 24A) and a LBD of an RORγ (FIG. 24B). (SEQ ID NOs:205-206)

FIG. 25A-25B depict amino acid sequences of an RXRα (FIG. 25A) and a LBD of an RXRα (FIG. 25B). (SEQ ID NOs:207-208)

FIG. 26A-26B depict amino acid sequences of a PXR (FIG. 26A) and a LBD of a PXR (FIG. 26B). (SEQ ID NOs:209-210)

FIG. 27 provides Table 1 (SEQ ID NOs:211-278).

FIG. 28 provides the amino acid sequence of *S. pyogenes* Cas9 (SEQ ID NO:279).

FIG. 29-51B provide amino acid sequences of various co-regulator polypeptides (SEQ ID NOs:280-302).

FIG. 52 depicts target cell killing by primary human CD8+ T cells expressing a CD19 specific ER-beta/CoRNR ON-switch CAR.

FIG. 53 provide the amino acid sequences of exemplary IL-2 family receptors (SEQ ID NOs:303-313).

FIG. 54 provide the amino acid sequences of exemplary IL-3 family receptors (SEQ ID NOs:314-317).

FIG. 55 provide the amino acid sequences of exemplary IL-6 family receptors (SEQ ID NOs:318-326).

FIG. 56 provide the amino acid sequences of exemplary IL-12 family receptors (SEQ ID NOs:327-329).

FIG. 57 provide the amino acid sequences of exemplary prolactin family receptors (SEQ ID NOs:330-334).

FIG. 58 provide the amino acid sequences of exemplary interferon family receptors (SEQ ID NOs:335-338).

FIG. 59 provide the amino acid sequences of exemplary IL-10 family receptors (SEQ ID NOs:339-345).

FIG. 60 provide the amino acid sequences of exemplary IL-17 family receptors (SEQ ID NOs:346-350).

FIG. 61 provide the amino acid sequences of exemplary immunoglobulin-like superfamily receptors (SEQ ID NOs:351-361).

FIG. 62 provide the amino acid sequences of exemplary tumor necrosis factor family receptors (SEQ ID NOs:362-392).

FIG. 63 provide the amino acid sequences of exemplary chemokine receptors (SEQ ID NOs:393-409).

FIG. 64 provide the amino acid sequences of exemplary TGF-beta family receptors (SEQ ID NOs:410-412).

FIG. 65 provide the amino acid sequences of exemplary receptor tyrosine kinases (RTKs) (SEQ ID NOs:413-469).

DEFINITIONS

[0019] The terms “polynucleotide” and “nucleic acid,” used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0020] "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression.

[0021] A "vector" or "expression vector" is a replicon, such as plasmid, phage, virus, or cosmid, to which another DNA segment, i.e. an "insert", may be attached so as to bring about the replication of the attached segment in a cell.

[0022] Heterologous," as used herein, means a nucleotide or polypeptide sequence that is not found in the native (e.g., naturally-occurring) nucleic acid or protein, respectively.

[0023] The terms "antibodies" and “immunoglobulin” include antibodies or immunoglobulins of any isotype, fragments of antibodies that retain specific binding to antigen, including, but not limited to, Fab, Fv, scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies (scAb), single domain antibodies (dAb), single domain heavy chain antibodies, a single domain light chain antibodies, bi-specific antibodies, multi-specific antibodies, and fusion proteins comprising an antigen-binding (also referred to herein as antigen binding) portion of an antibody and a non-antibody protein. The antibodies can be detectably labeled, *e.g.*, with a radioisotope, an enzyme that generates a detectable product, a fluorescent protein, and the like. The antibodies can be further conjugated to other moieties,

such as members of specific binding pairs, *e.g.*, biotin (member of biotin-avidin specific binding pair), and the like. The antibodies can also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like. Also encompassed by the term are Fab', Fv, $F(ab')_2$, and or other antibody fragments that retain specific binding to antigen, and monoclonal antibodies. As used herein, a monoclonal antibody is an antibody produced by a group of identical cells, all of which were produced from a single cell by repetitive cellular replication. That is, the clone of cells only produces a single antibody species. While a monoclonal antibody can be produced using hybridoma production technology, other production methods known to those skilled in the art can also be used (e.g., antibodies derived from antibody phage display libraries). An antibody can be monovalent or bivalent. An antibody can be an Ig monomer, which is a "Y-shaped" molecule that consists of four polypeptide chains: two heavy chains and two light chains connected by disulfide bonds.

[0024] The term "humanized immunoglobulin" as used herein refers to an immunoglobulin comprising portions of immunoglobulins of different origin, wherein at least one portion comprises amino acid sequences of human origin. For example, the humanized antibody can comprise portions derived from an immunoglobulin of nonhuman origin with the requisite specificity, such as a mouse, and from immunoglobulin sequences of human origin (e.g., chimeric immunoglobulin), joined together chemically by conventional techniques (e.g., synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques (e.g., DNA encoding the protein portions of the chimeric antibody can be expressed to produce a contiguous polypeptide chain). Another example of a humanized immunoglobulin is an immunoglobulin containing one or more immunoglobulin chains comprising a CDR derived from an antibody of nonhuman origin and a framework region derived from a light and/or heavy chain of human origin (e.g., CDR-grafted antibodies with or without framework changes). Chimeric or CDR-grafted single chain antibodies are also encompassed by the term humanized immunoglobulin. See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Cabilly et al., European Patent No. 0,125,023 B1; Boss et al., U.S. Pat. No. 4,816,397; Boss et al., European Patent No. 0,120,694 B1; Neuberger, M. S. et al., WO 86/01533; Neuberger, M. S. et al., European Patent No. 0,194,276 B1; Winter, U.S. Pat. No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Padlan, E. A. et al., European Patent Application No. 0,519,596 A1. See also, Ladner et al., U.S. Pat. No. 4,946,778; Huston, U.S. Pat. No. 5,476,786; and Bird, R. E. et al., Science, 242: 423-426 (1988)), regarding single chain antibodies.

[0025] The term "nanobody" (Nb), as used herein, refers to the smallest antigen binding fragment or single variable domain (V_{HH}) derived from naturally occurring heavy chain antibody and is known to the person skilled in the art. They are derived from heavy chain only antibodies, seen in camelids (Hamers-Casterman et al., 1993; Desmyter et al., 1996). In the family of "camelids" immunoglobulins devoid of light polypeptide chains are found. "Camelids" comprise old world camelids (*Camelus bactrianus* and

Camelus dromedarius) and new world camelids (for example, *Llama paccos*, *Llama glama*, *Llama guanicoe* and *Llama vicugna*). A single variable domain heavy chain antibody is referred to herein as a nanobody or a V_{HH} antibody.

[0026] "Antibody fragments" comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); domain antibodies (dAb; Holt et al. (2003) *Trends Biotechnol.* 21:484); single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

[0027] "Fv" is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRS of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0028] The "Fab" fragment also contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0029] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these classes can be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The subclasses can be further divided into types, e.g., IgG2a and IgG2b.

[0030] "Single-chain Fv" or "sFv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv

polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see *Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)*.

[0031] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448.

[0032] As used herein, the term “hinge region” refers to a flexible polypeptide connector region (also referred to herein as “hinge” or “spacer”) providing structural flexibility and spacing to flanking polypeptide regions and can consist of natural or synthetic polypeptides. A "hinge region" derived from an immunoglobulin (e.g., IgG1) is generally defined as stretching from Glu216 to Pro230 of human IgG1 (Burton (1985) Molec. Immunol., 22:161-206). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain disulfide (S-S) bonds in the same positions. The hinge region may be of natural occurrence or non-natural occurrence, including but not limited to an altered hinge region as described in U.S. Pat. No. 5,677,425. The hinge region can include complete hinge region derived from an antibody of a different class or subclass from that of the CH1 domain. The term “hinge region” can also include regions derived from CD8 and other receptors that provide a similar function in providing flexibility and spacing to flanking regions.

[0033] As used herein, the term "affinity" refers to the equilibrium constant for the reversible binding of two agents (e.g., an antibody and an antigen) and is expressed as a dissociation constant (K_D). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1,000-fold greater, or more, than the affinity of an antibody for unrelated amino acid sequences. Affinity of an antibody to a target protein can be, for example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM) or more. As used herein, the term “avidity” refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms “immunoreactive” and “preferentially binds” are used interchangeably herein with respect to antibodies and/or antigen-binding fragments.

[0034] The term "binding" refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. In some cases, the first member of a specific binding pair present in the extracellular domain of a conditionally active heterodimeric polypeptide of the present disclosure binds specifically to a second member of the specific binding pair. "Specific binding" refers to binding with an affinity of at least about 10^{-7} M or greater, e.g., 5x 10^{-7} M, 10^{-8} M, 5 x 10^{-8} M, and greater. "Non-specific binding" refers to binding with an affinity of less than about 10^{-7} M, e.g., binding with an affinity of 10^{-6} M, 10^{-5} M, 10^{-4} M, etc.

[0035] The terms "polypeptide," "peptide," and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like.

[0036] An "isolated" polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the polypeptide will be purified (1) to greater than 90%, greater than 95%, or greater than 98%, by weight of antibody as determined by the Lowry method, for example, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or nonreducing conditions using Coomassie blue or silver stain. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. In some instances, isolated polypeptide will be prepared by at least one purification step.

[0037] The term "module", as used herein, refers to a contiguous polypeptide sequence, or fragment thereof, that is associated with some function, particularly a biological function.

[0038] The terms "domain" and "motif", used interchangeably herein, refer to both structured domains having one or more particular functions and unstructured segments of a polypeptide that, although unstructured, retain one or more particular functions. For example, a structured domain may encompass but is not limited to a continuous or discontinuous plurality of amino acids, or portions thereof, in a folded polypeptide that comprise a three-dimensional structure which contributes to a particular function

of the polypeptide. In other instances, a domain may include an unstructured segment of a polypeptide comprising a plurality of two or more amino acids, or portions thereof, that maintains a particular function of the polypeptide unfolded or disordered. Also encompassed within this definition are domains that may be disordered or unstructured but become structured or ordered upon association with a target or binding partner. Non-limiting examples of intrinsically unstructured domains and domains of intrinsically unstructured proteins are described, e.g., in Dyson & Wright. *Nature Reviews Molecular Cell Biology* 6:197-208.

[0039] The terms "chimeric antigen receptor" and "CAR", used interchangeably herein, refer to artificial multi-module molecules capable of triggering or inhibiting the activation of an immune cell which generally but not exclusively comprise an extracellular domain (e.g., a ligand/antigen binding domain), a transmembrane domain and one or more intracellular signaling domains. The term "CAR" is not limited specifically to CAR molecules but also includes CAR variants. CAR variants include split CARs wherein the extracellular portion (e.g., the ligand binding portion) and the intracellular portion (e.g., the intracellular signaling portion) of a CAR are present on two separate molecules. CAR variants also include ON-switch CARs which are conditionally activatable CARs, e.g., comprising a split CAR wherein conditional hetero-dimerization of the two portions of the split CAR is pharmacologically controlled. CAR variants also include bispecific CARs, which include a secondary CAR binding domain that can either amplify or inhibit the activity of a primary CAR. CAR variants also include inhibitory chimeric antigen receptors (iCARs) which may, e.g., be used as a component of a bispecific CAR system, where binding of a secondary CAR binding domain results in inhibition of primary CAR activation. CAR molecules and derivatives thereof (i.e., CAR variants) are described, e.g., in PCT Application No. US2014/016527; Fedorov et al. *Sci Transl Med* (2013) ;5(215):215ra172; Glienke et al. *Front Pharmacol* (2015) 6:21; Kakarla & Gottschalk 52 *Cancer J* (2014) 20(2):151-5; Riddell et al. *Cancer J* (2014) 20(2):141-4; Pegram et al. *Cancer J* (2014) 20(2):127-33; Cheadle et al. *Immunol Rev* (2014) 257(1):91-106; Barrett et al. *Annu Rev Med* (2014) 65:333-47; Sadelain et al. *Cancer Discov* (2013) 3(4):388-98; Cartellieri et al., *J Biomed Biotechnol* (2010) 956304; the disclosures of which are incorporated herein by reference in their entirety.

[0040] As used herein, the term "immune cells" generally includes white blood cells (leukocytes) which are derived from hematopoietic stem cells (HSC) produced in the bone marrow. "Immune cells" includes, e.g., lymphocytes (T cells, B cells, natural killer (NK) cells) and myeloid-derived cells (neutrophil, eosinophil, basophil, monocyte, macrophage, dendritic cells).

[0041] "T cell" includes all types of immune cells expressing CD3 including T-helper cells ($CD4^+$ cells), cytotoxic T-cells ($CD8^+$ cells), T-regulatory cells (Treg) and gamma-delta T cells.

[0042] A "cytotoxic cell" includes $CD8^+$ T cells, natural-killer (NK) cells, and neutrophils, which cells are capable of mediating cytotoxicity responses.

[0043] As used herein, the term “stem cell” generally includes pluripotent or multipotent stem cells. “Stem cells” includes, e.g., embryonic stem cells (ES); mesenchymal stem cells (MSC); induced-pluripotent stem cells (iPS); and committed progenitor cells (hematopoietic stem cells (HSC); bone marrow derived cells, neural progenitor cells, etc.).

[0044] As used herein, the terms "treatment," "treating," “treat” and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect can be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or can be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which can be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0045] The terms “individual,” “subject,” “host,” and “patient,” used interchangeably herein, refer to a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), lagomorphs, etc. In some cases, the individual is a human. In some cases, the individual is a non-human primate. In some cases, the individual is a rodent, e.g., a rat or a mouse. In some cases, the individual is a lagomorph, e.g., a rabbit.

[0046] A “therapeutically effective amount” or “efficacious amount” refers to the amount of an agent, or combined amounts of two agents, that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The “therapeutically effective amount” will vary depending on the agent(s), the disease and its severity and the age, weight, etc., of the subject to be treated.

[0047] As used herein, the term “heteromeric” refers to a polypeptide or protein that contains more than one kind of subunit. Such heteromeric polypeptides may, in some instances, be referred to as “a heteromer”. Heteromeric polypeptides may contain two or more different polypeptides, wherein different polypeptides are defined at least as two polypeptides that are not identical, however, such different polypeptides may or may not include one or more portions of similar and/or identical amino acid sequence. In some instances, the two or more polypeptides of a heteromer share no identical amino acid sequence or share no identical domains. A heteromer may, in some instances, consist of two different polypeptides or two different types of polypeptides and may be referred to as a heterodimer. In some instances, a heteromer may consist of three different polypeptides or three different types of polypeptides and may be referred to as a heterotrimer. In some instances, a heteromer may consist of two or more different polypeptides or two or more different types of polypeptides, including but not limited to, e.g., three or more different polypeptides, four or more different polypeptides, five or more different

polypeptides, six or more different polypeptides, seven or more different polypeptides, eight or more different polypeptides, etc.

[0048] The term "synthetic" as used herein generally refers to an artificially derived polypeptide or polypeptide encoding nucleic acid that is not naturally occurring. Such synthetic polypeptides and/or nucleic acids may be assembled de novo from basic subunits including, e.g., single amino acids, single nucleotides, etc., or may be derived from pre-existing polypeptides or polynucleotides, whether naturally or artificially derived, e.g., as through recombinant methods.

[0049] The term "recombinant", as used herein describes a nucleic acid molecule, e.g., a polynucleotide of genomic, cDNA, viral, semisynthetic, and/or synthetic origin, which, by virtue of its origin or manipulation, is not associated with all or a portion of the polynucleotide sequences with which it is associated in nature. The term recombinant as used with respect to a protein or polypeptide means a polypeptide produced by expression from a recombinant polynucleotide. The term recombinant as used with respect to a host cell or a virus means a host cell or virus into which a recombinant polynucleotide has been introduced. Recombinant is also used herein to refer to, with reference to material (e.g., a cell, a nucleic acid, a protein, or a vector) that the material has been modified by the introduction of a heterologous material (e.g., a cell, a nucleic acid, a protein, or a vector).

[0050] A "biological sample" encompasses a variety of sample types obtained from an individual or a population of individuals and can be used in a diagnostic, monitoring or screening assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by mixing or pooling of individual samples, treatment with reagents, solubilization, or enrichment for certain components, such as cells, polynucleotides, polypeptides, etc. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples. The term "biological sample" includes urine, saliva, cerebrospinal fluid, interstitial fluid, ocular fluid, synovial fluid, blood fractions such as plasma and serum, and the like. The term "biological sample" also includes solid tissue samples, tissue culture samples, and cellular samples.

[0051] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0052] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0053] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0054] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a dimerizing agent" includes a plurality of such dimerizing agents and reference to "the antigen-binding domain" includes reference to one or more antigen-binding domains and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0055] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0056] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication

provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0057] The present disclosure provides conditionally active, heterodimeric polypeptides. The conditionally active, heterodimeric polypeptides are active in the presence of a dimerizing agent that induces dimerization of the polypeptides of the heterodimer. A conditionally active, heterodimeric polypeptide of the present disclosure is useful in a variety of research and treatment methods, which are also provided.

CONDITIONALLY ACTIVE HETERODIMERIC POLYPEPTIDES

[0058] The present disclosure provides a heterodimeric, conditionally active polypeptide comprising: a) a first chimeric polypeptide comprising a first member of a dimerization pair and a first heterologous polypeptide; and b) a second chimeric polypeptide comprising a second member of a dimerization pair and a second heterologous polypeptide. In some cases, the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor. In other cases, the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor. The first chimeric polypeptide and the second chimeric polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[0059] In some instances, the function of a conditionally active heterodimeric polypeptide of the present disclosure does not utilize a DNA-binding function or a transcription activating function of the nuclear hormone receptor from which the LBD is derived. Put another way, the function of the conditionally active heterodimeric polypeptide of the present disclosure may be independent of any DNA-binding function of the nuclear hormone receptor from which the LBD is derived. In some instances, activation of a conditionally active heterodimeric polypeptide of the present disclosure does not result in a transcription activating function attributable to a domain (e.g., a DNA-binding domain) of the nuclear hormone receptor from which a LBD of the conditionally active heterodimeric polypeptide is derived. In some instances, a conditionally active heterodimeric polypeptide of the present disclosure does not contain a DNA-binding domain of a nuclear hormone receptor, including e.g., the DNA-binding domain of the nuclear hormone receptor from which a LBD of a conditionally active heterodimeric polypeptide is derived.

[0060] A conditionally active heterodimeric polypeptide of the present disclosure can, in some instances, be activating. A conditionally active heterodimeric polypeptide of the present disclosure can, in some instances, have repressor activity. For example, where a conditionally active heterodimeric polypeptide of the present disclosure is present in the membrane of a cell, and the cell is contacted with a dimerizing

agent that induces binding of the LBD to the co-regulator, in some cases, the conditionally active heterodimeric polypeptide activates the cell. For example, where a conditionally active heterodimeric polypeptide of the present disclosure is present in the membrane of a cell, and the cell is contacted with a dimerizing agent that induces binding of the LBD to the co-regulator, in some cases, the conditionally active heterodimeric polypeptide represses an activity in or by the cell. In some cases, activation of a conditionally active heterodimeric polypeptide of the present disclosure depends on the presence of a dimerization agent. In some cases, activation of a conditionally active heterodimeric polypeptide of the present disclosure depends on the presence of a dimerization agent and a second agent (e.g., an antigen). Depending on the nature of the conditionally active heterodimeric polypeptide and/or depending on the nature of the dimerization agent, activation of a conditionally active heterodimeric polypeptide of the present disclosure can result in activation of an activity of a cell (where the conditionally active heterodimeric polypeptide is present in the cell), or repression of an activity of a cell (where the conditionally active heterodimeric polypeptide is present in the cell). Depending on the nature of the conditionally active heterodimeric polypeptide and/or depending on the nature of the dimerization agent, activation of a conditionally active heterodimeric polypeptide of the present disclosure can result in activation of an activity of a heterologous polypeptide present in the conditionally active heterodimeric polypeptide, or can result in inhibition of an activity of a heterologous polypeptide present in the conditionally active heterodimeric polypeptide.

[0061] By "conditionally active" is meant that the activity attributaible to the relevant heterodimeric polypeptide is dependent upon a condition, such as e.g., dimerization of the heterodimeric parts of the polypeptide. For instance, the activity of a heterodimeric polypeptide of the present disclosure may be conditionally dependent upon the presence of a dimerizing agent. In such instances, in the presence of the dimerizing agent, the heterodimeric polypeptide is "activatable", i.e., may be activated (e.g., through binding of the second member of a specific binding pair of the polypeptide, such as an antigen). Conditionally activatable polypeptides will generally not be activatable in the absesnce of dimerizing agent, i.e., the relevant polypeptide cannot be activated in the absence of the dimerizing agent regardless of whether some additional condition for function is met, e.g., the second member of a specific binding pair of the polypeptide (such as an antigen) is present and/or bound.

[0062] The activities attributaible to the relevant polypeptides that may be conditionally dependent, e.g., on the presence of the relevant dimerizing agent, may vary and will generally include any activity including both activating activities and repressive activities as noted above and described in greater detail below. As an example, a conditionally active on-switch chimeric antigen receptor (CAR), as described in more detail below, may be activatable in the presence of the relevant dimerization agent such that, in the presence of both the dimerization agent and the relevant member of the specific binding pair to which the polypeptide binds, signal transduction resulting from the on-switch CAR may activate a cell (e.g., an

immune cell) in which the on-switch CAR resides. In the contrary case, in the absence of the relevant dimerization agent, signal transduction which would lead to activation of a cell expressing the on-switch CAR does not occur regardless of the presence of the relevant member of the specific binding pair to which the on-switch CAR binds. As another example, the repression attributable to a conditionally active off-switch CAR, as described in more detail below, may be activatable in the presence of the relevant dimerization agent such that, in the presence of both the dimerization agent and the relevant member of the specific binding pair to which the polypeptide binds, the off-switch CAR may repress activation of a cell (e.g., an immune cell) in which the off-switch CAR resides. In the contrary case, in the absence of the relevant dimerization agent, activation of the off-switch CAR which would lead to repression of that activation of a cell expressing the off-switch CAR does not occur regardless of the presence of the relevant member of the specific binding pair to which the off-switch CAR binds.

Interacting polypeptides

[0063] The first heterologous polypeptide and the second heterologous polypeptide of the subject conditionally active heterodimeric polypeptides can be any polypeptides that exhibit an activity when brought into proximity to one another, e.g., when the first chimeric polypeptide and the second chimeric polypeptide are dimerized by the dimerization agent that induces binding of the LBD to the co-regulator. The first and the second heterologous polypeptides do not exhibit that activity individually, e.g., when not in proximity to one another. In other words, activity of the first heterologous polypeptide and the second heterologous polypeptide is dependent upon assembly of the two polypeptides. For example, in some cases, the first heterologous polypeptide is a receptor and the second heterologous polypeptide is a co-receptor. In some cases, the first heterologous polypeptide is a T-cell receptor (TCR) alpha chain; and the second heterologous polypeptide is a TCR beta chain. In some cases, the first heterologous polypeptide is a first polypeptide of a chimeric antigen receptor (CAR) heterodimer; and the second heterologous polypeptide is a second polypeptide of the CAR heterodimer. In some cases, the first heterologous polypeptide and the second heterologous polypeptide are caspases. In some cases, the first heterologous polypeptide is a first subunit of a polypeptide; and the second heterologous polypeptide is a second subunit of the polypeptide. In some cases, the first heterologous polypeptide is an enzyme; and the second heterologous polypeptide is a polypeptide substrate of the enzyme. In some cases, the first heterologous polypeptide is a scaffold polypeptide (e.g., LAT; SLP76; and the like); and the second heterologous polypeptide is a polypeptide that conditionally binds to the scaffold polypeptide. In some cases, the first heterologous polypeptide is a signal transducing adaptor protein (e.g., MyD88; Grb2; SHC1); and the second heterologous polypeptide is a polypeptide that conditionally binds the signal transducing adaptor protein. In some cases, the first and second heterologous polypeptides are dimerization-dependent cell-surface receptors (e.g., cytokine receptors, receptor tyrosine kinases (RTK), and the like) or a portion thereof. In some cases, the first and the second heterologous polypeptides are

the same dimerization-dependent cell-surface receptor (e.g., the same cytokine receptor, the same RTK, etc.) or the same portions of a dimerization-dependent cell-surface receptor.

[0064] In some cases, the first heterologous polypeptide and the second heterologous polypeptide have the same amino acid sequence. In some cases, the first heterologous polypeptide and the second heterologous polypeptide have different amino acid sequences.

Split products

[0065] In some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a single parent polypeptide that exhibit an activity of the parent polypeptide when brought into proximity to one another, e.g., when the first chimeric polypeptide and the second chimeric polypeptide are dimerized by the dimerization agent that induces binding of the LBD to the co-regulator. For example, in some cases, the first heterologous polypeptide is an N-terminal portion of a parent polypeptide; and the second heterologous polypeptide is the C-terminal portion of the parent polypeptide. The N-terminal portion and the C-terminal portion of the parent polypeptide, when split such that they are not in proximity to one another, do not individual exhibit the activity of the parent polypeptide. For example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a kinase. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a protease. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a fluorescent protein; see, e.g., Ghosh et al. (2000) *J. Am. Chem. Soc.*122:5658. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of ubiquitin; see, e.g., Johnsson and Varshavsky (1994) *Proc. Natl. Acad. Sci. USA* 91:10340. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a lactamase polypeptide; see, e.g., Galarneau et al. (2002) *Nat. Biotechnol.* 20:619. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of luciferase; see, e.g., Luker et al. (2004) *Proc. Natl. Acad. Sci. USA* 101:12288; Remy and Michnick (2006) *Nat. Methods* 3:977; and Paulmurugan and Gambhir (2003) *Anal. Chem.* 75:1584. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a caspase. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a phosphatase. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of an endonuclease. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of an RNA-guided endonuclease. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a Tobacco etch virus (TEV) protease; see, e.g., Gray et al. (2010) *Cell* 142:637. As another example, in some cases, the first heterologous polypeptide

and the second heterologous polypeptide are split products of a tyrosine phosphatase; see, e.g., Camacho-Soto et al. (2014) *J. Am. Chem. Soc.* 136:17078. As another example, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a Cas9 polypeptide; see, e.g., Zetche et al. (2015) *Nat. Biotechnol.* 33:139.

[0066] As noted above, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a kinase.As a non-limiting example, a tyrosine kinase can be the parent polypeptide; and split product of the parent tyrosine kinase can be the first heterologous polypeptide and the second heterologous polypeptide. For example, a tyrosine kinase can be split between amino acids E393 and D394 of the amino acid sequence depicted in FIG. 20A, or a corresponding site in a different tyrosine kinase, such that the first heterologous polypeptide is an N-terminal portion of the tyrosine kinase ending with E393, and the second heterologous polypeptide is a C-terminal portion of the tyrosine kinase beginning with D394. As an example, the first heterologous polypeptide can comprise the amino acid sequence depicted in FIG. 20B; and the second heterologous polypeptide can comprise the amino acid sequence depicted in FIG. 20C. As another example, a tyrosine kinase can be split between amino acids E572 and D573 of the amino acid sequence depicted in FIG. 21, or a corresponding site in a different tyrosine kinase, such that the first heterologous polypeptide is an N-terminal portion of the tyrosine kinase ending with E572, and the second heterologous polypeptide is a C-terminal portion of the tyrosine kinase beginning with D573. See, e.g., Camacho-Soto et al. (2014) J. Am. Chem. Soc. 136:3995. Tyrosine kinases include, e.g., Lyn, Fak, Src, and PKA. Tyrosine kinases include, e.g., ABL1, ABL1 (T315I), ACVR1, AKT1, ALK, AURKA, AXL, BTK, CAMK2D, CDK1B, CDK2A, CDK4D1, CSK, CSNK1G3, EGFR, EPHA4, EPHB4, ERBB4, FGFR1, FGFR2, FGFR3, FGFR3 (K650E), FGFR4, FLT3 (D835Y), IGF1R, GSK3B, INSR, IRAK4, JAK1, JAK2, JAK3, KDR, KIT, LCK, LYN, MAP3K8, MAPK1, MAPK10, MAPK14, MAPKAPK2, MAPKAPK5, MET, MKNK1, MKNK2, PAK2, PDGFRa, PDPK1, PIM2, PKN1, PKN2, PLK1, PRKACA, PRKCA, PRKCQ, RET, ROCK2, RPS6 KB1, SRC, SYK, TYK2, WNK1, ZAP70, PIKSCD, PIK3CG, MTOR, PIK3C3, PIK3CA, PIK3CB and PIK4CB. Suitable tyrosine kinases include SYK, LCK, IRK (=INSR=Insulin receptor), IGF-1 receptor, MK2, ZAP-70, Aurora-2, PRAK, ROCK, CAK, cMET, IRAK1, IRAK2, BLK, BMX, BTK, FRK, FGR, FYN, HCK, ITK, LYN, TEC, TXK, YES, ABL, SRC, EGF-R (=ErbB-1), ErbB-2 (=NEU=HER 2), ErbB-3, ErbB-4, FAK, FGF1R (=FGR-1), FGF2R (=FGR-2), IKK-1 (=IKK-α=CHUK), IKK-2 (=IKKβ), MET (=c-Met), NIK, PGDF receptor α, PDGF receptor β, TIE1, TIE2 (=TEK), VEGFR 1 (=FLT-1), VEGFR 2 (=KDR), FLT-3, FLT-4, KIT, CSK, JAK1, JAK2, JAK3, TYK2, RIP, RIP-2, LOK, TAKI, RET, ALK, MLK3, COT, TRKA, PYK2, EPHB4, RON, GSK3, UL13, ORF47, ATM, CDK (including all subtypes), PKA, PKB (including all PKB subtypes) (=AKT-1, AKT-2, AKT-3), PKC (including all PKC subtypes), REDK, SAPK, PIM, PDK, PIM, ERK and BARK, and all subtypes of these kinases.

[0067] As noted above, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a caspase. Chelur and Chalfie (2007) *Proc. Natl. Acad. Sci. USA* 104:2283. In some cases, the caspase is an apoptotic caspase.

[0068] As noted above, in some cases, the first heterologous polypeptide and the second heterologous polypeptide are split products of a Cas9 polypeptide. A Cas9 polypeptide can comprise an amino acid sequence having at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 28. In some cases, the Cas9 is a catalytically inactive Cas9 ("dCas9"); e.g., where the dCas9 comprises a D10A and an H840A substitution relative to the amino acid sequence depicted in FIG. 28, or corresponding amino acids of another Cas9 polypeptide.

Specific binding pairs and extracellular recognition domains

[0069] The present disclosure provides conditionally active, heterodimeric polypeptides. The conditionally active, heterodimeric polypeptides, when expressed on the plasma membrane of a cell, are active in the presence of: 1) an antigen or other moiety that interacts with the extracellular domain; and 2) a dimerizing agent that induces dimerization of the polypeptides of the heterodimer.

[0070] In some cases, a conditionally active heterodimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain. In some cases, the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor. In some cases, the first member of the dimerization pair comprises a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor.

[0071] Thus, in some cases, a conditionally active heterodimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair, wherein the first member of the dimerization pair comprises an LBD of a nuclear hormone receptor; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair, wherein the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor; and iv) an intracellular signaling domain. In other cases, a conditionally active heterodimeric polypeptide of the present disclosure comprises: a) a first

polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair, wherein the first member of the dimerization pair comprises a co-regulator of a nuclear hormone receptor; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair, wherein the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and iv) an intracellular signaling domain.

[0072] In some cases, a conditionally active heterodimeric polypeptide of the present disclosure comprises: a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a modulatory domain; iii) a first member of a dimerization pair; iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and b) a second polypeptide comprising: i) a second member of the dimerization pair; and ii) an intracellular signaling domain. In some cases, the first member of the dimerization pair comprises an LBD of a nuclear hormone receptor, and the second member of the dimerization pair is a co-regulator of the nuclear hormone receptor. In some cases, the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor.

[0073] In some cases, a conditionally active heterodimeric polypeptide of the present disclosure is a conditionally active on-switch chimeric antigen receptor (CAR). In some cases, a conditionally active heterodimeric polypeptide of the present disclosure is a conditionally active off-switch CAR. In some cases, a conditionally active heterodimeric polypeptide of the present disclosure is a conditionally active T-cell receptor (TCR).

[0074] The present disclosure provides a heterodimeric, conditionally repressible synthetic immune cell receptor (ICR) comprising: a synthetic stimulatory ICR comprising a first member of a dimerization pair linked to the synthetic stimulatory ICR; and a synthetic ICR repressor comprising a second member of the dimerization pair linked to an intracellular inhibitory domain, wherein the first member of the dimerization pair comprises an LBD of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-activator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-activator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor. The synthetic stimulatory ICR and the synthetic ICR repressor are dimerized in the presence of a ligand (dimerization agent) that induces binding of the LBD to the co-regulator.

[0075] The present disclosure provides a heterodimeric, conditionally repressible synthetic chimeric antigen receptor (CAR) comprising: a) a synthetic stimulatory CAR comprising: i) a extracellular recognition domain; ii) a transmembrane domain linked to the extracellular recognition domain; iii) a first member of a dimerization pair linked to the transmembrane domain; and iv) an intracellular stimulation domain;

and b) a synthetic CAR repressor comprising: i) a second member of the dimerization pair; and ii) an intracellular inhibitory domain linked to the second member of the dimerization pair. The first member of the dimerization pair comprises an LBD of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-activator of the nuclear hormone receptor; or the first member of the dimerization pair is a co-activator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor. The synthetic stimulatory CAR and the synthetic CAR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[0076] The present disclosure provides a heterodimeric, conditionally repressible synthetic T cell receptor (TCR) comprising: a) a synthetic stimulatory TCR comprising: i) a transmembrane domain; ii) a first member of a dimerization pair linked to the transmembrane domain; iii) an engineered TCR polypeptide comprising at least one TCR alpha or beta chain, wherein the at least one TCR alpha or beta chain is linked to the transmembrane domain or the first member of a dimerization pair; and b) a synthetic TCR repressor comprising: i) a second member of the dimerization pair; and ii) an intracellular inhibitory domain linked to the second member of the dimerization pair. The first member of the dimerization pair comprises an LBD of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-activator of the nuclear hormone receptor; or the first member of the dimerization pair is a co-activator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor. The synthetic stimulatory TCR and the synthetic TCR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[0077] The present disclosure provides a heterodimeric, conditionally active chimeric antigen receptor (CAR) comprising: I) a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain; or II) a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a modulatory domain; iii) a first member of a dimerization pair; iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and b) a second polypeptide comprising: i) a second member of the dimerization pair; and ii) an intracellular signaling domain. The first member of the dimerization pair comprises an LBD of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-activator of the nuclear hormone receptor; or the first member of the dimerization pair is a co-activator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor. The first polypeptide and the

second polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

CONDITIONALLY ACTIVE ON-SWITCH CAR

[0078] In some cases, a conditionally active heterodimeric polypeptide of the present disclosure is a conditionally active on-switch chimeric antigen receptor (CAR).

Member of a specific binding pair

[0079] As noted above, a conditionally active heterodimeric polypeptide of the present disclosure comprises a first polypeptide comprising a first member of a specific binding pair. The second member of the specific binding pair can be present on the surface of a cell. The second member of the specific binding pair can be immobilized on an insoluble support, expressed on the surface of a cell (e.g., a target cell, a non-target cell, etc.), or the like. The second member of the specific binding pair can be soluble. The second member of the specific binding pair can be present in an extracellular environment (e.g., extracellular matrix). The second member of the specific binding pair can be present in an artificial matrix. The second member of the specific binding pair can be present in an acellular environment.

[0080] Suitable first members of a specific binding pairs include, but are not limited to, antibody-based recognition scaffolds; antibodies (i.e., an antibody-based recognition scaffold, including antigen-binding antibody fragments); non-antibody-based recognition scaffolds; antigens (e.g., endogenous antigens; exogenous antigens; etc.); a ligand for a receptor; a receptor; a target of a non-antibody-based recognition scaffold; an Fc receptor (e.g., FcγRIIIa; FcγRIIIb; etc.); an extracellular matrix component; and the like.

[0081] Specific binding pairs include, e.g., antigen-antibody specific binding pairs, where the first member is an antibody (or antibody-based recognition scaffold) that binds specifically to the second member, which is an antigen, or where the first member is an antigen and the second member is an antibody (or antibody-based recognition scaffold) that binds specifically to the antigen; ligand-receptor specific binding pairs, where the first member is a ligand and the second member is a receptor to which the ligand binds, or where the first member is a receptor, and the second member is a ligand that binds to the receptor; non-antibody-based recognition scaffold-target specific binding pairs, where the first member is a non-antibody-based recognition scaffold and the second member is a target that binds to the non-antibody-based recognition scaffold, or where the first member is a target and the second member is a non-antibody-based recognition scaffold that binds to the target; adhesion molecule-extracellular matrix binding pairs; Fc receptor-Fc binding pairs, where the first member comprises an immunoglobulin Fc that binds to the second member, which is an Fc receptor, or where the first member is an Fc receptor that binds to the second member which comprises an immunoglobulin Fc; and receptor-co-receptor binding pairs, where the first member is a receptor that binds specifically to the second member which is

a co-receptor, or where the first member is a co-receptor that binds specifically to the second member which is a receptor.

Antigen-binding domain

[0082] An antigen-binding domain suitable for use in a conditionally active heterodimeric polypeptide of the present disclosure can be any antigen-binding polypeptide, a wide variety of which are known in the art. In some instances, the antigen-binding domain is a single chain Fv (scFv). Other antibody based recognition domains (cAb VHH (camelid antibody variable domains) and humanized versions, IgNAR VH (shark antibody variable domains) and humanized versions, sdAb VH (single domain antibody variable domains) and "camelized" antibody variable domains are suitable for use. In some instances, T-cell receptor (TCR) based recognition domains such as single chain TCR (scTv, single chain two-domain TCR containing VαVβ) are also suitable for use.

[0083] An antigen-binding domain suitable for use in a conditionally active heterodimeric polypeptide of the present disclosure can have a variety of antigen-binding specificities. In some cases, the antigen-binding domain is specific for an epitope present in an antigen that is expressed by (synthesized by) a cancer cell, i.e., a cancer cell associated antigen. The cancer cell associated antigen can be an antigen associated with, e.g., a breast cancer cell, a B cell lymphoma, a Hodgkin lymphoma cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma, a lung cancer cell (e.g., a small cell lung cancer cell), a non-Hodgkin B-cell lymphoma (B-NHL) cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma cell, a lung cancer cell (e.g., a small cell lung cancer cell), a melanoma cell, a chronic lymphocytic leukemia cell, an acute lymphocytic leukemia cell, a neuroblastoma cell, a glioma, a glioblastoma, a medulloblastoma, a colorectal cancer cell, etc. A cancer cell associated antigen may also be expressed by a non-cancerous cell.

[0084] Non-limiting examples of antigens to which an antigen-binding domain of a conditionally active heterodimeric polypeptide of the present disclosure can bind include, e.g., CD19, CD20, CD38, CD30, Her2/neu/ERBB2, CA125, MUC-1, prostate-specific membrane antigen (PSMA), CD44 surface adhesion molecule, mesothelin, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), EGFRvIII, vascular endothelial growth factor receptor-2 (VEGFR2), high molecular weight-melanoma associated antigen (HMW-MAA), MAGE-A1, IL-13R-a2, GD2, and the like.

[0085] In some instances, an antigen-binding domain of a conditionally active heterodimeric polypeptide of the instant disclosure may target a cancer-associated antigen. In some instances, an antigen-binding domain of the instant disclosure may include an antibody or portion thereof specific for a cancer associated antigen. Non-limiting examples of cancer associated antigens include but are not limited to e.g., CD19, CD20, CD38, CD30, Her2/neu, ERBB2, CA125, MUC-1, prostate-specific membrane antigen (PSMA), CD44 surface adhesion molecule, mesothelin, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), EGFRvIII, vascular endothelial growth factor receptor-2

(VEGFR2), high molecular weight-melanoma associated antigen (HMW-MAA), MAGE-A1, IL-13R-a2, GD2, and the like. Cancer-associated antigens also include, e.g., 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNTO888, CTLA-4, DRS, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin α5β1, integrin αvβ3, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, and vimentin.

[0086] In some instances, an antigen-binding domain may specifically bind a target comprising a fragment of a protein (e.g., a peptide) in conjunction with a major histocompatibility complex (MHC) molecule. As MHC molecules present peptide fragments of both intracellularly expressed and extracellularly expressed proteins, specific binding members directed to MHC-peptide complexes allows for the targeting of intracellular antigens as well as extracellularly expressed antigens.

[0087] Intracellularly expressed target proteins (e.g., cytoplasmically expressed (i.e., cytoplasmic proteins), nuclearly expressed (i.e., nuclear proteins), etc.) may be referred to as intracellular antigens (e.g., cytoplasmic antigens, nuclear antigens, etc.). Accordingly, antigen-binding domains of the subject disclosure may, in some instances, be specific for intracellular antigen fragments complexed with MHC, e.g., a peptide-MHC complex, also, in some instances, described as a human leukocyte antigen (HLA)-peptide complex.

[0088] Exemplary protein targets to which an antigen-binding domain targeting a peptide-MHC complex may be directed as well as exemplary peptides in the context of MHC for each protein target are provided in **Table 2** below.

Table 2: anti-peptide-MHC targets

Target	Exemplary Peptides	HLA	References
WT1	RMFPNAPYL (SEQ ID NO:470)	HLA-A2	Leukemia. (2015) 29(11):2238-47
KRAS and KRAS mutants (e.g., G12V & G12C)	KLVVVGAGGV (SEQ ID NO:471); KLVVVGAVGV (SEQ ID NO:742); KLVVVGACGV (SEQ ID NO:473); KLVVVGADGV (SEQ ID NO:744); VVGAVGVGK (SEQ ID NO:745); VVGACGVGK (SEQ ID NO:476); VVGAGGVGK (SEQ ID NO:477)	HLA-A2; HLA-A3	Proc Natl Acad Sci U S A. (2015) 112(32)
EGFP and EGFP	KITDFGLAK (SEQ ID NO:478);	HLA-A3	Proc Natl Acad Sci U S A. (2015)

mutants (e.g., L858R)	KITDFGRAK (SEQ ID NO:479);		112(32)
PR1	VLQELNVTV (SEQ ID NO:480)	HLA-A2	Cytotherapy. (2016) 18(8):985-94
MAGE-A1	EADPTGHSY (SEQ ID NO:481)	HLA-A1	Blood. (2011) 117(16):4262-4272
P53	LLGRNSFEV (SEQ ID NO:482); STTPPPGTRV (SEQ ID NO:483)	HLA-A2	Gene Ther. (2001) 8(21):1601-8
MART-1	ELAGIGILTV (SEQ ID NO:484)	HLA-A2	Biomark Med. (2010) 4(4):496-7
gp100	IMDQVPFSV (SEQ ID NO:485)	HLA-A2	Biomark Med. (2010) 4(4):496-7
CMV pp65	NLVPMVATV (SEQ ID NO:486)	HLA-A2	Biomark Med. (2010) 4(4):496-7
HIV Vpr	AIIRILQQL (SEQ ID NO:487)	HLA-A2	Biomark Med. (2010) 4(4):496-7
HA-1H	VLHDDLLEA (SEQ ID NO:488); VLRDDLLEA (SEQ ID NO:489)	HLA-A2	Biomark Med. (2010) 4(4):496-7
NY-ESO-1	SLLMWITQV (SEQ ID NO:490)	HLA-A2	Gene Ther. (2014) 21(6):575-84
EBNA3C	LLDFVRFMGV (SEQ ID NO:491)	HLA-A2	Proc Natl Acad Sci U S A. (2009) 106(14):5784-8
AFP	FMNKFIYEI (SEQ ID NO:492)	HLA-A2	Cancer Gene Ther. (2012) 19(2):84-100
Her2	KIFGSLAFL (SEQ ID NO:493)	HLA-A2	Clin Cancer Res. (2016) pii: clincanres 1203.2016
hCG-beta	GVLPALPQV (SEQ ID NO:494)	HLA-A2	J Natl Cancer Inst. (2013) 105(3):202-18
HBV Env183-91	FLLTRILTI (SEQ ID NO:495)	HLA-A2	J Immunol. (2006) 177(6):4187-95

[0089] In some instances, the antigen-binding domain of a conditionally active heterodimeric polypeptide of the instant disclosure is or includes a portion of an antibody (e.g., a scFv) that specifically binds a peptide-MHC having an intracellular cancer antigen peptide of **Table 2**.

[0090] In some instances, the antigen-binding domain of a conditionally active heterodimeric polypeptide of the instant disclosure is or includes a portion of an antibody (e.g., a scFv) that specifically binds a peptide-MHC described in Dhanik et al. *BMC Bioinformatics* (2016) 17:286, the disclosure of which is incorporated herein by reference in its entirety, including but not limited to e.g., a NLRP4 peptide (e.g., HLSPIDCEV (SEQ ID NO:496))-MHC complex, a UMODL1 peptide (e.g., LTSMWSPAV (SEQ ID NO:497))-MHC complex, a NLRP4 peptide (e.g., HLDHPHPAV (SEQ ID NO:498))-MHC complex, a MAGEC2 peptide (e.g., SLSVMSSNV (SEQ ID NO:499))-MHC complex, a NLRP4 peptide (e.g., MMAWSDNKI (SEQ ID NO:500))-MHC complex, a COX7B2 peptide (e.g., TQIGIEWNL (SEQ ID NO:501))-MHC complex, a NLRP4 peptide (e.g., CLFEMQDPA (SEQ ID NO:502))-MHC complex, a UMODL1 peptide (e.g., YLSHPSCNV (SEQ ID NO:503))-MHC complex, a COX7B2 peptide (e.g., GIEWNLSPV (SEQ ID NO:504))-MHC complex, a MAGEA11 peptide (e.g., GLGCSPASI (SEQ ID NO:505))-MHC complex, a RPE65 peptide (e.g., RQAFEFPQI (SEQ ID NO:506))-MHC complex, a RPE65 peptide (e.g., RQAFEFPQI (SEQ ID NO:507))-MHC complex, a NLRP4 peptide (e.g., GMWTDTFEF (SEQ ID NO:508))-MHC complex, a TRIM51 peptide (e.g., YLNWQDTAV (SEQ ID NO:509))-MHC complex, a MAGEA11 peptide (e.g., VLWGPITQI (SEQ ID NO:510))-MHC complex, a NLRP4 peptide (e.g., TLDHTGVVV (SEQ ID NO:511))-MHC complex, a RPE65 peptide (e.g., TMGVWLHIA (SEQ ID NO:512))-MHC complex, a RPE65 peptide (e.g., TMGVWLHIA (SEQ ID

NO:513))-MHC complex, a MAGEC2 peptide (e.g., KVWVQGHYL (SEQ ID NO:514))-MHC complex, a UMODL1 peptide (e.g., KINCNNFRL (SEQ ID NO:515))-MHC complex, etc.

Ligand

[0091] In some cases, a member of a specific binding pair suitable for use in a subject conditionally active heterodimeric polypeptide is a ligand for a receptor. Ligands include, but are not limited to, cytokines (e.g., IL-13, etc.); growth factors (e.g., heregulin; vascular endothelial growth factor (VEGF); and the like); an integrin-binding peptide (e.g., a peptide comprising the sequence Arg-Gly-Asp); and the like.

[0092] Where the member of a specific binding pair in a subject conditionally active heterodimeric polypeptide is a ligand, the conditionally active heterodimeric polypeptide can be activated in the presence of both a dimerizer agent and a second member of the specific binding pair, where the second member of the specific binding pair is a receptor for the ligand. For example, where the ligand is VEGF, the second member of the specific binding pair can be a VEGF receptor, including a soluble VEGF receptor. As another example, where the ligand is heregulin, the second member of the specific binding pair can be Her2.

Receptors

[0093] As noted above, in some cases, the member of a specific binding pair that is included in a subject conditionally active heterodimeric polypeptide is a receptor, e.g., a receptor for a ligand, a co-receptor, etc. The receptor can be a ligand-binding fragment of a receptor. Suitable receptors include, but are not limited to, a growth factor receptor (e.g., a VEGF receptor); a killer cell lectin-like receptor subfamily K, member 1 (NKG2D) polypeptide (receptor for MICA, MICB, and ULB6); a cytokine receptor (e.g., an IL-13 receptor; an IL-2 receptor; etc.); Her2; CD27; a natural cytotoxicity receptor (NCR) (e.g., NKP30 (NCR3/CD337) polypeptide (receptor for HLA-B–associated transcript 3 (BAT3) and B7-H6); etc.); etc.

Antibody-based recognition scaffolds

[0094] In some cases, the first member of the specific binding pair is an antibody. The antibody can be any antigen-binding antibody-based polypeptide, a wide variety of which are known in the art. In some instances, the antigen-binding domain is a single chain Fv (scFv). Other antibody based recognition domains (cAb VHH (camelid antibody variable domains) and humanized versions, IgNAR VH (shark antibody variable domains) and humanized versions, sdAb VH (single domain antibody variable domains) and "camelized" antibody variable domains are suitable for use. In some instances, T-cell receptor (TCR) based recognition domains such as single chain TCR (scTv, single chain two-domain TCR containing VαVβ) are also suitable for use.

[0095] Where the member of a specific binding pair in a conditionally active heterodimeric polypeptide of the present disclosure is an antibody-based recognition scaffold, the conditionally active heterodimeric polypeptide can be activated in the presence of a second member of the specific binding pair, where the

second member of the specific binding pair is an antigen that binds to the antibody-based recognition scaffold.

[0096] An antibody suitable for inclusion in a conditionally active heterodimeric polypeptide of the present disclosure can have a variety of antigen-binding specificities.

[0097] In some cases, the antigen-binding domain is specific for an epitope present in an antigen that is expressed by (synthesized by) a cancer cell, i.e., a cancer cell associated antigen. The cancer cell associated antigen can be an antigen associated with, e.g., a breast cancer cell, a B cell lymphoma, a pancreatic cancer, a Hodgkin lymphoma cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma, a lung cancer cell (e.g., a small cell lung cancer cell), a non-Hodgkin B-cell lymphoma (B-NHL) cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma cell, a lung cancer cell (e.g., a small cell lung cancer cell), a melanoma cell, a chronic lymphocytic leukemia cell, an acute lymphocytic leukemia cell, a neuroblastoma cell, a glioma, a glioblastoma, a medulloblastoma, a colorectal cancer cell, etc. A cancer cell associated antigen may also be expressed by a non-cancerous cell.

[0098] In some cases, the antigen-binding domain is specific for an epitope present in a tissue-specific antigen, including e.g., those antigens described herein. In some cases, the antigen-binding domain is specific for an epitope present in a disease-associated antigen, including e.g., those antigens described herein.

[0099] Non-limiting examples of antigens to which an antigen-binding domain of a subject conditionally active heterodimeric polypeptide can bind include, e.g., CD19, CD20, CD38, CD30, Her2/neu/ERBB2, CA125, MUC-1, prostate-specific membrane antigen (PSMA), CD44 surface adhesion molecule, mesothelin, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), EGFRvIII, vascular endothelial growth factor receptor-2 (VEGFR2), high molecular weight-melanoma associated antigen (HMW-MAA), MAGE-A1, IL-13R-a2, GD2, and the like.

[00100] Non-limiting examples of antigens to which an antigen-binding domain of a subject conditionally active heterodimeric polypeptide can bind include, e.g., Cadherins (CDH1-20), Integrins (alpha and beta isoforms), Ephrins, NCAMs, connexins, CD44, syndecan, CD47, DGalpha/beta, SV2, protocadherin, Fas, Dectin-1, CD7, CD40, Neuregulin, KIR, BTLA, Tim-2, Lag-3, CD19, CTLA4, CD28, TIGIT, and ICOS.

[00101] In some cases, the antibody is specific for a cytokine. In some cases, the antibody is specific for a cytokine receptor. In some cases, the antibody is specific for a growth factor. In some cases, the antibody is specific for a growth factor receptor. In some cases, the antibody is specific for a cell-surface receptor.

[00102] In some cases, the antibody is specific for a cell surface target, where non-limiting examples of cell surface targets include CD19, CD30, Her2, CD22, ENPP3, EGFR, CD20, CD52, CD 11a, and alpha-integrin.

[00103] In some cases, the antigen (second member of the specific binding pair) bound by the antibody-based scaffold is soluble. In some cases, the antigen is membrane-bound, e.g., in some cases, the antigen is present on the surface of a cell. In some cases, the antigen is immobilized on an insoluble support, where an insoluble support can comprise any of a variety of materials (e.g., polyethylene, polystyrene, polyvinylpyrrolidone, polycarbonate, nitrocellulose, and the like); and where an insoluble support can take a variety of forms, e.g., a plate, a tissue culture dish, a column, and the like. In some cases, the antigen is present in an extracellular matrix (ECM) (e.g., the antigen is an ECM component). In some cases, the antigen is present in an artificial matrix. In some cases, the antigen is present in an acellular environment.

Non-antibody-based recognition scaffolds

[00104] In some cases, the first member of the specific binding pair is a non-antibody-based recognition scaffold. Where the member of a specific binding pair in a conditionally active heterodimeric polypeptide of the present disclosure is a non-antibody-based recognition scaffold, the conditionally active heterodimeric polypeptide can be activated in the presence of a second member of the specific binding pair, where the second member of the specific binding pair is a target that binds to the non-antibody-based recognition scaffold.

[00105] Non-antibody-based recognition scaffolds include, e.g., affibodies; engineered Kunitz domains; monobodies (adnectins); anticalins; designed ankyrin repeat domains (DARPins); a binding site of a cysteine-rich polypeptide (e.g., cysteine-rich knottin peptides); avimers; afflins; and the like. See, e.g., Gebauer and Skerra (2009) *Curr. Opin. Chem. Biol.* 13:245.

[00106] Non-antibody-based scaffolds (also referred to herein as "antibody mimic molecules") may be identified by selection or isolation of a target-binding variant from a library of binding molecules having artificially diversified binding sites. Diversified libraries can be generated using completely random approaches (e.g., error-prone polymerase chain reaction (PCR), exon shuffling, or directed evolution) or aided by art-recognized design strategies. For example, amino acid positions that are usually involved when the binding site interacts with its cognate target molecule can be randomized by insertion of degenerate codons, trinucleotides, random peptides, or entire loops at corresponding positions within the nucleic acid which encodes the binding site (see e.g., U.S. Pub. No. 20040132028). The location of the amino acid positions can be identified by investigation of the crystal structure of the binding site in protein entity with the target molecule. Candidate positions for randomization include loops, flat surfaces, helices, and binding cavities of the binding site. In certain embodiments, amino acids within the

binding site that are likely candidates for diversification can be identified by their homology with the immunoglobulin fold. For example, residues within the CDR-like loops of fibronectin may be randomized to generate a library of fibronectin binding molecules (see, e.g., Koide et al., J. Mol. Biol., 284: 1141-1151 (1998)). Other portions of the binding site which may be randomized include flat surfaces. Following randomization, the diversified library may then be subjected to a selection or screening procedure to obtain binding molecules with the desired binding characteristics. For example, selection can be achieved by art-recognized methods such as phage display, yeast display, or ribosome display.

[00107] For example, in some cases, the non-antibody-based scaffold comprises a binding site from a fibronectin binding molecule. Fibronectin binding molecules (e.g., molecules comprising the Fibronectin type I, II, or III domains) display CDR-like loops which, in contrast to immunoglobulins, do not rely on intra-chain disulfide bonds. The FnIII loops comprise regions that may be subjected to random mutation and directed evolutionary schemes of iterative rounds of target binding, selection, and further mutation in order to develop useful therapeutic tools. Fibronectin-based "addressable" therapeutic binding molecules ("FATBIM") can be developed to specifically bind the target antigen or epitope. Methods for making fibronectin binding polypeptides are described, for example, in WO 01/64942 and in U.S. Pat. Nos. 6,673,901, 6,703,199, 7,078,490, and 7,119,171.

[00108] As another example, in some cases, the non-antibody-based scaffold comprises a binding site from an affibody. Affibodies are derived from the immunoglobulin binding domains of staphylococcal Protein A (SPA) (see e.g., Nord et al., Nat. Biotechnol., 15: 772-777 (1997)). An affibody is an antibody mimic that has unique binding sites that bind specific targets. Affibodies can be small (e.g., consisting of three alpha helices with 58 amino acids and having a molar mass of about 6 kDa), have an inert format (no Fc function), and have been successfully tested in humans as targeting moieties. Affibody binding sites can be synthesized by mutagenizing an SPA-related protein (e.g., Protein Z) derived from a domain of SPA (e.g., domain B) and selecting for mutant SPA-related polypeptides having binding affinity for a target antigen or epitope. Other methods for making affibody binding sites are described in U.S. Pat. Nos. 6,740,734 and 6,602,977 and in WO 00/63243.

[00109] As another example, in some cases, the non-antibody-based scaffold comprises a binding site from an anticalin. An anticalin is an antibody functional mimetic derived from a human lipocalin. Lipocalins are a family of naturally-occurring binding proteins that bind and transport small hydrophobic molecules such as steroids, bilins, retinoids, and lipids. The main structure of an anticalin is similar to wild type lipocalins. The central element of this protein architecture is a beta-barrel structure of eight antiparallel strands, which supports four loops at its open end. These loops form the natural binding site of the lipocalins and can be reshaped in vitro by extensive amino acid replacement, thus creating novel binding specificities. Anticalins possess high affinity and specificity for their ligands as well as fast binding

kinetics, so that their functional properties are similar to those of antibodies. Anticalins are described in, e.g., U.S. Pat. No. 7,723,476.

[00110] As another example, in some cases, the non-antibody-based scaffold comprises a binding site from a cysteine-rich polypeptide. Cysteine-rich domains in some cases do not form an alpha-helix, a beta-sheet, or a beta-barrel structure. In some cases, the disulfide bonds promote folding of the domain into a three-dimensional structure. In some cases, cysteine-rich domains have at least two disulfide bonds, e.g., at least three disulfide bonds. An exemplary cysteine-rich polypeptide is an A domain protein. A-domains (sometimes called "complement-type repeats") contain about 30-50 or 30-65 amino acids. In some cases, the domains comprise about 35-45 amino acids and in some cases about 40 amino acids. Within the 30-50 amino acids, there are about 6 cysteine residues. Of the six cysteines, disulfide bonds typically are found between the following cysteines: C1 and C3, C2 and C5, C4 and C6. The A domain constitutes a ligand binding moiety. The cysteine residues of the domain are disulfide linked to form a compact, stable, functionally independent moiety. Clusters of these repeats make up a ligand binding domain, and differential clustering can impart specificity with respect to the ligand binding. Exemplary proteins containing A-domains include, e.g., complement components (e.g., C6, C7, C8, C9, and Factor I), serine proteases (e.g., enteropeptidase, matriptase, and corin), transmembrane proteins (e.g., ST7, LRP3, LRP5 and LRP6) and endocytic receptors (e.g. Sortilin-related receptor, LDL-receptor, VLDLR, LRP1, LRP2, and ApoER2). Methods for making A-domain proteins of a desired binding specificity are disclosed, for example, in WO 02/088171 and WO 04/044011.

[00111] As another example, in some cases, the non-antibody-based scaffold comprises a binding site from a repeat protein. Repeat proteins are proteins that contain consecutive copies of small (e.g., about 20 to about 40 amino acid residues) structural units or repeats that stack together to form contiguous domains. Repeat proteins can be modified to suit a particular target binding site by adjusting the number of repeats in the protein. Exemplary repeat proteins include designed ankyrin repeat proteins (i.e., a DARPins) (see e.g., Binz et al., Nat. Biotechnol., 22: 575-582 (2004)) or leucine-rich repeat proteins (i.e., LRRPs) (see e.g., Pancer et al., Nature, 430: 174-180 (2004)). As another example, in some cases, the non-antibody-based scaffold comprises a DARPin.

[00112] As used herein, the term "DARPin" refers to a genetically engineered antibody mimetic protein that typically exhibits highly specific and high-affinity target protein binding. DARPins were first derived from natural ankyrin proteins. In some cases, DARPins comprise three, four or five repeat motifs of an ankyrin protein. In some cases, a unit of an ankyrin repeat consists of 30-34 amino acid residues and functions to mediate protein-protein interactions. In some cases, each ankyrin repeat exhibits a helix-turn-helix conformation, and strings of such tandem repeats are packed in a nearly linear array to form helix-turn-helix bundles connected by relatively flexible loops. In some cases, the global structure of an ankyrin repeat protein is stabilized by intra- and inter-repeat hydrophobic and hydrogen bonding

interactions. The repetitive and elongated nature of the ankyrin repeats provides the molecular bases for the unique characteristics of ankyrin repeat proteins in protein stability, folding and unfolding, and binding specificity. The molecular mass of a DARPin domain can be from about 14 or 18 kDa for four- or five-repeat DARPins, respectively. DARPins are described in, e.g., U.S. Pat. No. 7,417,130. In some cases, tertiary structures of ankyrin repeat units share a characteristic composed of a beta-hairpin followed by two antiparallel alpha-helices and ending with a loop connecting the repeat unit with the next one. Domains built of ankyrin repeat units can be formed by stacking the repeat units to an extended and curved structure. LRRP binding sites from part of the adaptive immune system of sea lampreys and other jawless fishes and resemble antibodies in that they are formed by recombination of a suite of leucine-rich repeat genes during lymphocyte maturation. Methods for making DARpin or LRRP binding sites are described in WO 02/20565 and WO 06/083275.

[00113] As another example, in some cases, the non-antibody-based scaffold comprises a binding site derived from Src homology domains (e.g. SH2 or SH3 domains), PDZ domains, beta-lactamase, high affinity protease inhibitors, or small disulfide binding protein scaffolds such as scorpion toxins. Methods for making binding sites derived from these molecules have been disclosed in the art, see e.g., Panni et al., J. Biol. Chem., 277: 21666-21674 (2002), Schneider et al., Nat. Biotechnol., 17: 170-175 (1999); Legendre et al., Protein Sci., 11:1506-1518 (2002); Stoop et al., Nat. Biotechnol., 21: 1063-1068 (2003); and Vita et al., PNAS, 92: 6404-6408 (1995). Yet other binding sites may be derived from a binding domain selected from the group consisting of an EGF-like domain, a Kringle-domain, a PAN domain, a Gla domain, a SRCR domain, a Kunitz/Bovine pancreatic trypsin Inhibitor domain, a Kazal-type serine protease inhibitor domain, a Trefoil (P-type) domain, a von Willebrand factor type C domain, an Anaphylatoxin-like domain, a CUB domain, a thyroglobulin type I repeat, LDL-receptor class A domain, a Sushi domain, a Link domain, a Thrombospondin type I domain, an Immunoglobulin-like domain, a C-type lectin domain, a MAM domain, a von Willebrand factor type A domain, a Somatomedin B domain, a WAP-type four disulfide core domain, a F5/8 type C domain, a Hemopexin domain, a Laminin-type EGF-like domain, a C2 domain, a binding domain derived from tetranectin in its monomeric or trimeric form, and other such domains known to those of ordinary skill in the art, as well as derivatives and/or variants thereof. Exemplary non-antibody-based scaffolds, and methods of making the same, can also be found in Stemmer et al., "Protein scaffolds and uses thereof", U.S. Patent Publication No. 20060234299 (Oct. 19, 2006) and Hey, et al., Artificial, Non-Antibody Binding Proteins for Pharmaceutical and Industrial Applications, TRENDS in Biotechnology, vol. 23, No. 10, Table 2 and pp. 514-522 (October 2005).

[00114] As another example, in some cases, the non-antibody-based scaffold comprises a Kunitz domain. The term "Kunitz domains" as used herein, refers to conserved protein domains that inhibit certain proteases, e.g., serine proteases. Kunitz domains are relatively small, typically being about 50 to 60

amino acids long and having a molecular weight of about 6 kDa. Kunitz domains typically carry a basic charge and are characterized by the placement of two, four, six or eight or more that form disulfide linkages that contribute to the compact and stable nature of the folded peptide. For example, many Kunitz domains have six conserved cysteine residues that form three disulfide linkages. The disulfide-rich α/β fold of a Kunitz domain can include two, three (typically), or four or more disulfide bonds.

[00115] Kunitz domains have a pear-shaped structure that is stabilized the, e.g., three disulfide bonds, and that contains a reactive site region featuring the principal determinant P1 residue in a rigid confirmation. These inhibitors competitively prevent access of a target protein (e.g., a serine protease) for its physiologically relevant macromolecular substrate through insertion of the P1 residue into the active site cleft. The P1 residue in the proteinase-inhibitory loop provides the primary specificity determinant and dictates much of the inhibitory activity that particular Kunitz protein has toward a targeted proteinase. In general, the N-terminal side of the reactive site (P) is energetically more important that the P' C-terminal side. In most cases, lysine or arginine occupy the P1 position to inhibit proteinases that cleave adjacent to those residues in the protein substrate. Other residues, particularly in the inhibitor loop region, contribute to the strength of binding. Generally, about 10-12 amino acid residues in the target protein and 20-25 residues in the proteinase are in direct contact in the formation of a stable proteinase-inhibitor protein entity and provide a buried area of about 600 to 900 A. By modifying the residues in the P site and surrounding residues Kunitz domains can be designed to target a protein of choice. Kunitz domains are described in, e.g., U.S. Pat. No. 6,057,287.

[00116] As another example, in some cases, the non-antibody-based scaffold is an affilin. Affilins are small antibody-mimic proteins which are designed for specific affinities towards proteins and small compounds. New affilins can be very quickly selected from two libraries, each of which is based on a different human derived scaffold protein. Affilins do not show any structural homology to immunoglobulin proteins. There are two commonly-used affilin scaffolds, one of which is gamma crystalline, a human structural eye lens protein and the other is "ubiquitin" superfamily proteins. Both human scaffolds are very small, show high temperature stability and are almost resistant to pH changes and denaturing agents. This high stability is mainly due to the expanded beta sheet structure of the proteins. Examples of gamma crystalline derived proteins are described in WO200104144 and examples of "ubiquitin-like" proteins are described in WO2004106368.

[00117] As another example, in some cases, the non-antibody-based scaffold is an Avimer. Avimers are evolved from a large family of human extracellular receptor domains by in vitro exon shuffling and phage display, generating multidomain proteins with binding and inhibitory properties. Linking multiple independent binding domains has been shown to create avidity and results in improved affinity and specificity compared with conventional single-epitope binding proteins. In certain embodiments, Avimers consist of two or more peptide sequences of 30 to 35 amino acids each, connected by spacer

region peptides. The individual sequences are derived from A domains of various membrane receptors and have a rigid structure, stabilized by disulfide bonds and calcium. Each A domain can bind to a certain epitope of the target protein. The combination of domains binding to different epitopes of the same protein increases affinity to this protein, an effect known as avidity (hence the name). Avimers with sub-nanomolar affinities have been obtained against a variety of targets. Alternatively, the domains can be directed against epitopes on different target proteins. Additional information regarding avimers can be found in U.S. patent application Publication Nos. 2006/0286603, 2006/0234299, 2006/0223114, 2006/0177831, 2006/0008844, 2005/0221384, 2005/0164301, 2005/0089932, 2005/0053973, 2005/0048512, 2004/0175756.

[00118] Suitable targets of a non-antibody-based scaffold include any of the above-mentioned antigens to which an antibody-based scaffold can bind.

[00119] In some cases, the target (second member of the specific binding pair) bound by the non-antibody-based scaffold is soluble. In some cases, the target is membrane-bound, e.g., in some cases, the target is present on the surface of a cell. In some cases, the target is immobilized on an insoluble support, where an insoluble support can comprise any of a variety of materials (e.g., polyethylene, polystyrene, polyvinylpyrrolidone, polycarbonate, nitrocellulose, and the like); and where an insoluble support can take a variety of forms, e.g., a plate, a tissue culture dish, a column, and the like. In some cases, the target is present in an extracellular matrix (ECM) (e.g., the antigen is an ECM component). In some cases, the target is present in an artificial matrix. In some cases, the target is present in an acellular environment.

Cell adhesion molecules

[00120] In some cases, the first member of the specific binding pair is a cell adhesion molecule (CAM), i.e., a polypeptide that binds a component of an extracellular matrix (ECM) or that binds a cell surface molecule. For example, in some cases, the first member of the specific binding pair is the extracellular region of a CAM. In some cases, the CAM is a calcium-independent adhesion molecule; for example, in some cases, the CAM is an immunoglobulin superfamily CAM. In some cases, the CAM is a calcium-dependent adhesion molecule; e.g., the CAM is an integrin, a cadherin, or a selectin. In some cases, the first member of the specific binding pair is an integrin. In some cases, the first member of the specific binding pair is a cadherin, e.g., an E-cadherin, a P-cadherin, an N-cadherin, an R-cadherin, an M-cadherin, etc. In some cases, the first member of the specific binding pair is a selectin, e.g., an E-selectin, an L-selectin, or a P-selectin. Binding fragments of a CAM can be used as the first member of the specific binding pair.

[00121] Where the first member of the specific binding pair is a CAM, the second member of the specific binding pair is a component of ECM or a cell surface molecule that binds the CAM. For example, where the first member of the specific binding pair is an integrin, the second member of the specific binding pair is a component of collagen, fibrinogen, fibronectin, or vitronectin. As another example, where the

first member of the specific binding pair is cadherin, the second member of the specific binding pair is cell surface antigen bound by the cadherin. As another example, where the first member of the specific binding pair is a selectin, the second member of the specific binding pair is a fucosylated carbohydrate.

Ligands

[00122] In some cases, the first member of the specific binding pair is a ligand for a receptor. Ligands include polypeptides, nucleic acids, glycoproteins, small molecules, carbohydrates, lipids, glycolipids, lipoproteins, lipopolysaccharides, etc. In some cases, the ligand is soluble.

[00123] Ligands include, but are not limited to, cytokines (e.g., IL-13, etc.); growth factors (e.g., heregulin; vascular endothelial growth factor (VEGF); and the like); peptide hormones; an integrin-binding peptide (e.g., a peptide comprising the sequence Arg-Gly-Asp); an N-glycan; and the like.

[00124] Where the member of a specific binding pair in a conditionally active heterodimeric polypeptide of the present disclosure is a ligand, the conditionally active heterodimeric polypeptide can be activated in the presence of a second member of the specific binding pair, where the second member of the specific binding pair is a receptor for the ligand. For example, where the ligand is VEGF, the second member of the specific binding pair can be a VEGF receptor, including a soluble VEGF receptor. Alternatively, the first member of the specific binding pair can be a VEGF receptor; and the first member of the specific binding pair can be VEGF. As another example, where the ligand is heregulin, the second member of the specific binding pair can be Her2.

[00125] Where the first member of the specific binding pair is a ligand, the second member of the specific binding pair is a molecule that binds the ligand, e.g., the second member of the specific binding pair is an antibody that specifically binds the ligand, a receptor for the ligand, etc.

[00126] Where the first member of the specific binding pair is a ligand, in some cases, the second member of the specific binding pair (the molecule that binds the ligand) is soluble. In some cases, the second member of the specific binding pair is membrane-bound, e.g., in some cases, the second member of the specific binding pair is present on the surface of a cell. In some cases, the second member of the specific binding pair is immobilized on an insoluble support, where an insoluble support can comprise any of a variety of materials (e.g., polyethylene, polystyrene, polyvinylpyrrolidone, polycarbonate, nitrocellulose, and the like); and where an insoluble support can take a variety of forms, e.g., a plate, a tissue culture dish, a column, and the like. In some cases, the second member of the specific binding pair is present in an acellular environment.

Antigens

[00127] In some cases, the first member of the specific binding pair is an antigen to which an antibody specifically binds. The antigen can be any antigen, e.g., a naturally-occurring (endogenous) antigen; a

synthetic (e.g., modified in such a way that it is no longer the same as a naturally-occurring antigen; modified from its natural state; etc.) antigen; etc.

[00128] Where the member of a specific binding pair in a conditionally active heterodimeric polypeptide of the present disclosure is an antigen, the conditionally active heterodimeric polypeptide can be activated in the presence of a second member of the specific binding pair, where the second member of the specific binding pair is an antibody (antibody-based recognition scaffold) that binds to the antigen.

[00129] In some cases, the antigen is a disease-associated antigen, e.g., a cancer-associated antigen, an autoimmune disease-associated antigen, a pathogen-associated antigen, an inflammation-associated antigen, or the like.

[00130] For example, where the second member of the specific binding pair is an antibody specific for a cancer-associated antigen, the antigen can be a cancer-associated antigen, where cancer-associated antigens include, e.g., CD19, CD20, CD38, CD30, Her2/neu/ERBB2, CA125, MUC-1, prostate-specific membrane antigen (PSMA), CD44 surface adhesion molecule, mesothelin, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), EGFRvIII, vascular endothelial growth factor receptor-2 (VEGFR2), high molecular weight-melanoma associated antigen (HMW-MAA), MAGE-A1, IL-13R-a2, GD2, and the like. Cancer-associated antigens also include, e.g., 4-1BB, 5T4, adenocarcinoma antigen, alpha-fetoprotein, BAFF, B-lymphoma cell, C242 antigen, CA-125, carbonic anhydrase 9 (CA-IX), C-MET, CCR4, CD152, CD19, CD20, CD200, CD22, CD221, CD23 (IgE receptor), CD28, CD30 (TNFRSF8), CD33, CD4, CD40, CD44 v6, CD51, CD52, CD56, CD74, CD80, CEA, CNTO888, CTLA-4, DRS, EGFR, EpCAM, CD3, FAP, fibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptor kinase, IGF-1 receptor, IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factor I receptor, integrin $\alpha 5\beta 1$, integrin $\alpha v\beta 3$, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, VEGF-A, VEGFR-1, VEGFR2, and vimentin.

[00131] The antigen can be associated with an inflammatory disease. Non-limiting examples of antigens associated with inflammatory disease include, e.g., AOC3 (VAP-1), CAM-3001, CCL11 (eotaxin-1), CD125, CD147 (basigin), CD154 (CD40L), CD2, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD3, CD4, CD5, IFN-α, IFN-γ, IgE, IgE Fc region, IL-1, IL-12, IL-23, IL-13, IL-17, IL-17A, IL-22, IL-4, IL-5, IL-5, IL-6, IL-6 receptor, integrin $\alpha 4$, integrin $\alpha 4\beta 7$, LFA-1 (CD11a), myostatin, OX-40, scleroscin, SOST, TGF beta 1, TNF-α, and VEGF-A.

[00132] Where the first member of the specific binding pair is an antigen, the second member of the specific binding pair can be an antibody-based scaffold (e.g., an antibody) or a non-antibody-based scaffold. In

some cases, the second member of the specific binding pair is present on the surface of a cell. In some cases, the second member of the specific binding pair is immobilized on an insoluble support. In some cases, the second member of the specific binding pair is soluble. In some cases, the second member of the specific binding pair is present in an extracellular environment (e.g., extracellular matrix). In some cases, the second member of the specific binding pair is present in an artificial matrix. In some cases, the second member of the specific binding pair is present in an acellular environment.

Targets of non-antibody-based recognition scaffolds

[00133] In some cases, the first member of the specific binding pair is a target of a non-antibody-based scaffold. Targets include, e.g., polypeptides, nucleic acids, glycoproteins, small molecules, carbohydrates, lipids, glycolipids, lipoproteins, lipopolysaccharides, etc.

[00134] Where the first member of the specific binding pair is a target of a non-antibody-based scaffold, the second member of the specific binding pair is a non-antibody-based scaffold.

Receptors

[00135] In some cases, the first member of the specific binding pair is a receptor. In some cases, the receptor is a growth factor receptor. In some cases, the receptor is a cytokine receptor. In some cases, the receptor is a cell surface receptor that binds to a co-receptor on a cell. In some cases, the receptor is a neurotransmitter receptor. In some cases, the receptor binds to an extracellular matrix component. In some cases, the receptor is an immunoglobulin Fc receptor.

[00136] Suitable receptors include, but are not limited to, a growth factor receptor (e.g., a VEGF receptor); a killer cell lectin-like receptor subfamily K, member 1 (NKG2D) polypeptide (receptor for MICA, MICB, and ULB6); a cytokine receptor (e.g., an IL-13 receptor; an IL-2 receptor; etc.); an epidermal growth factor (EGF) receptor; Her2; CD27; a natural cytotoxicity receptor (NCR) (e.g., NKP30 (NCR3/CD337) polypeptide (receptor for HLA-B–associated transcript 3 (BAT3) and B7-H6); etc.); a T cell antigen receptor; a dihydrofolate receptor; a chimeric cytokine receptor; an Fc receptor; an extracellular matrix receptor (e.g. an integrin); a cell adhesion receptor (e.g. a cadherin); an immunoregulatory receptor including both positive co-receptors (e.g. CD28) and negative (immunosuppressive) co-receptors (e.g., PD1); a cytokine receptor; and a receptor for a immunoregulatory molecule (e.g. TGFβ), etc. In some cases, the receptor is truncated, relative to the wild-type receptor.

[00137] Where the first member of the specific binding pair is a receptor, the second member of the specific binding pair is target of the receptor, where the target can be a ligand for the receptor, or a co-receptor. In some cases, the second member of the specific binding pair is present on the surface of a cell. In some cases, the second member of the specific binding pair is immobilized on an insoluble support. In some cases, the second member of the specific binding pair is soluble. In some cases, the second member of the specific binding pair is present in an extracellular environment (e.g., extracellular matrix). In some

cases, the second member of the specific binding pair is present in an artificial matrix. In some cases, the second member of the specific binding pair is present in an acellular environment.

Hinge region

[00138] In some cases, the first polypeptide of a subject conditionally active heterodimeric polypeptide comprises a hinge region (also referred to herein as a "spacer"), where the hinge region is interposed between the antigen-binding domain and the transmembrane domain. In some cases, the hinge region is an immunoglobulin heavy chain hinge region. In some cases, the hinge region is a hinge region polypeptide derived from a receptor (e.g., a CD8-derived hinge region).

[00139] The hinge region can have a length of from about 4 amino acids to about 50 amino acids, e.g., from about 4 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 40 aa, or from about 40 aa to about 50 aa.

[00140] Suitable spacers can be readily selected and can be of any of a number of suitable lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and can be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[00141] Exemplary spacers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO:516) and $(GGGS)_n$ (SEQ ID NO:517), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary spacers can comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO:518), GGSGG (SEQ ID NO:519), GSGSG (SEQ ID NO:520), GSGGG (SEQ ID NO:521), GGGSG (SEQ ID NO:522), GSSSG (SEQ ID NO:523), and the like.

[00142] In some cases, the hinge region in the first polypeptide of a subject conditionally active heterodimeric polypeptide includes at least one cysteine. For example, in some cases, the hinge region can include the sequence Cys-Pro-Pro-Cys. If present, a cysteine in the hinge region of a first conditionally active heterodimeric polypeptide can be available to form a disulfide bond with a hinge region in a second conditionally active heterodimeric polypeptide.

[00143] Immunoglobulin hinge region amino acid sequences are known in the art; see, e.g., Tan et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:162; and Huck et al. (1986) *Nucl. Acids Res.* 14:1779. As non-limiting examples, an immunoglobulin hinge region can include one of the following amino acid sequences:

DKTHT (SEQ ID NO:524); CPPC (SEQ ID NO:525); CPEPKSCDTPPPCPR (SEQ ID NO:526) (see, e.g., Glaser et al. (2005) *J. Biol. Chem.* 280:41494); ELKTPLGDTTHT (SEQ ID NO:527); KSCDKTHTCP (SEQ ID NO:528); KCCVDCP (SEQ ID NO:529); KYGPPCP (SEQ ID NO:530); EPKSCDKTHTCPPCP (SEQ ID NO:531) (human IgG1 hinge); ERKCCVECPPCP (SEQ ID NO:532) (human IgG2 hinge); ELKTPLGDTTHTCPRCP (SEQ ID NO:533) (human IgG3 hinge); SPNMVPHAHHAQ (SEQ ID NO:534) (human IgG4 hinge); and the like.

[00144] The hinge region can comprise an amino acid sequence of a human IgG1, IgG2, IgG3, or IgG4, hinge region. The hinge region can include one or more amino acid substitutions and/or insertions and/or deletions compared to a wild-type (naturally-occurring) hinge region. For example, His_{229} of human IgG1 hinge can be substituted with Tyr, so that the hinge region comprises the sequence EPKSCDKTYTCPPCP (SEQ ID NO:535); see, e.g., Yan et al. (2012) *J. Biol. Chem.* 287:5891.

[00145] The hinge region can comprise an amino acid sequence derived from human CD8; e.g., the hinge region can comprise the amino acid sequence: TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:536), or a variant thereof.

Transmembrane domain

[00146] The first and the second polypeptides of a CAR of the present disclosure include transmembrane domains for insertion into a eukaryotic cell membrane. The transmembrane domain of the first polypeptide is interposed between the antigen-binding domain and the co-stimulatory domain. Where the first polypeptide includes a hinge region, the transmembrane domain is interposed between the hinge region and the co-stimulatory domain, such that the first polypeptide comprises, in order from the amino terminus (N-terminus) to the carboxyl terminus (C-terminus): an antigen-binding domain; a hinge region; a transmembrane domain; a first co-stimulatory domain; and a first member of a dimerizer-binding pair.

[00147] The transmembrane domain of the second polypeptide is at or near the N-terminus of the polypeptide, such that the second polypeptide comprises, in order from N-terminus to C-terminus: a transmembrane domain; a second co-stimulatory domain; a second member of the dimerizer-binding pair; and an intracellular signaling domain.

[00148] Any transmembrane (TM) domain that provides for insertion of a polypeptide into the cell membrane of a eukaryotic (e.g., mammalian) cell is suitable for use. As one non-limiting example, the TM sequence IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:537) can be used. Additional non-limiting examples of suitable TM sequences include: a) CD8 beta derived : LGLLVAGVLVLLVSLGVAIHLCC (SEQ ID NO:538); b) CD4 derived: ALIVLGGVAGLLLFIGLGIFFCVRC (SEQ ID NO:539); c) CD3 zeta derived: LCYLLDGILFIYGVILTALFLRV (SEQ ID NO:540); d) CD28 derived: WVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:541); e) CD134 (OX40) derived:

VAAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO:542); and f) CD7 derived: ALPAALAVISFLLGLGLGVACVLA (SEQ ID NO:543).

Linkers

[00149] In some cases, a first polypeptide of a subject CAR includes a linker between any two adjacent domains. For example, a linker can be disposed between the transmembrane domain and the first co-stimulatory domain of the first polypeptide. As another example, a linker can be disposed between the first co-stimulatory domain and the first member of a dimerizer-binding pair of the first polypeptide. As another example, a linker can be disposed between the transmembrane domain and the second co-stimulatory domain of the second polypeptide. As another example, a linker can be disposed between the second co-stimulatory domain and the second member of the dimerizer-binding pair of the second polypeptide. As another example, a linker can be disposed between the second member of the dimerizer-binding pair and the intracellular signaling domain of the second polypeptide.

[00150] The linker peptide may have any of a variety of amino acid sequences. Proteins can be joined by a spacer peptide, generally of a flexible nature, although other chemical linkages are not excluded. A linker can be a peptide of between about 6 and about 40 amino acids in length, or between about 6 and about 25 amino acids in length. These linkers can be produced by using synthetic, linker-encoding oligonucleotides to couple the proteins. Peptide linkers with a degree of flexibility can be used. The linking peptides may have virtually any amino acid sequence, bearing in mind that suitable linkers will have a sequence that results in a generally flexible peptide. The use of small amino acids, such as glycine and alanine, are of use in creating a flexible peptide. The creation of such sequences is routine to those of skill in the art.

[00151] Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[00152] Exemplary flexible linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $GSGGS_n$ (SEQ ID NO:516) and $GGGS_n$ (SEQ ID NO:517), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are of interest since both of these amino acids are relatively unstructured, and therefore may serve as a neutral tether between components. Glycine polymers are of particular interest since glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary flexible linkers include, but are not limited GGSG (SEQ ID NO:518), GGSGG (SEQ ID NO:519), GSGSG (SEQ ID NO:520), GSGGG (SEQ ID NO:521), GGGSG (SEQ ID NO:522),

GSSSG (SEQ ID NO:523), and the like. The ordinarily skilled artisan will recognize that design of a peptide conjugated to any elements described above can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

Modulatory domains

[00153] Modulatory domains suitable for use in a CAR of the present disclosure include co-stimulatory domains. Modulatory domains may be present or absent in a subject conditionally acitive heterodimeric polypeptide of the present disclosure. As such, a particular conditionally acitive heterodimeric polypeptide of the present disclosure (or one or more polypeptide chains of a multichain conditionally acitive heterodimeric polypeptide of the present disclosure) may include anywhere from 0 to 6 or more modulatory domains. For example, in some instances, the polypeptide chains of a conditionally acitive heterodimeric polypeptide of the present disclosure may collectively include 0, 1, 2, 3, 4, 5, 6 or more modulatory domains. In some instances, a polypeptide chain of a conditionally acitive heterodimeric polypeptide of the present disclosure may individually include 0, 1, 2, 3, 4, 5, 6 or more modulatory domains. A particular modulatory domain may be present in one polypeptide chain of a conditionally acitive heterodimeric polypeptide but absent from another. In some instances, a particular modulatory domain may be present in both chains of a two chain conditionally acitive heterodimeric polypeptide. Furthermore, position of a modulatory domains on or within a polypeptide may vary greatly as a particular modulatory domain may be positioned at an end (e.g., the N-termal or C-terminal end) of the polypeptide or essentially any appropriate position within the polypeptide, e.g., adjacent to a transmembrane domain, adjacent to an intracellular signaling domain, adjacent to one or more other modulatory domains, etc.

[00154] In some cases, the modulatory domain on the first polypeptide of a subject CAR has substantially the same amino acid sequence as the modulatory domain on the second polypeptide of the CAR. For example, in some cases, the modulatory domain on the first polypeptide of a CAR comprises an amino acid sequence that is at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, identical to the amino acid sequence of the modulatory domain on the second polypeptide of the CAR. The modulatory domain of the first polypeptide of a subject CAR can have substantially the same length as the modulatory domain of the second polypeptide of a subject CAR; e.g., the first and second modulatory domains can differ in length from one another by fewer than 10 amino acids, or fewer than 5 amino acids. In some cases, the first and second modulatory domains have the same length.

[00155] A modulatory domain suitable for inclusion in the first and the second polypeptide of a subject CAR can have a length of from about 30 amino acids to about 70 amino acids (aa), e.g., a modulatory domain can have a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to

about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa. In other cases, modulatory domain can have a length of from about 70 aa to about 100 aa, from about 100 aa to about 200 aa, or greater than 200 aa.

[00156] Co-stimulatory domains suitable for use in a CAR of the present disclosure are generally polypeptides derived from receptors. In some embodiments, co-stimulatory domains homodimerize. A subject co-stimulatory domain can be an intracellular portion of a transmembrane protein (i.e., the co-stimulatory domain can be derived from a transmembrane protein). Non-limiting examples of suitable co-stimulatory polypeptides include, but are not limited to, 4-1BB (CD137), CD28, ICOS, OX-40, BTLA, CD27, CD30, GITR, and HVEM.

[00157] In some cases, the co-stimulatory domain on the first polypeptide of a subject CAR has substantially the same amino acid sequence as the co-stimulatory domain on the second polypeptide of the CAR. For example, in some cases, the co-stimulatory domain on the first polypeptide of a CAR comprises an amino acid sequence that is at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, identical to the amino acid sequence of the co-stimulatory domain on the second polypeptide of the CAR. The co-stimulatory domain of the first polypeptide of a subject CAR can have substantially the same length as the co-stimulatory domain of the second polypeptide of a subject CAR; e.g., the first and second co-stimulatory domains can differ in length from one another by fewer than 10 amino acids, or fewer than 5 amino acids. In some cases, the first and second co-stimulatory domains have the same length.

[00158] A co-stimulatory domain suitable for inclusion in the first and the second polypeptide of a subject CAR can have a length of from about 30 amino acids to about 70 amino acids (aa), e.g., a co-stimulatory domain can have a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa. In other cases, the co-stimulatory domain can have a length of from about 70 aa to about 100 aa, from about 100 aa to about 200 aa, or greater than 200 aa.

[00159] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein 4-1BB (also known as TNFRSF9; CD137; 4-1BB; CDw137; ILA; etc.). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:544). In some of these embodiments, the co-stimulatory domain of both the first and the second polypeptide has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from

about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00160] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein CD28 (also known as Tp44). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:545). In some of these embodiments, the co-stimulatory domain of both the first and the second polypeptide has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00161] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein ICOS (also known as AILIM, CD278, and CVID1). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: TKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL (SEQ ID NO:546). In some of these embodiments, the co-stimulatory domain of both the first and the second polypeptide has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00162] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein OX-40 (also known as TNFRSF4, RP5-902P8.3, ACT35, CD134, OX40, TXGP1L). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO:547). In some of these embodiments, the co-stimulatory domain of both the first and the second polypeptide has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00163] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein BTLA (also known as BTLA1 and CD272). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%,

at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: CCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCFRMQEG SEVYSNPCLEENKPGIVYASLNHSVIGPNSRLARNVKEAPTEYASICVRS (SEQ ID NO:548).

[00164] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein CD27 (also known as S152, T14, TNFRSF7, and Tp55). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: HQRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO:549). In some of these embodiments, the co-stimulatory domain of both the first and the second polypeptide has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00165] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein CD30 (also known as TNFRSF8, D1S166E, and Ki-1). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, from about 150 aa to about 160 aa, or from about 160 aa to about 185 aa of the following amino acid sequence: RRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETC HSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEG RGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK (SEQ ID NO:550).

[00166] In some cases, the co-stimulatory domain is derived from an intracellular portion of the transmembrane protein GITR (also known as TNFRSF18, RP5-902P8.2, AITR, CD357, and GITR-D). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: HIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPEEERGERSAEEKGRLGDLWV (SEQ ID NO:551). In some of these embodiments, the co-stimulatory domain of both the first and the second polypeptide has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about

40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00167] In some cases, the co-stimulatory domain derived from an intracellular portion of the transmembrane protein HVEM (also known as TNFRSF14, RP3-395M20.6, ATAR, CD270, HVEA, HVEM, LIGHTR, and TR2). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: CVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRSPNH (SEQ ID NO:552). In some of these embodiments, the co-stimulatory domain of both the first and the second polypeptide has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

Dimerization pairs

[00168] As noted above, a conditionally active, heterodimeric polypeptide of the present disclosure comprises two polypeptide chains, one of which comprises a first member of a dimerization pair, and the second of which comprises a second member of a dimerization pair. One of the members of the dimerization pair will comprise a ligand-binding domain (LBD) of a nuclear hormone receptor; the other member of the dimerization pair will comprise a co-regulator peptide of the same nuclear hormone receptor. In the presence of a dimerization agent (e.g., a nuclear hormone, or a functional derivative or analog of the nuclear hormone; also referred to herein as a "dimerizer"), the first and second members of the dimerization pair will bind to one another, and will effect dimerization of the two polypeptide chains of the conditionally active, heterodimeric polypeptide of the present disclosure. A first member of a dimerization pair, or a second member of a dimerization pair, can also be referred to as a "dimerization domain."

Ligand-binding domain (LBD)

[00169] A ligand-binding domain of a nuclear hormone receptor can be from any of a variety of nuclear hormone receptors, including, but not limited to, ERα, ERβ, PR, AR, GR, MR, RARα, RARβ, RARγ, TRα, TRβ, VDR, EcR, RXRα, RXRβ, RXRγ, PPARα, PPARβ, PPARγ, LXRα, LXRβ, FXR, PXR, SXR, CAR, SF-1, LRH-1, DAX-1, SHP, TLX, PNR, NGF1-Bα, NGF1-Bβ, NGF1-Bγ, RORα, RORβ, RORγ, ERRα, ERRβ, ERRγ, GCNF, TR2/4, HNF-4, COUP-TFα, COUP-TFβ and COUP-TFγ.

[00170] Abbreviations for nuclear hormone receptors are as follows. ER: Estrogen Receptor; PR: Progesterone Receptor; AR: Androgen Receptor; GR: Glucocorticoid Receptor; MR: Mineralocorticoid Receptor; RAR: Retinoic Acid Receptor; TRα, β: Thyroid Receptor; VDR: Vitamin D3 Receptor; EcR: Ecdysone Receptor; RXR: Retinoic Acid X Receptor; PPAR: Peroxisome Proliferator Activated Receptor; LXR: Liver X Receptor; FXR: Farnesoid X Receptor; PXR/SXR: Pregnane X

Receptor/Steroid and Xenobiotic Receptor; CAR: Constitutive Adrostrane Receptor; SF-1: Steroidogenic Factor 1; DAX-1: Dosage sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1; LRH-1: Liver Receptor Homolog 1; SHP: Small Heterodimer Partner; TLX: Tail-less Gene; PNR: Photoreceptor-Specific Nuclear Receptor; NGF1-B: Nerve Growth Factor; ROR: RAR related orphan receptor; ERR: Estrogen Related Receptor; GCNF: Germ Cell Nuclear Factor; TR2/4: Testicular Receptor; HNF-4: Hepatocyte Nuclear Factor; COUP-TF: Chicken Ovalbumin Upstream Promoter, Transcription Factor.

[00171] In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises a single LBD of a nuclear hormone receptor. In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises multiple (two or more) LBD of a nuclear hormone receptor. In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises two LBD of a nuclear hormone receptor. In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises three LBD of a nuclear hormone receptor. Where a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises multiple (two or more) LBD of a nuclear hormone receptor, in some cases the multiple LBD comprise identical amino acid sequences. In some cases, the two or more LBD are in tandem, either directly or separated by a linker.

Mineralocorticoid receptor

[00172] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a mineralocorticoid receptor (MR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an MR having the amino acid sequence depicted in FIG. 1A.

[00173] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 1F; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00174] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1B; and has a length of from about 250 amino acids to 299 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 299 amino acids).

[00175] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1C; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00176] As another non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1D, and has an S810L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 1A); and has a length of from about 250 amino acids to 299 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 299 amino acids).

[00177] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1C, and has an S810L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 1A); and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00178] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00179] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the second member of the dimerization pair is

a co-regulator peptide comprising the amino acid sequence QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00180] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Androgen receptor

[00181] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an androgen receptor (AR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an AR having the amino acid sequence depicted in FIG. 2A.

[00182] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B; and has a length of from

about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00183] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2C; and has a length of from about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00184] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, and has a T877A substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00185] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2C, and has a T877A substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00186] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, and has an F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00187] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2C, and has an F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00188] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, and has a T877A and an

F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00189] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2C, and has a T877A and an F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00190] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an AR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3), where the co-regulator peptide has a length of from about 19 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 19 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an AR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3), where the co-regulator peptide has a length of from about 19 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 19 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Progesterone receptor

[00191] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a progesterone receptor (PR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a PR having the amino acid sequence depicted in FIG. 3A.

[00192] As one non-limiting example, the LBD of a PR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 3D; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00193] As one non-limiting example, the LBD of a PR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 3B; and has a length of from about 200 amino acids to 256 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 256 amino acids; e.g., has a length of 256 amino acids).

[00194] As one non-limiting example, the LBD of a PR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 3C; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00195] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8), where the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8), where the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Thyroid hormone receptor-β

[00196] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of thyroid hormone receptor-beta (TRβ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a TRβ having the amino acid sequence depicted in FIG. 4A.

[00197] As one non-limiting example, the LBD of a TRβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 4D; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino

acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 240 amino acids, or from 240 amino acids to 250 amino acids).

[00198] As one non-limiting example, the LBD of a TRβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 4B; and has a length of from about 200 amino acids to 260 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 260 amino acids; e.g., has a length of 260 amino acids).

[00199] As one non-limiting example, the LBD of a TRβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 4B; and has a length of from about 200 amino acids to 246 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 246 amino acids; e.g., has a length of 246 amino acids).

[00200] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is an NCOA3/SRC3 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is a co-regulator peptide comprises the amino acid sequence CSSDDRGHSSLTNSPLDSSCKESSVSVTSPSGVSSSTSGGVSSTSNMHGSLLQEKHRILHKLLQNG NSPAEVAKITAEATGKDTSSITSCGDGNVVKQEQLSPKKKENNALLRYLLDRDDPSDALSKELQ PQVEGVDNKMSQCTSSTIPSSSQEKDPKIKTETSEEGSGDLDNLDAILGDLTSSDFYNNSISSNGS HLGTKQQ (SEQ ID NO:553). In other cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the first member of the dimerization pair is an NCOA3/SRC3 polypeptide. In some cases, the co-regulator peptide comprises the amino acid sequence: MHGSLLQEKHRILHKLLQNGNSPAEVAKITAEATGKDTSSITSCGDGNVVKQEQLSPKKKENNA LLRYLLDRDDPSDA (SEQ ID NO:554).

[00201] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is a co-regulator peptide comprises the amino acid sequence STAPGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERLDSKTDPASNTKLIAMKT EKEEMSFEPGDQPGSELDNLEEILDDLQNSQLPQLFPDTRPGAPAGSVDKQAIINDLMQLTAENS PVTPVGAQKTALRISQSTFNNPRPGQLGRLLPNQNLPLDITLQSPTGAGPFPPIRNSSPYSVIPQPG MMGNQGMIGNQGNLGNSSTGMIGNSASRPTMPSGEWAPQSSAVRVTCAATTSAMNRPVQGG

MIRNPAASIPMRPSSQPGQRQTLQSQVMNIGPSELEMNMGGP (SEQ ID NO:555). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the first member of the dimerization pair is an NCOA2/SRC2 polypeptide.

Estrogen receptor-alpha

[00202] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of estrogen receptor-alpha (ERα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an ERα having the amino acid sequence depicted in FIG. 5A.

[00203] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 5H; and has a length of from about 200 amino acids to 240 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 235 amino acids, or from 235 amino acids to 240 amino acids).

[00204] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5B; and has a length of from about 180 amino acids to 229 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, or from 200 amino acids to 229 amino acids; e.g., has a length of 229 amino acids).

[00205] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5C; and has a length of from about 250 amino acids to 314 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 314 amino acids; e.g., has a length of 314 amino acids).

[00206] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5D; and has a length of from about 190 amino acids to 238 amino acids (e.g., has a length of from 190 amino acids to 220 amino acids, or from 220 amino acids to 238 amino acids; e.g., has a length of 238 amino acids).

[00207] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%,

amino acid sequence identity to the amino acid sequence depicted in FIG. 5E, and has a D351Y substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 5A); and has a length of from about 180 amino acids to 229 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, or from 200 amino acids to 229 amino acids; e.g., has a length of 229 amino acids).

[00208] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5F, and has a D351Y substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 5A); and has a length of from about 250 amino acids to 314 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 314 amino acids; e.g., has a length of 314 amino acids).

[00209] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5G, and has a D351Y substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 5A); and has a length of from about 190 amino acids to 238 amino acids (e.g., has a length of from 190 amino acids to 220 amino acids, or from 220 amino acids to 238 amino acids; e.g., has a length of 238 amino acids).

[00210] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERα, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence DAFQLRQLILRGLQDD (SEQ ID NO:12), where the co-regulator peptide has a length of from about 16 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 16 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERα, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence DAFQLRQLILRGLQDD (SEQ ID NO:12), where the co-regulator peptide has a length of from about from about 16 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 16 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00211] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERα, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SPGSREWFKDMLS (SEQ ID NO:13), where the co-regulator peptide has a length of from about 13 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 13 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERα, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SPGSREWFKDMLS (SEQ ID NO:13), where the co-regulator peptide has a length of from about from about 13 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 13 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Estrogen receptor-beta (ERβ)

[00212] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of estrogen receptor-alpha (ERβ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an ERβ having the amino acid sequence depicted in FIG. 6A.

[00213] As one non-limiting example, the LBD of an ERβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 6C; and has a length of from about 200 amino acids to 243 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 235 amino acids, or from 235 amino acids to 243 amino acids).

[00214] As one non-limiting example, the LBD of an ERβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 6B; and has a length of from about 200 amino acids to 243 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 235 amino acids, or from 235 amino acids to 243 amino acids).

[00215] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERβ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PRQGSILYSMLTSAKQT (SEQ ID NO:9), where the co-regulator peptide has a length of from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 17 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERβ, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PRQGSILYSMLTSAKQT (SEQ ID NO:9), where the co-regulator peptide has a length of from about from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 17 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Peroxisome proliferator-activated receptor-gamma

[00216] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of peroxisome proliferator-activated receptor-gamma (PPAR-γ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a PPAR-γ having the amino acid sequence depicted in FIG. 7A.

[00217] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 7E; and has a length of from about 200 amino acids to 269 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 250 amino acids, or from 250 amino acids to 269 amino acids).

[00218] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7B; and has a length of from about 150 amino acids to 202 amino acids (e.g., has a length of from 150 amino acids to 160 amino acids, from 160 amino acids to 170 amino acids, from 170 amino acids to 190 amino acids, or from 190 amino acids to 202 amino acids).

[00219] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7C; and has a length of from about 200 amino acids to 269 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 250 amino acids, or from 250 amino acids to 269 amino acids).

[00220] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7D; and has a length of from about 200 amino acids to 269 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 250 amino acids, or from 250 amino acids to 271 amino acids).

[00221] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1), where the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 28 amino acids, from 28 amino acids to 29 amino acids, from 29 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1), where the co-regulator peptide has a length of from about from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 28 amino acids, from 28 amino acids to 29 amino acids, from 29 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00222] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the first

member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4), where the co-regulator peptide has a length of from about from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00223] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11), where the co-regulator peptide has a length of from about from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Glucocorticoid receptor

[00224] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of glucocorticoid receptor (GR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a GR having the amino acid sequence depicted in FIG. 8A.

[00225] As one non-limiting example, the LBD of a GR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 8C; and has a length of from about 200 amino acids to 247 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 240 amino acids, or from 240 amino acids to 247 amino acids).

[00226] As one non-limiting example, the LBD of a GR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 8B; and has a length of from about 200 amino acids to 247 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 247 amino acids; e.g., has a length of 247 amino acids).

[00227] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide comprising the amino acid sequence NYGTNPGTPPASTSPFSQLAANPEASLANRNSMVSRGMTGNIGGQFGTGINPQMQQNVFQYPG AGMVPQGEANFAPSLSPGSSMVPMPIPPPQSSLLQQTPPASGYQSPDMKAWQQGAIGNNNVFSQ AVQNQPTPAQPGVYNNMSITVSMAGGNTNVQNMNPMMAQMQMSSLQMPGMNTVCPEQIND PALRHTGLYCNQLSSTDLLKTEADGTQQVQQVQVFADVQCTVNLVGGDPYLNQPGPLGTQKP TSGPQTPQAQQKSLLQQLLTE (SEQ ID NO:556) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the first member of the dimerization pair is an NCOA1/SRC1 polypeptide.

[00228] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide comprising the amino acid sequence KRHHHEVLRQGLAFSQIYRFSLSDGTLVAAQTKSKLIRSQTTNEPQLVISLHMLHREQNVCVMN PDLTGQTMGKPLNPISSNSPAHQALCSGNPGQDMTLSSNINFPINGPKEQMGMPMGRFGGSGG MNHVSGMQATTPQGSNYALKMNSPSQSSPGMNPGQPTSMLSPRHRMSPGVAGSPRIPPSQFSPA GSLHSPVGVCSSTGNSHSYTNSSLNALQALSEGHGVSLGSSLASPDLKMGNLQNSPVNMNPPPL SKMGSLDSKDCFGLYGEPSEGTTGQAESSCHPGEQKETNDPNLPPAVSSERADGQSRLHDSKGQ TKLLQLLTTKSDQMEPSPLASSLSDTNKDSTGSLPGSGSTHGTSLKEKHKILHRLLQDSSSPVDL AKLTAEATGKDLSQESSSTAPGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERL DSKTDPASNTKLIAMKTEKEEMSFEPGDQPGSELDNLEEILDDLQNSQLPQLFPDTRPGAPAGSV DKQAIINDLMQLTAENSPVTPVGAQKTALRISQSTFNNPRPGQLGRLLPNQNLPLDITLQSPTGA GPFPPIRNSSPYSVIPQPGMMGNQGMIGNQGNLGNSSTGMIGNSASRPTMPSGEWAPQSSAVRV TCAATTSAMNRPVQGGMIRNPAASIPMRPSSQPGQRQTLQSQVMNIGPSELEMNMGGP (SEQ ID

NO:557) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the first member of the dimerization pair is an NCOA2/SRC2 polypeptide.

Vitamin D receptor

[00229] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of vitamin D receptor (VDR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a VDR having the amino acid sequence depicted in FIG. 9A.

[00230] As one non-limiting example, the LBD of a VDR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 9C; and has a length of from about 250 amino acids to 310 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 310 amino acids).

[00231] As one non-limiting example, the LBD of a VDR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 9B; and has a length of from about 250 amino acids to 303 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 303 amino acids).

[00232] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide comprising the amino acid sequence

NYGTNPGTPPASTSPFSQLAANPEASLANRNSMVSRGMTGNIGGQFGTGINPQMQQNVFQYPG
AGMVPQGEANFAPSLSPGSSMVPMPIPPPQSSLLQQTPPASGYQSPDMKAWQQGAIGNNNVFSQ
AVQNQPTPAQPGVYNNMSITVSMAGGNTNVQNMNPMMAQMQMSSLQMPGMNTVCPEQIND
PALRHTGLYCNQLSSTDLLKTEADGTQQVQQVQVFADVQCTVNLVGGDPYLNQPGPLGTQKP
TSGPQTPQAQQKSLLQQLLTE (SEQ ID NO:558) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the first member of the dimerization pair is an NCOA1/SRC1 polypeptide.

[00233] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide comprising the amino acid sequence KRHHHEVLRQGLAFSQIYRFSLSDGTLVAAQTKSKLIRSQTTNEPQLVISLHMLHREQNVCVMN PDLTGQTMGKPLNPISSNSPAHQALCSGNPGQDMTLSSNINFPINGPKEQMGMPMGRFGGSGG MNHVSGMQATTPQGSNYALKMNSPSQSSPGMNPGQPTSMLSPRHRMSPGVAGSPRIPPSQFSPA GSLHSPVGVCSSTGNSHSYTNSSLNALQALSEGHGVSLGSSLASPDLKMGNLQNSPVNMNPPPL SKMGSLDSKDCFGLYGEPSEGTTGQAESSCHPGEQKETNDPNLPPAVSSERADGQSRLHDSKGQ TKLLQLLTTKSDQMEPSPLASSLSDTNKDSTGSLPGSGSTHGTSLKEKHKILHRLLQDSSSPVDL AKLTAEATGKDLSQESSSTAPGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERL DSKTDPASNTKLIAMKTEKEEMSFEPGDQPGSELDNLEEILDDLQNSQLPQLFPDTRPGAPAGSV DKQAIINDLMQLTAENSPVTPVGAQKTALRISQSTFNNPRPGQLGRLLPNQNLPLDITLQSPTGA GPFPPIRNSSPYSVIPQPGMMGNQGMIGNQGNLGNSSTGMIGNSASRPTMPSGEWAPQSSAVRV TCAATTSAMNRPVQGGMIRNPAASIPMRPSSQPGQRQTLQSQVMNIGPSELEMNMGGP (SEQ ID NO:559) or a fragment thereof. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide comprising the amino acid sequence LLRYLLDK (SEQ ID NO:560), where the co-regulator peptide has a length of from about from about 8 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the first member of the dimerization pair is an NCOA2/SRC2 polypeptide.

Thyroid hormone receptor-alpha

[00234] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of thyroid hormone receptor-alpha (TRα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a TRα having the amino acid sequence depicted in FIG. 10A.

[00235] As one non-limiting example, the LBD of a TRα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 10C; and has a length of from about 190 amino acids to about 245 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, from 210 amino acids to 230 amino acids, or from 230 amino acids to 245 amino acids).

[00236] As one non-limiting example, the LBD of a TRα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 10B; and has a length of from about 190 amino acids to about 243 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, from 210 amino acids to 230 amino acids, or from 230 amino acids to 243 amino acids).

[00237] A suitable co-regulator peptide for TRα is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

Retinoic acid receptor-beta

[00238] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of retinoic acid receptor-beta (RARβ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a RARβ having the amino acid sequence depicted in FIG. 11A.

[00239] As one non-limiting example, the LBD of a RARβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 11C; and has a length of from about 180 amino acids to about 235 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, from 200 amino acids to 220 amino acids, or from 220 amino acids to 235 amino acids).

[00240] As one non-limiting example, the LBD of a RARβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 11B; and has a length of from about 180 amino acids to about 231 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, from 200 amino acids to 220 amino acids, or from 220 amino acids to 231 amino acids).

[00241] A suitable co-regulator peptide for RARβ is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

[00242] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a RARβ, the second member of the dimerization pair

is an NCOA1/SRC1 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a RARβ, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide comprising the amino acid sequence

NYGTNPGTPPASTSPFSQLAANPEASLANRNSMVSRGMTGNIGGQFGTGINPQMQQNVFQYPG AGMVPQGEANFAPSLSPGSSMVPMPIPPPQSSLLQQTPPASGYQSPDMKAWQQGAIGNNNVFSQ AVQNQPTPAQPGVYNNMSITVSMAGGNTNVQNMNPMMAQMQMSSLQMPGMNTVCPEQIND PALRHTGLYCNQLSSTDLLKTEADGTQQVQQVQVFADVQCTVNLVGGDPYLNQPGPLGTQKP TSGPQTPQAQQKSLLQQLLTE (SEQ ID NO:561) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a RARβ, the first member of the dimerization pair is an NCOA1/SRC1 polypeptide.

[00243] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a RARβ, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a RARβ, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide comprising the amino acid sequence

KRHHHEVLRQGLAFSQIYRFSLSDGTLVAAQTKSKLIRSQTTNEPQLVISLHMLHREQNVCVMN PDLTGQTMGKPLNPISSNSPAHQALCSGNPGQDMTLSSNINFPINGPKEQMGMPMGRFGGSGG MNHVSGMQATTPQGSNYALKMNSPSQSSPGMNPGQPTSMLSPRHRMSPGVAGSPRIPPSQFSPA GSLHSPVGVCSSTGNSHSYTNSSLNALQALSEGHGVSLGSSLASPDLKMGNLQNSPVNMNPPPL SKMGSLDSKDCFGLYGEPSEGTTGQAESSCHPGEQKETNDPNLPPAVSSERADGQSRLHDSKGQ TKLLQLLTTKSDQMEPSPLASSLSDTNKDSTGSLPGSGSTHGTSLKEKHKILHRLLQDSSSPVDL AKLTAEATGKDLSQESSSTAPGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERL DSKTDPASNTKLIAMKTEKEEMSFEPGDQPGSELDNLEEILDDLQNSQLPQLFPDTRPGAPAGSV DKQAIINDLMQLTAENSPVTPVGAQKTALRISQSTFNNPRPGQLGRLLPNQNLPLDITLQSPTGA GPFPPIRNSSPYSVIPQPGMMGNQGMIGNQGNLGNSSTGMIGNSASRPTMPSGEWAPQSSAVRV TCAATTSAMNRPVQGGMIRNPAASIPMRPSSQPGQRQTLQSQVMNIGPSELEMNMGGP (SEQ ID NO:562) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a RARβ, the first member of the dimerization pair is an NCOA2/SRC2 polypeptide.

Farnesoid X receptor

[00244] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of farnesoid X receptor (FXR. For

example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an FXR having the amino acid sequence depicted in FIG. 22A.

[00245] As one non-limiting example, the LBD of an FXR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 22B; and has a length of from about 100 amino acids to about 136 amino acids (e.g., has a length of from 100 amino acids to 110 amino acids, from 110 amino acids to 120 amino acids, or from 120 amino acids to 136 amino acids).

[00246] A suitable co-regulator peptide for an FXR is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

LXR-alpha

[00247] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of liver X receptor-alpha (LRXα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an LRXα having the amino acid sequence depicted in FIG. 23A.

[00248] As one non-limiting example, the LBD of an LRXα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 23B; and has a length of from about 200 amino acids to about 266 amino acids (e.g., has a length of from 200 amino acids to 220 amino acids, from 220 amino acids to 240 amino acids, or from 240 amino acids to 266 amino acids).

[00249] A suitable co-regulator peptide for an LRXα is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

RORgamma

[00250] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a retinoid-related orphan receptor gamma (RORγ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an RORγ having the amino acid sequence depicted in FIG. 24A.

[00251] As one non-limiting example, the LBD of an RORγ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 24B; and has a length of from

about 200 amino acids to about 261 amino acids (e.g., has a length of from 200 amino acids to 220 amino acids, from 220 amino acids to 240 amino acids, or from 240 amino acids to 261 amino acids).

[00252] A suitable co-regulator for an RORγ is an NCORNR peptide (CDPASNLGLEDIIRKALMGSFDDK, SEQ ID NO:563).

[00253] A suitable co-regulator peptide for an RORγ is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

RXR-alpha

[00254] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a retinoid-X receptor-alpha (RXRα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an RXRα having the amino acid sequence depicted in FIG. 25A.

[00255] As one non-limiting example, the LBD of an RORγ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 25B; and has a length of from about 190 amino acids to about 238 amino acids (e.g., has a length of from 190 amino acids to 200 amino acids, from 200 amino acids to 210 amino acids, or from 210 amino acids to 238 amino acids).

[00256] A suitable co-regulator peptide for an RXRα is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

PXR

[00257] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a Pregnane X Receptor (PXR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a PXR having the amino acid sequence depicted in FIG. 26A. In some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 143-428 of the amino acid sequence depicted in FIG. 26A. In some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 205-434 of the amino acid sequence depicted in FIG. 26A.

[00258] As one non-limiting example, the LBD of a PXR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 26B; and has a length of from

about 250 amino acids to about 302 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 290 amino acids, or from 290 amino acids to 302 amino acids).

[00259] A suitable co-regulator peptide for a PXR is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

Co-regulator polypeptides

[00260] Suitable co-regulator polypeptides include full-length naturally-occurring nuclear hormone co-regulator polypeptides. Suitable co-regulator polypeptides include fragments of naturally-occurring nuclear hormone co-regulator polypeptides. Suitable co-regulator polypeptides include synthetic or recombinant nuclear hormone co-regulator polypeptides.

[00261] Suitable co-regulator polypeptides can have a length of from 8 amino acids to 2000 amino acids. Suitable co-regulator polypeptides can have a length of from 8 amino acids to 50 amino acids, e.g., from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids. Suitable co-regulator polypeptides can have a length of from 50 amino acids to 100 amino acids, e.g., from 50 amino acids to 60 amino acids, from 60 amino acids to 70 amino acids, from 70 amino acids to 80 amino acids, from 80 amino acids to 90 amino acids, or from 90 amino acids to 100 amino acids. Suitable co-regulator polypeptides can have a length of from 100 amino acids to 200 amino acids, from 200 amino acids to 300 amino acids, from 300 amino acids to 400 amino acids, from 400 amino acids to 500 amino acids, from 500 amino acids to 600 amino acids, from 600 amino acids to 700 amino acids, from 700 amino acids to 800 amino acids, from 800 amino acids to 900 amino acids, or from 900 amino acids to 1000 amino acids. Suitable co-regulator polypeptides can have a length of from 1000 amino acids to 2000 amino acids.

[00262] Suitable co-regulator polypeptides include, but are not limited to, SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

[00263] National Center for Biotechnology Information (NCBI) accession numbers for such co-regulators include the following: SRC1 (NP_003734), GRIP1 (NP_006531), AIB1 (NP_006525), PGC1a (NP_037393), PGC1b (NP_573570), PRC (NP_055877), TRAP220 (NP_004765), ASC2 (NP_054790), CBP (NP_004371), P300 (NP_001420), CIA (NP_066018), ARA70 (NP_005428), TIF1 (NP_003843), NSD1 (NP_071900), SMAP (NP_006687), Tip60 (NP_006379), ERAP140 (NP_861447), Nix1 (NP_113662), LCoR (NP_115816), N-CoR (NP_006302), SMRT (NP_006303), RIP140 (NP_003480) and PRIC285 (NP_208384).

[00264] Examples of suitable co-regulator polypeptides are provided in FIGs. 29-51B.

[00265] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 29.

[00266] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 30.

[00267] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 31.

[00268] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 32.

[00269] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at

least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 33.

[00270] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 34.

[00271] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 35.

[00272] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 36A-36B.

[00273] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 37A-37B.

[00274] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino

acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 36A-36B.

[00275] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 39.

[00276] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 40.

[00277] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 41.

[00278] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 42A-42B.

[00279] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino

acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 43.

[00280] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 44.

[00281] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 45.

[00282] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 46.

[00283] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 47.

[00284] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20

amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 48A-48B.

[00285] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 49A-49B.

[00286] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 50.

[00287] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 51A-51B.

[00288] Suitable co-regulator peptides include, but are not limited to, Steroid Receptor Coactivator (SRC)-1, SRC-2, SRC-3, TRAP220-1, TRAP220-2, NR0B1, NRIP1, CoRNR box, αβV, TIF1, TIF2, EA2, TA1, EAB1, SRC1-1, SRC1-2, SRC1-3, SRC1-4a, SRC1-4b, GRIP1-1, GRIP1-2, GRIP1-3, AIB1-1, AIB1-2, AIB1-3, PGC1a, PGC1b, PRC, ASC2-1, ASC2-2, CBP-1, CBP-2, P300, CIA, ARA70-1, ARA70-2, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, CoRNR1 (N-CoR), CoRNR2, SMRT, RIP140-C, RIP140-1, RIP140-2, RIP140-3, RIP140-4, RIP140-5, RIP140-6, RIP140-7, RIP140-8, RIP140-9, PRIC285-1, PRIC285-2, PRIC285-3, PRIC285-4, and PRIC285-5.

[00289] In some cases, a suitable co-regulator peptide comprises an LXXLL motif, where X is any amino acid; where the co-regulator peptide has a length of from 8 amino acids to 50 amino acids, e.g., from 8 amino acids to 10 amino acids, from 10 amino acids to 12 amino acids, from 12 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids.

[00290] Non-limiting examples of suitable co-regulator peptides are as follows:

SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

abV: SPGSREWFKDMLS (SEQ ID NO:13);

TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);

SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);
AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);
PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);
PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);
PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);
TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);
TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);
ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);
ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);
CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);
CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);
P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);
CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);
ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);
ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);
TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);
NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);
SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);
Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);
ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);
Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);
LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);
CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);
CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);
CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);
CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);
RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);
RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);
RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);
RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);
RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);
RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);
RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);
RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);
RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);

RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);

PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[00291] Further examples are provided in Example 1, Example 2 and Example 3.

[00292] In some cases, a given LBD can be paired with two or more different co-regulator polypeptides. For example, as depicted in FIG. 19, PPARγ can be paired with SRC1, SRC2, SRC3, or TRAP220. As another example, ERα can be paired with CoRNR, αβV, or TA1. As another example, ERβ can be paired with CoRNR, αβV, or TA1. As another example, AR can be paired with SRC1, SRC2, SRC3, or TRAP220. As another example, PR can be paired with SRC1, SRC2, SRC3, TRAP220, NR0B1, PGC1B, NRIP1, EA2, or EAB1. As another example, TRβ can be paired with SRC1, SRC2, SRC3, or TRAP220.

[00293] In some cases, a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides. Where a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides, the multiple co-regulator peptides can be in tandem, directly or separated by a linker. In some cases, the two or more co-regulator peptides present in the polypeptide chain are identical in amino acid sequence to one another. In some cases, where a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides, the polypeptide chain comprises two co-regulator peptides. In some cases, where a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides, the polypeptide chain comprises three co-regulator peptides. In such cases, the second polypeptide chain can comprise multiple (two or more) LBD of a nuclear hormone receptor. For example, where the second polypeptide chain comprises two LBD of a nuclear hormone receptor, the two LBD can be identical in amino acid sequence to one another.

Intracellular signaling domain

[00294] Intracellular signaling domains suitable for use in a conditionally active heterodimeric polypeptide of the present disclosure include any desired signaling domain that provides a distinct and detectable signal (e.g., increased production of one or more cytokines by the cell; change in transcription of a target gene; change in activity of a protein; change in cell behavior, e.g., cell death; cellular proliferation; cellular differentiation; cell survival; modulation of cellular signaling responses; etc.) in response to activation of the conditionally active heterodimeric polypeptide (i.e., activated by antigen and dimerizing agent). In some instances, the intracellular signaling domain(s) of a conditionally active heterodimeric polypeptide

of the present disclosure provides a signal transduction function when activated and may thus, in some instances, be referred to as a signal transducing domain. In some embodiments, the intracellular signaling domain includes at least one (e.g., one, two, three, four, five, six, etc.) ITAM motifs as described below. In some embodiments, the intracellular signaling domain includes DAP10/CD28 type signaling chains. In some embodiments, the intracellular signaling domain is not covalently attached to the membrane bound conditionally active heterodimeric polypeptide, but is instead diffused in the cytoplasm.

ITAM

[00295] Intracellular signaling domains suitable for use in a conditionally active heterodimeric polypeptide of the present disclosure include immunoreceptor tyrosine-based activation motif (ITAM)-containing intracellular signaling polypeptides. An ITAM motif is YX_1X_2L/I, where X_1 and X_2 are independently any amino acid (SEQ ID NO:564). In some cases, the intracellular signaling domain of a subject CAR comprises 1, 2, 3, 4, or 5 ITAM motifs. In some cases, an ITAM motif is repeated twice in an intracellular signaling domain, where the first and second instances of the ITAM motif are separated from one another by 6 to 8 amino acids, e.g., $(YX_1X_2L/I)(X_3)_n(YX_1X_2L/I)$, where n is an integer from 6 to 8, and each of the 6-8 X_3 can be any amino acid (SEQ ID NO:565). In some cases, the intracellular signaling domain of a subject CAR comprises 3 ITAM motifs.

[00296] A suitable intracellular signaling domain can be an ITAM motif-containing portion that is derived from a polypeptide that contains an ITAM motif. For example, a suitable intracellular signaling domain can be an ITAM motif-containing domain from any ITAM motif-containing protein. Thus, a suitable intracellular signaling domain need not contain the entire sequence of the entire protein from which it is derived. Examples of suitable ITAM motif-containing polypeptides include, but are not limited to: DAP12; FCER1G (Fc epsilon receptor I gamma chain); CD3D (CD3 delta); CD3E (CD3 epsilon); CD3G (CD3 gamma); CD3Z (CD3 zeta); and CD79A (antigen receptor complex-associated protein alpha chain).

[00297] In some cases, the intracellular signaling domain is derived from DAP12 (also known as TYROBP; TYRO protein tyrosine kinase binding protein; KARAP; PLOSL; DNAX-activation protein 12; KAR-associated protein; TYRO protein tyrosine kinase-binding protein; killer activating receptor associated protein; killer-activating receptor-associated protein; etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to any of the following amino acid sequences (4 isoforms):
MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFL GRLVPRGRGAAEAATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:566);
MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFL GRLVPRGRGAAEATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:567);

MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAA EAATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:568); or MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAA EATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:569), where the ITAM motifs are in bold and are underlined.

[00298] Likewise, a suitable intracellular signaling domain polypeptide can comprise an ITAM motif-containing portion of the full length DAP12 amino acid sequence. Thus, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: ESP**YQEL**QGQRSDV**YSDL**NTQ (SEQ ID NO:570), where the ITAM motifs are in bold and are underlined.

[00299] In some cases, the intracellular signaling domain is derived from FCER1G (also known as FCRG; Fc epsilon receptor I gamma chain; Fc receptor gamma-chain; fc-epsilon RI-gamma; fcRgamma; fceRI gamma; high affinity immunoglobulin epsilon receptor subunit gamma; immunoglobulin E receptor, high affinity, gamma chain; etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITSYEKSDGV**YT GL**STRNQET**YETL**KHEKPPQ (SEQ ID NO:571), where the ITAM motifs are in bold and are underlined.

[00300] Likewise, a suitable intracellular signaling domain polypeptide can comprise an ITAM motif-containing portion of the full length FCER1G amino acid sequence. Thus, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: DGV**YTGL**STRNQET**YETL**KHE (SEQ ID NO:572), where the ITAM motifs are in bold and are underlined.

[00301] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 delta chain (also known as CD3D; CD3-DELTA; T3D; CD3 antigen, delta subunit; CD3 delta; CD3d antigen, delta polypeptide (TiT3 complex); OKT3, delta chain; T-cell receptor T3 delta chain; T-cell surface glycoprotein CD3 delta chain; etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence

identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 170 aa, of either of the following amino acid sequences (2 isoforms):
MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDP
RGIYRCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLLLALGVFCFAGHETG
RLSGAADTQALLRNDQV**YQPL**RDRDDAQ**YSHL**GGNWARNK (SEQ ID NO:573) or
MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDP
RGIYRCNGTDIYKDKESTVQVHYRTADTQALLRNDQV**YQPL**RDRDDAQ**YSHL**GGNWARNK
(SEQ ID NO:574) , where the ITAM motifs are in bold and are underlined.

[00302] Likewise, a suitable intracellular signaling domain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 delta amino acid sequence. Thus, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence:
DQV**YQPL**RDRDDAQ**YSHL**GGN (SEQ ID NO:575), where the ITAM motifs are in bold and are underlined.

[00303] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 epsilon chain (also known as CD3e, T-cell surface antigen T3/Leu-4 epsilon chain, T-cell surface glycoprotein CD3 epsilon chain, AI504783, CD3, CD3epsilon, T3e, etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 205 aa, of the following amino acid sequence:
MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHND
KNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVCENCMEMD
VMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVTRGAGAGGRQRGQNKERPPPVPNPD**YE**
PIRKGQRDL**YSGL**NQRRI (SEQ ID NO:576), where the ITAM motifs are in bold and are underlined.

[00304] Likewise, a suitable intracellular signaling domain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 epsilon amino acid sequence. Thus, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence:

NPD**YEPI**RKGQRDL**YSGL**NQR (SEQ ID NO:577), where the ITAM motifs are in bold and are underlined.

[00305] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 gamma chain (also known as CD3G, T-cell receptor T3 gamma chain, CD3-GAMMA, T3G, gamma polypeptide (TiT3 complex), etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 180 aa, of the following amino acid sequence:
MEQGKGLAVLILAIILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGF
LTEDKKKWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNAATISGFLFAEIVSIFV
LAVGVYFIAGQDGVRQSRASDKQTLLPNDQL**YQPL**KDREDDQ**YSHL**QGNQLRRN (SEQ ID NO:578), where the ITAM motifs are in bold and are underlined.

[00306] Likewise, a suitable intracellular signaling domain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 gamma amino acid sequence. Thus, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence:
DQL**YQPL**KDREDDQ**YSHL**QGN (SEQ ID NO:579), where the ITAM motifs are in bold and are underlined.

[00307] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 zeta chain (also known as CD3Z, T-cell receptor T3 zeta chain, CD247, CD3-ZETA, CD3H, CD3Q, T3Z, TCRZ, etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 160 aa, of either of the following amino acid sequences (2 isoforms):
MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQ
QGQNQL**YNEL**NLGRREE**YDVL**DKRRGRDPEMGGKPRRKNPQEGL**YNEL**QKDKMAEA**YSEI**G
MKGERRRGKGHDGL**YQGL**STATKDT**YDAL**HMQALPPR (SEQ ID NO:580) or
MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQ

QGQNQL**YNEL**NLGRREE**YDVL**DKRRGRDPEMGGKPQRRKNPQEGL**YNEL**QKDKMAEA**YSEI**GMKGERRRGKGHDGL**YQGL**STATKDT**YDAL**HMQALPPR(SEQ ID NO:581), where the ITAM motifs are in bold and are underlined.

[00308] Likewise, a suitable intracellular signaling domain polypeptide can comprise an ITAM motif-containing portion of the full length CD3 zeta amino acid sequence. Thus, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to any of the following amino acid sequences: RVKFSRSADAPAYQQGQNQL**YNEL**NLGRREE**YDVL**DKRRGRDPEMGGKPRRKNPQEGL**YNEL**QKDKMAEA**YSEI**GMKGERRRGKGHDGL**YQGL**STATKDT**YDAL**HMQALPPR (SEQ ID NO:582); NQL**YNEL**NLGRREE**YDVL**DKR (SEQ ID NO:583); EGL**YNEL**QKDKMAEA**YSEI**GMK (SEQ ID NO:534); or DGL**YQGL**STATKDT**YDAL**HMQ (SEQ ID NO:585), where the ITAM motifs are in bold and are underlined.

[00309] In some cases, the intracellular signaling domain is derived from CD79A (also known as B-cell antigen receptor complex-associated protein alpha chain; CD79a antigen (immunoglobulin-associated alpha); MB-1 membrane glycoprotein; ig-alpha; membrane-bound immunoglobulin-associated protein; surface IgM-associated protein; etc.). For example, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 150 aa, from about 150 aa to about 200 aa, or from about 200 aa to about 220 aa, of either of the following amino acid sequences (2 isoforms):

[00310] MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSN NANVTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKSHGGIYVCRVQEGNESYQQSCGTYL RVRQPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQNEKLGLDAGDEYEDENL**Y EGL**NLDDCSM**YEDI**SRGLQGTYQDVGSLNIGDVQLEKP (SEQ ID NO:586); or

[00311] MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSN NANVTWWRVLHGNYTWPPEFLGPGEDPNEPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLL LFRKRWQNEKLGLDAGDEYEDENLYEGLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKP (SEQ ID NO:587), where the ITAM motifs are in bold and are underlined.

[00312] Likewise, a suitable intracellular signaling domain polypeptide can comprise an ITAM motif-containing portion of the full length CD79A amino acid sequence. Thus, a suitable intracellular signaling domain polypeptide can comprise an amino acid sequence having at least about 75%, at least about 80%,

at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: ENL**YEGL**NLDDCSM**YEDI**SRG (SEQ ID NO:588), where the ITAM motifs are in bold and are underlined.

DAP10/CD28

[00313] Intracellular signaling domains suitable for use in a conditionally active heterodimeric polypeptide of the present disclosure include a DAP10/CD28 type signaling chain.

[00314] An example of a DAP10 signaling chain is the amino acid sequence is: RPRRSPAQDGKV**YINM**PGRG (SEQ ID NO:589). In some embodiments, a suitable intracellular signaling domain comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, amino acid sequence identity to the entire length of the amino acid sequence RPRRSPAQDGKV**YINM**PGRG (SEQ ID NO:590).

[00315] An example of a CD28 signaling chain is the amino acid sequence is FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSD**YMNM**TPRRPGPTRKHYQPYAPPRDF AAYRS (SEQ ID NO:591). In some embodiments, a suitable intracellular signaling domain comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, amino acid sequence identity to the entire length of the amino acid sequence FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSD**YMNM**TPRRPGPTRKHYQPYAPPRDF AAYRS (SEQ ID NO:592).

ZAP70

[00316] Intracellular signaling domains suitable for use in a conditionally active heterodimeric polypeptide of the present disclosure include a ZAP70 polypeptide, e.g., a polypeptide comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 400 amino acids, from about 400 amino acids to about 500 amino acids, or from about 500 amino acids to 619 amino acids, of the following amino acid sequence:

[00317] MPDPAAHLPFFYGSISRAEAEEHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFHHFPIE RQLNGTYAIAGGKAHCGPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPGVFDCLRDAMVRD YVRQTWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSLTREEAERKLYSGAQTDGKFLL RPRKEQGTYALSLIYGKTVYHYLISQDKAGKYCIPEGTKFDTLWQLVEYLKLKADGLIYCLKEA CPNSSASNASGAAAPTLPAHPSTLTHPQRRIDTLNSDGYTPEPARITSPDKPRPMPMDTSVYESPY SDPEELKDKKLFLKRDNLLIADIELGCGNFGSVRQGVYRMRKKQIDVAIKVLKQGTEKADTEE MMREAQIMHQLDNPYIVRLIGVCQAEALMLVMEMAGGGPLHKFLVGKREEIPVSNVAELLHQ VSMGMKYLEEKNFVHRDLAARNVLLVNRHYAKISDFGLSKALGADDSYYTARSAGKWPLKW YAPECINFRKFSSRSDVWSYGVTMWEALSYGQKPYKKMKGPEVMAFIEQGKRMECPPECPPEL

YALMSDCWIYKWEDRPDFLTVEQRMRACYYSLASKVEGPPGSTQKAEAACA (SEQ ID NO:593).

Additional sequences

[00318] The first and/or the second polypeptide of a subject conditionally active heterodimeric polypeptide can further include one or more additional polypeptide domains, where such domains include, but are not limited to, a signal sequence; an epitope tag; an affinity domain; and a polypeptide that produces a detectable signal.

Signal sequences

[00319] Signal sequences that are suitable for use in a subject CAR, e.g., in the first polypeptide of a subject CAR, include any eukaryotic signal sequence, including a naturally-occurring signal sequence, a synthetic (e.g., man-made) signal sequence, etc.

Epitope tag

[00320] Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:594); FLAG (e.g., DYKDDDDK (SEQ ID NO:595); c-myc (e.g., EQKLISEEDL; SEQ ID NO:596), and the like.

Affinity domain

[00321] Affinity domains include peptide sequences that can interact with a binding partner, e.g., such as one immobilized on a solid support, useful for identification or purification. DNA sequences encoding multiple consecutive single amino acids, such as histidine, when fused to the expressed protein, may be used for one-step purification of the recombinant protein by high affinity binding to a resin column, such as nickel sepharose. Exemplary affinity domains include His5 (HHHHH) (SEQ ID NO:597), HisX6 (HHHHHH) (SEQ ID NO:598), C-myc (EQKLISEEDL) (SEQ ID NO:599), Flag (DYKDDDDK) (SEQ ID NO:600), StrepTag (WSHPQFEK) (SEQ ID NO:601), hemagglutinin, e.g., HA Tag (YPYDVPDYA) (SEQ ID NO:602), GST, thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:603), Phe-His-His-Thr (SEQ ID NO:064), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:605), metal binding domains, e.g., zinc binding domains or calcium binding domains such as those from calcium-binding proteins, e.g., calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins, parvalbumin, calbindin D9K, calbindin D28K, and calretinin, inteins, biotin, streptavidin, MyoD, Id, leucine zipper sequences, and maltose binding protein.

Detectable signal-producing polypeptides

[00322] Suitable detectable signal-producing proteins include, e.g., fluorescent proteins; enzymes that catalyze a reaction that generates a detectable signal as a product; and the like.

[00323] Suitable fluorescent proteins include, but are not limited to, green fluorescent protein (GFP) or variants thereof, blue fluorescent variant of GFP (BFP), cyan fluorescent variant of GFP (CFP), yellow fluorescent variant of GFP (YFP), enhanced GFP (EGFP), enhanced CFP (ECFP), enhanced YFP (EYFP), GFPS65T, Emerald, Topaz (TYFP), Venus, Citrine, mCitrine, GFPuv, destabilized EGFP (dEGFP), destabilized ECFP (dECFP), destabilized EYFP (dEYFP), mCFPm, Cerulean, T-Sapphire, CyPet, YPet, mKO, HcRed, t-HcRed, DsRed, DsRed2, DsRed-monomer, J-Red, dimer2, t-dimer2(12), mRFP1, pocilloporin, Renilla GFP, Monster GFP, paGFP, Kaede protein and kindling protein, Phycobiliproteins and Phycobiliprotein conjugates including B-Phycoerythrin, R-Phycoerythrin and Allophycocyanin. Other examples of fluorescent proteins include mHoneydew, mBanana, mOrange, dTomato, tdTomato, mTangerine, mStrawberry, mCherry, mGrape1, mRaspberry, mGrape2, mPlum (Shaner et al. (2005) *Nat. Methods* 2:905-909), and the like. Any of a variety of fluorescent and colored proteins from Anthozoan species, as described in, e.g., Matz et al. (1999) *Nature Biotechnol.* 17:969-973, is suitable for use.

[00324] Suitable enzymes include, but are not limited to, horse radish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase (GAL), glucose-6-phosphate dehydrogenase, beta-N-acetylglucosaminidase, β-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase, glucose oxidase (GO), and the like.

Exemplary conditionally active heterodimeric polypeptides

[00325] In some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises: a) a first chimeric polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second chimeric polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain, where i) the first and second modulatory domains are derived from 4-1BB; ii) the first and second members of the dimerization pair are SRC3 and a PPARγ LBD, respectively; and iii) the signaling domain comprises an ITAM. In some cases, the first member of the dimerization pair comprises one copy of an SRC3 co-regulator peptide. In some cases, the SRC3 co-regulator peptide has a length of from 20 amino acids to 25 amino acids. In some cases, the SRC3 co-regulator peptide has a length of from 75 amino acids to 80 amino acids. In some cases, the first chimeric polypeptide comprises a single SRC3 co-regulator peptide. In some cases, the first chimeric polypeptide comprises 2 copies of an SRC3 co-regulator peptide. In some cases, the first chimeric polypeptide comprises 3 copies of an SRC3 co-regulator peptide. In some cases, each of the copies of the SRC3 co-regulator peptide has a length of from 20 amino acids to 25 amino acids.

[00326] For example, in some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises a first polypeptide chain comprising: i) a single chain Fv; ii) a transmembrane polypeptide; and iii) a polypeptide comprising the amino acid sequence: ***KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL***SRGSGSGSTSPKKENNALLRYLLDRDDPSDAGS* (SEQ ID NO:606), where the 4-1BB sequence is in italics and bold text, and the co-regulator peptide is underlined (single underline). In some cases, the second polypeptide chain comprises the following amino acid sequence:

[00327] IYIWAPLAGTCGVLLLSLVITLYCSL**KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL**GSGSGSGSGSGSGSTSESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKIKFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDLYGSGSGSGSSL***RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*** (SEQ ID NO:607), where the PPARγ LBD is underlined (single underline); the CD8α TM domain is double underlined; 4-1BB is in bold text; and CD3 zeta is in bold and italics.

[00328] In some cases, the first member of a specific binding pair is an scFv specific for CD19, and the first polypeptide chain comprises a transmembrane domain between the anti-CD19 scFv and the first modulatory domain, where the first modulatory domain is 4-1BB. For example, in some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises a first polypeptide chain comprising the following amino acid sequence:

[00329] DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSL*KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL*SRGSGSGSTSPKKENNALLRYLLDRDDPSDAGS* (SEQ ID NO:608), where the anti-CD19 sequence is in bold, the CD8α transmembrane (TM) domain is double underlined, the 4-1BB sequence is in italics and bold text, and the co-regulator peptide is underlined (single underline). In some cases, the second polypeptide chain comprises the following amino acid sequence:

[00330] IYIWAPLAGTCGVLLLSLVITLYCSL**KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL**GSGSGSGSGSGSGSTSESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPF

VIYDMNSLMMGEDKIKFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLN DQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNA LELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDL RQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDLYGSGSGSGSSL***RVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR*** (SEQ ID NO:609), where the PPARγ LBD is underlined (single underline); the CD8α TM domain is double underlined; 4-1BB is in bold text; and CD3 zeta is in bold and italics.

[00331] As another example, in some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises a first polypeptide chain comprising the following amino acid sequence: **DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRF SGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSE VKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSA LKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSTT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD**IYIWAPLAGTCGVLLLSLVIT LYCSL***KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL***SRGSGSGSTSMHGSLLQ EKHRILHKLLQNGNSPAEVAKITAEATGKDTSSITSCGDGNVVKQEQLSPKKKENNALLRYLLD RDDPSDAGS* (SEQ ID NO:610), where the anti-CD19 sequence is in bold, the CD8α transmembrane (TM) domain is double underlined, the 4-1BB sequence is in italics and bold text, and the co-regulator peptide is underlined (single underline). In some cases, the second polypeptide chain comprises the following amino acid sequence:

[00332] IYIWAPLAGTCGVLLLSLVITLYCSL**KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL**GSGSGSGSGSGSGSTSESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPF VIYDMNSLMMGEDKIKFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLN DQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNA LELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDL RQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDLYGSGSGSGSSL***RVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR*** (SEQ ID NO:611), where the PPARγ LBD is underlined (single underline); the CD8α TM domain is double underlined; 4-1BB is in bold text; and CD3 zeta is in bold and italics.

[00333] As another example, in some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises a first polypeptide chain comprising the following amino acid sequence: **DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRF SGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSE**

VKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSA LKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSTT TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT LYCSL***KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL***SRGSGSGSTSPKKENNA LLRYLLDRDDPSDAGGGSGGGSPKKENNALLRYLLDRDDPSDAGGGSGGGSKKENNALLRYLL DRDDPSDAGS* (SEQ ID NO:612), where the anti-CD19 sequence is in bold, the CD8α transmembrane (TM) domain is double underlined, the 4-1BB sequence is in italics and bold text, and the co-regulator peptide is underlined (single underline). In some cases, the second polypeptide chain comprises the following amino acid sequence:

[00334] IYIWAPLAGTCGVLLLSLVITLYCSL**KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL**GSGSGSGSGSGSGSTSESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPF VIYDMNSLMMGEDKIKFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLN DQVTLLKYGVHEIIYTMLASLMNKDGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNA LELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDL RQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDLYGSGSGSGSSL***RVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR*** (SEQ ID NO:613), where the PPARγ LBD is underlined (single underline); the CD8α TM domain is double underlined; 4-1BB is in bold text; and CD3 zeta is in bold and italics.

[00335] As another example, in some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises: a) a first chimeric polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second chimeric polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain, where i) the first and second modulatory domains are derived from 4-1BB; ii) the first and second members of the dimerization pair are CoRNR and an ERα LBD, respectively; and iii) the signaling domain comprises an ITAM. In some cases, the first member of the dimerization pair comprises one copy of a CoRNR co-regulator peptide. In some cases, the CoRNR co-regulator peptide has a length of from 60 amino acids to 70 amino acids. In some cases, the first chimeric polypeptide comprises a single CoRNR co-regulator peptide. In some cases, the first chimeric polypeptide comprises 2 copies of a CoRNR co-regulator peptide. In some cases, the first chimeric polypeptide comprises 3 copies of a CoRNR co-regulator peptide. In some cases, each of the copies of the CoRNR co-regulator peptide has a length of from 15 amino acids to 20 amino acids.

[00336] For example, in some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises a first polypeptide chain comprising: i) a single chain Fv; ii) a transmembrane polypeptide; and iii) a polypeptide comprising the amino acid sequence: ***KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL***SRGSGSGSTSDAFQLRQLILRG LQDDGGGSGGGSDAFQLRQLILRGLQDDGGGSGGGSDAFQLRQLILRGLQDDG (SEQ ID NO:614), where the 4-1BB amino acid sequence is in bold and italics, and the 3x CoRNR co-regulator peptide is underlined. In some cases, the second polypeptide chain comprises the following amino acid sequence: IYIWAPLAGTCGVLLLSLVITLYCSL***KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG CEL***GSGSGSGSGSGSGSTSDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKR SKKNSLALSLTADQMVSALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKR VPGFVDLTLHDQVHLLECAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFD MLLATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAK AGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTS (SEQ ID NO:615), where the TM polypeptide is double underlined, the 4-1BB amino acid sequence is in bold and italics, and the ER LBD is underlined (single underlined).

[00337] In some cases, the first member of a specific binding pair is an scFv specific for CD19, and the first polypeptide chain comprises a transmembrane domain between the anti-CD19 scFv and the first modulatory domain, where the first modulatory domain is 4-1BB. For example, in some cases, a conditionally active, heterodimeric polypeptide of the present disclosure comprises a first polypeptide chain comprising the following amino acid sequence:

[00338] DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFS GSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQE SGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIK DNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCSL*KRGRKKLLY IFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL*SRGSGSGSTSDAFQLRQLILRGLQDDGGGSGG GSDAFQLRQLILRGLQDDGGGSGGGSDAFQLRQLILRGLQDDGS (SEQ ID NO:616), where the anti-CD19 scFv is in bold, the TM region is double underlined, the 4-1BB polypeptide is in bold and italics, and the 3x CoRNR co-regulator peptide is underlined (single underlined). In some cases, the second polypeptide chain comprises the following amino acid sequence: MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLLSL VITLYCSL*KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL*GSGSGSGSGSGSTSD RRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMVSAL LDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECA

WLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEF VCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLLLIL SHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTS (SEQ ID NO:617), where the TM polypeptide is double underlined, the 4-1BB amino acid sequence is in bold and italics, and the ER LBD is underlined (single underlined).

Dimerization agents

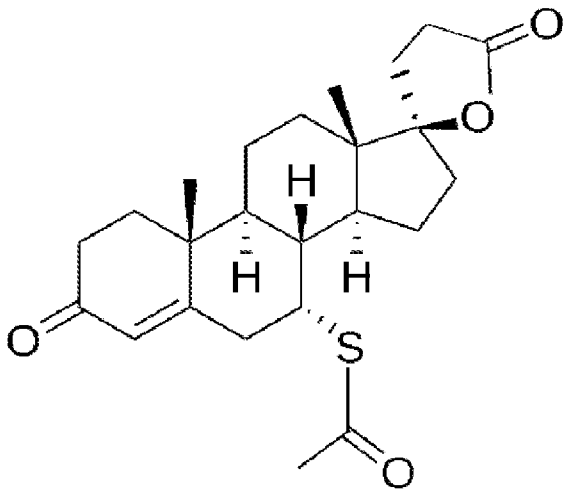
[00339] Suitable dimerization agents (also referred to as dimerizing agents; dimerizers) bind the LBD of a nuclear hormone receptor in a first polypeptide of a conditionally active, heterodimeric polypeptide of the present disclosure, and bind the co-regulator peptide in a second polypeptide of the conditionally active, heterodimeric polypeptide. Binding of to dimerization agent to the LBD and the co-regulator peptide functions to dimerize the first and second polypeptides of a conditionally active, heterodimeric polypeptide of the present disclosure.

[00340] Suitable dimerization agents are known in the art; any known dimerization agent can be used.

[00341] Examples of dimerization agents include corticosterone (11beta,21-dihydroxy-4-pregnene-3,20-dione); deoxycorticosterone (21-hydroxy-4-pregnene-3,20-dione); cortisol (11beta,17,21-trihydroxy-4-pregnene-3,20-dione); 11-deoxycortisol (17,21-dihydroxy-4-pregnene-3,20-dione); cortisone (17,21-dihydroxy-4-pregnene-3,11,20-trione); 18-hydroxycorticosterone (11beta,18,21-trihydroxy-4-pregnene-3,20-dione); 1.alpha.-hydroxycorticosterone (1alpha,11beta,21-trihydroxy-4-pregnene-3,20-dione); aldosterone 18,11-hemiacetal of 11beta,21-dihydroxy-3,20-dioxo-4-pregnen-18-al, androstenedione (4-androstene-3,17-dione); 4-hydroxy-androstenedione; 11β-hydroxyandrostenedione (11 beta-4-androstene-3,17-dione); androstanediol (3-beta,17-beta-Androstanediol); androsterone (3alpha-hydroxy-5alpha-androstan-17-one); epiandrosterone (3beta-hydroxy-5alpha-androstan-17-one); adrenosterone (4-androstene-3,11,17-trione); dehydroepiandrosterone (3beta-hydroxy-5-androsten-17-one); dehydroepiandrosterone sulphate (3beta-sulfoxy-5-androsten-17-one); testosterone (17beta-hydroxy-4-androsten-3-one); epitestosterone (17alpha-hydroxy-4-androsten-3-one); 5α-dihydrotestosterone (17beta-hydroxy-5alpha-androstan-3-one 5β-dihydrotestosterone; 5-beta-dihydroxy testosterone (17beta-hydroxy-5beta-androstan-3-one); 11β-hydroxytestosterone (11beta,17beta-dihydroxy-4-androsten-3-one); 11-ketotestosterone (17beta-hydroxy-4-androsten-3,17-dione), estrone (3-hydroxy-1,3,5(10)-estratrien-17-one); estradiol (1,3,5(10)-estratriene-3,17beta-diol); estriol 1,3,5(10)-estratriene-3,16alpha,17beta-triol; pregnenolone (3-beta-hydroxy-5-pregnen-20-one); 17-hydroxypregnenolone (3-beta,17-dihydroxy-5-pregnen-20-one); progesterone (4-pregnene-3,20-dione); 17-hydroxyprogesterone (17-hydroxy-4-pregnene-3,20-dione); progesterone (pregn-4-ene-3,20-dione); T3 and T4.

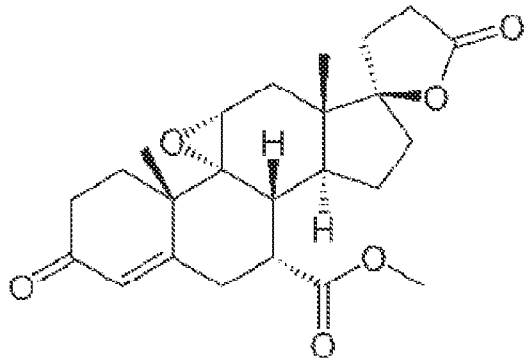
[00342] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of a mineralocorticoid receptor (MR) and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, spironolactone, and eplerenone.

[00343] Spironolactone is a compound of the following structure:



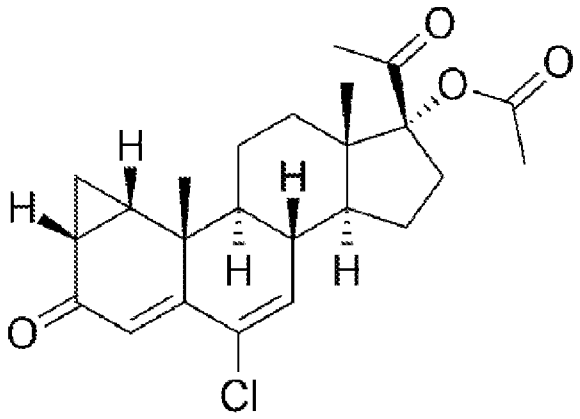
[00344] Spironolactone can be administered at a dose ranging from 10 to 35 mg per day, e.g., 25 mg per day.

[00345] Eplerenone is a compound of the following structure:

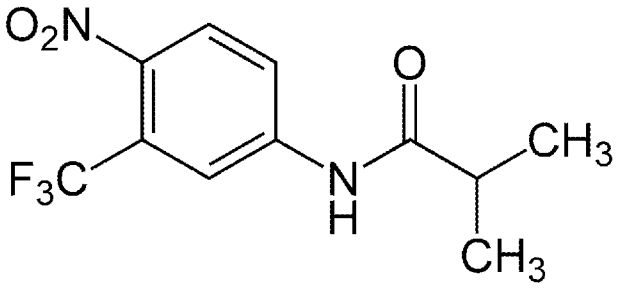


[00346] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of an androgen receptor (AR) and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, cyproterone acetate, hydroxyflutamide, enzalutamide, ARN-509, 3,3'-diindolylmethane (DIM), bexlosteride, bicalutamide, N-butylbenzene-sulfonamide (NBBS), dutasteride, epristeride, finasteride, flutamide, izonsteride, ketoconazole, N-butylbenzene-sulfonamide, nilutamide, megestrol, a steroidal antiandrogen, and turosteride.

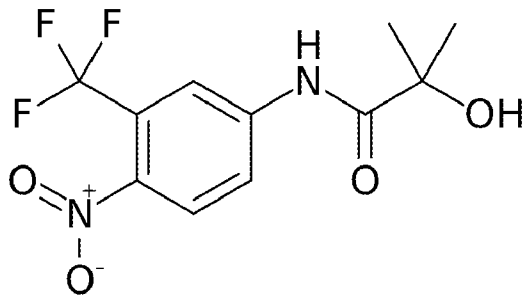
[00347] Cyproterone acetate is a compound of the formula:



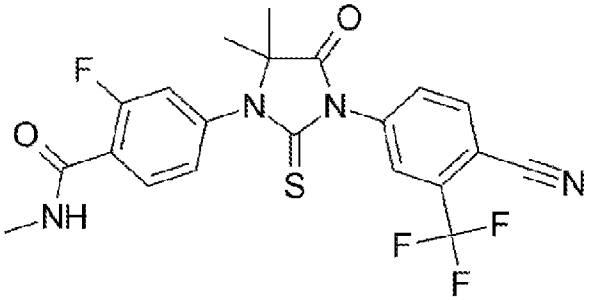
[00348] Flutamide is a compound of the formula:



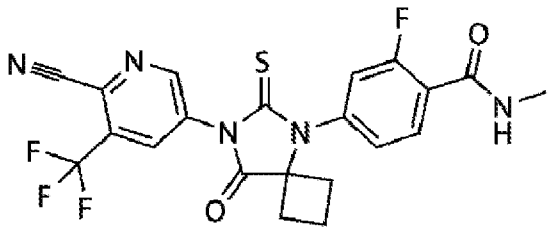
[00349] Hydroxyflutamide is a compound of the formula:



[00350] Enzalutamide is a compound of the formula:



[00351] ARN-509 is a compound of the formula:

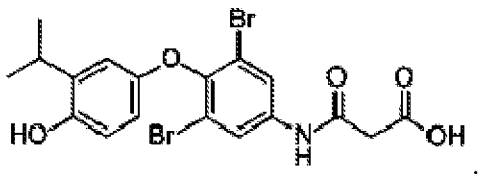


[00352] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of progesterone receptor (PR) and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, mifepristone (RU-486; 11β-[4 N,N-dimethylaminophenyl]-17β -hydroxy-17-(1-propynyl)-estra-4,9-dien-- 3-one); Lilopristone (11β-(4 N,N-dimethylaminophenyl)-17β-hydroxy-17-((Z)-3-hydroxypropenyl)estra-4,9-dien-3-one); onapristone (11β-(4 N,N-dimethylaminophenyl)-17α-hydroxy-17-(3-hydroxypropyl)-13α- estra-4,9-dien-3-one); asoprisnil (benzaldehyde, 4-[(11β,17β)-17-methoxy-17-(methoxymethyl)-3-oxoestra-4,9-dien- 11-yl]-1-(E)-oxim; J867); J912 (4-[17β-Hydroxy-17α-(methoxymethyl)-3-oxoestra-4,9-dien-11β-yl]benzaldehyd-(1E)-oxim); and CDB-2914 (17α-acetoxy-11β-(4-N,N-dimethylaminophenyl)-19-norpregna-4,9- dien-3,20-dione). Other suitable dimerization agents include, e.g., JNJ-1250132, (6α,11β,17β)-11-(4-dimethylaminophenyl)-6-methyl-4',5'-dihydrospiro[estra-4,9-diene-17,2'(3'H)-furan]-3-one (ORG-31710); (11β,17α)-11-(4-acetylphenyl)-17,23-epoxy-19,24-dinorchola-4,9-,20-trien-3-one (ORG-33628); (7β,11β,17β)-11-(4-dimethylaminophenyl-7-methyl]-4',5'-dihydrospiro[estra-4,9-diene-17,2'(3'H)-furan]-3-one (ORG-31806); ZK-112993; ORG-31376; ORG-33245; ORG-31167; ORG-31343; RU-2992; RU-1479; RU-25056; RU-49295; RU-46556; RU-26819; LG1127; LG120753; LG120830; LG1447; LG121046; CGP-19984A; RTI-3021-012; RTI-3021-022; RTI-3021-020; RWJ-25333; ZK-136796; ZK-114043; ZK-230211; ZK-136798; ZK-98229; ZK-98734; ZK-137316; 4-[17β-Methoxy-17α-(methoxymethyl)-3-oxoestra-4,9-dien-11β-yl]benzaldehyde-1-(E)-oxime; 4-[17β-Methoxy-17α-(methoxymethyl)-3-oxoestra-4,9-dien-11β-yl]benzaldehyde-1-(E)-[O-(ethylamino)carbonyl]oxime; 4-[17β-Methoxy-17α-(methoxymethyl)-3-oxoestra-4,9-dien-11β-yl]benzaldehyde-1-(E)-[O-(ethylthio)carbonyl]oxime; (Z)-6'-(4-cyanophenyl)-9,11α-dihydro-17β-hydroxy-17α-[4--(1-oxo-3-methylbutoxy)-1-butenyl]4'H-naphtho[3',2',1';10,9,11]estr-4-en-3- one; 11β-(4-acetylphenyl)-17β-hydroxy-17α-(1,1,2,2,2-penta-fluoroethyl)estra-4,9-dien-3-one; 11β-(4-Acetylphenyl)-19,24-dinor-17,23-epoxy-17alpha-chola-4,9,20-trie- n-3-one; (Z)-11beta,19-[4-(3-Pyridinyl)-o-phenylene]-17beta-hydroxy-17α-[3-hydroxy-1-propenyl]-4-androsten-3-one; 11beta-[4-(1-methylethenyl)phenyl]-17α-hydroxy-17beta-β-hydroxypropyl)-13α-

estra-4,9-dien-3-one; 4',5'-Dihydro-11beta-[4-(dimethylamino)phenyl]-6beta-methylspiro[estra-4,- 9-dien-17beta,2'(3'H)-furan]-3-one, and drospirenone.

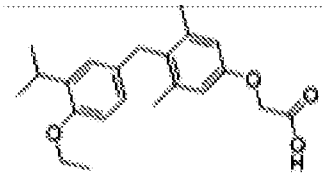
[00353] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of thyroid receptor-beta (TRβ) and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, T_3 (3,5,3'-triiodo-L-thyronine); KB-141 (3,5-dichloro-4-(4-hydroxy-3-isopropylphenoxy)phenylacetic acid); sobetirome (also known as GC-1) (3,5-dimethyl-4-(4'-hydroxy-3'-isopropylbenzyl)-phenoxy acetic acid); GC-24 (3,5-dimethyl-4-(4'-hydroxy-3'-benzyl)benzylphenoxyacetic acid); 4-OH-PCB106 (4-OH-2',3,3',4',5'-pentachlorobiphenyl); eprotirome; MB07811 ((2*R*,4*S*)-4-(3-chlorophenyl)-2-[(3,5-dimethyl-4-(4′-hydroxy-3′-isopropylbenzyl)phenoxy)methyl]-2-oxido-[1,3,2]-dioxaphosphonane); QH2; and (3,5-dimethyl-4-(4′-hydroxy-3′-isopropylbenzyl)phenoxy)methylphosphonic acid (MB07344).

[00354] Eprotirome has the following structure:



.

[00355] QH2 has the following structure:



.

[00356] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of estrogen receptor-alpha (ERα) and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, tamoxifen, 4-OH-tamoxifen, raloxifene, lasofoxifene, bazedoxifene, falsodex, clomifene, femarelle, ormeloxifene, toremifiene, ospemifene, and ethinyl estradiol.

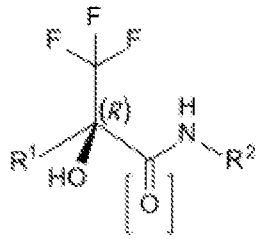
[00357] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of estrogen receptor-beta (ERβ) and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, estradiol (E2; or 17-beta-estradiol), and ethinyl estradiol.

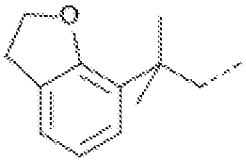
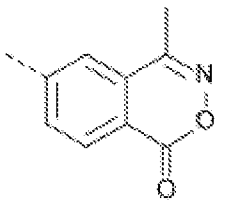
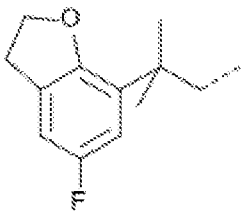
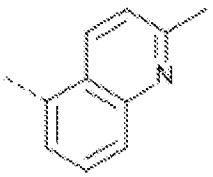
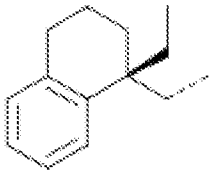
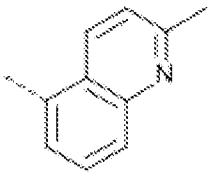
[00358] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of a PPARγ and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, a thiazolidinedione (e.g., rosiglitazone, pioglitazone, lobeglitazone, troglitazone), farglitazar, aleglitazar, and fenofibric acid.

[00359] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of a GR and a corresponding co-regulator peptide, a suitable dimerization agent can be a selective GR

agonist (SEGRA) or a selective GR modulator (SEGRM). Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of a GR and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, benzopyranoquinoline A 276575, Mapracorat, ZK 216348, 55D1E1, dexamethasone, prednisolone, prednisone, methylprednisolone, fluticasone propionate, beclomethasone-17-monopropionate, betamethasone, rimexolone, paramethasone, and hydrocortisone.

[00360] Non-limiting examples are shown below.



designation	R^1	R^2	
ZK 216348			amide
Mapracorat			amine
55D1E1			amine

[00361] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of a VDR and a corresponding co-regulator peptide, a suitable dimerization agent can be 1,25-dihydroxyvitamin D3 (calcitriol), paricalitol, doxercalciferol, 25-hydroxyvitamin D3 (calcifediol), cholecalciferol, ergocalciferol, tacalciol, 22-dihydroergocalciferol, (6Z)-Tacalciol, 2-methylene-19-nor-

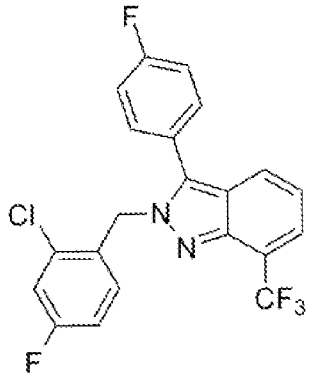
20(S)-1α-hydroxy-bishomopregnacalciferol, 19-nor-26,27-dimethylene-20(S)-2-methylene-1α,25-dihydroxyvitamin D3, 2-methylene-1α,25-dihydroxy-(17E)-17(20)-dehydro-19-nor-vi- tamin D3, 2-methylene-19-nor-(24R)-1α,25-dihydroxyvitamin D2, 2-methylene-(20R,25S)-19,26-dinor-1α,25-dihydroxyvitamin D3, 2-methylene-19-nor-1α-hydroxy-pregnacalciferol, 1α-hydroxy-2-methylene-19-nor-homopregnacalciferol, (20R)-1α-hydroxy-2-methylene-19-nor-bishomopregnacalciferol, 2-methylene-19-nor-(20S)-1α-hydroxy-trishomopregnacalciferol, 2-methylene-23,23-difluoro-1α-hydroxy-19-nor-bishomopregnacalcifero- l, 2-methylene-(20S)-23,23-difluoro-1α-hydroxy-19-nor-bishomopregnan- calciferol, (2-(3' hydroxypropyl-1',2'-idene)-19,23,24-trinor-(20S)-1α-hydroxyvitamin D3, 2-methylene-18,19-dinor-(20S)-1α,25-dihydroxyvitamin D3, and the like.

[00362] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of a RARβ and a corresponding co-regulator peptide, a suitable dimerization agent can be retinoic acid, all-trans-retinoic acid, 9-cis-retinoic acid, tamibarotene, 13-cis-retinoic acid, (2E,4E,6Z,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexeneyl)nona-2,4,6,- 8-tetraenoic acid, 9-(4-methoxy-2,3,6-trimethyl-phenyl)-3,7-dimethyl-nona-2,4,6,8-tetraenoic acid, 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-napthoic acid, 4-[1-(3,5,5,8,8-pentamethyl-tetralin-2-yl)ethenyl]benzoic acid, retinobenzoic acid, ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)ethynyl]pyridine-3-carboxylate, retinoyl t-butyrate, retinoyl pinacol, and retinoyl cholesterol.

[00363] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of an FXR and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, obeticholic acid, LY2562175 (6-(4-((5-cyclopropyl-3-(2,6-dichlorophenyl)isoxazol-4-yl)methoxy)piperidin-1-yl)-1-methyl-1H-indole-3-carboxylic acid), and GW4064 (3-[2-[2-Chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]ethenyl]benzoic acid).

[00364] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of an LXRα and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, T0901317 (*N*-(2,2,2-Trifluoroethyl)-*N*-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]benzenesulfonamide), GW3965 (3-[3-[[[2-Chloro-3-(trifluoromethyl)phenyl]methyl](2,2-diphenylethyl)amino]propoxy]benzeneacetic acid hydrochloride), and LXR-623.

[00365] LXR-632 has the following structure:

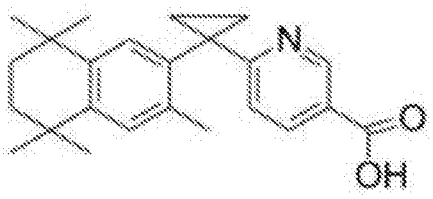


[00366] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of an RORγ and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, GNE-3500 (27, 1-{4-[3-fluoro-4-((3S,6R)-3-methyl-1,1-dioxo-6-phenyl-[1,2]thiazinan-2-ylmethyl)-phenyl]-piperazin-1-yl}-ethanone).

[00367] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of an RORγ and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, 7β, 27-dihydroxycholesterol, and 7α, 27-dihydroxycholesterol.

[00368] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of an RXRα and a corresponding co-regulator peptide, a suitable dimerization agent includes, but is not limited to, 9-cis retinoic acid, LGD100268, CD3254 (3-[4-Hydroxy-3-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)phenyl]-2-propenoic acid), and CD2915 (Sorensen et al. (1997) *Skin Pharmacol.* 10:144).

[00369] LGD100268 has the following structure:



[00370] Where a conditionally active, heterodimeric polypeptide of the present disclosure comprises an LBD of a PXR and a corresponding co-regulator peptide, a suitable dimerization agent can be rifampicin, chlotrimazole, and lovastatin.

CONDITIONALLY ACTIVE OFF-SWITCH CAR

[00371] In some cases, a conditionally active heterodimeric polypeptide of the present disclosure is a conditionally active off-switch CAR. A conditionally active off-switch CAR of the present disclosure is also referred to as a heteromeric, conditionally repressible synthetic immune cell receptor (ICR). A conditionally active off-switch CAR of the present disclosure comprises: a synthetic stimulatory ICR comprising a first member of a dimerization pair linked to the synthetic stimulatory ICR; and a synthetic

ICR repressor comprising a second member of the dimerization pair linked to an intracellular inhibitory domain. A heteromeric, conditionally repressible synthetic ICR of the present disclosure will generally include a synthetic stimulatory ICR and a synthetic ICR repressor configured such that upon introduction of a dimerizing agent, the synthetic ICR repressor dimerizes with the synthetic stimulatory ICR to repress activation due to the synthetic stimulatory ICR. Examples of heterodimeric conditionally active off-switch CAR polypeptides include but are not limited to e.g., those described in PCT International Application No. PCT/US2016/062612; the disclosure of which is incorporated herein by reference in its entirety.

[00372] The configuration of the heteromeric, conditionally repressible synthetic ICR will vary depending on the particular context within which repression of a synthetic stimulatory ICR is desired. In some instances, the stimulatory portion of the heteromeric, conditionally repressible synthetic ICR may be referred to as Part 1 of the heteromeric, conditionally repressible synthetic ICR. In some instances, the repressor portion of the heteromeric, conditionally repressible synthetic ICR may be referred to as Part 2 of the heteromeric, conditionally repressible synthetic ICR. Thus, a heteromeric, conditionally repressible synthetic ICR collectively refers to a multi-modular protein or protein complex that includes various modules including the stimulatory portion (e.g., synthetic stimulatory ICR, Part 1, etc.) and the repressor portion (e.g., synthetic ICR repressor, Part 2, etc.) whether or not the various modules are or are not present or were or were not present at some point within the same protein and whether or not the various modules are expressed from the same or different nucleic acid constructs.

[00373] One of skill in the art will readily recognize from the instant disclosure that first and second parts (e.g., stimulatory and inhibitory parts) will individually include first and second portions of a dimerizing pair and that such portions of the dimerizing pair may be interchangeable between the first and second parts of the heteromeric, conditionally repressible synthetic ICR. One of skill in the art will also readily recognize from the instant disclosure that individual domains of heteromeric, conditionally repressible synthetic ICR may be rearranged in many instances, in order and/or orientation, while maintaining the functions of being activatable and repressible as described herein. As such, description of a particular configuration of a heteromeric, conditionally repressible synthetic ICR described herein also includes wherein the modules of the heteromeric, conditionally repressible synthetic ICR are rearranged without abolishing the primary functions of the heteromeric, conditionally repressible synthetic ICR. Such rearrangements may also include the inclusion or exclusion of particular optional modules (including e.g., linkers, reporters, etc.) that do not result in abolishment of the primary functions of the heteromeric, conditionally repressible synthetic ICR due to their inclusion or exclusion from the heteromeric, conditionally repressible synthetic ICR.

Synthetic Stimulatory ICR

[00374] As described herein, a heteromeric, conditionally repressible synthetic ICR includes a synthetic stimulatory ICR, also referred to herein as a "stimulatory ICR" or "stimulatory part" for simplicity. Such stimulatory ICRs will vary depending on the particular context of immune cell stimulation to which the construct is directed and will generally function to mediate activation of the immune cell expressing the stimulatory ICR. Thus, a stimulatory ICR includes an extracellular domain that upon reception of a specific signal functions to transduce the signal to intracellularly to activate the immune cell expressing the stimulatory ICR.

[00375] In some instances, the extracellular component of a stimulatory ICR therefore may include an extracellular recognition domain, described in more detail below, which contains one member of a specific binding pair. Specific binding pairs include, but are not limited to, antigen-antibody binding pairs; ligand-receptor binding pairs; and the like. Thus, a member of a specific binding pair suitable for use in an extracellular recognition domain of the present disclosure includes an antigen; an antibody; a ligand; and a ligand-binding receptor. Suitable extracellular recognition domains for use in a stimulatory ICR include but are not limited to e.g., members of specific binding pairs (e.g., antigen-binding domain containing members of specific binding pairs) including e.g., those described above with relationship to conditionally acitive heterodimeric On-switch CARs above.

[00376] A stimulatory ICR further includes one or more intracellular stimulation domains that, upon activation of one or more extracellular domains, mediates intracellular signaling leading to activation of the immune cell expressing the stimulatory ICR. Domains useful as signaling domains will vary depending on the particular context of immune cell activation, including e.g., the particular type of cell to be activated and the desired degree of activation. Exemplary non-limited examples of stimulatory domains, described in greater detail below, include but are not limited to domains and motifs thereof derived from immune stimulatory molecules including, e.g., co-stimulatory molecules, immune receptors and the like. Suitable intracellular stimulation domains for use in a stimulatory ICR include but are not limited to e.g., modulatory domains and portions thereof including e.g., those described above with relationship to conditionally acitive heterodimeric On-switch CARs above.

[00377] In some instances, stimulatory ICRs may be or may be derived from engineered or synthetic immune regulatory constructs designed for therapeutic immune system modulation including but not limited to e.g., chimeric antigen receptors (CARs) and derivatives, engineered T cell receptors (TCRs) and derivatives and the like. Engineered CARs, TCRs and derivatives thereof useful as the basis for a synthetic ICR include those CARs, TCRs and derivatives thereof that are activatable, e.g., are activated upon binding of a binding partner to the CAR, TCR or derivative thereof, and upon activation transduce the signal intracellularly to activate the immune cell expressing the CAR, TCR or derivative thereof. In some instances, a stimulatory ICR may be conditionally activatable such that activation upon binding of

a binding partner to the stimulatory ICR requires an additional event for transduction of the activation signal including e.g., dimerization of components of the stimulatory ICR.

[00378] A stimulatory ICR further includes, as described in more detail below, a domain (e.g., a first member or a second member) of a dimerization pair. Useful dimerization pairs will vary depending on the desired dimerizer and the desired relative position of the member of the dimerization pair within the stimulatory ICR. Generally, the presence of a first member of a dimerization pair within the stimulatory ICR mediates the dimerization, upon introduction of the dimerizer, with a second member of the dimerization pair present in the ICR repressor such that upon dimerization the ICR repressor represses any immune cell activation due to the stimulatory ICR.

[00379] In some instances, a stimulatory ICR may further include additional domains. Such additional domains may be functional, e.g., they directly contribute to the immune cell activation function of the stimulatory ICR, or non-functional, e.g., they do not directly contribute to the activation function of the stimulatory ICR. Non-functional additional domains may include domains having various purposes that do not directly affect the ability of the stimulatory ICR to activate immune cell function including, but not limited to, e.g., structural functions, linker functions, etc.

Chimeric Antigen Receptor (CAR)

[00380] In some instances, a heteromeric, conditionally repressible synthetic ICR may include, in part or in whole, a CAR or may essentially be a modified CAR such that by modification the CAR is conditionally repressible. In such instances, the CAR containing heteromeric, conditionally repressible synthetic ICR may be referred to as a heterodimeric, conditionally repressible synthetic CAR or, for simplicity, a repressible CAR. Any CAR having immune cell activation function may find use in a heteromeric, conditionally repressible synthetic ICR as described herein including but not limited to, e.g., those CAR variants described herein.

[00381] In some instances, a CAR may be modified for use as a component of a heterodimeric, conditionally repressible synthetic ICR through introduction or insertion of a dimerization domain (e.g., a member of a dimerization pair) into the CAR and, in such instances, following modification, the CAR may be referred to as a dimerizer-domain containing CAR or a dimerizable CAR.

[00382] A dimerizer domain may be inserted into the CAR amino acid sequence, e.g., by introducing a coding sequence for the dimerizer domain into the coding sequence of the CAR, at any convenient location provided the insertion does not negatively impact the primary functional domains of the CAR (including e.g., the extracellular recognition domain, the immune activation domain(s), etc.) and/or the negatively impact the dimerization function of the dimerizer domain.

[00383] In some instances, the dimerizer may be inserted into an extracellular portion of the CAR. In some instances the dimerizer may be inserted into an intracellular portion of the CAR. In some instances, the

dimerizer may be inserted such that following insertion the dimerizer is linked to an extracellular recognition domain of the CAR. In some instances, the dimerizer may be inserted such that following insertion the dimerizer is linked to a transmembrane domain of the CAR. In some instances, the dimerizer may be inserted such that following insertion the dimerizer is linked to the extracellular side of a transmembrane domain of the CAR. In some instances, the dimerizer may be inserted such that following insertion the dimerizer is linked to the intracellular side of a transmembrane domain of the CAR. In some instances, the dimerizer may be inserted such that following insertion the dimerizer is linked to an immune stimulatory domain of the CAR. In some instances, the dimerizer may be inserted such that following insertion the dimerizer is linked to a co-stimulation domain of the CAR. In some instances, the dimerizer may be inserted such that following insertion the dimerizer is at the N-terminal end of the CAR. In some instances, the dimerizer may be inserted such that following insertion the dimerizer is at the C-terminal end of the CAR.

[00384] In instances where a heteromeric, conditionally repressible synthetic ICR includes, in part or in whole, or the heteromeric, conditionally repressible synthetic ICR is essentially a modified CAR, the CAR may contain an extracellular recognition domain, a stimulatory domain and a transmembrane domain. Such a CAR may optionally include linker regions and/or hinge regions. CARs as part of a heteromeric, conditionally repressible synthetic ICR may be encompassed within a single polypeptide or may be "split" across two or more polypeptides.

Extracellular Recognition Domain

[00385] A repressible CAR includes a member of a specific binding pair. Specific binding pairs include, but are not limited to, antigen-antibody binding pairs; ligand-receptor binding pairs; and the like. Thus, a member of a specific binding pair suitable for use in a repressible CAR of the present disclosure includes an antigen; an antibody; a ligand; and a ligand-binding receptor.

Antigen binding domain

[00386] An antigen-binding domain suitable for use in a repressible CAR of the present disclosure can be any antigen-binding polypeptide, a wide variety of which are known in the art. In some instances, the antigen-binding domain is a single chain Fv (scFv). Other antibody based recognition domains (cAb VHH (camelid antibody variable domains) and humanized versions, IgNAR VH (shark antibody variable domains) and humanized versions, sdAb VH (single domain antibody variable domains) and "camelized" antibody variable domains are suitable for use. In some instances, T-cell receptor (TCR) based recognition domains such as single chain TCR (scTv, single chain two-domain TCR containing VαVβ) are also suitable for use. Such TCR recognition domains when present as a repressible engineered TCR rather than a component of a repressible CAR as described in more detail below.

[00387] An antigen-binding domain suitable for use in a repressible CAR of the present disclosure can have a variety of antigen-binding specificities. In some cases, the antigen-binding domain is specific for an

epitope present in an antigen that is expressed by (synthesized by) a cancer cell, i.e., a cancer cell associated antigen. The cancer cell associated antigen can be an antigen associated with, e.g., a breast cancer cell, a B cell lymphoma, a Hodgkin lymphoma cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma, a lung cancer cell (e.g., a small cell lung cancer cell), a non-Hodgkin B-cell lymphoma (B-NHL) cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma cell, a lung cancer cell (e.g., a small cell lung cancer cell), a melanoma cell, a chronic lymphocytic leukemia cell, an acute lymphocytic leukemia cell, a neuroblastoma cell, a glioma, a glioblastoma, a medulloblastoma, a colorectal cancer cell, etc. A cancer cell associated antigen may also be expressed by a non-cancerous cell.

[00388] Non-limiting examples of antigens to which an antigen-binding domain of a subject repressible CAR can bind include, e.g., CD19, CD20, CD38, CD30, Her2/neu/ERBB2, CA125, MUC-1, prostate-specific membrane antigen (PSMA), CD44 surface adhesion molecule, mesothelin, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), EGFRvIII, vascular endothelial growth factor receptor-2 (VEGFR2), high molecular weight-melanoma associated antigen (HMW-MAA), MAGE-A1, IL-13R-a2, GD2, and the like. Further examples of antigens to which an antigen-binding domain of a subject repressible CAR can bind include but are not limited to those described above with relevance to conditionally active on-switch CARs.

Ligand

[00389] In some cases, a member of a specific binding pair suitable for use in a subject repressible CAR is a ligand for a receptor. Ligands include, but are not limited to, cytokines (e.g., IL-13, etc.); growth factors (e.g., heregulin; vascular endothelial growth factor (VEGF); and the like); an integrin-binding peptide (e.g., a peptide comprising the sequence Arg-Gly-Asp); and the like.

[00390] Where the member of a specific binding pair in a subject repressible CAR is a ligand, the repressible CAR can be activated in the presence of both a dimerizer agent and a second member of the specific binding pair, where the second member of the specific binding pair is a receptor for the ligand. For example, where the ligand is VEGF, the second member of the specific binding pair can be a VEGF receptor, including a soluble VEGF receptor. As another example, where the ligand is heregulin, the second member of the specific binding pair can be Her2.

Receptors

[00391] As noted above, in some cases, the member of a specific binding pair that is included in a subject repressible CAR is a receptor, e.g., a receptor for a ligand, a co-receptor, etc. The receptor can be a ligand-binding fragment of a receptor. Suitable receptors include, but are not limited to, a growth factor receptor (e.g., a VEGF receptor); a killer cell lectin-like receptor subfamily K, member 1 (NKG2D) polypeptide (receptor for MICA, MICB, and ULB6); a cytokine receptor (e.g., an IL-13 receptor; an IL-2

receptor; etc.); Her2; CD27; a natural cytotoxicity receptor (NCR) (e.g., NKP30 (NCR3/CD337) polypeptide (receptor for HLA-B–associated transcript 3 (BAT3) and B7-H6); etc.); etc.

Stimulatory Domain

[00392] A stimulatory domain suitable for use in a stimulatory CAR of a subject repressible ICR may be any functional unit of a polypeptide as short as a 3 amino acid linear motif and as long as an entire protein, where size of the stimulatory domain is restricted only in that the domain must be sufficiently large as to retain its function and sufficiently small so as to be compatible with the other components of the repressible CAR. Accordingly, a stimulatory domain may range in size from 3 amino acids in length to 1000 amino acids or more and, in some instances, can have a length of from about 30 amino acids to about 70 amino acids (aa), e.g., a stimulatory domain can have a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa. In other cases, stimulatory domain can have a length of from about 70 aa to about 100 aa, from about 100 aa to about 200 aa, or greater than 200 aa.

[00393] In some instances, "co-stimulatory domains" find use as stimulatory domains of a repressible CAR of the present disclosure. Co-stimulation generally refers to a secondary non-specific activation mechanism through which a primary specific stimulation is propagated. Examples of co-stimulation include antigen nonspecific T cell co-stimulation following antigen specific signaling through the T cell receptor and antigen nonspecific B cell co-stimulation following signaling through the B cell receptor. Co-stimulation, e.g., T cell co-stimulation, and the factors involved have been described in Chen & Flies. *Nat Rev Immunol* (2013) 13(4):227-42, the disclosure of which are incorporated herein by reference in their entirety. Co-stimulatory domains are generally polypeptides derived from receptors. In some embodiments, co-stimulatory domains homodimerize. A subject co-stimulatory domain can be an intracellular portion of a transmembrane protein (i.e., the co-stimulatory domain can be derived from a transmembrane protein). Non-limiting examples of suitable co-stimulatory polypeptides include, but are not limited to, 4-1BB (CD137), CD28, ICOS, OX-40, BTLA, CD27, CD30, GITR, and HVEM. In some instances, a co-stimulatory domain, e.g., as used in repressible CAR of the instant disclosure may include a co-stimulatory domain listed in **Table 1**. In some instances, a co-stimulatory domain of a repressible CAR comprises a an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to a co-stimulatory domain as described herein.

[00394] In some instances, a stimulatory CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of a transmembrane protein listed in **Table 1**. For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about

98%, or 100% amino acid sequence identity to an amino acid sequence listed in **Table 1**. In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, from about 95 aa to about 100 aa, from about 100 aa to about 105 aa, from about 105 aa to about 110 aa, from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 125 aa, from about 125 aa to about 130 aa, from about 130 aa to about 135 aa, from about 135 aa to about 140 aa, from about 140 aa to about 145 aa, from about 145 aa to about 150 aa, from about 150 aa to about 155 aa, from about 155 aa to about 160 aa, from about 160 aa to about 165, aa from about 165 aa to about 170 aa, from about 170 aa to about 175 aa, from about 175 aa to about 180 aa, from about 180 aa to about 185 aa, or from about 185 aa to about 190 aa.

[00395] In some cases, a repressible CAR may contain two more stimulatory domains, present on the same or different polypeptides. In some instances, where the repressible CAR contains two more stimulatory domains, the stimulatory domains may have substantially the same amino acid sequences. For example, in some cases, the first stimulatory domain comprises an amino acid sequence that is at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, identical to the amino acid sequence of the second stimulatory domain. In some instances, where the repressible CAR contains two more stimulatory domains, the stimulatory domains of the subject repressible CAR can have substantially the same length; e.g., the first and second stimulatory domains can differ in length from one another by fewer than 10 amino acids, or fewer than 5 amino acids. In some instances, where the repressible CAR contains two more stimulatory domains, the first and second stimulatory domains have the same length. In some instances, where the repressible CAR contains two more stimulatory domains, the two stimulatory domains are the same.

[00396] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein 4-1BB (also known as TNFRSF9; CD137; 4-1BB; CDw137; ILA; etc.). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:618). In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to

about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00397] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein CD28 (also known as Tp44). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:619). In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00398] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein ICOS (also known as AILIM, CD278, and CVID1). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: TKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL (SEQ ID NO:620). In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00399] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein OX-40 (also known as TNFRSF4, RP5-902P8.3, ACT35, CD134, OX40, TXGP1L). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: RRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO:621). In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00400] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein BTLA (also

known as BTLA1 and CD272). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence:
CCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCFRMQEG
SEVYSNPCLEENKPGIVYASLNHSVIGPNSRLARNVKEAPTEYASICVRS (SEQ ID NO:622).

[00401] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein CD27 (also known as S152, T14, TNFRSF7, and Tp55). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence:
HQRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO:623). In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00402] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein CD30 (also known as TNFRSF8, D1S166E, and Ki-1). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, from about 150 aa to about 160 aa, or from about 160 aa to about 185 aa of the following amino acid sequence:
RRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETC
HSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEG
RGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK (SEQ ID NO:624).

[00403] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein GITR (also known as TNFRSF18, RP5-902P8.2, AITR, CD357, and GITR-D). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid

sequence identity to the following amino acid sequence: HIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPEEERGERSAEEKGRLGDLWV (SEQ ID NO:625). In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00404] In some instances, a repressible CAR may contain an intracellular signaling domain, e.g., a co-stimulatory domain, derived from an intracellular portion of the transmembrane protein HVEM (also known as TNFRSF14, RP3-395M20.6, ATAR, CD270, HVEA, HVEM, LIGHTR, and TR2). For example, a suitable co-stimulatory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: CVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRSPNH (SEQ ID NO:626). In some of these embodiments, the co-stimulatory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00405] In some instances, a repressible CAR may contain an intracellular signaling domain that includes at least one (e.g., one, two, three, four, five, six, etc.) intracellular signaling motif. In some instances, the intracellular signaling motif may be an immunoreceptor tyrosine-based activation motif (ITAM). In some instances, the intracellular signaling motif, e.g., an ITAM motif is within an intracellular signaling domain derived from a signaling molecule that contains one or more ITAM motifs. In other instances, the ITAM is derived, e.g., synthetically produced, within an amino acid sequence de novo, e.g., through mutation of the amino acid sequence.

[00406] An ITAM motif is YX_1X_2L/I, where X_1 and X_2 are independently any amino acid (SEQ ID NO:564). In some cases, the intracellular signaling domain of a subject repressible CAR comprises 1, 2, 3, 4, or 5 ITAM motifs. In some cases, an ITAM motif is repeated twice in an intracellular signaling domain, where the first and second instances of the ITAM motif are separated from one another by 6 to 8 amino acids, e.g., $(YX_1X_2L/I)(X_3)_n(YX_1X_2L/I)$, where n is an integer from 6 to 8, and each of the 6-8 X_3 can be any amino acid (SEQ ID NO:565). In some cases, the intracellular signaling domain of a subject repressible CAR comprises 3 ITAM motifs.

[00407] A suitable intracellular signaling domain can be an ITAM motif-containing portion that is derived from a polypeptide that contains an ITAM motif. For example, a suitable intracellular signaling domain can be an ITAM motif-containing domain from any ITAM motif-containing protein. Thus, a suitable intracellular signaling domain need not contain the entire sequence of the entire protein from which it is

derived. Examples of suitable ITAM motif-containing polypeptides include, but are not limited to: DAP12; FCER1G (Fc epsilon receptor I gamma chain); CD3D (CD3 delta); CD3E (CD3 epsilon); CD3G (CD3 gamma); CD3Z (CD3 zeta); and CD79A (antigen receptor complex-associated protein alpha chain).

[00408] In some cases, the intracellular signaling domain is derived from DAP12 (also known as TYROBP; TYRO protein tyrosine kinase binding protein; KARAP; PLOSL; DNAX-activation protein 12; KAR-associated protein; TYRO protein tyrosine kinase-binding protein; killer activating receptor associated protein; killer-activating receptor-associated protein; etc.). For example, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to any of the following amino acid sequences (4 isoforms): MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFL GRLVPRGRGAAEAATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:627); MGGLEPCSRLLLLPLLLAVSGLRPVQAQAQSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFL GRLVPRGRGAAEATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:628); MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAA EAATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:629); or MGGLEPCSRLLLLPLLLAVSDCSCSTVSPGVLAGIVMGDLVLTVLIALAVYFLGRLVPRGRGAA EATRKQRITETESP**YQEL**QGQRSDV**YSDL**NTQRPYYK (SEQ ID NO:630), where the ITAM motifs are in bold and are underlined.

[00409] Likewise, a suitable intracellular signaling domain can comprise an ITAM motif-containing portion of the full length DAP12 amino acid sequence. Thus, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: ESP**YQEL**QGQRSDV**YSDL**NTQ (SEQ ID NO:631), where the ITAM motifs are in bold and are underlined.

[00410] In some cases, the intracellular signaling domain is derived from FCER1G (also known as FCRG; Fc epsilon receptor I gamma chain; Fc receptor gamma-chain; fc-epsilon RI-gamma; fcRgamma; fceRI gamma; high affinity immunoglobulin epsilon receptor subunit gamma; immunoglobulin E receptor, high affinity, gamma chain; etc.). For example, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITSYEKSDGV**YT**

GLSTRNQET**YETL**KHEKPPQ (SEQ ID NO:632), where the ITAM motifs are in bold and are underlined.

[00411] Likewise, a suitable intracellular signaling domain can comprise an ITAM motif-containing portion of the full length FCER1G amino acid sequence. Thus, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: DGV**YTGL**STRNQET**YETL**KHE (SEQ ID NO:633), where the ITAM motifs are in bold and are underlined.

[00412] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 delta chain (also known as CD3D; CD3-DELTA; T3D; CD3 antigen, delta subunit; CD3 delta; CD3d antigen, delta polypeptide (TiT3 complex); OKT3, delta chain; T-cell receptor T3 delta chain; T-cell surface glycoprotein CD3 delta chain; etc.). For example, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 170 aa, of either of the following amino acid sequences (2 isoforms):
MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDP
RGIYRCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVIATLLLALGVFCFAGHETG
RLSGAADTQALLRNDQV**YQPL**RDRDDAQ**YSHL**GGNWARNK (SEQ ID NO:634) or
MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDP
RGIYRCNGTDIYKDKESTVQVHYRTADTQALLRNDQV**YQPL**RDRDDAQ**YSHL**GGNWARNK
(SEQ ID NO:635) , where the ITAM motifs are in bold and are underlined.

[00413] Likewise, a suitable intracellular signaling domain can comprise an ITAM motif-containing portion of the full length CD3 delta amino acid sequence. Thus, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: DQV**YQPL**RDRDDAQ**YSHL**GGN (SEQ ID NO:636), where the ITAM motifs are in bold and are underlined.

[00414] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 epsilon chain (also known as CD3e, T-cell surface antigen T3/Leu-4 epsilon chain, T-cell surface glycoprotein CD3 epsilon chain, AI504783, CD3, CD3epsilon, T3e, etc.). For example, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%,

amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 205 aa, of the following amino acid sequence:

[00415] MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQH NDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVCENCME MDVMSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVTRGAGAGGRQRGQNKERPPPVPNPD **YEPI**RKGQRDL**YSGL**NQRRI (SEQ ID NO:637), where the ITAM motifs are in bold and are underlined.

[00416] Likewise, a suitable intracellular signaling domain can comprise an ITAM motif-containing portion of the full length CD3 epsilon amino acid sequence. Thus, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: NPD**YEPI**RKGQRDL**YSGL**NQR (SEQ ID NO:638), where the ITAM motifs are in bold and are underlined.

[00417] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 gamma chain (also known as CD3G, T-cell receptor T3 gamma chain, CD3-GAMMA, T3G, gamma polypeptide (TiT3 complex), etc.). For example, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 180 aa, of the following amino acid sequence:
MEQGKGLAVLILAIILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGF LTEDKKKWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRMCQNCIELNAATISGFLFAEIVSIFV LAVGVYFIAGQDGVRQSRASDKQTLLPNDQL**YQPL**KDREDDQ**YSHL**QGNQLRRN (SEQ ID NO:639), where the ITAM motifs are in bold and are underlined.

[00418] Likewise, a suitable intracellular signaling domain can comprise an ITAM motif-containing portion of the full length CD3 gamma amino acid sequence. Thus, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: DQL**YQPL**KDREDDQ**YSHL**QGN (SEQ ID NO:640), where the ITAM motifs are in bold and are underlined.

[00419] In some cases, the intracellular signaling domain is derived from T-cell surface glycoprotein CD3 zeta chain (also known as CD3Z, T-cell receptor T3 zeta chain, CD247, CD3-ZETA, CD3H, CD3Q, T3Z, TCRZ, etc.). For example, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 140 aa, from about 140 aa to about 150 aa, or from about 150 aa to about 160 aa, of either of the following amino acid sequences (2 isoforms):
MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQ QGQNQL**YNEL**NLGRREE**YDVL**DKRRGRDPEMGGKPRRKNPQEGL**YNEL**QKDKMAEA**YSEI**G MKGERRRGKGHDGL**YQGL**STATKDT**YDAL**HMQALPPR (SEQ ID NO:641) or
MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSADAPAYQ QGQNQL**YNEL**NLGRREE**YDVL**DKRRGRDPEMGGKPQRRKNPQEGL**YNEL**QKDKMAEA**YSEI** GMKGERRRGKGHDGL**YQGL**STATKDT**YDAL**HMQALPPR(SEQ ID NO:642) , where the ITAM motifs are in bold and are underlined.

[00420] Likewise, a suitable intracellular signaling domain can comprise an ITAM motif-containing portion of the full length CD3 zeta amino acid sequence. Thus, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to any of the following amino acid sequences:
RVKFSRSADAPAYQQGQNQL**YNEL**NLGRREE**YDVL**DKRRGRDPEMGGKPRRKNPQEGL**YNE L**QKDKMAEA**YSEI**GMKGERRRGKGHDGL**YQGL**STATKDT**YDAL**HMQALPPR (SEQ ID NO:643); NQL**YNEL**NLGRREE**YDVL**DKR (SEQ ID NO:644); EGL**YNEL**QKDKMAEA**YSEI**GMK (SEQ ID NO:645); or DGL**YQGL**STATKDT**YDAL**HMQ (SEQ ID NO:646), where the ITAM motifs are in bold and are underlined.

[00421] In some cases, the intracellular signaling domain is derived from CD79A (also known as B-cell antigen receptor complex-associated protein alpha chain; CD79a antigen (immunoglobulin-associated alpha); MB-1 membrane glycoprotein; ig-alpha; membrane-bound immunoglobulin-associated protein; surface IgM-associated protein; etc.). For example, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to a contiguous stretch of from about 100 amino acids to about 110 amino acids (aa), from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 130 aa, from about 130 aa to about 150 aa, from about 150 aa to about 200 aa, or from about 200 aa to about 220 aa, of either of the

following amino acid sequences (2 isoforms):
MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSNNAN
VTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKSHGGIYVCRVQEGNESYQQSCGTYLRVR
QPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQNEKLGLDAGDEYEDENL**YEGL**
NLDDCSM**YEDI**SRGLQGTYQDVGSLNIGDVQLEKP (SEQ ID NO:647); or
MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSNNAN
VTWWRVLHGNYTWPPEFLGPGEDPNEPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFR
KRWQNEKLGLDAGDEYEDENLYEGLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKP
(SEQ ID NO:648) , where the ITAM motifs are in bold and are underlined.

[00422] Likewise, a suitable intracellular signaling domain can comprise an ITAM motif-containing portion of the full length CD79A amino acid sequence. Thus, a suitable intracellular signaling domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity to the following amino acid sequence: ENL**YEGL**NLDDCSM**YEDI**SRG (SEQ ID NO:649), where the ITAM motifs are in bold and are underlined.

[00423] In some instances, a repressible CAR may contain an intracellular signaling domain derived from a DAP10/CD28 type signaling chain. Intracellular signaling domains suitable for use in a repressible CAR of the present disclosure include a DAP10/CD28 type signaling chain.

[00424] An example of a DAP10 signaling chain is the amino acid sequence is: RPRRSPAQDGKV**YINM**PGRG (SEQ ID NO:650). In some embodiments, a suitable intracellular signaling domain comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, amino acid sequence identity to the entire length of the amino acid sequence RPRRSPAQDGKV**YINM**PGRG (SEQ ID NO:651).

[00425] An example of a CD28 signaling chain is the amino acid sequence is FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSD**YMNM**TPRRPGPTRKHYQPYAPPRDF
AAYRS (SEQ ID NO:652). In some embodiments, a suitable intracellular signaling domain comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, amino acid sequence identity to the entire length of the amino acid sequence FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSD**YMNM**TPRRPGPTRKHYQPYAPPRDF
AAYRS (SEQ ID NO:653).

[00426] Intracellular signaling domains suitable for use in a CAR of the present disclosure include a ZAP70 polypeptide, e.g., a polypeptide comprising an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of from about 300 amino acids to about 400 amino acids, from about 400

amino acids to about 500 amino acids, or from about 500 amino acids to 619 amino acids, of the following amino acid sequence:

MPDPAAHLPFFYGSISRAEAEEHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFHHFPIERQ LNGTYAIAGGKAHCGPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPGVFDCLRDAMVRDYV RQTWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSLTREEAERKLYSGAQTDGKFLLRP RKEQGTYALSLIYGKTVYHYLISQDKAGKYCIPEGTKFDTLWQLVEYLKLKADGLIYCLKEACP NSSASNASGAAAPTLPAHPSTLTHPQRRIDTLNSDGYTPEPARITSPDKPRPMPMDTSVYESPYSD PEELKDKKLFLKRDNLLIADIELGCGNFGSVRQGVYRMRKKQIDVAIKVLKQGTEKADTEEMM REAQIMHQLDNPYIVRLIGVCQAEALMLVMEMAGGGPLHKFLVGKREEIPVSNVAELLHQVSM GMKYLEEKNFVHRDLAARNVLLVNRHYAKISDFGLSKALGADDSYYTARSAGKWPLKWYAP ECINFRKFSSRSDVWSYGVTMWEALSYGQKPYKKMKGPEVMAFIEQGKRMECPPECPPELYAL MSDCWIYKWEDRPDFLTVEQRMRACYYSLASKVEGPPGSTQKAEAACA (SEQ ID NO:654).

Transmembrane Domain

[00427] Any transmembrane (TM) domain that provides for insertion of a polypeptide into the cell membrane of a eukaryotic (e.g., mammalian) cell is suitable for use. As one non-limiting example, the TM sequence IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:655) can be used. Additional non-limiting examples of suitable TM sequences include: a) CD8 beta derived : LGLLVAGVLVLLVSLGVAIHLCC (SEQ ID NO:656); b) CD4 derived: ALIVLGGVAGLLLFIGLGIFFCVRC (SEQ ID NO:657); c) CD3 zeta derived: LCYLLDGILFIYGVILTALFLRV (SEQ ID NO:658); d) CD28 derived: WVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:659); e) CD134 (OX40) derived: VAAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO:660); and f) CD7 derived: ALPAALAVISFLLGLGLGVACVLA (SEQ ID NO:661).

Linkers

[00428] In some cases, a subject repressible CAR includes a linker between any two adjacent domains. For example, a linker can be disposed between the transmembrane domain and the first intracellular functional domain, e.g., a co-stimulatory domain, of the repressible CAR. As another example, a linker can be disposed between a first intracellular functional domain and the member of the dimerization domain of the repressible CAR. As another example, a linker can be disposed between the member of the dimerization domain and a second intracellular functional domain, e.g., an immune cell activation domain. As another example, a linker can be disposed between any domain of the repressible CAR and any additional domain including e.g., a domain not involved in the primary immune activation functions of the CAR including but not limited to e.g., a reporter domain, a tag domain, etc.

[00429] Linkers may be utilized in a suitable configuration in the repressible CAR provided they do not abolish the primary activities of the repressible CAR including, e.g., the ability of the repressible CAR to

become activated upon extracellular binding, the ability of the dimerization domain of the repressible CAR to bind the dimerization domain of the ICR repressor.

[00430] A linker peptide may have any of a variety of amino acid sequences. Proteins can be joined by a spacer peptide, generally of a flexible nature, although other chemical linkages are not excluded. A linker can be a peptide of between about 6 and about 40 amino acids in length, or between about 6 and about 25 amino acids in length. These linkers can be produced by using synthetic, linker-encoding oligonucleotides to couple the proteins. Peptide linkers with a degree of flexibility can be used. The linking peptides may have virtually any amino acid sequence, bearing in mind that suitable linkers will have a sequence that results in a generally flexible peptide. The use of small amino acids, such as glycine and alanine, are of use in creating a flexible peptide. The creation of such sequences is routine to those of skill in the art.

[00431] Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[00432] Exemplary flexible linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $GSGGS_n$ (SEQ ID NO:516) and $GGGS_n$ (SEQ ID NO:517), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are of interest since both of these amino acids are relatively unstructured, and therefore may serve as a neutral tether between components. Glycine polymers are of particular interest since glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary flexible linkers include, but are not limited GGSG (SEQ ID NO:518), GGSGG (SEQ ID NO:519), GSGSG (SEQ ID NO:520), GSGGG (SEQ ID NO:521), GGGSG (SEQ ID NO:522), GSSSG (SEQ ID NO:523), and the like. The ordinarily skilled artisan will recognize that design of a peptide conjugated to any elements described above can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

Hinge Regions

[00433] In some cases, the first polypeptide of a subject repressible CAR comprises a hinge region (also referred to herein as a "spacer"), where the hinge region is interposed between the antigen-binding domain and the transmembrane domain. In some cases, the hinge region is an immunoglobulin heavy chain hinge region. In some cases, the hinge region is a hinge region polypeptide derived from a receptor (e.g., a CD8-derived hinge region).

[00434] The hinge region can have a length of from about 4 amino acids to about 50 amino acids, e.g., from about 4 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 40 aa, or from about 40 aa to about 50 aa.

[00435] Suitable spacers can be readily selected and can be of any of a number of suitable lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and can be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[00436] Exemplary spacers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$ (SEQ ID NO:516) and $(GGGS)_n$ (SEQ ID NO:517), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers can be used; both Gly and Ser are relatively unstructured, and therefore can serve as a neutral tether between components. Glycine polymers can be used; glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary spacers can comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO:518), GGSGG (SEQ ID NO:519), GSGSG (SEQ ID NO:520), GSGGG (SEQ ID NO:521), GGGSG (SEQ ID NO:522), GSSSG (SEQ ID NO:523), and the like.

[00437] In some cases, e.g., when the stimulatory ICR portion of a repressible CAR is split between two or more polypeptides the repressible CAR may include a hinge region that includes at least one cysteine. For example, in some cases, the hinge region can include the sequence Cys-Pro-Pro-Cys. If present, a cysteine in the hinge region of a first polypeptide, e.g., a first portion of a repressible CAR, can be available to form a disulfide bond with a hinge region in a second polypeptide, e.g., a second portion of a repressible CAR.

[00438] Immunoglobulin hinge region amino acid sequences are known in the art; see, e.g., Tan et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:162; and Huck et al. (1986) *Nucl. Acids Res.* 14:1779. As non-limiting examples, an immunoglobulin hinge region can include one of the following amino acid sequences: DKTHT (SEQ ID NO:524); CPPC (SEQ ID NO:525); CPEPKSCDTPPPCPR (SEQ ID NO:526) (see, e.g., Glaser et al. (2005) *J. Biol. Chem.* 280:41494); ELKTPLGDTTHT (SEQ ID NO:527); KSCDKTHTCP (SEQ ID NO:528); KCCVDCP (SEQ ID NO:529); KYGPPCP (SEQ ID NO:530); EPKSCDKTHTCPPCP (SEQ ID NO:531) (human IgG1 hinge); ERKCCVECPPCP (SEQ ID NO:532) (human IgG2 hinge); ELKTPLGDTTHTCPRCP (SEQ ID NO:533) (human IgG3 hinge); SPNMVPHAHHAQ (SEQ ID NO:534) (human IgG4 hinge); and the like.

[00439] The hinge region can comprise an amino acid sequence of a human IgG1, IgG2, IgG3, or IgG4, hinge region. The hinge region can include one or more amino acid substitutions and/or insertions and/or deletions compared to a wild-type (naturally-occurring) hinge region. For example, His_{229} of human IgG1 hinge can be substituted with Tyr, so that the hinge region comprises the sequence EPKSCDKTYTCPPCP (SEQ ID NO:535); see, e.g., Yan et al. (2012) *J. Biol. Chem.* 287:5891.

[00440] The hinge region can comprise an amino acid sequence derived from human CD8; e.g., the hinge region can comprise the amino acid sequence: TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:536), or a variant thereof.

Dimerization domain

[00441] As noted above, heterodimeric, conditionally repressible synthetic immune cell receptor (ICR) of the present disclosure comprises a first member of a dimerization pair, and a second member of a dimerization pair. One of the members of the dimerization pair will comprise an LBD of a nuclear hormone receptor; the other member of the dimerization pair will comprise a co-regulator peptide of the same nuclear hormone receptor. In the presence of a dimerization agent (e.g., a nuclear hormone, or a functional derivative or analog of the nuclear hormone), the members of the dimerization pair will bind to one another, and will effect dimerization of the two polypeptide chains of the conditionally repressible synthetic ICR of the present disclosure. A first member of a dimerization pair, or a second member of a dimerization pair, can also be referred to as a "dimerization domain."

Ligand-binding domain (LBD)

[00442] A ligand-binding domain of a nuclear hormone receptor can be from any of a variety of nuclear hormone receptors, including, but not limited to, ERα, ERβ, PR, AR, GR, MR, RARα, RARβ, RARγ, TRα, TRβ, VDR, EcR, RXRα, RXRβ, RXRγ, PPARα, PPARβ, PPARγ, LXRα, LXRβ, FXR, PXR, SXR, CAR, SF-1, LRH-1, DAX-1, SHP, TLX, PNR, NGF1-Bα, NGF1-Bβ, NGF1-Bγ, RORα, RORβ, RORγ, ERRα, ERRβ, ERRγ, GCNF, TR2/4, HNF-4, COUP-TFα, COUP-TFβ and COUP-TFγ.

[00443] Abbreviations for nuclear hormone receptors are as follows. ER: Estrogen Receptor; PR: Progesterone Receptor; AR: Androgen Receptor; GR: Glucocorticoid Receptor; MR: Mineralocorticoid Receptor; RAR: Retinoic Acid Receptor; TRα, β: Thyroid Receptor; VDR: Vitamin D3 Receptor; EcR: Ecdysone Receptor; RXR: Retinoic Acid X Receptor; PPAR: Peroxisome Proliferator Activated Receptor; LXR: Liver X Receptor; FXR: Farnesoid X Receptor; PXR/SXR: Pregnane X Receptor/Steroid and Xenobiotic Receptor; CAR: Constitutive Adrostrane Receptor; SF-1: Steroidogenic Factor 1; DAX-1: Dosage sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1; LRH-1: Liver Receptor Homolog 1; SHP: Small Heterodimer Partner; TLX: Tail-less Gene; PNR: Photoreceptor-Specific Nuclear Receptor; NGF1-B: Nerve Growth Factor; ROR: RAR

related orphan receptor; ERR: Estrogen Related Receptor; GCNF: Germ Cell Nuclear Factor; TR2/4: Testicular Receptor; HNF-4: Hepatocyte Nuclear Factor; COUP-TF: Chicken Ovalbumin Upstream Promoter, Transcription Factor.

[00444] In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises a single LBD of a nuclear hormone receptor. In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises multiple (two or more) LBD of a nuclear hormone receptor. In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises two LBD of a nuclear hormone receptor. In some cases, a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises three LBD of a nuclear hormone receptor. Where a polypeptide chain of a heterodimeric polypeptide of the present disclosure comprises multiple (two or more) LBD of a nuclear hormone receptor, in some cases the multiple LBD comprise identical amino acid sequences. In some cases, the two or more LBD are in tandem, either directly or separated by a linker.

Mineralocorticoid receptor

[00445] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a mineralocorticoid receptor (MR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an MR having the amino acid sequence depicted in FIG. 1A.

[00446] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 1F; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00447] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1B; and has a length of from about 250 amino acids to 299 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 299 amino acids).

[00448] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1C; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00449] As another non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1D, and has an S810L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 1A); and has a length of from about 250 amino acids to 299 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 299 amino acids).

[00450] As one non-limiting example, the LBD of a MR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1C, and has an S810L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 1A); and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00451] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00452] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the

present disclosure is an LBD of an MR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00453] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an MR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Androgen receptor

[00454] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an androgen receptor (AR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an AR having the amino acid sequence depicted in FIG. 2A.

[00455] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B; and has a length of from about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00456] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2C; and has a length of from

about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00457] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, and has a T877A substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00458] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2C, and has a T877A substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00459] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, and has an F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00460] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2C, and has an F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00461] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, and has a T877A and an F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 250 amino acids to 301 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, or from 275 amino acids to 301 amino acids).

[00462] As one non-limiting example, the LBD of an AR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%,

amino acid sequence identity to the amino acid sequence depicted in FIG. 2C, and has a T877A and an F876L substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 2A); and has a length of from about 190 amino acids to 230 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, or from 210 amino acids to 230 amino acids).

[00463] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an AR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3), where the co-regulator peptide has a length of from about 19 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 19 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an AR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3), where the co-regulator peptide has a length of from about 19 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 19 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Progesterone receptor

[00464] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a progesterone receptor (PR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a PR having the amino acid sequence depicted in FIG. 3A.

[00465] As one non-limiting example, the LBD of a PR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 3D; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00466] As one non-limiting example, the LBD of a PR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 3B; and has a length of from about 200 amino acids to 256 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 256 amino acids; e.g., has a length of 256 amino acids).

[00467] As one non-limiting example, the LBD of a PR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 3C; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 250 amino acids; e.g., has a length of 248 amino acids).

[00468] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PR, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8), where the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PR, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8), where the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Thyroid hormone receptor-β

[00469] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of thyroid hormone receptor-beta (TRβ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a TRβ having the amino acid sequence depicted in FIG. 4A.

[00470] As one non-limiting example, the LBD of a TRβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 4D; and has a length of from about 200 amino acids to 250 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 240 amino acids, or from 240 amino acids to 250 amino acids).

[00471] As one non-limiting example, the LBD of a TRβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 4B; and has a length of from

about 200 amino acids to 260 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 260 amino acids; e.g., has a length of 260 amino acids).

[00472] As one non-limiting example, the LBD of a TRβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 4B; and has a length of from about 200 amino acids to 246 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 246 amino acids; e.g., has a length of 246 amino acids).

[00473] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is an NCOA3/SRC3 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is a co-regulator peptide comprises the amino acid sequence CSSDDRGHSSLTNSPLDSSCKESSVSVTSPSGVSSSTSGGVSSTSNMHGSLLQEKHRILHKLLQNG NSPAEVAKITAEATGKDTSSITSCGDGNVVKQEQLSPKKKENNALLRYLLDRDDPSDALSKELQ PQVEGVDNKMSQCTSSTIPSSSQEKDPKIKTETSEEGSGDLDNLDAILGDLTSSDFYNNSISSNGS HLGTKQQ (SEQ ID NO:662). In other cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the first member of the dimerization pair is an NCOA3/SRC3 polypeptide.

[00474] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the second member of the dimerization pair is a co-regulator peptide comprises the amino acid sequence STAPGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERLDSKTDPASNTKLIAMKT EKEEMSFEPGDQPGSELDNLEEILDDLQNSQLPQLFPDTRPGAPAGSVDKQAIINDLMQLTAENS PVTPVGAQKTALRISQSTFNNPRPGQLGRLLPNQNLPLDITLQSPTGAGPFPPIRNSSPYSVIPQPG MMGNQGMIGNQGNLGNSSTGMIGNSASRPTMPSGEWAPQSSAVRVTCAATTSAMNRPVQGG MIRNPAASIPMRPSSQPGQRQTLQSQVMNIGPSELEMNMGGP (SEQ ID NO:663). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a TRβ, the first member of the dimerization pair is an NCOA2/SRC2 polypeptide.

Estrogen receptor-alpha

[00475] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of estrogen receptor-alpha (ERα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least

80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an ERα having the amino acid sequence depicted in FIG. 5A.

[00476] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 5H; and has a length of from about 200 amino acids to 240 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 235 amino acids, or from 235 amino acids to 240 amino acids).

[00477] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5B; and has a length of from about 180 amino acids to 229 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, or from 200 amino acids to 229 amino acids; e.g., has a length of 229 amino acids).

[00478] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5C; and has a length of from about 250 amino acids to 314 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 314 amino acids; e.g., has a length of 314 amino acids).

[00479] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5D; and has a length of from about 190 amino acids to 238 amino acids (e.g., has a length of from 190 amino acids to 220 amino acids, or from 220 amino acids to 238 amino acids; e.g., has a length of 238 amino acids).

[00480] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5E, and has a D351Y substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 5A); and has a length of from about 180 amino acids to 229 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, or from 200 amino acids to 229 amino acids; e.g., has a length of 229 amino acids).

[00481] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5F, and has a D351Y

substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 5A); and has a length of from about 250 amino acids to 314 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 314 amino acids; e.g., has a length of 314 amino acids).

[00482] As one non-limiting example, the LBD of an ERα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 5G, and has a D351Y substitution (where the amino acid numbering is based on the amino acid sequence depicted in FIG. 5A); and has a length of from about 190 amino acids to 238 amino acids (e.g., has a length of from 190 amino acids to 220 amino acids, or from 220 amino acids to 238 amino acids; e.g., has a length of 238 amino acids).

[00483] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERα, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence DAFQLRQLILRGLQDD (SEQ ID NO:12), where the co-regulator peptide has a length of from about 16 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 16 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERα, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence DAFQLRQLILRGLQDD (SEQ ID NO:12), where the co-regulator peptide has a length of from about from about 16 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 16 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00484] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERα, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SPGSREWFKDMLS (SEQ ID NO:13), where the co-regulator peptide has a length of from about 13 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 13 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD

of an ERα, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence SPGSREWFKDMLS (SEQ ID NO:13), where the co-regulator peptide has a length of from about from about 13 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 13 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Estrogen receptor-beta (ERβ)

[00485] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of estrogen receptor-alpha (ERβ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an ERβ having the amino acid sequence depicted in FIG. 6A.

[00486] As one non-limiting example, the LBD of an ERβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to an amino acid sequence depicted in FIG. 6C; and has a length of from about 200 amino acids to 243 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 235 amino acids, or from 235 amino acids to 243 amino acids).

[00487] As one non-limiting example, the LBD of an ERβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 6B; and has a length of from about 200 amino acids to 243 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 235 amino acids, or from 235 amino acids to 243 amino acids).

[00488] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERβ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PRQGSILYSMLTSAKQT (SEQ ID NO:9), where the co-regulator peptide has a length of from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 17 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of an ERβ, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence

PRQGSILYSMLTSAKQT (SEQ ID NO:9), where the co-regulator peptide has a length of from about from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 17 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Peroxisome proliferator-activated receptor-gamma

[00489] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of peroxisome proliferator-activated receptor-gamma (PPAR-γ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a PPAR-γ having the amino acid sequence depicted in FIG. 7A.

[00490] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 7E; and has a length of from about 200 amino acids to 269 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 250 amino acids, or from 250 amino acids to 269 amino acids).

[00491] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7B; and has a length of from about 150 amino acids to 202 amino acids (e.g., has a length of from 150 amino acids to 160 amino acids, from 160 amino acids to 170 amino acids, from 170 amino acids to 190 amino acids, or from 190 amino acids to 202 amino acids).

[00492] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7C; and has a length of from about 200 amino acids to 269 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 250 amino acids, or from 250 amino acids to 269 amino acids).

[00493] As one non-limiting example, the LBD of a PPAR-γ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 7D; and has a length of from about 200 amino acids to 269 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 250 amino acids, or from 250 amino acids to 271 amino acids).

[00494] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1), where the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 28 amino acids, from 28 amino acids to 29 amino acids, from 29 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1), where the co-regulator peptide has a length of from about from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 25 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 25 amino acids to 28 amino acids, from 28 amino acids to 29 amino acids, from 29 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00495] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4), where the co-regulator peptide has a length of from about from about 17 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

[00496] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the second member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKKENALLRYLLDKDDTKDI

(SEQ ID NO:11), where the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a PPAR-γ, the first member of the dimerization pair is a co-regulator peptide comprising the amino acid sequence PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11), where the co-regulator peptide has a length of from about from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 21 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from 21 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids).

Glucocorticoid receptor

[00497] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of glucocorticoid receptor (GR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a GR having the amino acid sequence depicted in FIG. 8A.

[00498] As one non-limiting example, the LBD of a GR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 8C; and has a length of from about 200 amino acids to 247 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, from 225 amino acids to 230 amino acids, from 230 amino acids to 240 amino acids, or from 240 amino acids to 247 amino acids).

[00499] As one non-limiting example, the LBD of a GR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 8B; and has a length of from about 200 amino acids to 247 amino acids (e.g., has a length of from 200 amino acids to 225 amino acids, or from 225 amino acids to 247 amino acids; e.g., has a length of 247 amino acids).

[00500] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide comprising the amino acid

sequence
NYGTNPGTPPASTSPFSQLAANPEASLANRNSMVSRGMTGNIGGQFGTGINPQMQQNVFQYPG
AGMVPQGEANFAPSLSPGSSMVPMPIPPPQSSLLQQTPPASGYQSPDMKAWQQGAIGNNNVFSQ
AVQNQPTPAQPGVYNNMSITVSMAGGNTNVQNMNPMMAQMQMSSLQMPGMNTVCPEQIND
PALRHTGLYCNQLSSTDLLKTEADGTQQVQQVQVFADVQCTVNLVGGDPYLNQPGPLGTQKP
TSGPQTPQAQQKSLLQQLLTE (SEQ ID NO:664) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the first member of the dimerization pair is an NCOA1/SRC1 polypeptide.

[00501] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide comprising the amino acid sequence
KRHHHEVLRQGLAFSQIYRFSLSDGTLVAAQTKSKLIRSQTTNEPQLVISLHMLHREQNVCVMN
PDLTGQTMGKPLNPISSNSPAHQALCSGNPGQDMTLSSNINFPINGPKEQMGMPMGRFGGSGG
MNHVSGMQATTPQGSNYALKMNSPSQSSPGMNPGQPTSMLSPRHRMSPGVAGSPRIPPSQFSPA
GSLHSPVGVCSSTGNSHSYTNSSLNALQALSEGHGVSLGSSLASPDLKMGNLQNSPVNMNPPPL
SKMGSLDSKDCFGLYGEPSEGTTGQAESSCHPGEQKETNDPNLPPAVSSERADGQSRLHDSKGQ
TKLLQLLTTKSDQMEPSPLASSLSDTNKDSTGSLPGSGSTHGTSLKEKHKILHRLLQDSSSPVDL
AKLTAEATGKDLSQESSSTAPGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERL
DSKTDPASNTKLIAMKTEKEEMSFEPGDQPGSELDNLEEILDDLQNSQLPQLFPDTRPGAPAGSV
DKQAIINDLMQLTAENSPVTPVGAQKTALRISQSTFNNPRPGQLGRLLPNQNLPLDITLQSPTGA
GPFPPIRNSSPYSVIPQPGMMGNQGMIGNQGNLGNSSTGMIGNSASRPTMPSGEWAPQSSAVRV
TCAATTSAMNRPVQGGMIRNPAASIPMRPSSQPGQRQTLQSQVMNIGPSELEMNMGGP (SEQ ID NO:665) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a GR, the first member of the dimerization pair is an NCOA2/SRC2 polypeptide.

Vitamin D receptor

[00502] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of vitamin D receptor (VDR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a VDR having the amino acid sequence depicted in FIG. 9A.

[00503] As one non-limiting example, the LBD of a VDR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 9C; and has a length of from about 250 amino acids to 310 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 310 amino acids).

[00504] As one non-limiting example, the LBD of a VDR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 9B; and has a length of from about 250 amino acids to 303 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 300 amino acids, or from 300 amino acids to 303 amino acids).

[00505] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA1/SRC1 polypeptide comprising the amino acid sequence NYGTNPGTPPASTSPFSQLAANPEASLANRNSMVSRGMTGNIGGQFGTGINPQMQQNVFQYPG AGMVPQGEANFAPSLSPGSSMVPMPIPPPQSSLLQQTPPASGYQSPDMKAWQQGAIGNNNVFSQ AVQNQPTPAQPGVYNNMSITVSMAGGNTNVQNMNPMMAQMQMSSLQMPGMNTVCPEQIND PALRHTGLYCNQLSSTDLLKTEADGTQQVQQVQVFADVQCTVNLVGGDPYLNQPGPLGTQKP TSGPQTPQAQQKSLLQQLLTE (SEQ ID NO:666) or a fragment thereof. In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the first member of the dimerization pair is an NCOA1/SRC1 polypeptide.

[00506] In some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide comprising the amino acid sequence KRHHHEVLRQGLAFSQIYRFSLSDGTLVAAQTKSKLIRSQTTNEPQLVISLHMLHREQNVCVMN PDLTGQTMGKPLNPISSNSPAHQALCSGNPGQDMTLSSNINFPINGPKEQMGMPMGRFGGSGG MNHVSGMQATTPQGSNYALKMNSPSQSSPGMNPGQPTSMLSPRHRMSPGVAGSPRIPPSQFSPA GSLHSPVGVCSSTGNSHSYTNSSLNALQALSEGHGVSLGSSLASPDLKMGNLQNSPVNMNPPPL SKMGSLDSKDCFGLYGEPSEGTTGQAESSCHPGEQKETNDPNLPPAVSSERADGQSRLHDSKGQ

TKLLQLLTTKSDQMEPSPLASSLSDTNKDSTGSLPGSGSTHGTSLKEKHKILHRLLQDSSSPVDL AKLTAEATGKDLSQESSSTAPGSEVTIKQEPVSPKKKENALLRYLLDKDDTKDIGLPEITPKLERL DSKTDPASNTKLIAMKTEKEEMSFEPGDQPGSELDNLEEILDDLQNSQLPQLFPDTRPGAPAGSV DKQAIINDLMQLTAENSPVTPVGAQKTALRISQSTFNNPRPGQLGRLLPNQNLPLDITLQSPTGA GPFPPIRNSSPYSVIPQPGMMGNQGMIGNQGNLGNSSTGMIGNSASRPTMPSGEWAPQSSAVRV TCAATTSAMNRPVQGGMIRNPAASIPMRPSSQPGQRQTLQSQVMNIGPSELEMNMGGP (SEQ ID NO:667) or a fragment thereof. For example, in some cases, where the first member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the second member of the dimerization pair is an NCOA2/SRC2 polypeptide comprising the amino acid sequence LLRYLLDK (SEQ ID NO:668), where the co-regulator peptide has a length of from about from about 8 amino acids to about 50 amino acids (e.g., the co-regulator peptide has a length of from about 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 23 amino acids, from 23 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids). In some cases, where the second member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a VDR, the first member of the dimerization pair is an NCOA2/SRC2 polypeptide.

Thyroid hormone receptor-alpha

[00507] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of thyroid hormone receptor-alpha (TRα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a TRα having the amino acid sequence depicted in FIG. 10A.

[00508] As one non-limiting example, the LBD of a TRα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 10C; and has a length of from about 190 amino acids to about 245 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, from 210 amino acids to 230 amino acids, or from 230 amino acids to 245 amino acids).

[00509] As one non-limiting example, the LBD of a TRα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 10B; and has a length of from about 190 amino acids to about 243 amino acids (e.g., has a length of from 190 amino acids to 210 amino acids, from 210 amino acids to 230 amino acids, or from 230 amino acids to 243 amino acids).

Retinoic acid receptor-beta

[00510] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of retinoic acid receptor-beta (RARβ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a RARβ having the amino acid sequence depicted in FIG. 11A.

[00511] As one non-limiting example, the LBD of a RARβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to one of the amino acid sequences depicted in FIG. 11C; and has a length of from about 180 amino acids to about 235 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, from 200 amino acids to 220 amino acids, or from 220 amino acids to 235 amino acids).

[00512] As one non-limiting example, the LBD of a RARβ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 11B; and has a length of from about 180 amino acids to about 231 amino acids (e.g., has a length of from 180 amino acids to 200 amino acids, from 200 amino acids to 220 amino acids, or from 220 amino acids to 231 amino acids).

[00513] A suitable co-regulator peptide for a RARβ is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

Farnesoid X receptor

[00514] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of farnesoid X receptor (FXR. For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an FXR having the amino acid sequence depicted in FIG. 22A.

[00515] As one non-limiting example, the LBD of an FXR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 22B; and has a length of from about 100 amino acids to about 136 amino acids (e.g., has a length of from 100 amino acids to 110 amino acids, from 110 amino acids to 120 amino acids, or from 120 amino acids to 136 amino acids).

[00516] A suitable co-regulator peptide for an FXR is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

LXR-alpha

[00517] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of liver X receptor-alpha (LRXα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an LRXα having the amino acid sequence depicted in FIG. 23A.

[00518] As one non-limiting example, the LBD of an LRXα can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 23B; and has a length of from about 200 amino acids to about 266 amino acids (e.g., has a length of from 200 amino acids to 220 amino acids, from 220 amino acids to 240 amino acids, or from 240 amino acids to 266 amino acids).

[00519] A suitable co-regulator peptide for an LXRα is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

RORgamma

[00520] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a retinoid-related orphan receptor gamma (RORγ). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an RORγ having the amino acid sequence depicted in FIG. 24A.

[00521] As one non-limiting example, the LBD of an RORγ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 24B; and has a length of from about 200 amino acids to about 261 amino acids (e.g., has a length of from 200 amino acids to 220 amino acids, from 220 amino acids to 240 amino acids, or from 240 amino acids to 261 amino acids).

[00522] A suitable co-regulator for an RORγ is an NCORNR peptide (CDPASNLGLEDIIRKALMGSFDDK, SEQ ID NO:669).

[00523] A suitable co-regulator peptide for an RORγ is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

RXR-alpha

[00524] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a retinoid-X receptor-alpha (RXRα). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of an RXRα having the amino acid sequence depicted in FIG. 25A.

[00525] As one non-limiting example, the LBD of an RORγ can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 25B; and has a length of from about 190 amino acids to about 238 amino acids (e.g., has a length of from 190 amino acids to 200 amino acids, from 200 amino acids to 210 amino acids, or from 210 amino acids to 238 amino acids).

[00526] A suitable co-regulator peptide for an RXRα is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

PXR

[00527] In some cases, an LBD suitable for inclusion as a member of a dimerization pair of a conditionally active, heterodimeric polypeptide of the present disclosure is an LBD of a Pregnane X Receptor (PXR). For example, in some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the LBD of a PXR having the amino acid sequence depicted in FIG. 26A. In some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 143-428 of the amino acid sequence depicted in FIG. 26A. In some cases, the LBD comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to amino acids 205-434 of the amino acid sequence depicted in FIG. 26A.

[00528] As one non-limiting example, the LBD of a PXR can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIG. 26B; and has a length of from about 250 amino acids to about 302 amino acids (e.g., has a length of from 250 amino acids to 275 amino acids, from 275 amino acids to 290 amino acids, or from 290 amino acids to 302 amino acids).

[00529] A suitable co-regulator peptide for a PXR is an SRC1 polypeptide, or a fragment thereof (e.g., a peptide of from 8 amino acids to 50 amino acids in length, derived from an SRC1 polypeptide).

Co-regulator peptides

[00530] Suitable co-regulator polypeptides include full-length naturally-occurring nuclear hormone co-regulator polypeptides. Suitable co-regulator polypeptides include fragments of naturally-occurring nuclear hormone co-regulator polypeptides. Suitable co-regulator polypeptides include synthetic or recombinant nuclear hormone co-regulator polypeptides.

[00531] Suitable co-regulator polypeptides can have a length of from 8 amino acids to 2000 amino acids. Suitable co-regulator polypeptides can have a length of from 8 amino acids to 50 amino acids, e.g., from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids. Suitable co-regulator polypeptides can have a length of from 50 amino acids to 100 amino acids, e.g., from 50 amino acids to 60 amino acids, from 60 amino acids to 70 amino acids, from 70 amino acids to 80 amino acids, from 80 amino acids to 90 amino acids, or from 90 amino acids to 100 amino acids. Suitable co-regulator polypeptides can have a length of from 100 amino acids to 200 amino acids, from 200 amino acids to 300 amino acids, from 300 amino acids to 400 amino acids, from 400 amino acids to 500 amino acids, from 500 amino acids to 600 amino acids, from 600 amino acids to 700 amino acids, from 700 amino acids to 800 amino acids, from 800 amino acids to 900 amino acids, or from 900 amino acids to 1000 amino acids. Suitable co-regulator polypeptides can have a length of from 1000 amino acids to 2000 amino acids.

[00532] Suitable co-regulator polypeptides include, but are not limited to, SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

[00533] National Center for Biotechnology Information (NCBI) accession numbers for such co-regulators include the following: SRC1 (NP_003734), GRIP1 (NP_006531), AIB1 (NP_006525), PGC1a (NP_037393), PGC1b (NP_573570), PRC (NP_055877), TRAP220 (NP_004765), ASC2 (NP_054790), CBP (NP_004371), P300 (NP_001420), CIA (NP_066018), ARA70 (NP_005428), TIF1 (NP_003843), NSD1 (NP_071900), SMAP (NP_006687), Tip60 (NP_006379), ERAP140 (NP_861447), Nix1 (NP_113662), LCoR (NP_115816), N-CoR (NP_006302), SMRT (NP_006303), RIP140 (NP_003480) and PRIC285 (NP_208384).

[00534] Examples of suitable co-regulator polypeptides are provided in FIGs. 29-51B.

[00535] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 29.

[00536] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20

amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 30.

[00537] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 31.

[00538] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 32.

[00539] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 33.

[00540] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 34.

[00541] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 35.

[00542] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 36A-36B.

[00543] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 37A-37B.

[00544] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 36A-36B.

[00545] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at

least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 39.

[00546] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 40.

[00547] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 41.

[00548] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 42A-42B.

[00549] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 43.

[00550] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino

acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 44.

[00551] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 45.

[00552] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 46.

[00553] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 47.

[00554] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 48A-48B.

[00555] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino

acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 49A-49B.

[00556] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 50.

[00557] In some cases, a suitable suitable co-regulator polypeptide has a length of from 8 amino acids to 10 amino acids, from 10 amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids; and has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to a stretch of from 8 to 50 contiguous amino acids of the amino acid sequence depicted in FIG. 51A-51B.

[00558] Suitable co-regulator peptides include, but are not limited to, Steroid Receptor Coactivator (SRC)-1, SRC-2, SRC-3, TRAP220-1, TRAP220-2, NR0B1, NRIP1, CoRNR box, abV, TIF1, TIF2, EA2, TA1, EAB1, SRC1-1, SRC1-2, SRC1-3, SRC1-4a, SRC1-4b, GRIP1-1, GRIP1-2, GRIP1-3, AIB1-1, AIB1-2, AIB1-3, PGC1a, PGC1b, PRC, ASC2-1, ASC2-2, CBP-1, CBP-2, P300, CIA, ARA70-1, ARA70-2, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, CoRNR1 (N-CoR), CoRNR2, SMRT, RIP140-C, RIP140-1, RIP140-2, RIP140-3, RIP140-4, RIP140-5, RIP140-6, RIP140-7, RIP140-8, RIP140-9, PRIC285-1, PRIC285-2, PRIC285-3, PRIC285-4, and PRIC285-5.

[00559] National Center for Biotechnology Information (NCBI) accession numbers for such co-regulators include the following: SRC1 (NP_003734), GRIP1 (NP_006531), AIB1 (NP_006525), PGC1a (NP_037393), PGC1b (NP_573570), PRC (NP_055877), TRAP220 (NP_004765), ASC2 (NP_054790), CBP (NP_004371), P300 (NP_001420), CIA (NP_066018), ARA70 (NP_005428), TIF1 (NP_003843), NSD1 (NP_071900), SMAP (NP_006687), Tip60 (NP_006379), ERAP140 (NP_861447), Nix1 (NP_113662), LCoR (NP_115816), N-CoR (NP_006302), SMRT (NP_006303), RIP140 (NP_003480) and PRIC285 (NP_208384).

[00560] In some cases, a suitable co-regulator peptide comprises an LXXLL motif, where X is any amino acid; where the co-regulator peptide has a length of from 12 amino acids to 50 amino acids, e.g., from 12

amino acids to 15 amino acids, from 15 amino acids to 20 amino acids, from 20 amino acids to 25 amino acids, from 25 amino acids to 30 amino acids, from 30 amino acids to 35 amino acids, from 35 amino acids to 40 amino acids, from 40 amino acids to 45 amino acids, or from 45 amino acids to 50 amino acids.

[00561] Non-limiting examples of suitable co-regulator peptides are as follows:

SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

abV: SPGSREWFKDMLS (SEQ ID NO:13);

TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);

SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);
PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);
PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);
TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);
TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);
ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);
ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);
CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);
CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);
P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);
CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);
ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);
ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);
TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);
NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);
SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);
Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);
ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);
Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);
LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);
CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);
CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);
CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);
CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);
RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);
RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);
RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);
RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);
RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);
RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);
RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);
RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);
RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);
RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);
PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[00562] In some cases, a given LBD can be paired with two or more different co-regulator polypeptides. For example, as depicted in FIG. 19, PPARγ can be paired with SRC1, SRC2, SRC3, or TRAP220. As another example, ERα can be paired with CoRNR, αβV, or TA1. As another example, ERβ can be paired with CoRNR, αβV, or TA1. As another example, AR can be paired with SRC1, SRC2, SRC3, or TRAP220. As another example, PR can be paired with SRC1, SRC2, SRC3, TRAP220, NR0B1, PGC1B, NRIP1, EA2, or EAB1. As another example, TRβ can be paired with SRC1, SRC2, SRC3, or TRAP220.

[00563] In some cases, a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides. Where a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides, the multiple co-regulator peptides can be in tandem, directly or separated by a linker. In some cases, the two or more co-regulator peptides present in the polypeptide chain are identical in amino acid sequence to one another. In some cases, where a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides, the polypeptide chain comprises two co-regulator peptides. In some cases, where a heterodimeric polypeptide of the present disclosure comprises a polypeptide chain comprising multiple (two or more) co-regulator peptides, the polypeptide chain comprises three co-regulator peptides. In such cases, the second polypeptide chain can comprise multiple (two or more) LBD of a nuclear hormone receptor. For example, where the second polypeptide chain comprises two LBD of a nuclear hormone receptor, the two LBD can be identical in amino acid sequence to one another.

[00564] Suitable dimerization agents are as described above.

Engineered T Cell Receptor (TCR)

[00565] In some instances, a heteromeric, conditionally repressible synthetic ICR may include, in part or in whole, an engineered T cell receptor (TCR) or may essentially be a modified engineered TCR such that by modification the engineered TCR is conditionally repressible. In such instances, the engineered TCR containing heteromeric, conditionally repressible synthetic ICR may be referred to as a heteromeric, conditionally repressible synthetic TCR or, for simplicity, a repressible TCR.

[00566] Any engineered TCR having immune cell activation function may find use in a heteromeric, conditionally repressible synthetic ICR as described herein including but not limited to, e.g., antigen-specific TCRs, Monoclonal TCRs (MTCRs), Single chain MTCRs, High Affinity CDR2 Mutant TCRs,

CD1-binding MTCRs, High Affinity NY-ESO TCRs, VYG HLA-A24 Telomerase TCRs, including e.g., those described in PCT Pub Nos. WO 2003/020763, WO 2004/033685, WO 2004/044004, WO 2005/114215, WO 2006/000830, WO 2008/038002, WO 2008/039818, WO 2004/074322, WO 2005/113595, WO 2006/125962; Strommes et al. Immunol Rev. 2014; 257(1):145-64; Schmitt et al. Blood. 2013; 122(3):348-56; Chapuls et al. Sci Transl Med. 2013; 5(174):174ra27; Thaxton et al. Hum Vaccin Immunother. 2014; 10(11):3313-21 (PMID:25483644); Gschweng et al. Immunol Rev. 2014; 257(1):237-49 (PMID:24329801); Hinrichs et al. Immunol Rev. 2014; 257(1):56-71 (PMID:24329789); Zoete et al. Front Immunol. 2013; 4:268 (PMID:24062738); Marr et al. Clin Exp Immunol. 2012; 167(2):216-25 (PMID:22235997); Zhang et al. Adv Drug Deliv Rev. 2012; 64(8):756-62 (PMID:22178904); Chhabra et al. Scientific World Journal. 2011; 11:121-9 (PMID:21218269); Boulter et al. Clin Exp Immunol. 2005; 142(3):454-60 (PMID:16297157); Sami et al. Protein Eng Des Sel. 2007; 20(8):397-403; Boulter et al. Protein Eng. 2003; 16(9):707-11; Ashfield et al. IDrugs. 2006; 9(8):554-9; Li et al. Nat Biotechnol. 2005; 23(3):349-54; Dunn et al. Protein Sci. 2006; 15(4):710-21; Liddy et al. Mol Biotechnol. 2010; 45(2); Liddy et al. Nat Med. 2012; 18(6):980-7; Oates, et al. Oncoimmunology. 2013; 2(2):e22891; McCormack, et al. Cancer Immunol Immunother. 2013 Apr;62(4):773-85; Bossi et al. Cancer Immunol Immunother. 2014; 63(5):437-48 and Oates, et al. Mol Immunol. 2015 Oct;67(2 Pt A):67-74; the disclosures of which are incorporated herein by reference in their entirety.

[00567] In some instances, an engineered TCR useful in a heteromeric, conditionally repressible synthetic ICR as described herein may include, e.g., a NY-ESO-1-binding TCR or a TCR that binds to NY-ESO-1 or a peptide derived therefrom. For example, in some instances a NY-ESO-1-binding TCR may be an engineered TCR that binds to a peptide having the amino acid sequence: SLLMWITQC (SEQ ID NO:670).

[00568] In some instances, an engineered TCR useful in a heteromeric, conditionally repressible synthetic ICR as described herein may be or may be derived from an engineered TCR having high affinity for its ligand including but not limited to, e.g., a K_D of less than or equal to 100 μM, including but not limited to e.g., a K_D of less than or equal to 10 μM or a K_D of less than or equal to 1 μM. In some instances, an engineered TCR useful in a heteromeric, conditionally repressible synthetic ICR as described herein may be or may be derived from an engineered TCR having high affinity for the peptide SLLMWITQC (SEQ ID NO:671), including but not limited to, e.g., a K_D of less than or equal to 100 μM, including but not limited to e.g., a K_D of less than or equal to 10 μM or a K_D of less than or equal to 1 μM for the peptide SLLMWITQC (SEQ ID NO:672). The K_D measurement can be made by any known method, including but not limited to e.g., Surface Plasmon Resonance (Biacore).

[00569] In some instances, an engineered TCR useful in a heteromeric, conditionally repressible synthetic ICR as described herein may be or may be derived from an engineered TCR having a slow off-rate (k_{off}) from its ligand including but not limited to, e.g., a k_{off} of 0.1 S^{-1} or slower, including but not limited to

e.g., a k_{off} of 1 x 10^{-2} S^{-1} or slower or a k_{off} of 1 x 10^{-3} S^{-1} or slower. In some instances, an engineered TCR useful in a heteromeric, conditionally repressible synthetic ICR as described herein may be or may be derived from an engineered TCR having a slow off-rate from the peptide SLLMWITQC (SEQ ID NO:673), including but not limited to, e.g., a K_D of less than or equal to 100 μM, including but not limited to e.g., a k_{off} of 0.1 S^{-1} or slower, including but not limited to e.g., a k_{off} of 1 x 10^{-2} S^{-1} or slower or a k_{off} of 1 x 10^{-3} S^{-1} or slower from the peptide SLLMWITQC (SEQ ID NO:674). The k_{off} measurement can be made by any known method, including but not limited to e.g., Surface Plasmon Resonance (Biacore).

[00570] In some instances, an engineered TCR may be modified for use as a component of a heteromeric, conditionally repressible synthetic ICR through introduction or insertion of a dimerization domain (e.g., a member of a dimerizer pair) into the engineered TCR and, in such instances, following modification, the engineered TCR may be referred to as a dimerizer-domain containing TCR or a dimerizable TCR.

[00571] A dimerizer domain may be inserted into the engineered TCR amino acid sequence, e.g., by introducing a coding sequence for the dimerizer domain into the coding sequence of the engineered TCR, at any convenient location provided the insertion does not negatively impact the primary functional domains of the engineered TCR (including e.g., a TCR alpha chain domain, a TCR beta chain domain, a TCR CD3 chain domain, a TCR zeta chain domain, a TCR CD3-zeta chain domain a TCR extracellular domain, a TCR intracellular domain, a TCR variable region domain, a TCR constant region domain, a TCR IgSF domain, etc., or a function thereof) and/or the negatively impact the dimerization function of the dimerizer domain.

[00572] An engineered TCR may include one or more epsilon, sigma, or gamma chains, or in some instances, an engineered TCR may not include one or more epsilon, sigma, or gamma chains and may instead rely upon endogenously expressed epsilon, sigma, or gamma chains. In some instances, an engineered TCR may not include one or more CD3-zeta chains and may instead rely on endogenously expressed CD3-zeta.

[00573] In some instances, the dimerizer domain may be inserted into an extracellular portion of the engineered TCR. In some instances the dimerizer domain (first or second member of the dimerization pair) may be inserted into an intracellular portion of the engineered TCR.

[00574] In some instances, the dimerizer domain may be inserted into or linked to an alpha chain of the engineered TCR. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked intracellularly to the alpha chain including e.g., where the dimerizer domain is linked to the cytoplasmic side of the alpha chain transmembrane domain. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked extracellularly to the alpha chain including e.g., where the dimerizer domain

is linked to the extracellular side of the alpha chain transmembrane domain, where the dimerizer domain is inserted between the alpha chain transmembrane domain and the alpha chain constant region domain, etc.

[00575] In some instances, the dimerizer domain may be inserted into or linked to a beta chain of the engineered TCR. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked intracellularly to the beta chain including e.g., where the dimerizer domain is linked to the cytoplasmic side of the beta chain transmembrane. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked extracellularly to the beta chain including e.g., where the dimerizer domain is linked to the extracellular side of the beta chain transmembrane domain, where the dimerizer domain is inserted between the beta chain transmembrane domain and the beta chain constant region domain, etc..

[00576] In some instances, the dimerizer domain may be inserted into or linked to a fused alpha-CD3-zeta chain, e.g., where the CD3-zeta chain is full-length CD3-zeta (e.g., a TCR:zeta fusion) of the engineered TCR. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked intracellularly to the fused alpha-CD3-zeta chain including e.g., where the dimerizer domain is inserted between the CD3-zeta transmembrane domain and other intracellular domains of the fused alpha-CD3-zeta chain, including e.g., one or more intracellular ITAM domains. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked extracellularly to the fused alpha-CD3-zeta chain including e.g., where the dimerizer domain is linked to the extracellular side of the CD3-zeta transmembrane domain, where the dimerizer domain is inserted between the extracellular alpha chain domain and the transmembrane domain of the fused CD3-zeta, etc.

[00577] In some instances, the dimerizer domain may be inserted into or linked to a fused beta-CD3-zeta chain, e.g., where the CD3-zeta chain is full-length CD3-zeta (e.g., a TCR:zeta fusion) of the engineered TCR. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked intracellularly to the fused beta-CD3-zeta chain including e.g., where the dimerizer domain is inserted between the CD3-zeta transmembrane domain and other intracellular domains of the fused beta-CD3-zeta chain, including e.g., one or more intracellular ITAM domains. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked extracellularly to the fused beta-CD3-zeta chain including e.g., where the dimerizer domain is linked to the extracellular side of the CD3-zeta transmembrane domain, where the dimerizer domain is inserted between the extracellular beta chain domain and the transmembrane domain of the fused CD3-zeta, etc.

[00578] In some instances, the dimerizer domain may be inserted into or linked to a fused alpha-CD3-zeta domain (e.g., in an engineered TCR alpha-zeta + beta-zeta fusion) of the engineered TCR. In some

instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked intracellularly to the fused alpha-CD3-zeta domain including e.g., where the dimerizer domain is inserted between one or more domains of the CD3-zeta domain and the transmembrane domain of the alpha chain. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked extracellularly to the fused alpha-CD3-zeta domain including e.g., where the dimerizer domain is linked to the extracellular side of the alpha chain transmembrane domain, where the dimerizer domain is inserted between the alpha chain transmembrane domain and the alpha chain constant region domain, etc.

[00579] In some instances, the dimerizer domain may be inserted into or linked to a fused beta-CD3-zeta domain (e.g., in an engineered TCR alpha-zeta + beta-zeta fusion) of the engineered TCR. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked intracellularly to the fused beta-CD3-zeta domain including e.g., where the dimerizer domain is inserted between one or more domains of the CD3-zeta domain and the transmembrane domain of the beta chain. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked extracellularly to the fused beta-CD3-zeta domain including e.g., where the dimerizer domain is linked to the extracellular side of the beta chain transmembrane domain, where the dimerizer domain is inserted between the beta chain transmembrane domain and the beta chain constant region domain, etc..

[00580] In some instances, the dimerizer domain may be inserted into or linked to a chain of an engineered single chain TCR (e.g., in an engineered single chain TCR:zeta fusion, e.g., where a TCR alpha chain variable domain is linked to a TCR beta chain which is fused to a full-length CD3-zeta chain). In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked intracellularly to the engineered single chain TCR including e.g., where the dimerizer domain is inserted between one or more domains of the CD3-zeta chain and the transmembrane domain of the CD3-zeta chain. In some instances, the dimerizer domain is inserted or linked such that following the insertion or linking the dimerizer domain is linked extracellularly to the engineered single chain TCR including e.g., where the dimerizer domain is linked to the extracellular side of the CD3-zeta chain transmembrane domain, where the dimerizer domain is inserted between the CD3-zeta chain transmembrane domain and the beta chain constant region domain, etc..

[00581] In some instances, only a single dimerizer domain may be present in a conditionally repressible engineered TCR, e.g., where a single dimerizer domain is linked or inserted into an alpha chain of the engineered TCR, where a single dimerizer domain is linked or inserted into a beta chain of the engineered TCR, where a single dimerizer domain is linked or inserted into a CD3-zeta chain of the engineered TCR, etc.

[00582] In some instances, two or more dimerizer domains may be present in a conditionally repressible engineered TCR. For example, two dimerizer domains may be present in a conditionally repressible engineered TCR, e.g., where a first dimerizer domain is linked or inserted into an alpha chain of the engineered TCR and a second dimerizer domain is linked or inserted into a beta chain of the engineered TCR, where a first dimerizer domain is linked or inserted into a first CD3-zeta chain of the engineered TCR and a second dimerizer domain is linked or inserted into a second CD3-zeta chain of the engineered TCR, etc.

[00583] In some instances, the engineered TCR of a conditionally repressible TCR may be an engineered TCR variant including but not limited to, e.g., TCR variants that include one or more variant or mutant TCR chains. In some instances, the engineered TCR of a conditionally repressible TCR may include one or more non-modified chains, including but not limited to a non-modified alpha chain, a non-modified beta chain, etc. In some instances, the engineered TCR of a conditionally repressible TCR may include one or more murinized chains, including but not limited to, e.g., a murinized alpha chain, a murinized beta chain, etc. In some instances, the engineered TCR of a conditionally repressible TCR may include one or more cysteine modified chains, including but not limited to, e.g., a cysteine modified alpha chain, a cysteine modified beta chain, etc. In some instances, the engineered TCR of a conditionally repressible TCR may include a combination of variant TCR chains, including but not limited to a combination of murinized and cysteine-modified chains, including but not limited to, e.g., a murinized and cysteine-modified alpha chain, a murinized and cysteine-modified beta chain, a murinized alpha chain and cysteine-modified beta chain, a murinized beta chain and cysteine-modified alpha chain, etc.

[00584] In instances where a heteromeric, conditionally repressible synthetic ICR includes, in part or in whole, or the heteromeric, conditionally repressible synthetic ICR is essentially a modified TCR, the TCR may contain non-modified TRC chains having extracellular domains or the extracellular domains therefore present in modified TCR chains, one or more intracellular stimulatory domains present in non-modified or modified TCR chains and the transmembrane domains of such extracellular domain-containing or intracellular domain-containing chains. Such a TCR may optionally include linker regions and/or hinge regions. TCRs as part of a heteromeric, conditionally repressible synthetic ICR may be encompassed within a single polypeptide (e.g., as in engineered single chain TCRs) or various chains and portions thereof may be "split" across two or more polypeptides.

TCR Chains

[00585] Many native TCRs exist in heterodimeric αβ or γδ forms. However, recombinant or engineered TCR may include a single TCR α or TCR β chain and may bind to peptide MHC molecules. In certain embodiments, an engineered TCR of a repressible ICR includes both an α chain variable domain and an TCR β chain variable domain. The chains of an engineered TCR useful in a repressible ICR of the instant

disclosure may vary and may include any suitable native or synthetic or recombinant or mutant TCR chain or chains or combination thereof.

[00586] As will be apparent to those skilled in the art the mutation(s) in TCR chain sequence, including e.g., α chain sequence and/or TCR β chain sequence, may be one or more of substitution(s), deletion(s) or insertion(s). These mutations can be carried out using any appropriate method including, but not limited to, those based on polymerase chain reaction (PCR), restriction enzyme-based cloning, or ligation independent cloning (LIC) procedures. These methods are detailed in many standard molecular biology texts, including but not limited to e.g., Sambrook & Russell, (2001) Molecular Cloning—A Laboratory Manual (3rd Ed.) CSHL Press and Rashtchian, (1995) *Curr Opin Biotechnol* 6 (1): 30-6.

[00587] As used herein the term "variable domain" is understood to encompass all amino acids of a given TCR which are not included within the constant domain as encoded by the TRAC gene for TCR α chains and either the TRBC1 or TRBC2 for TCR β chains as described in, e.g., *T cell receptor Factsbook*, (2001) LeFranc and LeFranc, Academic Press.

[00588] In some instances, an engineered TCR has at least one TCR α chain domain having or derived from an amino acid sequence that is at least 70% identical, including at least 75% identical to, including at least 80% identical to, including at least 85% identical to, including at least 90% identical to, including at least 95% identical to or is 100% identical to the IG4 α chain amino acid sequence: METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLT SLLLIQSSQREQTSGRLNASLDKSSGRSTLYIAASQPGDSATYLCAVRPTSGGSYIPTFGRGTSLIV HPPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKV AGFNLLMTLRLWSS (SEQ ID NO:675).

[00589] In some instances, an engineered TCR has at least one TCR α chain domain having or derived from an amino acid sequence that is at least 70% identical, including at least 75% identical to, including at least 80% identical to, including at least 85% identical to, including at least 90% identical to, including at least 95% identical to or is 100% identical to the IG4 α chain A95:LY mutant amino acid sequence: METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLT SLLLIQSSQREQTSGRLNASLDKSSGRSTLYIAASQPGDSATYLCAVRPLYGGSYIPTFGRGTSLIV HPPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKV AGFNLLMTLRLWSS (SEQ ID NO:676).

[00590] In some instances, an engineered TCR has at least one TCR β chain domain having or derived from an amino acid sequence that is at least 70% identical, including at least 75% identical to, including at least 80% identical to, including at least 85% identical to, including at least 90% identical to, including at

least 95% identical to or is 100% identical to the IG4 β chain amino acid sequence: MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMG LRLIHYSVGAGITDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNTGELFFGEG SRLTVLEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIV SAEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO:677).

[00591] In some instances, an engineered TCR has at least one TCR β chain domain having or derived from an amino acid sequence that is at least 70% identical, including at least 75% identical to, including at least 80% identical to, including at least 85% identical to, including at least 90% identical to, including at least 95% identical to or is 100% identical to the IG4 β chain G51A mutant amino acid sequence: MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMG LRLIHYSVAAGITDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNTGELFFGEG SRLTVLEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIV SAEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF (SEQ ID NO:678).

[00592] In some instances, a NY-ESO-1-binding TCR has at least one TCR α chain variable domain having an amino acid sequence that is at least 70% identical, including at least 75% identical to, including at least 80% identical to, including at least 85% identical to, including at least 90% identical to, including at least 95% identical to or is 100% identical to the α chain extracellular sequence: MQEVTQIPAALSVPEGENLVLNCSFTDSAIYNLQWFRQDPGKGLTSLLLIQSSQREQTSGRLNAS LDKSSGRSTLYIAASQPGDSATYLCAVRPTSGGSYIPTFGRGTSLIVHPYIQNPDPAVYQLRDSKS SDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNS IIPEDTFFPSPESS (SEQ ID NO:679). In some instances, the α chain extracellular sequence contains one or more of the following amino acid substitutions: T95L and S96Y.

[00593] In some instances, a NY-ESO-1-binding TCR has at least one TCR β chain variable domain having an amino acid sequence that is at least 70% identical, including at least 75% identical to, including at least 80% identical to, including at least 85% identical to, including at least 90% identical to, including at least 95% identical to or is 100% identical to the β chain extracellular sequence: MGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQGEVPN GYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYVGNTGELFFGEGSRLTVLEDLKNVFPPEVAVF EPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYALSS RLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRAD (SEQ ID NO:680).

[00594] In some instances, the engineered TCR include an introduced disulfide bond between cysteines. For example, disulfide bond between cysteines may be introduced between substitute amino acids of two chains of the engineered including but not limited to, e.g., between an α chain and a β chain of the TCR. In some instances an engineered TCR may be a NY-ESO-1-binding TCR that includes a disulfide bond between cysteines of substitute amino acids of two chains of the engineered NY-ESO-1-binding TCR including but not limited to, e.g., between an α chain and a β chain of the engineered NY-ESO-1-binding TCR. For example, in some instances, an engineered NY-ESO-1-binding TCR may include a disulfide bond between cysteines substituted for alpha chain T162 and beta chain S169 of SEQ ID NOs:679-680. Linkers

[00595] In some cases, a subject conditionally repressible TCR includes a linker between any two adjacent domains or artificially linked chains. For example, a linker can be disposed between the intracellular potion of a transmembrane domain of an alpha chain and a dimerizer domain of the conditionally repressible TCR. In some instances, a linker can be disposed between the intracellular potion of a transmembrane domain of a beta chain and a dimerizer domain of the conditionally repressible TCR. In some instances, a linker can be disposed between the transmembrane domain of an alpha chain and the first intracellular functional domain of a linked CD3-zeta chain of the conditionally repressible TCR. In some instances, a linker can be disposed between the transmembrane domain of a beta chain and the first intracellular functional domain of a linked CD3-zeta chain of the conditionally repressible TCR. As another example, a linker can be disposed between any domain of the conditionally repressible TCR and any additional domain including e.g., a domain not involved in the primary immune activation functions of the conditionally repressible TCR including but not limited to e.g., a reporter domain, a tag domain, etc.

[00596] Linkers may be utilized in a suitable configuration in the conditionally repressible TCR provided they do not abolish the primary activities of the conditionally repressible TCR including, e.g., the ability of the conditionally repressible TCR to activate an immune cell, the ability of the dimerization domain of the conditionally repressible TCR to bind the dimerization domain of the synthetic ICR repressor, etc.

[00597] Any suitable linker, including two or more linkers (e.g., where the two or more linkers are the same or different and including where the multiple linkers are three or more, four or more , five or more, six or more, etc. and including where all the linkers are different and where the multiple linkers include an mix of some linkers utilized in more than one location and some linkers utilized specifically in only one location and the like) may be utilized in the subject conditionally repressible TCRs including e.g., those linkers described herein for acceptable use in a CAR.

Synthetic ICR Repressor

[00598] As described herein, a heteromeric, conditionally repressible synthetic ICR includes a synthetic ICR repressor, also referred to herein as an "ICR repressor" or "inhibitory part" for simplicity. Such

inhibitory ICRs will vary depending on the particular context of immune cell repression to which the construct is directed and will generally function to mediate repression of an activated or activatable immune cell expressing a stimulatory ICR and the ICR repressor. Thus, an ICR repressor includes an inhibitory domain that functions to repress immune cell activation attributed to the stimulatory ICR upon dimerization of reciprocal dimerizer domains present in the ICR repressor and the stimulatory ICR when dimerizer is present.

[00599] A ICR repressor therefore includes one or more intracellular inhibitory domains that mediates intracellular signaling leading to inhibition of immune cell activation in immune cells expressing the stimulatory ICR. Domains useful as inhibitory domains will vary depending on the particular context of immune cell activation and repression, including e.g., the particular type of activated cell to be repressed and the desired degree of repression. Exemplary non-limited examples of inhibitory domains, described in greater detail below, include but are not limited to domains and motifs thereof derived from immune receptors including, e.g., co-inhibitory molecules, immune checkpoint molecules, immune tolerance molecules, and the like.

[00600] An ICR repressor further includes, as described in more detail below, a domain of a dimerization pair. Useful dimerization domains will vary depending on the desired dimerizer and the desired relative position of the dimerization domain within the ICR repressor. Generally, the presence of a first domain of a dimerization pair within the stimulatory ICR mediates the dimerization, upon introduction of the dimerizer, with a second domain of the dimerization pair present in the ICR repressor such that upon dimerization the ICR repressor represses immune cell activation due to the stimulatory ICR.

[00601] An ICR repressor may, optionally, include a transmembrane domain. As such, ICR repressors as described herein may or may not be membrane tethered. As such, an ICR repressor may contain a transmembrane domain, or portion thereof, and thus may be a membrane-bound ICR repressor. In other instances, an ICR repressor may lack a transmembrane domain and thus may be a cytosolic ICR repressor. Such transmembrane domains useful in an ICR repressor of the instant disclosure are described further herein.

[00602] In some instances, an ICR repressor may further include additional domains. Such additional domains may be functional, e.g., they directly contribute to the immune cell activation inhibition function of the ICR repressor, or non-functional, e.g., they do not directly contribute to the repression function of the ICR repressor. Non-functional additional domains may include domains having various purposes that do not directly affect the ability of the ICR repressor to repress immune cell activation including, but not limited to, e.g., structural functions, linker functions, etc.

Intracellular Inhibitory Domain

[00603] A stimulatory domain suitable for use in a synthetic ICR repressor of a subject repressible ICR may be any functional unit of a polypeptide as short as a 3 amino acid linear motif and as long as an entire protein, where size of the stimulatory domain is restricted only in that the domain must be sufficiently large as to retain its function and sufficiently small so as to be compatible with the other components of the repressible ICR. Accordingly, an inhibitory domain may range in size from 3 amino acids in length to 1000 amino acids or more and, in some instances, can have a length of from about 30 amino acids to about 70 amino acids (aa), e.g., an inhibitory domain can have a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa. In other cases, stimulatory domain can have a length of from about 70 aa to about 100 aa, from about 100 aa to about 200 aa, or greater than 200 aa.

[00604] In some instances, "co-inhibitory domains" find use in the synthetic ICR repressor of the present disclosure. Such co- inhibitory domains are generally polypeptides derived from receptors. Co-inhibition generally refers to the secondary inhibition of primary antigen-specific activation mechanisms which prevents co-stimulation. Co-inhibition, e.g., T cell co-inhibition, and the factors involved have been described in Chen & Flies. *Nat Rev Immunol* (2013) 13(4):227-42 and Thaventhiran et al. *J Clin Cell Immunol* (2012) S12, the disclosures of which are incorporated herein by reference in their entirety. In some embodiments, co-inhibitory domains homodimerize. A subject co-inhibitory domain can be an intracellular portion of a transmembrane protein (i.e., the co-inhibitory domain can be derived from a transmembrane protein). Non-limiting examples of suitable co-inhibitory polypeptides include, but are not limited to, CTLA-4 and PD-1. In some instances, a co-inhibitory domain, e.g., as used in a synthetic ICR repressor of the instant disclosure may include a co-inhibitory domain listed in FIG. 27, which provides **Table 1**. In some instances, a co- inhibitory domain of a synthetic ICR repressor comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to a co-inhibitory domain as described herein.

[00605] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of the transmembrane protein PD-1 (also known as CD279, programed cell death 1; etc.). For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence:
ICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPS
GMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL (SEQ ID NO:681). In some of these

embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, or from about 95 aa to about 100 aa.

[00606] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of the transmembrane protein CTLA4 (also known as CD152, Cytotoxic T-lymphocyte protein 4, Cytotoxic T-lymphocyte-associated antigen 4 ; etc.). For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: SLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPYFIPIN (SEQ ID NO:682). In some of these embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa.

[00607] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of the transmembrane protein HPK1 (also known as MAP4K1, Mitogen-activated protein kinase kinase kinase kinase 1, Hematopoietic progenitor kinase, MAPK/ERK kinase kinase kinase 1, MEK kinase kinase 1, MEKKK 1; etc.). For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: YDLLQRLGGGTYGEVFKARDKVSGDLVALKMVKMEPDDDVSTLQKEILILKTCRHANIVAYH GSYLWLQKLWICMEFCGAGSLQDIYQVTGSLSELQISYVCREVLQGLAYLHSQKKIHRDIKGAN ILINDAGEVRLADFGISAQIGATLARRLSFIGTPYWMAPEVAAVALKGGYNELCDIWSLGITAIEL AELQPPLFDVHPLRVLFLMTKSGYQPPRLKEKGKWSAAFHNFIKVTLTKSPKKRPSATKMLSHQ LV (SEQ ID NO:683). In some of these embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, from about 95 aa to about 100 aa, from about 100 aa to about 105 aa, from about 105 aa to about 110 aa, from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 125 aa, from about 125 aa to about 130 aa, from about 130 aa to about 135 aa, from about 135 aa

to about 140 aa, from about 140 aa to about 145 aa, from about 145 aa to about 150 aa, from about 150 aa to about 155 aa, from about 155 aa to about 160 aa, from about 160 aa to about 165 aa, from about 165 aa to about 170 aa, from about 170 aa to about 175 aa, from about 175 aa to about 180 aa, from about 180 aa to about 185 aa, from about 185 aa to about 190 aa, from about 190 aa to about 195 aa, from about 195 aa to about 200 aa, from about 200 aa to about 205 aa, from about 205 aa to about 210 aa, from about 210 aa to about 215 aa, from about 215 aa to about 220 aa, from about 220 aa to about 225 aa, from about 225 aa to about 230 aa, from about 230 aa to about 235 aa, from about 235 aa to about 240 aa, from about 240 aa to about 245 aa, from about 245 aa to about 250 aa, from about 250 aa to about 255 aa or from about 255 aa to about 258 aa.

[00608] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of the transmembrane protein SHP1 (also known as PTN6, Tyrosine-protein phosphatase non-receptor type 6, Hematopoietic cell protein-tyrosine phosphatase, Protein-tyrosine phosphatase 1C, PTP-1C, SH-PTP1, HCP, PTP1C; etc.). For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: FWEEFESLQKQEVKNLHQRLEGQRPENKGKNRYKNILPFDHSRVILQGRDSNIPGSDYINANYIK NQLLGPDENAKTYIASQGCLEATVNDFWQMAWQENSRVIVMTTREVEKGRNKCVPYWPEVG MQRAYGPYSVTNCGEHDTTEYKLRTLQVSPLDNGDLIREIWHYQYLSWPDHGVPSEPGGVLSF LDQINQRQESLPHAGPIIVHCSAGIGRTGTIIVIDMLMENISTKGLDCDIDIQKTIQMVRAQRSGM VQTEAQYKFIYVAIAQF (SEQ ID NO:684). In some of these embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, from about 95 aa to about 100 aa, from about 100 aa to about 105 aa, from about 105 aa to about 110 aa, from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 125 aa, from about 125 aa to about 130 aa, from about 130 aa to about 135 aa, from about 135 aa to about 140 aa, from about 140 aa to about 145 aa, from about 145 aa to about 150 aa, from about 150 aa to about 155 aa, from about 155 aa to about 160 aa, from about 160 aa to about 165 aa, from about 165 aa to about 170 aa, from about 170 aa to about 175 aa, from about 175 aa to about 180 aa, from about 180 aa to about 185 aa, from about 185 aa to about 190 aa, from about 190 aa to about 195 aa, from about 195 aa to about 200 aa, from about 200 aa to about 205 aa, from about 205 aa to about 210 aa, from about 210 aa to about 215 aa, from about 215 aa to about 220 aa, from about 220 aa to about 225 aa, from about 225 aa to about 230 aa, from about 230 aa to about 235

aa, from about 235 aa to about 240 aa, from about 240 aa to about 245 aa, from about 245 aa to about 250 aa, from about 250 aa to about 255 aa, from about 255 aa to about 260 aa, from about 260 aa to about 265 aa, from about 265 aa to about 270 aa or from about 270 aa to about 272 aa.

[00609] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of the transmembrane protein SHP2 (also known as PTN11, Tyrosine-protein phosphatase non-receptor type 11, Protein-tyrosine phosphatase 1D, PTP-1D, Protein-tyrosine phosphatase 2C, PTP-2C, SH-PTP2, SHP-2, SH-PTP3, PTP2C, SHPTP2; etc.). For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence: FWEEFETLQQQECKLLYSRKEGQRQENKNKNRYKNILPFDHTRVVLHDGDPNEPVSDYINANII MPEFETKCNNSKPKKSYIATQGCLQNTVNDFWRMVFQENSRVIVMTTKEVERGKSKCVKYWP DEYALKEYGVMRVRNVKESAAHDYTLRELKLSKVGQALLQGNTERTVWQYHFRTWPDHGVP SDPGGVLDFLEEVHHKQESIMDAGPVVVHCSAGIGRTGTFIVIDILIDIIREKGVDCDIDVPKTIQM VRSQRSGMVQTEAQYRFIYMA (SEQ ID NO:685). In some of these embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, from about 95 aa to about 100 aa, from about 100 aa to about 105 aa, from about 105 aa to about 110 aa, from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 125 aa, from about 125 aa to about 130 aa, from about 130 aa to about 135 aa, from about 135 aa to about 140 aa, from about 140 aa to about 145 aa, from about 145 aa to about 150 aa, from about 150 aa to about 155 aa, from about 155 aa to about 160 aa, from about 160 aa to about 165 aa, from about 165 aa to about 170 aa, from about 170 aa to about 175 aa, from about 175 aa to about 180 aa, from about 180 aa to about 185 aa, from about 185 aa to about 190 aa, from about 190 aa to about 195 aa, from about 195 aa to about 200 aa, from about 200 aa to about 205 aa, from about 205 aa to about 210 aa, from about 210 aa to about 215 aa, from about 215 aa to about 220 aa, from about 220 aa to about 225 aa, from about 225 aa to about 230 aa, from about 230 aa to about 235 aa, from about 235 aa to about 240 aa, from about 240 aa to about 245 aa, from about 245 aa to about 250 aa, from about 250 aa to about 255 aa, from about 255 aa to about 260 aa, from about 260 aa to about 265 aa, from about 265 aa to about 270 aa or from about 270 aa to about 275 aa.

[00610] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of the transmembrane protein Sts1 (also known as UBS3B, Ubiquitin-associated and SH3 domain-containing protein B, Cbl-interacting protein

p70, Suppressor of T-cell receptor signaling 1, STS-1, T-cell ubiquitin ligand 2, TULA-2, Tyrosine-protein phosphatase STS1/TULA2, UBASH3B, KIAA1959; etc.). For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence:
GPQKRCLFVCRHGERMDVVFGKYWLSQCFDAKGRYIRTNLNMPHSLPQRSGGFRDYEKDAPIT
VFGCMQARLVGEALLESNTIIDHVYCSPSLRCVQTAHNILKGLQQENHLKIRVEPGLFEWTKWV
AGSTLPAWIPPSELAAANLSVDTTYRPHIPISKLVVSESYDTYISRSFQVTKEIISECKSKGNNILIV
AHASSLEACTCQLQGLSPQNSKDFVQMVRKIPYLGFCSCEELGETGIWQLTDPPILPLTHGPTGG
FNWRETLLQE (SEQ ID NO:686). In some of these embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, from about 95 aa to about 100 aa, from about 100 aa to about 105 aa, from about 105 aa to about 110 aa, from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 125 aa, from about 125 aa to about 130 aa, from about 130 aa to about 135 aa, from about 135 aa to about 140 aa, from about 140 aa to about 145 aa, from about 145 aa to about 150 aa, from about 150 aa to about 155 aa, from about 155 aa to about 160 aa, from about 160 aa to about 165 aa, from about 165 aa to about 170 aa, from about 170 aa to about 175 aa, from about 175 aa to about 180 aa, from about 180 aa to about 185 aa, from about 185 aa to about 190 aa, from about 190 aa to about 195 aa, from about 195 aa to about 200 aa, from about 200 aa to about 205 aa, from about 205 aa to about 210 aa, from about 210 aa to about 215 aa, from about 215 aa to about 220 aa, from about 220 aa to about 225 aa, from about 225 aa to about 230 aa, from about 230 aa to about 235 aa, from about 235 aa to about 240 aa, from about 240 aa to about 245 aa, from about 245 aa to about 250 aa, from about 250 aa to about 255 aa, from about 255 aa to about 260 aa, from about 260 aa to about 265 aa or from about 265 aa to about 270 aa.

[00611] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of the transmembrane protein Csk (also known as Tyrosine-protein kinase CSK, C-Src kinase, Protein-tyrosine kinase CYL; etc.). For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to the following amino acid sequence:
LKLLQTIGKGEFGDVMLGDYRGNKVAVKCIKNDATAQAFLAEASVMTQLRHSNLVQLLGVIVE
EKGGLYIVTEYMAKGSLVDYLRSRGRSVLGGDCLLKFSLDVCEAMEYLEGNNFVHRDLAARN

VLVSEDNVAKVSDFGLTKEASSTQDTGKLPVKWTAPEALREKKFSTKSDVWSFGILLWEIYSFG RVPYPRIPLKDVVPRVEKGYKMDAPDGCPPAVYEVMKNCWHLDAAMRPSFLQLREQLEHIKT HELH (SEQ ID NO:687). In some of these embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, from about 95 aa to about 100 aa, from about 100 aa to about 105 aa, from about 105 aa to about 110 aa, from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 125 aa, from about 125 aa to about 130 aa, from about 130 aa to about 135 aa, from about 135 aa to about 140 aa, from about 140 aa to about 145 aa, from about 145 aa to about 150 aa, from about 150 aa to about 155 aa, from about 155 aa to about 160 aa, from about 160 aa to about 165 aa, from about 165 aa to about 170 aa, from about 170 aa to about 175 aa, from about 175 aa to about 180 aa, from about 180 aa to about 185 aa, from about 185 aa to about 190 aa, from about 190 aa to about 195 aa, from about 195 aa to about 200 aa, from about 200 aa to about 205 aa, from about 205 aa to about 210 aa, from about 210 aa to about 215 aa, from about 215 aa to about 220 aa, from about 220 aa to about 225 aa, from about 225 aa to about 230 aa, from about 230 aa to about 235 aa, from about 235 aa to about 240 aa, from about 240 aa to about 245 aa, from about 245 aa to about 250 aa or from about 250 aa to about 255 aa.

[00612] In some instances, a synthetic ICR repressor may contain an intracellular signaling domain, e.g., a co-inhibitory domain, derived from an intracellular portion of a transmembrane protein listed in **Table 1**. For example, a suitable co-inhibitory domain can comprise an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100% amino acid sequence identity to an amino acid sequence listed in **Table 1**. In some of these embodiments, the co-inhibitory domain has a length of from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, from about 45 aa to about 50 aa, from about 50 aa to about 55 aa, from about 55 aa to about 60 aa, from about 60 aa to about 65 aa, or from about 65 aa to about 70 aa, from about 70 aa to about 75 aa, from about 75 aa to about 80 aa, from about 80 aa to about 85 aa, from about 85 aa to about 90 aa, from about 90 aa to about 95 aa, from about 95 aa to about 100 aa, from about 100 aa to about 105 aa, from about 105 aa to about 110 aa, from about 110 aa to about 115 aa, from about 115 aa to about 120 aa, from about 120 aa to about 125 aa, from about 125 aa to about 130 aa, from about 130 aa to about 135 aa, from about 135 aa to about 140 aa, from about 140 aa to about 145 aa, from about 145 aa to about 150 aa, from about 150 aa to about 155 aa, from about 155 aa to about 160 aa, from about 160 aa to about 165, aa from about 165 aa to about 170 aa, from about

170 aa to about 175 aa, from about 175 aa to about 180 aa, from about 180 aa to about 185 aa, or from about 185 aa to about 190 aa.

Transmembrane Domain

[00613] Any transmembrane (TM) domain that provides for insertion of a polypeptide into the cell membrane of a eukaryotic (e.g., mammalian) cell is suitable for use. As one non-limiting example, the TM sequence IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:688) can be used. Additional non-limiting examples of suitable TM sequences include: a) CD8 beta derived : LGLLVAGVLVLLVSLGVAIHLCC (SEQ ID NO:689); b) CD4 derived: ALIVLGGVAGLLLFIGLGIFFCVRC (SEQ ID NO:690); c) CD3 zeta derived: LCYLLDGILFIYGVILTALFLRV (SEQ ID NO:691); d) CD28 derived: WVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:692); e) CD134 (OX40) derived: VAAILGLGLVLGLLGPLAILLALYLL (SEQ ID NO:693); and f) CD7 derived: ALPAALAVISFLLGLGLGVACVLA (SEQ ID NO:694).

Linkers

[00614] In some cases, a subject synthetic ICR repressor includes a linker between any two adjacent domains. For example, a linker can be disposed between the transmembrane domain, where present, and the first intracellular functional domain, e.g., a co-inhibitory domain, of the synthetic ICR repressor. As another example, a linker can be disposed between a first intracellular functional domain and the member of the dimerization domain of the synthetic ICR repressor. As another example, a linker can be disposed the transmembrane domain, where present, and the member of the dimerization domain of the synthetic ICR repressor. As another example, a linker can be disposed between the member of the dimerization domain and a second intracellular functional domain, e.g., an immune cell negative regulatory domain. As another example, a linker can be disposed between any domain of the synthetic ICR repressor and any additional domain including e.g., a domain not involved in the primary immune repression functions of the synthetic ICR repressor including but not limited to e.g., a reporter domain, a tag domain, etc.

[00615] Linkers may be utilized in a suitable configuration in the synthetic ICR repressor provided they do not abolish the primary activities of the synthetic ICR repressor including, e.g., the ability of the synthetic ICR repressor to repress an activated ICR, the ability of the dimerization domain of the synthetic ICR repressor to bind the dimerization domain of the repressible ICR.

[00616] Any suitable linker, including two or more linkers (e.g., where the two or more linkers are the same or different and including where the multiple linkers are three or more, four or more , five or more, six or more, etc. and including where all the linkers are different and where the multiple linkers include an mix of some linkers utilized in more than one location and some linkers utilized specifically in only one location and the like) may be utilized in the subject synthetic ICR repressors including e.g., those linkers described herein for acceptable use in a CAR.

Additional Sequences

[00617] The heteromeric, conditionally repressible synthetic ICR of the instant disclosure may further include one or more additional polypeptide domains, where such domains include, but are not limited to, a signal sequence; an epitope tag; an affinity domain; and a polypeptide that produces a detectable signal.

Signal sequences

[00618] Signal sequences that are suitable for use in a subject repressible synthetic ICR, e.g., in the stimulatory ICR or the ICR repressor, include any eukaryotic signal sequence, including a naturally-occurring signal sequence, a synthetic (e.g., man-made) signal sequence, etc.

Epitope tag

[00619] Suitable epitope tags include, but are not limited to, hemagglutinin (HA; e.g., YPYDVPDYA (SEQ ID NO:594); FLAG (e.g., DYKDDDDK (SEQ ID NO:595); c-myc (e.g., EQKLISEEDL; SEQ ID NO:596), and the like.

Affinity domain

[00620] Affinity domains include peptide sequences that can interact with a binding partner, e.g., such as one immobilized on a solid support, useful for identification or purification. DNA sequences encoding multiple consecutive single amino acids, such as histidine, when fused to the expressed protein, may be used for one-step purification of the recombinant protein by high affinity binding to a resin column, such as nickel sepharose. Exemplary affinity domains include His5 (HHHHH) (SEQ ID NO:597), HisX6 (HHHHHH) (SEQ ID NO:598), C-myc (EQKLISEEDL) (SEQ ID NO:599), Flag (DYKDDDDK) (SEQ ID NO:600), StrepTag (WSHPQFEK) (SEQ ID NO:601), hemagglutinin, e.g., HA Tag (YPYDVPDYA) (SEQ ID NO:602), GST, thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO:603), Phe-His-His-Thr (SEQ ID NO:604), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO:605), metal binding domains, e.g., zinc binding domains or calcium binding domains such as those from calcium-binding proteins, e.g., calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins, parvalbumin, calbindin D9K, calbindin D28K, and calretinin, inteins, biotin, streptavidin, MyoD, Id, leucine zipper sequences, and maltose binding protein.

Detectable signal-producing polypeptides

[00621] Suitable detectable signal-producing proteins include, e.g., fluorescent proteins; enzymes that catalyze a reaction that generates a detectable signal as a product; and the like.

[00622] Suitable fluorescent proteins include, but are not limited to, green fluorescent protein (GFP) or variants thereof, blue fluorescent variant of GFP (BFP), cyan fluorescent variant of GFP (CFP), yellow fluorescent variant of GFP (YFP), enhanced GFP (EGFP), enhanced CFP (ECFP), enhanced YFP (EYFP), GFPS65T, Emerald, Topaz (TYFP), Venus, Citrine, mCitrine, GFPuv, destabilised EGFP

(dEGFP), destabilised ECFP (dECFP), destabilised EYFP (dEYFP), mCFPm, Cerulean, T-Sapphire, CyPet, YPet, mKO, HcRed, t-HcRed, DsRed, DsRed2, DsRed-monomer, J-Red, dimer2, t-dimer2(12), mRFP1, pocilloporin, Renilla GFP, Monster GFP, paGFP, Kaede protein and kindling protein, Phycobiliproteins and Phycobiliprotein conjugates including B-Phycoerythrin, R-Phycoerythrin and Allophycocyanin. Other examples of fluorescent proteins include mHoneydew, mBanana, mOrange, dTomato, tdTomato, mTangerine, mStrawberry, mCherry, mGrape1, mRaspberry, mGrape2, mPlum (Shaner et al. (2005) *Nat. Methods* 2:905-909), and the like. Any of a variety of fluorescent and colored proteins from Anthozoan species, as described in, e.g., Matz et al. (1999) *Nature Biotechnol.* 17:969-973, is suitable for use.

[00623] Suitable enzymes include, but are not limited to, horse radish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase (GAL), glucose-6-phosphate dehydrogenase, beta-N-acetylglucosaminidase, β-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase, glucose oxidase (GO), and the like.

CONDITIONALLY ACTIVE DIMERIZATION-DEPENDENT CELL-SURFACE RECEPTORS

[00624] In some cases, a conditionally active heterodimeric polypeptide of the present disclosure is a conditionally active dimerization-dependent cell-surface receptor. By "conditionally active dimerization-dependent cell-surface receptor" is meant a variant of a cell surface receptor that is naturally dependent on dimerization for signal propagation, e.g., through ligand-binding induced dimerization, where the variant comprises a modification (e.g., a modification of a naturally-occurring cell surface receptor or other parental cell surface receptor) such that it includes one member of a dimerization pair and is conditionally dependent on dimerization of the dimerization pair induced by the presence of a dimerizing agent. As such, a conditionally acitive dimerization-dependent cell-surface receptor of the present disclosure will generally include a cell-surface receptor polypeptide which comprises a first member of a dimerization pair paired with a cell-surface receptor polypeptide which comprises a second member of the dimerization pair. For example, in some embodiments, a first dimerization-dependent cell-surface receptor that naturally forms a dimer in the presence of a ligand is modified to include a LBD of a nuclear hormone receptor and a second dimerization-dependent cell-surface receptor is modified to include a co-regulator of the nuclear hormone receptor such that the first dimerization-dependent cell-surface receptor and the second dimerization-dependent cell-surface receptor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[00625] In some cases, the first and second dimerization-dependent cell-surface receptor may be the same dimerization-dependent cell-surface receptor, i.e., the first and second dimerization-dependent cell-surface receptors naturally form a homodimer in the presence of a dimerizing ligand. Accordingly, in some instances, the first and second conditionally acitive dimerization-dependent cell-surface receptors

may only differ in that one includes a LBD of a nuclear hormone receptor and the other includes a co-regulator of the nuclear hormone receptor.

[00626] In some cases, the first and second dimerization-dependent cell-surface receptor may be different dimerization-dependent cell-surface receptors, i.e., the first and second dimerization-dependent cell-surface receptors naturally form a heterodimer in the presence of a dimerizing ligand. Accordingly, in some instances, the first and second conditionally acitive dimerization-dependent cell-surface receptors may differ in one or more domains besides the LBD of a nuclear hormone receptor present in one member and the co-regulator of the nuclear hormone receptor present in the other member.

[00627] By "dimerization-dependent cell-surface receptor" is meant any cell-surface receptor polypeptide which depends upon dimerization with a second polypeptide to propagate an intracellular signal. In its natural context, a dimerization-dependent cell-surface receptor will generally dimerize in response to binding a ligand of the receptor. Useful dimerization-dependent cell-surface receptors include those that dimerize to form homodimers, those that dimerize to form heterodimers, as well as those that dimerize to form homodimers or heterodimers depending on the context.

[00628] Conditionally active dimerization-dependent cell-surface receptors of the present disclosure may be constructed in various ways. In some cases, a LBD of a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor is appended to or recombinantly inserted into the otherwise unmodified dimerization-dependent cell-surface receptor. In some cases, one or more domains of the dimerization-dependent cell-surface receptor are replaced with a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor. In some cases, a LBD of a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor may be appended to or recombinantly inserted into a modified dimerization-dependent cell-surface receptor, e.g., a dimerization-dependent cell-surface receptor that has been modified to remove its natural ligand binding activity or other render it unable to bind its natural receptor ligand (e.g., by deletion of all or a potion of the endogenous ligand binding domain, buy mutation of the ligand binding domain, etc.). In some cases, the endogenous ligand binding domain of a dimerization-dependent cell-surface receptor may be replaced with a LBD of a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor.

[00629] For example, in some embodiments, the endogengous ligand binding domain of a cytokine receptor may be replaced with a LBD of a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor of the subject disclosure to generate a conditionally active dimerization-dependent cytokine receptor. In some embodiments, the endogengous ligand binding domain of a cytokine receptor may be completely or partially removed and a LBD of a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor of the subject disclosure may be appended to or inserted into the modified cytokine receptor to generate a conditionally active dimerization-dependent cytokine receptor.

[00630] In some embodiments, the endogengous ligand binding domain of a RTK may be replaced with a LBD of a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor of the subject disclosure to generate a conditionally active dimerization-dependent RTK. In some embodiments, the endogengous ligand binding domain of a RTK may be completely or partially removed and a LBD of a nuclear hormone receptor or a co-regulator of a nuclear hormone receptor of the subject disclosure may be appended to or inserted into the modified RTK to generate a conditionally active dimerization-dependent RTK.

Cytokine receptors

[00631] As noted above, useful non-limiting examples of dimerization-dependent cell-surface receptors include cytokine receptors, including e.g., cytokine receptors that form homodimers and cytokine receptors that form heterodimers. Useful cytokine receptors include but are not limited to e.g., IL-2 family receptors, IL-3 family receptors, IL-6 family receptors, IL-12 family receptors, prolactin family receptors, interferon family receptors, IL-10 family receptors, IL-17 family receptors, immunoglobulin-like superfamily receptors, tumor necrosis factor family receptors, chemokine receptors, TGF-beta family receptors, and the like.

[00632] Non-limiting examples of IL-2 family receptors include Interleukin 13 receptor, α2; Interleukin-2 receptor subunit α; Interleukin-2 receptor subunit β; Interleukin-2 receptor subunit γ; Interleukin-4 receptor subunit α ; Interleukin-7 receptor subunit α; Interleukin 9 receptor; Interleukin-13 receptor subunit α1; Interleukin-15 receptor subunit α; Interleukin 21 receptor; Cytokine receptor-like factor 2, and the like. The amino acid sequences of such examples are provided in FIG. 53.

[00633] Non-limiting examples of IL-3 family receptors include Interleukin 3 receptor, α subunit ; Interleukin 5 receptor, α subunit; GM-CSF receptor, α subunit; Cytokine receptor common β subunit; and the like. The amino acid sequences of such examples are provided in FIG. 54.

[00634] Non-limiting examples of IL-6 family receptors include Leptin receptor; IL6R (Interleukin-6 receptor, α subunit / interleukin 6 receptor); IL6ST (Interleukin-6 receptor, β subunit / interleukin 6 signal transducer); Interleukin-11 receptor, α subunit; Interleukin 27 receptor, alpha; Interleukin-31 receptor, α subunit; Ciliary neurotrophic factor receptor α subunit; Leukemia inhibitory factor receptor; Oncostatin M-specific receptor, β subunit; and the like. The amino acid sequences of such examples are provided in FIG. 55.

[00635] Non-limiting examples of IL-12 family receptors include Interleukin-12 receptor, β1 subunit; Interleukin-12 receptor, β2 subunit; Interleukin 23 receptor; and the like. The amino acid sequences of such examples are provided in FIG. 56.

[00636] Non-limiting examples of prolactin family receptors include Eythropoietin receptor; Granulocyte colony-stimulating factor receptor; Growth hormone receptor; Prolactin receptor; Thrombopoietin receptor; and the like. The amino acid sequences of such examples are provided in FIG. 57.

[00637] Non-limiting examples of interferon family receptors include interferon α/β receptor 1; Interferon α/β receptor 2; Interferon γ receptor 1; Interferon γ receptor 2; and the like. The amino acid sequences of such examples are provided in FIG. 58.

[00638] Non-limiting examples of IL-10 family receptors include Interleukin-22 receptor α2; Interleukin 10 receptor, α subunit; Interleukin 10 receptor, β subunit; Interleukin 20 receptor, α subunit; Interleukin 20 receptor, β subunit; Interleukin 22 receptor, α1 subunit; Interferon-λ receptor subunit 1; and the like. The amino acid sequences of such examples are provided in FIG. 59.

[00639] Non-limiting examples of IL-17 family receptors include Interleukin 17 receptor A; Interleukin 17 receptor B; interleukin 17 receptor C; Interleukin-17 receptor D; Interleukin 17 receptor E; and the like. The amino acid sequences of such examples are provided in FIG. 60.

[00640] Non-limiting examples of immunoglobulin-like superfamily receptors include Interleukin 1 receptor, type I ; Interleukin 1 receptor, type II; Interleukin-1 receptor-like 1; Interleukin-1 receptor-like 2; Interleukin-18 1; IL-1 receptor accessory protein; IL-18 receptor accessory protein; PDGFRα (platelet derived growth factor receptor alpha); PDGFRβ (platelet derived growth factor receptor beta); KIT proto-oncogene receptor tyrosine kinase; CSFR (colony stimulating factor 1 receptor); and the like. The amino acid sequences of such examples are provided in FIG. 61.

[00641] Non-limiting examples of tumor necrosis factor family receptors include TNFR1 (tumor necrosis factor receptor 1 / TNFRSF1A); TNFR2 (tumor necrosis factor receptor 2 / TNFRSF1B); lymphotoxin β receptor / TNFRSF3; OX40 / TNFRSF4; CD40 / TNFRSF5; Fas / TNFRSF6; decoy receptor 3 / TNFRSF6B; CD27 / TNFRSF7; CD30 / TNFRSF8; 4-1BB / TNFRSF9; DR4 (death receptor 4 / TNFRSF10A); DR5 (death receptor 5 / TNFRSF10B); decoy receptor 1 / TNFRSF10C; decoy receptor 2 / TNFRSF10D; RANK (receptor activator of NF-kappa B / TNFRSF11A); OPG (osteoprotegerin / TNFRSF11B); DR3 (death receptor 3 / TNFRSF25); TWEAK receptor / TNFRSF12A; TACI / TNFRSF13B; BAFF-R (BAFF receptor / TNFRSF13C); HVEM (herpes virus entry mediator / TNFRSF14); nerve growth factor receptor / TNFRSF16; BCMA (B cell maturation antigen / TNFRSF17); GITR (glucocorticoid-induced TNF receptor / TNFRSF18); TAJ (toxicity and JNK inducer / TNFRSF19); RELT / TNFRSF19L; DR6 (death receptor 6 / TNFRSF21); TNFRSF22; TNFRSF23; ectodysplasin A2 isoform receptor / TNFRS27; ectodysplasin 1, anhidrotic receptor; and the like. The amino acid sequences of such examples are provided in FIG. 62.

[00642] Non-limiting examples of chemokine receptors include CCR1; CCR2; CCR3; CCR4; CCR5; CCR6; CCR7; CCR8; CCR9; CCR10; CXCR1 (IL8Ra); CXCR2 (IL8Rb); CXCR3; CXCR4; CXCR5; CXCR6; CX3CR1; and the like. The amino acid sequences of such examples are provided in FIG. 63.

[00643] Non-limiting examples of TGF-beta family receptors include transforming growth factor beta, receptor type I (TGFBR1 (ALK5)); transforming growth factor beta, receptor type II (TGFBR2 (MFS2))

and transforming growth factor beta, receptor type III (TGFBR3 (β-Glycan)). The amino acid sequences of such examples are provided in FIG. 64.

Receptor tyrosine kinases

[00644] As noted above, useful non-limiting examples of dimerization-dependent cell-surface receptors include receptor tyrosine kinases (RTKs), including e.g., RTKs that form homodimers and RTKs that form heterodimers. Useful RTKs include but are not limited to e.g., MERTK (RefSeq Accession NP_006334); LMTK3 (RefSeq Accession NP_001073903); CSF1R (RefSeq Accession NP_001275634); EGFR (RefSeq Accession NP_005219); EPHA2 (RefSeq Accession NP_004422); EPHA1 (RefSeq Accession NP_005223); EPHA3 (RefSeq Accession NP_005224); EPHA4 (RefSeq Accession NP_001291465); EPHA5 (RefSeq Accession NP_004430); EPHA7 (RefSeq Accession NP_004431); EPHA8 (RefSeq Accession NP_065387); EPHB1 (RefSeq Accession NP_004432); EPHB2 (RefSeq Accession NP_001296122); EPHB3 (RefSeq Accession NP_004434); EPHB4 (RefSeq Accession NP_004435); EPHB6 (RefSeq Accession NP_004436); ERBB2 (RefSeq Accession NP_004439); ERBB3 (RefSeq Accession NP_001973); ERBB4 (RefSeq Accession NP_005226); FGFR1 (RefSeq Accession NP_075598); FGFR3 (RefSeq Accession NP_000133); FGFR2 (RefSeq Accession NP_000132); FGFR4 (RefSeq Accession NP_002002); LMTK2 (RefSeq Accession NP_055731); FLT1 (RefSeq Accession NP_002010); FLT3 (RefSeq Accession NP_004110); FLT4 (RefSeq Accession NP_891555); ALK (RefSeq Accession NP_004295); EPHA10 (RefSeq Accession NP_001092909); EPHA6 (RefSeq Accession NP_001265229); IGF1R (RefSeq Accession NP_000866); INSR (RefSeq Accession NP_000199); INSRR (RefSeq Accession NP_055030); KDR (RefSeq Accession NP_002244); KIT (RefSeq Accession NP_000213); LTK (RefSeq Accession NP_002335); MET (RefSeq Accession NP_000236); MST1R (RefSeq Accession NP_002438); MUSK (RefSeq Accession NP_005583); NTRK1 (RefSeq Accession NP_002520); NTRK2 (RefSeq Accession NP_001018074); NTRK3 (RefSeq Accession NP_001012338); ROR1 (RefSeq Accession NP_005003); ROR2 (RefSeq Accession NP_004551); DDR2 (RefSeq Accession NP_001014796); PDGFRA (RefSeq Accession NP_006197); PDGFRB (RefSeq Accession NP_002600); AXL (RefSeq Accession NP_068713); PTK7 (RefSeq Accession NP_002812); RET (RefSeq Accession NP_066124); ROS1 (RefSeq Accession NP_002935); RYK (RefSeq Accession NP_002949); TEK (RefSeq Accession NP_000450); TIE1 (RefSeq Accession NP_005415); TYRO3 (RefSeq Accession NP_006284); DDR1 (RefSeq Accession NP_001284583); AATK (RefSeq Accession NP_001073864); and the like. The amino acid sequences of such examples are provided in FIG. 65.

Dimerization pairs

[00645] As noted above, conditionally active dimerization-dependent cell-surface receptor of the present disclosure will generally include a cell-surface receptor polypeptide that comprises a first member of a dimerization pair that is conditionally dimerizable with a second cell-surface receptor polypeptide that

comprises the second member of a dimerization pair. Thus, a conditionally active dimerization-dependent cell-surface receptor of the present disclosure will comprise a member of a dimerization pair that includes a LBD of a nuclear hormone receptor or a co-regulator peptide. Two conditionally active dimerization-dependent cell-surface receptors may be utilized as a system where one conditionally active dimerization-dependent cell-surface receptor comprises a first member of a dimerization pair that includes a LBD of a nuclear hormone receptor and the other conditionally active dimerization-dependent cell-surface receptor comprises the second member of the dimerization pair that includes a co-regulator peptide of the same nuclear hormone receptor. In the presence of a dimerization agent (e.g., a nuclear hormone, or a functional derivative or analog of the nuclear hormone; also referred to herein as a "dimerizer"), the first and second members of the dimerization pair will bind to one another, and will effect dimerization of the two conditionally active dimerization-dependent cell-surface receptor polypeptides. A first member of a dimerization pair, or a second member of a dimerization pair, can also be referred to as a "dimerization domain".

[00646] A ligand-binding domain of a nuclear hormone receptor can be from any of a variety of nuclear hormone receptors, including, but not limited to, those described above. Suitable co-regulator polypeptides include full-length naturally-occurring nuclear hormone co-regulator polypeptides. Suitable co-regulator polypeptides include fragments of naturally-occurring nuclear hormone co-regulator polypeptides. Suitable co-regulator polypeptides include synthetic or recombinant nuclear hormone co-regulator polypeptides. Non-limiting examples of suitable co-regulator polypeptides include those described above.

NUCLEIC ACIDS

[00647] The present disclosure provides a nucleic acid that comprises a nucleotide sequence encoding a heterodimeric, conditionally active polypeptide of the present disclosure. A single nucleic acid molecule may include multiple sequences encoding two or more portions of a heterodimeric, conditionally active polypeptide of the present disclosure. In some instances, two or more portions of a heterodimeric, conditionally active polypeptide of the present disclosure may be separated across multiple individual nucleic acid molecules (e.g., multiple nucleic acid vectors). A nucleic acid comprising a nucleotide sequence encoding a heterodimeric, conditionally active polypeptide of the present disclosure will in some embodiments be DNA, including, e.g., a recombinant expression vector. A nucleic acid comprising a nucleotide sequence encoding a heterodimeric, conditionally active polypeptide of the present disclosure will in some embodiments be RNA, e.g., *in vitro* synthesized RNA.

[00648] In some cases, a nucleic acid of the present disclosure comprises a nucleotide sequence encoding only a first portion, e.g., a first polypeptide chain of a heterodimeric, conditionally active polypeptide of the present disclosure. In some cases, a nucleic acid of the present disclosure comprises a nucleotide sequence encoding only a second portion, e.g., a second polypeptide chain, of a heterodimeric,

conditionally active polypeptide of the present disclosure. In some cases, a nucleic acid of the present disclosure comprises a nucleotide sequence encoding both polypeptide chains of a heterodimeric, conditionally active polypeptide of the present disclosure.

[00649] In some cases, a single nucleic acid of the present disclosure may comprise one or more nucleotide sequences encoding two or more conditionally active polypeptides of the present disclosure. For example, in some instances, a nucleic acid of the present disclosure may encode a first conditionally acitive polypeptide comprising a first member of a dimerization pair and a second conditionally acitive polypeptide comprising the second member of a dimerization pair. In some embodiments, a nucleic acid of the present disclosure may comprise a sequence or multiple sequences that encode a first conditionally active dimerization-dependent cell-surface receptor comprising a first member of a dimerization pair and a second conditionally active dimerization-dependent cell-surface receptor comprising the second member of a dimerization pair. In some embodiments, two conditionally active dimerization-dependent cell-surface receptors, although each containing half of a dimerization pair, may be encoded by sequences present on separate nucleic acids.

[00650] In some cases, a subject nucleic acid provides for production of a heterodimeric, conditionally active polypeptide of the present disclosure, e.g., in a mammalian cell. In other cases, a subject nucleic acid provides for amplification of the heterodimeric, conditionally active polypeptide-encoding nucleic acid.

[00651] A nucleotide sequence encoding a heterodimeric, conditionally active polypeptide of the present disclosure can be operably linked to a transcriptional control element, e.g., a promoter, and enhancer, etc. In some instances, the heterodimeric, conditionally active polypeptide encoding nucleic acid is operably linked to a tissue specific promoter for expression in a particular cell type of interest. For example, a heterodimeric, conditionally active polypeptide may be operably linked to an immune cell specific promoter for specific expression in one or more immune cell types. In other instances, a heterodimeric, conditionally active polypeptide may be operably linked to a general (i.e., non-immune cell specific) promoter including e.g., a ubiquitous promoter, a constitutive promoter, a heterologous promoter, a regulatable promoters (e.g., inducible promoters, reversible promoters, etc.), etc.

General Promoters

[00652] Suitable promoter and enhancer elements are known in the art. For expression in a bacterial cell, suitable promoters include, but are not limited to, lacI, lacZ, T3, T7, gpt, lambda P and trc. For expression in a eukaryotic cell, suitable promoters include, but are not limited to; cytomegalovirus immediate early promoter; herpes simplex virus thymidine kinase promoter; early and late SV40 promoters; promoter present in long terminal repeats from a retrovirus; mouse metallothionein-I promoter; and various art-known promoters.

[00653] Suitable promoters for use in prokaryotic host cells include, but are not limited to, a bacteriophage T7 RNA polymerase promoter; a trp promoter; a lac operon promoter; a hybrid promoter, e.g., a lac/tac hybrid promoter, a tac/trc hybrid promoter, a trp/lac promoter, a T7/lac promoter; a trc promoter; a tac promoter, and the like; an araBAD promoter; in vivo regulated promoters, such as an ssaG promoter or a related promoter (see, e.g., U.S. Patent Publication No. 20040131637), a pagC promoter (Pulkkinen and Miller, J. Bacteriol., 1991: 173(1): 86-93; Alpuche-Aranda et al., PNAS, 1992; 89(21): 10079-83), a nirB promoter (Harborne et al. (1992) Mol. Micro. 6:2805-2813), and the like (see, e.g., Dunstan et al. (1999) Infect. Immun. 67:5133-5141; McKelvie et al. (2004) Vaccine 22:3243-3255; and Chatfield et al. (1992) Biotechnol. 10:888-892); a sigma70 promoter, e.g., a consensus sigma70 promoter (see, e.g., GenBank Accession Nos. AX798980, AX798961, and AX798183); a stationary phase promoter, e.g., a dps promoter, an spv promoter, and the like; a promoter derived from the pathogenicity island SPI-2 (see, e.g., WO96/17951); an actA promoter (see, e.g., Shetron-Rama et al. (2002) Infect. Immun. 70:1087-1096); an rpsM promoter (see, e.g., Valdivia and Falkow (1996). Mol. Microbiol. 22:367); a tet promoter (see, e.g., Hillen,W. and Wissmann,A. (1989) In Saenger,W. and Heinemann,U. (eds), Topics in Molecular and Structural Biology, Protein–Nucleic Acid Interaction. Macmillan, London, UK, Vol. 10, pp. 143–162); an SP6 promoter (see, e.g., Melton et al. (1984) Nucl. Acids Res. 12:7035); and the like. Suitable strong promoters for use in prokaryotes such as Escherichia coli include, but are not limited to Trc, Tac, T5, T7, and PLambda. Non-limiting examples of operators for use in bacterial host cells include a lactose promoter operator (LacI repressor protein changes conformation when contacted with lactose, thereby preventing the LacI repressor protein from binding to the operator), a tryptophan promoter operator (when complexed with tryptophan, TrpR repressor protein has a conformation that binds the operator; in the absence of tryptophan, the TrpR repressor protein has a conformation that does not bind to the operator), and a tac promoter operator (see, for example, deBoer et al. (1983) Proc. Natl. Acad. Sci. U.S.A. 80:21-25).

[00654] Suitable reversible promoters, including reversible inducible promoters are known in the art. Such reversible promoters may be isolated and derived from many organisms, e.g., eukaryotes and prokaryotes. Modification of reversible promoters derived from a first organism for use in a second organism, e.g., a first prokaryote and a second a eukaryote, a first eukaryote and a second a prokaryote, etc., is well known in the art. Such reversible promoters, and systems based on such reversible promoters but also comprising additional control proteins, include, but are not limited to, alcohol regulated promoters (e.g., alcohol dehydrogenase I (alcA) gene promoter, promoters responsive to alcohol transactivator proteins (AlcR), etc.), tetracycline regulated promoters, (e.g., promoter systems including TetActivators, TetON, TetOFF, etc.), steroid regulated promoters (e.g., rat glucocorticoid receptor promoter systems, human estrogen receptor promoter systems, retinoid promoter systems, thyroid promoter systems, ecdysone promoter systems, mifepristone promoter systems, etc.), metal regulated

promoters (e.g., metallothionein promoter systems, etc.), pathogenesis-related regulated promoters (e.g., salicylic acid regulated promoters, ethylene regulated promoters, benzothiadiazole regulated promoters, etc.), temperature regulated promoters (e.g., heat shock inducible promoters (e.g., HSP-70, HSP-90, soybean heat shock promoter, etc.), light regulated promoters, synthetic inducible promoters, and the like.

[00655] In some embodiments, e.g., for expression in a yeast cell, a suitable promoter is a constitutive promoter such as an ADH1 promoter, a PGK1 promoter, an ENO promoter, a PYK1 promoter and the like; or a regulatable promoter such as a GAL1 promoter, a GAL10 promoter, an ADH2 promoter, a PHO5 promoter, a CUP1 promoter, a GAL7 promoter, a MET25 promoter, a MET3 promoter, a CYC1 promoter, a HIS3 promoter, an ADH1 promoter, a PGK promoter, a GAPDH promoter, an ADC1 promoter, a TRP1 promoter, a URA3 promoter, a LEU2 promoter, an ENO promoter, a TP1 promoter, and AOX1 (e.g., for use in Pichia). Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

Immune Cell Promoters

[00656] In some instances, nucleic acids of the present disclosure include immune cell specific promoters that are expressed in one or more immune cell types, including but not limited to lymphocytes, hematopoietic stem cells and/or progeny thereof (i.e., immune cell progenitors), etc. Any convenient and appropriate promoter of an immune cell specific gene may find use in nucleic acids of the present disclosure. In some instances, an immune cell specific promoter of a nucleic acid of the present disclosure may be a T cell specific promoter. In some instances, an immune cell specific promoter of a nucleic acid of the present disclosure may be a light and/or heavy chain immunoglobulin gene promoter and may or may not include one or more related enhancer elements.

[00657] In some instances, an immune cell specific promoter of a nucleic acid of the present disclosure may be a promoter of a B29 gene promoter, a CD14 gene promoter, a CD43 gene promoter, a CD45 gene promoter, a CD68 gene promoter, a IFN-β gene promoter, a WASP gene promoter, a T-cell receptor β -chain gene promoter, a V9 γ (TRGV9) gene promoter, a V2 δ (TRDV2) gene promoter, and the like.

[00658] In some instances, an immune cell specific promoter of a nucleic acid of the present disclosure may be a viral promoter expressed in immune cells. As such, in some instances, viral promoters useful in nucleic acids of the present disclosure include viral promoters derived from immune cells viruses, including but not limited to, e.g., lentivirus promoters (e.g., HIV, SIV, FIV, EIAV, or Visna promoters) including e.g., LTR promoter, etc., Retroviridae promoters including, e.g., HTLV-I promoter, HTLV-II promoter, etc., and the like.

[00659] In some cases, the promoter is a CD8 cell-specific promoter, a CD4 cell-specific promoter, a neutrophil-specific promoter, or an NK-specific promoter. For example, a CD4 gene promoter can be

used; see, e.g., Salmon et al. (1993) Proc. Natl. Acad. Sci. USA 90:7739; and Marodon et al. (2003) Blood 101:3416. As another example, a CD8 gene promoter can be used. NK cell-specific expression can be achieved by use of an Ncr1 (p46) promoter; see, e.g., Eckelhart et al. (2011) Blood 117:1565.

Additional Nucleic Acid Components, Constructs and Use Thereof

[00660] In some instances, the locus or construct or transgene containing the suitable promoter is irreversibly switched through the induction of an inducible system. Suitable systems for induction of an irreversible switch are well known in the art, e.g., induction of an irreversible switch may make use of a Cre-lox-mediated recombination (see, e.g., Fuhrmann-Benzakein, et al., PNAS (2000) 28:e99, the disclosure of which is incorporated herein by reference). Any suitable combination of recombinase, endonuclease, ligase, recombination sites, etc. known to the art may be used in generating an irreversibly switchable promoter. Methods, mechanisms, and requirements for performing site-specific recombination, described elsewhere herein, find use in generating irreversibly switched promoters and are well known in the art, see, e.g., Grindley et al. (2006) Annual Review of Biochemistry, 567-605 and Tropp (2012) Molecular Biology (Jones & Bartlett Publishers, Sudbury, MA), the disclosures of which are incorporated herein by reference.

[00661] A nucleotide sequence encoding a subject heterodimeric, conditionally active polypeptide can be present in an expression vector and/or a cloning vector. Where a subject heterodimeric, conditionally active polypeptide is split between two or more separate polypeptides, nucleotide sequences encoding the two or more polypeptides can be cloned in the same or separate vectors. An expression vector can include a selectable marker, an origin of replication, and other features that provide for replication and/or maintenance of the vector. Suitable expression vectors include, e.g., plasmids, viral vectors, and the like.

[00662] Large numbers of suitable vectors and promoters are known to those of skill in the art; many are commercially available for generating a subject recombinant constructs. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia).

[00663] Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins. A selectable marker operative in the expression host may be present. Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Opthalmol Vis Sci 35:2543 2549, 1994; Borras et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998, Flannery et al., PNAS 94:6916 6921, 1997; Bennett

et al., Invest Opthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997, Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

[00664] As noted above, in some embodiments, a nucleic acid comprising a nucleotide sequence encoding a heterodimeric, conditionally active polypeptide of the present disclosure will in some embodiments be RNA, e.g., in vitro synthesized RNA. Methods for in vitro synthesis of RNA are known in the art; any known method can be used to synthesize RNA comprising a nucleotide sequence encoding the first and/or the second polypeptide of a heterodimeric, conditionally active polypeptide of the present disclosure. Methods for introducing RNA into a host cell are known in the art. See, e.g., Zhao et al. (2010) Cancer Res. 15:9053. Introducing RNA comprising a nucleotide sequence encoding the first and/or the second polypeptide of a heterodimeric, conditionally active polypeptide of the present disclosure into a host cell can be carried out in vitro or ex vivo or in vivo. For example, a host cell (e.g., an NK cell, a cytotoxic T lymphocyte, etc.) can be electroporated in vitro or ex vivo with RNA comprising a nucleotide sequence encoding the first and/or the second polypeptide of a heterodimeric, conditionally active polypeptide of the present disclosure.

CELLS

[00665] The present disclosure provides a mammalian cell that is genetically modified to produce a heterodimeric, conditionally active polypeptide of the present disclosure.

[00666] Suitable mammalian cells include primary cells and immortalized cell lines. Suitable mammalian cell lines include human cell lines, non-human primate cell lines, rodent (e.g., mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, Hut-78, Jurkat, HL-60, NK cell lines (e.g., NKL, NK92, and YTS), and the like.

[00667] In some instances, suitable cells include those described in Themeli et al. Cell Stem Cell. 2015 Apr 2;16(4):357-66; the disclosure of which is incorporated herein by reference in its entirety.

[00668] In some instances, the cell is not an immortalized cell line, but is instead a cell (e.g., a primary cell) obtained from an individual. For example, in some cases, the cell is an immune cell, immune cell progenitor or immune stem cell obtained from an individual. As an example, the cell is a T lymphocyte, or progenitor thereof, obtained from an individual. As another example, the cell is a cytotoxic cell, or progenitor thereof, obtained from an individual. As another example, the cell is a stem cell or progenitor cell obtained from an individual.

UTILITY

[00669] A heterodimeric, conditionally active polypeptide of the present disclosure finds use in a variety of research and treatment methods, which are provided herein.

[00670] In some cases, where a heterodimeric, conditionally active polypeptide of the present disclosure comprises a first heterologous polypeptide and a second heterologous polypeptide that individually do not exhibit an activity but do exhibit the activity when present in a heterodimeric, conditionally active polypeptide of the present disclosure and in the presence of a dimerization agent that induces binding of the LBD to the co-regulator peptide in the heterodimeric, conditionally active polypeptide, the present disclosure provides a method of activating the activity. Thus, e.g., the present disclosure provides a method of activating a polypeptide (inducing an activity of a polypeptide), the method comprising contacting a heterodimeric, conditionally active polypeptide of the present disclosure with a dimerization agent, where the polypeptide is present in the heterodimeric polypeptide as a first heterologous polypeptide and a second heterologous polypeptide, where the first heterologous polypeptide and a second heterologous polypeptide that individually do not exhibit an activity but do exhibit the activity when present in a heterodimeric, conditionally active polypeptide of the present disclosure and in the presence of the dimerization agent. The activity that is induced depends on the nature of the first heterologous polypeptide and a second heterologous polypeptide. For example, where the first heterologous polypeptide and a second heterologous polypeptide are a receptor and a co-receptor, respectively, the activity can be binding of the receptor to the co-receptor, or a downstream activity that results from binding of the receptor to the co-receptor. In some cases, contacting a heterodimeric, conditionally active polypeptide of the present disclosure occurs *in vitro*, where the heterodimeric, conditionally active polypeptide of the present disclosure is not in a cell. In some cases, contacting a heterodimeric, conditionally active polypeptide of the present disclosure occurs in a cell *in vitro*, where the heterodimeric, conditionally active polypeptide of the present disclosure is in the cell. In some cases, contacting a heterodimeric, conditionally active polypeptide of the present disclosure occurs in a cell *in vivo*, where the heterodimeric, conditionally active polypeptide of the present disclosure is in the cell.

METHODS FOR MODULATING THE ACTIVITY OF A CELL

[00671] The present disclosure provides methods for modulating (activating; repressing) an activity of a cell. The methods generally involve contacting a cell that expresses a heterodimeric, conditionally active

polypeptide of the present disclosure with a dimerizing agent. In some cases, the methods involve contacting a cell that expresses a heterodimeric, conditionally active polypeptide of the present disclosure with a dimerizing agent and a second agent (e.g., an antigen).

METHODS OF ACTIVATING AN IMMUNE CELL

[00672] The present disclosure provides methods of activating an immune cell, where the immune cell expresses an On-switch CAR of the present disclosure. The present disclosure provides methods of activating an immune cell *in vitro*, *in vivo*, or *ex vivo*. The methods generally involve contacting an immune cell (*in vitro*, *in vivo*, or *ex vivo*) with a dimerizing agent and an antigen, where the immune cell is genetically modified to produce a heterodimeric, conditionally active CAR of the present disclosure. In the presence of the dimerizing agent and the antigen, the heterodimeric, conditionally active CAR dimerizes and activates the immune cell, thereby producing an activated immune cell. Immune cells include, e.g., a cytotoxic T lymphocyte, an NK cell, a $CD4^+$ T cell, a T regulatory (Treg) cell, etc.

[00673] Contacting the genetically modified immune cell (e.g., a T lymphocyte, an NK cell) with a dimerizing agent and a second member of a specific binding pair (e.g., an antigen, a ligand, a receptor) can increase production of a cytokine by the immune cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared with the amount of cytokine produced by the immune cell in the absence of the second member of a specific binding pair and/or the dimerizing agent. Cytokines whose production can be increased include, but are not limited to, IL-2 and IFN-γ.

[00674] Contacting the genetically modified immune cell (e.g., a T lymphocyte, an NK cell) with a dimerizing agent and an antigen can increase production of a cytokine by the immune cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared with the amount of cytokine produced by the immune cell in the absence of the antigen and/or the dimerizing agent. Cytokines whose production can be increased include, but are not limited to, IL-2 and IFN-γ.

[00675] Contacting a genetically modified cytotoxic cell (e.g., cytotoxic T lymphocyte) with a dimerizing agent and a second member of a specific binding pair (e.g., an antigen, a ligand, a receptor) can increase cytotoxic activity of the cytotoxic cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared to the cytotoxic activity of the cytotoxic cell in the absence of the dimerizing agent.

[00676] Contacting a genetically modified cytotoxic cell (e.g., cytotoxic T lymphocyte) with a dimerizing agent and an antigen can increase cytotoxic activity of the cytotoxic cell by at least about 10%, at least

about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared to the cytotoxic activity of the cytotoxic cell in the absence of the dimerizing agent.

[00677] In other embodiments, e.g., depending on the host immune cell, contacting a genetically modified host cell with a dimerizing agent and an antigen can increase or decrease cell proliferation, cell survival, cell death, and the like.

[00678] The present disclosure provides a method of generating a conditionally activatable cell. The method generally involves genetically modifying a mammalian cell with an expression vector, or an RNA (e.g., *in vitro* transcribed RNA), comprising nucleotide sequences encoding a heterodimeric, conditionally active CAR of the present disclosure. The genetically modified cell is conditionally activatable in the presence of: a) an antigen to which the first polypeptide of the CAR binds; and b) a dimerizer (a dimerizing agent). The genetic modification can be carried out *in vivo*, *in vitro*, or *ex vivo*. The cell can be an immune cell (e.g., a T lymphocyte or NK cell), a stem cell, a progenitor cell, etc.

[00679] In some cases, the genetic modification is carried out *ex vivo*. For example, a T lymphocyte, a stem cell, or an NK cell is obtained from an individual; and the cell obtained from the individual is genetically modified to express a CAR of the present disclosure. The genetically modified cell is conditionally activatable in the presence of: a) an antigen to which the first polypeptide of the CAR binds; and b) a dimerizer. In some cases, the genetically modified cell is activated *ex vivo*. In other cases, the genetically modified cell is introduced into an individual (e.g., the individual from whom the cell was obtained); and the genetically modified cell is activated *in vivo*, e.g., by administering to the individual a dimerizer. For example, where the antigen is present on the surface of a cell in the individual, there is no need to administer the antigen. The genetically modified cell comes into contact with the antigen present on the surface of a cell in the individual; and, upon administration to the individual of a dimerizer, the genetically modified cell is activated. For example, where the genetically modified cell is a T lymphocyte, the genetically modified cell can exhibit cytotoxicity toward a cell that presents an antigen on its surface to which the CAR binds.

METHODS OF REPRESSING IMMUNE CELL ACTIVATION

[00680] The present disclosure provides methods of repressing immune cell activation, such methods being applicable in vitro, in vivo, or ex vivo. The methods generally involve contacting an immune cell (in vitro, in vivo, or ex vivo) with a dimerizing agent, where the immune cell is genetically modified to produce a heteromeric, conditionally repressible synthetic ICR of the present disclosure. In the presence of the dimerizing agent, the heteromeric, conditionally repressible ICR dimerizes and represses activation of the immune cell, thereby producing a repressed immune cell. Immune cells include, e.g., a cytotoxic T lymphocyte, an NK cell, a CD4+ T cell, a T regulatory (Treg) cell, etc.

[00681] Contacting the genetically modified immune cell (e.g., a T lymphocyte, an NK cell) with a dimerizing agent can repress the expression of a lymphocyte cell surface antigen, e.g., a cell surface antigen indicative of immune cell activation, T cell activation, etc., by the immune cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared with the amount of the cell surface antigen expressed by the activated immune cell in the absence of the dimerizing agent. Lymphocyte cell surface antigens whose production can be repressed include, but are not limited to e.g., CD69.

[00682] Contacting the genetically modified immune cell (e.g., a T lymphocyte, an NK cell) with a dimerizing agent can repress the production of a cytokine by the immune cell by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared with the amount of cytokine produced by the activated immune cell in the absence of the dimerizing agent. Cytokines whose production can be repressed include, but are not limited to, IL-2 and IFN-γ.

FORMULATIONS, DOSAGES, AND ROUTES OF ADMINISTRATION

[00683] As discussed above, a treatment method of the present disclosure involves administration to an individual in need thereof of an effective amount of a dimerizer agent, and may also involve administration of an antigen.

[00684] An "effective amount" of a dimerizer agent is in some cases an amount that, when administered in one or more doses to an individual in need thereof, increases the level of cytotoxic activity of a T lymphocyte expressing a subject CAR by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared to the cytotoxic activity of the T lymphocyte in the absence of the dimerizing agent.

[00685] An "effective amount" of a dimerizer agent is in some cases an amount that, when administered in one or more doses to an individual in need thereof, increases the level of cytotoxic activity of an NK cell expressing a subject CAR by at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, at least about 2-fold, at least about 2.5-fold, at least about 5-fold, at least about 10-fold, or more than 10-fold, compared to the cytotoxic activity of the NK cell in the absence of the dimerizing agent.

[00686] An "effective amount" of a dimerizer agent is in some cases an amount that, when administered in one or more doses to an individual in need thereof, reduces the number of cancer cells in the individual and/or reduces tumor mass in the individual, by at least about 10%, at least about 15%, at least about

20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 75%, or more than 75%, compared to the number of cancer cells and/or tumor mass in the absence of the dimerizing agent.

[00687] In some embodiments, an effective amount of a dimerizer is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce one or more of tumor growth rate, cancer cell number, and tumor mass, by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the tumor growth rate, cancer cell number, or tumor mass in the absence of treatment with the dimerizer.

Formulations

[00688] In the subject methods, a dimerizer can be administered to the host using any convenient means capable of resulting in the desired therapeutic effect or diagnostic effect. Thus, the dimerizer can be incorporated into a variety of formulations for therapeutic administration. More particularly, a dimerizer can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

[00689] In pharmaceutical dosage forms, a dimerizer can be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

[00690] Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of a dimerizer adequate to achieve the desired state in the subject being treated.

[00691] The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

[00692] For oral preparations, a dimerizer can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose,

mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

[00693] A dimerizer can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[00694] Pharmaceutical compositions comprising a dimerizer are prepared by mixing the dimerizer having the desired degree of purity with optional physiologically acceptable carriers, excipients, stabilizers, surfactants, buffers and/or tonicity agents. Acceptable carriers, excipients and/or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid, glutathione, cysteine, methionine and citric acid; preservatives (such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, or combinations thereof); amino acids such as arginine, glycine, ornithine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophan, methionine, serine, proline and combinations thereof; monosaccharides, disaccharides and other carbohydrates; low molecular weight (less than about 10 residues) polypeptides; proteins, such as gelatin or serum albumin; chelating agents such as EDTA; sugars such as trehalose, sucrose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, N-methylglucosamine, galactosamine, and neuraminic acid; and/or non-ionic surfactants such as Tween, Brij Pluronics, Triton-X, or polyethylene glycol (PEG).

[00695] The pharmaceutical composition may be in a liquid form, a lyophilized form or a liquid form reconstituted from a lyophilized form, wherein the lyophilized preparation is to be reconstituted with a sterile solution prior to administration. The standard procedure for reconstituting a lyophilized composition is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization); however solutions comprising antibacterial agents may be used for the production of pharmaceutical compositions for parenteral administration; see also Chen (1992) Drug Dev Ind Pharm 18, 1311-54.

[00696] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity a dimerizer calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for a given dimerizer may depend on the

particular dimerizer employed and the effect to be achieved, and the pharmacodynamics associated with each dimerizer in the host.

[00697] In some embodiments, a dimerizer is formulated in a controlled release formulation. Sustained-release preparations may be prepared using methods well known in the art. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the dimerizer in which the matrices are in the form of shaped articles, e.g. films or microcapsules. Examples of sustained-release matrices include polyesters, copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, hydrogels, polylactides, degradable lactic acid-glycolic acid copolymers and poly-D-(-)-3-hydroxybutyric acid. Possible loss of biological activity may be prevented by using appropriate additives, by controlling moisture content and by developing specific polymer matrix compositions.

Dosages

[00698] A suitable dosage can be determined by an attending physician or other qualified medical personnel, based on various clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular dimerizer to be administered, sex of the patient, time, and route of administration, general health, and other drugs being administered concurrently. A dimerizer may be administered in amounts between 1 ng/kg body weight and 20 mg/kg body weight per dose, e.g. between 0.1 mg/kg body weight to 10 mg/kg body weight, e.g. between 0.5 mg/kg body weight to 5 mg/kg body weight; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it can also be in the range of 1 μg to 10 mg per kilogram of body weight per minute.

[00699] Those of skill will readily appreciate that dose levels can vary as a function of the specific dimerizer, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Routes of administration

[00700] A dimerizer is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[00701] Conventional and pharmaceutically acceptable routes of administration include intratumoral, peritumoral, intramuscular, intratracheal, intracranial, subcutaneous, intradermal, topical application, intravenous, intraarterial, rectal, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the dimerizer and/or the desired effect. A dimerizer can be administered in a single dose or in multiple doses. In some embodiments, a dimerizer is administered orally. In some embodiments, a dimerizer is administered via

an inhalational route. In some embodiments, a dimerizer is administered intranasally. In some embodiments, a dimerizer is administered locally. In some embodiments, a dimerizer is administered intratumorally. In some embodiments, a dimerizer is administered peritumorally. In some embodiments, a dimerizer is administered intracranially. In some embodiments, a dimerizer is administered intravenously.

[00702] The agent can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

[00703] Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intratumoral, peritumoral, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of a dimerizer. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

[00704] A dimerizer can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

[00705] By treatment is meant at least an amelioration of the symptoms associated with the pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, *e.g.* symptom, associated with the pathological condition being treated, such as cancer. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, *e.g.* prevented from happening, or stopped, *e.g.* terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

[00706] In some embodiments, a dimerizer is administered by injection and/or delivery, e.g., to a site in a brain artery or directly into brain tissue. A dimerizer can also be administered directly to a target site e.g., by direct injection, by implantation of a drug delivery device such as an osmotic pump or slow release particle, by biolistic delivery to the target site, etc.

[00707]

EXAMPLES OF NON-LIMITING ASPECTS OF THE DISCLOSURE

[00708] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing

description, certain non-limiting aspects of the disclosure numbered 1-45 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

[00709] 1. A heterodimeric, conditionally active polypeptide comprising: a) a first chimeric polypeptide comprising a first member of a dimerization pair and a first heterologous polypeptide; and b) a second chimeric polypeptide comprising a second member of a dimerization pair and a second heterologous polypeptide, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the first chimeric polypeptide and the second chimeric polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[00710] 2. The heterodimeric, conditionally active polypeptide of aspect 1, wherein: a) the first heterologous polypeptide is a T-cell receptor (TCR) alpha chain; and b) the second heterologous polypeptide is a TCR beta chain.

[00711] 3. The heterodimeric, conditionally active polypeptide of aspect 1, wherein: a) the first heterologous polypeptide is a first polypeptide of a chimeric antigen receptor (CAR) heterodimer; and b) the second heterologous polypeptide is a second polypeptide of a CAR heterodimer.

[00712] 4. The heterodimeric, conditionally active polypeptide of aspect 1, wherein: a) the first heterologous polypeptide is an N-terminal portion of an RNA-guided endonuclease; and b) the second heterologous polypeptide is a C-terminal portion of the RNA-guided endonuclease, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide, mediated by the dimerization agent that induces binding of the LBD to the co-regulator, restores enzymatic function of the RNA-guided endonuclease.

[00713] 5. The heterodimeric, conditionally active polypeptide of aspect 4, wherein the RNA-guided endonuclease is a class 2 CRISPR/Cas endonuclease.

[00714] 6. The heterodimeric, conditionally active polypeptide of aspect 5, wherein the class 2 CRISPR/Cas endonuclease is a type II CRISPR/Cas protein, a type V CRISPR/Cas protein, or a type VI CRISPR/Cas protein.

[00715] 7. The heterodimeric, conditionally active polypeptide of aspect 1, wherein: a) the first heterologous polypeptide is an N-terminal portion of an enzyme; and b) the second heterologous

polypeptide is a C-terminal portion of the enzyme, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide restore enzymatic activity of the enzyme.

[00716] 8. The heterodimeric, conditionally active polypeptide of aspect 7, wherein the enzyme is a kinase, a protease, a phosphatase, or a caspase.

[00717] 9. The heterodimeric, conditionally active polypeptide of aspect 1, wherein the first polypeptide and the second polypeptide exhibit an activity when brought into proximity upon dimerization mediated by the dimerization agent, but do not exhibit the activity individually.

[00718] 10. The heterodimeric, conditionally active polypeptide of aspect 1, wherein a) the first heterologous polypeptide is an N-terminal portion of an antigen receptor; and b) the second heterologous polypeptide is a C-terminal portion of the antigen receptor, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide restore signaling activity of the antigen receptor.

[00719] 11. The heterodimeric, conditionally active polypeptide of aspect 1, wherein: a) the first heterologous polypeptide is an N-terminal portion of a receptor; and b) the second heterologous polypeptide is a C-terminal portion of the antigen receptor, wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide mediated by the dimerization agent restores signaling activity of the receptor.

[00720] 12. The heterodimeric, conditionally active polypeptide of any one of aspects 1-11, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from an estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

[00721] 13. The heterodimeric, conditionally active polypeptide of any one of aspects 1-12, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

[00722] 14. The heterodimeric, conditionally active polypeptide of any one of aspects 1-12, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);

ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);

eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);

fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);

ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);

hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);

iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);

jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);

kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);

lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);

mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[00723] 15. A heterodimeric, conditionally active receptor comprising: a) a first chimeric polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second chimeric polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain; or comprising: a) a first chimeric polypeptide comprising:

i) a first member of a specific binding pair; ii) a modulatory domain; iii) a first member of a dimerization pair; iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and b) a second chimeric polypeptide comprising: i) a second member of the dimerization pair; and ii) an intracellular signaling domain, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor, wherein the first chimeric polypeptide and the second chimeric polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[00724] 16. The heterodimeric, conditionally active receptor of aspect 15, wherein the first polypeptide comprises a hinge region interposed between the first member of the specific binding pair and the transmembrane domain.

[00725] 17. The heterodimeric, conditionally active receptor of aspect 15, wherein the first member of the specific binding pair is an antibody or antibody fragment, a ligand, a receptor, or a non-antibody-based recognition scaffold.

[00726] 18. The heterodimeric, conditionally active receptor of aspect 17, wherein the hinge region is an immunoglobulin IgG hinge region or a hinge derived from CD8.

[00727] 19. The heterodimeric, conditionally active receptor of aspect 15, wherein the first and second modulatory domains are selected from 4-1BB (CD137), CD28, ICOS, BTLA, OX-40, CD27, CD30, GITR, HVEM, DAP10, DAP12, and CD28.

[00728] 20. The heterodimeric, conditionally active receptor of aspect 15, wherein the intracellular signaling domain is selected from ZAP70 and CD3-zeta.

[00729] 21. The heterodimeric, conditionally active receptor of aspect 15, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM).

[00730] 22. The heterodimeric, conditionally active receptor of any one of aspects 15-21, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

[00731] 23. The heterodimeric, conditionally active receptor of any one of aspects 15-22, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC,

TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

[00732] 24. The heterodimeric, conditionally active receptor of any one of aspects 15-22, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);

ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);

eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);

fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);

ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);

hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);

iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);

jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);

kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);

lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);

mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[00733] 25. The heterodimeric, conditionally active receptor of aspect 18, wherein: i) the first and second modulatory domains are derived from 4-1BB; ii) the first and second members of the dimerization pair are PPARγ and SRC3; and ii) the signaling domain comprises an ITAM.

[00734] 26. The heterodimeric, conditionally active receptor of aspect 15, wherein the first member of the specific binding pair is a single-chain Fv.

[00735] 27. The heterodimeric, conditionally active receptor of aspect 15, wherein the first member of the specific binding pair binds an epitope present on a cell, on a solid surface, or a lipid bilayer.

[00736] 28. The heterodimeric, conditionally active receptor of aspect 27, wherein the cell is a cancer cell.

[00737] 29. The heterodimeric, conditionally active receptor of aspect 15, wherein the intracellular signaling domain is an intracellular inhibitory domain.

[00738] 30. The heterodimeric, conditionally active receptor of aspect 29, wherein the intracellular inhibitory domain is derived from a protein selected from the group consisting of: PD-1, CTLA4, HPK1, SHP1, SHP2, Sts1, and Csk.

[00739] 31. A heterodimeric, conditionally repressible synthetic immune cell receptor (ICR) comprising: a synthetic stimulatory ICR comprising a first member of a dimerization pair linked to the synthetic stimulatory ICR; and a synthetic ICR repressor comprising a second member of the dimerization pair linked to an intracellular inhibitory domain, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor, wherein the synthetic stimulatory ICR and the synthetic ICR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[00740] 32. The conditionally repressible synthetic ICR of Aspect 31, wherein the synthetic stimulatory ICR comprises an intracellular co-stimulatory domain.

[00741] 33. The conditionally repressible synthetic ICR of Aspect 32, wherein the intracellular co-stimulatory domain is selected from the group consisting of: 4-1BB (CD137), CD28, ICOS, OX-40, BTLA, CD27, CD30, GITR, and HVEM.

[00742] 34. The conditionally repressible synthetic ICR of any one of aspects 31-33, wherein the first member of a dimerization pair is linked intracellularly to the synthetic stimulatory ICR and the second member of the dimerization pair is linked intracellularly to the intracellular inhibitory domain.

[00743] 35. The conditionally repressible synthetic ICR of any one of aspects 31-34, wherein the synthetic ICR repressor further comprises a transmembrane domain.

[00744] 36. The conditionally repressible synthetic ICR of Aspect 35, wherein the second member of the dimerization pair is linked intracellularly to the transmembrane domain.

[00745] 37. The conditionally repressible synthetic ICR of Aspect 35, wherein the second member of the dimerization pair is extracellular and linked to the intracellular inhibitory domain by way of the transmembrane domain.

[00746] 38. The conditionally repressible synthetic ICR of any one of aspects 31-37, wherein the stimulatory ICR binds a soluble antigen.

[00747] 39. The conditionally repressible synthetic ICR of any one of aspects 31-38, wherein the stimulatory ICR binds a cell surface antigen.

[00748] 40. The conditionally repressible synthetic ICR of any one aspects 31-39, wherein the intracellular inhibitory domain is an inhibitory domain derived from a protein selected from the group consisting of: PD-1, CTLA4, HPK1, SHP1, SHP2, Sts1 and Csk.

[00749] 41. The conditionally repressible synthetic ICR of any one of aspects 31-40, wherein the synthetic stimulatory ICR comprises an intracellular signaling domain selected from the group consisting of: a CD3-zeta signaling domain, a ZAP70 signaling domain and an immunoreceptor tyrosine-based activation motif (ITAM).

[00750] 42. The conditionally repressible synthetic ICR of any one of aspects 31-41, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

[00751] 43. The conditionally repressible synthetic ICR of any one of aspects 41-42, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

[00752] 44. The conditionally repressible synthetic ICR of any one of aspects 41-42, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);

ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);

eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);

fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);

ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);

hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);

iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);

jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);

kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);

lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);

mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[00753] 45. The conditionally repressible synthetic ICR of any one of aspects 31-44, wherein the synthetic stimulatory ICR is a synthetic chimeric antigen receptor (CAR) or portion thereof.

[00754] 46. The conditionally repressible synthetic ICR of any one of aspects 31-45, wherein the synthetic stimulatory ICR is a synthetic T cell receptor (TCR) or portion thereof.

[00755] 47. A heterodimeric, conditionally repressible synthetic chimeric antigen receptor (CAR) comprising: a) a synthetic stimulatory CAR comprising: i) a extracellular recognition domain; ii) a transmembrane domain linked to the extracellular recognition domain; iii) a first member of a dimerization pair linked to the transmembrane domain; and iv) an intracellular stimulation domain; and b) a synthetic CAR repressor comprising: i) a second member of the dimerization pair; and ii) an intracellular inhibitory domain linked to the second member of the dimerization pair, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the synthetic stimulatory CAR and the synthetic CAR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[00756] 48. The heterodimeric, conditionally repressible synthetic CAR of Aspect 47, wherein the synthetic CAR repressor further comprises a transmembrane domain linked to the second member of the dimerization pair, the intracellular inhibitory domain or both.

[00757] 49. A heterodimeric, conditionally repressible synthetic T cell receptor (TCR) comprising: a) a synthetic stimulatory TCR comprising: i) a transmembrane domain; ii) a first member of a dimerization pair linked to the transmembrane domain; iii) an engineered TCR polypeptide comprising at least one TCR alpha or beta chain, wherein the at least one TCR alpha or beta chain is linked to the transmembrane domain or the first member of a dimerization pair; and b) a synthetic TCR repressor comprising: i) a second member of the dimerization pair; and ii) an intracellular inhibitory domain linked to the second member of the dimerization pair, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the synthetic stimulatory TCR and the synthetic TCR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[00758] 50. The heterodimeric, conditionally repressible synthetic TCR of Aspect 49, wherein the synthetic TCR repressor further comprises a transmembrane domain linked to the second member of the dimerization pair, the intracellular inhibitory domain or both.

[00759] 51. The heterodimeric, conditionally repressible synthetic TCR of Aspects 49 or 50, wherein the engineered TCR polypeptide further comprises a TCR gamma chain.

[00760] 52. A heterodimeric, conditionally active chimeric antigen receptor (CAR) comprising: a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a first modulatory domain; iii) a first member of a dimerization pair; and iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and b) a second polypeptide comprising: i) a transmembrane domain; ii) a second modulatory domain; iii) a second member of the dimerization pair; and iv) an intracellular signaling domain; or comprising: a) a first polypeptide comprising: i) a first member of a specific binding pair; ii) a modulatory domain; iii) a first member of a dimerization pair; iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and b) a second polypeptide comprising: i) a second member of the dimerization pair; and ii) an intracellular signaling domain, wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and wherein the first polypeptide and the second polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

[00761] 53. The heterodimeric, conditionally active CAR of aspect 52, wherein the first polypeptide comprises a hinge region interposed between the first member of the specific binding pair and the transmembrane domain.

[00762] 54. The heterodimeric, conditionally active CAR of aspect 52, wherein the first member of the specific binding pair is an antibody or antibody fragment, a ligand, or a receptor.

[00763] 55. The heterodimeric, conditionally active CAR of aspect 53, wherein the hinge region is an immunoglobulin IgG hinge region or a hinge derived from CD8.

[00764] 56. The heterodimeric, conditionally active CAR of aspect 52, wherein the first and second modulatory domains are selected from 4-1BB (CD137), CD28, ICOS, BTLA, OX-40, CD27, CD30, GITR, HVEM, DAP10, DAP12, and CD28.

[00765] 57. The heterodimeric, conditionally active CAR of aspect 52, wherein the intracellular signaling domain is selected from ZAP70 and CD3-zeta.

[00766] 58. The heterodimeric, conditionally active CAR of aspect 52, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM).

[00767] 59. The heterodimeric, conditionally active CAR of any one of aspects 52-58, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

[00768] 60. The heterodimeric, conditionally active CAR of any one of aspects 52-59, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

[00769] 61. The heterodimeric, conditionally active CAR of any one of aspects 52-59, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);

ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);

eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);

fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);

ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);

hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);

iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);

jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);

kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);

lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);

mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

[00770] 63. The heterodimeric, conditionally active CAR of any one of aspects 52-62, wherein the first member of the specific binding pair is a single-chain Fv.

[00771] 64. The heterodimeric, conditionally active CAR of any one of aspects 52-63, wherein the first member of the specific binding pair binds an epitope present on a cell, on a solid surface, or a lipid bilayer.

[00772] 65. The heterodimeric, conditionally active CAR of aspect 64, wherein the cell is a cancer cell.

[00773] 66. A mammalian cell genetically modified to produce the heterodimeric, conditionally active polypeptide or receptor of any one of aspects 1-65.

[00774] 67. The cell of aspect 66, wherein the cell is a stem cell, a progenitor cell, or a cell derived from a stem cell or a progenitor cell.

[00775] 68. The cell of aspect 66, wherein the cell is a T lymphocyte or an NK cell.

[00776] 69. A nucleic acid comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide of any one of aspects 1-65.

[00777] 70. The nucleic acid of aspect 69, wherein the nucleotide sequences are operably linked to a promoter.

[00778] 71. The nucleic acid of aspect 69, wherein the promoter is an inducible promoter.

[00779] 72. The nucleic acid of aspect 69, wherein the promoter is a cell type-specific or tissue-specific promoter.

[00780] 73. The nucleic acid of aspect 72, wherein the promoter is a T lymphocyte-specific promoter or an NK cell-specific promoter.

[00781] 74. The nucleic acid of any one of aspects 69-73, wherein the nucleic acid is in vitro transcribed RNA.

[00782] 75. A recombinant expression vector comprising the nucleic acid of any one of aspects 69-74.

[00783] 76. A method of modulating an activity of a eukaryotic cell, the method comprising: a) expressing the heterodimeric, conditionally active polypeptide or receptor of any one of aspects 1-65 in the eukaryotic cell; and b) contacting the cell with the ligand.

[00784] 77. A method of modulating an activity of a T lymphocyte, the method comprising contacting the T lymphocyte with a dimerizing agent and a second member of a specific binding pair, wherein the T lymphocyte is genetically modified to produce a heterodimeric, conditionally active receptor of any one of aspects 15-65, and wherein, in the presence of the dimerizing agent and the second member of a specific binding pair, the heterodimeric, conditionally active receptor dimerizes and modulates an activity of the T lymphocyte, thereby producing a modulated T lymphocyte.

[00785] 78. The method of aspect 77, wherein the second member of a specific binding pair is an antigen.

[00786] 79. The method of aspect 77, wherein said contacting occurs in vivo.

[00787] 80. The method of aspect 77, wherein the T lymphocyte is activated, thereby producing an activated T lymphocyte.

[00788] 81. The method of aspect 80, wherein the activated T lymphocyte mediates killing of a target cell.

[00789] 82. The method of aspect 80, wherein the activated T lymphocyte produces IL-2 and/or IFN-γ.

[00790] 83. The method of aspect 81, wherein the target cell is a cancer cell.

[00791] 84. A method of making the cell of any one of aspects 66-68, the method comprising genetically modifying a mammalian cell with an expression vector comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide of any one of aspects 1-65, or genetically modifying a mammalian cell with an RNA comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide of any one of aspects 1-65.

[00792] 85. The method of aspect 84, wherein said genetic modification is carried out ex vivo.

[00793] 86. The method of aspect 84 wherein the cell is a T lymphocyte, a stem cell, an NK cell, a progenitor cell, a cell derived from a stem cell, or a cell derived from a progenitor cell.

[00794] 87. A method of treating a cancer in an individual, the method comprising: i) genetically modifying T lymphocytes obtained from the individual with an expression vector comprising nucleotide sequences encoding the heterodimeric, conditionally active chimeric antigen receptor (CAR) of any one of aspects 49-65, wherein the antigen-binding domain of the heterodimeric, conditionally active CAR is specific for an epitope on a cancer cell in the individual, and wherein said genetic modification is carried out ex vivo; ii) introducing the genetically modified T lymphocytes into the individual; and iii) administering to the individual an effective amount of a dimerizing agent, wherein the dimerizing agent induces dimerization

of the heterodimeric, conditionally active receptor, wherein said dimerization provides for activation of the genetically modified T lymphocytes and killing of the cancer cell, thereby treating the cancer.

[00795] 88. The method of aspect 87, wherein the dimerizing agent is a nuclear hormone that binds the LBD of the nuclear hormone receptor and the co-regulator.

[00796] 89. A method of modulating the activity of a host cell, the method comprising contacting the host cell with a dimerizing agent and a second member of a specific binding pair, wherein the T lymphocyte is genetically modified to produce a heterodimeric, conditionally active receptor of any one of aspects 49-65, and wherein, in the presence of the dimerizing agent and the second member of a specific binding pair, the heterodimeric, conditionally active receptor dimerizes and modulates at least one activity of the host cell.

[00797] 90. The method of aspect 89, wherein the activity is proliferation, cell survival, apoptosis, gene expression, or immune activation.

[00798] 91. The method of aspect 89, wherein the second member of a specific binding pair is an antigen.

EXAMPLES

[00799] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

MATERIALS AND METHODS

[00800] The following Materials and Methods were used for the experiments described in Examples 1 and 2.

Construction of PPARγ-based ON-switch CARs

[00801] The sequence encoding the anti-human CD19 scFv was cloned into expression vectors. The human 4-1BB co-stimulation and CD3 zeta ITAM signaling chains were cloned from cDNAs supplied by Open Biosystems. PPARgamma LBD and SRC3 co-activator peptide encoding sequences were custom synthesized by IDT DNA. Standard molecular cloning techniques (polymerase chain reaction (PCR), restriction digestion, ligation, homology-based DNA recombination *etc.*) were applied to generate lentiviral expression plasmids for each ON-switch CAR molecule.

Effector and target cell culturing conditions

[00802] A Jurkat cell line expressing Green Fluorescent Protein upon NFAT activation was used. The cell line was maintained in RPMI-1640 medium supplemented with 10% FBS, penicillin and streptomycin. K562 target cells (CD19+/-) from U. Penn were cultured in IMDM supplemented with 10% FBS.

Effector cell engineering with lentivirus

[00803] Pantropic VSV-G pseudotyped lentivirus was produced from Lenti-X 293T cells (Clontech Laboratories #632180) co-transfected with a pHR'SIN:CSW transgene expression vector, viral packaging plasmids pCMVdR8.91 and pMD2.G using Lipofectamine LTX (Life Technologies #15338). Infection medium supernatant was collected 48 hours after transfection and used directly for transduction.

[00804] Jurkat cells were split 1~2 days prior to transduction to ensure that the cell culture would be in log phase at the time of transduction. Transduced cells were cultured for at least 7 days and sorted for abundant CAR expression as needed before experiments were conducted. Expression levels of ON-switch CAR molecules were quantified by flow cytometry. Part 1 of the ON-switch CAR (featuring the scFv) was stained with a fluorophore-conjugated anti-myc antibody, which recognized a myc epitope upstream of the anti-CD19 scFv. Part 2 of the ON-switch CAR (featuring the CD3zeta ITAMs) was quantified using fluorescence of mCherry, which was downstream of the ITAMs.

Quantitation of IL-2 production by ON-switch Jurkat cells

[00805] Jurkat T cells expressing ON-switch CARs were co-cultured with CD19+/- K562 target cells at a 1:2 effector:target ratio. Rosiglitazone (Sigma-Aldrich #R2408) was dissolved in DMSO and added to samples to a final concentration of 10μM. Sample without dimerizer contained the respective vehicle control (1:3000 dilution of dimethyl sulfoxide (DMSO) or 1:1000 dilution of ethanol). After 18 hours of incubation, medium supernatants were collected and analyzed with BD OptEIA Human IL-2 ELISA Set (BD Biosciences #555190).

Construction of Hormone Receptor-based ON-switch CARs

[00806] The sequence encoding the anti-human CD19 scFv was cloned. The human 4-1BB co-stimulation and CD3 zeta ITAM signaling chains were cloned from cDNAs supplied by Open Biosystems. Estrogen receptor LBD and co-activator peptide encoding sequences were custom synthetized by IDT DNA. The sequences of the coactivator peptides were obtained from Heldring et al J Biol Chem. 2007 Apr 6;282(14):10449-55, PMID 17283072. Standard molecular cloning techniques (PCR, restriction digestion, ligation, homology-based DNA recombination *etc.*) were applied to generate lenti-viral expression plasmids for each ON-switch CAR molecule.

Quantitation of CD-69 expression by ON-switch Jurkat cells

[00807] A Jurkat cell line expressing Green Fluorescent Protein upon NFAT activation was used. The cell line was maintained in RPMI-1640 medium supplemented with 10% FBS and glutamine. K562 target cells (CD19+/-) were cultured in DMEM supplemented with 10% FBS. Jurkat cells infected with lentivirus with the CAR constructs were co-cultured with K562 target cells that expressed the target antigen (CD19) or an irrelevant antigen (mesothelin). 4-Hydroxytamoxifen (Sigma-Aldrich #H7904) was dissolved in DMSO and added to samples to a final concentration of 0-10 μM. The Rapalog A/C Heterodimerizer (Clontech Laboratories #635055) was dissolved in ethanol and added to a final concentration of 500 nM. The cells were cultured for 24 hours, stained for CD69 expression, and analyzed on a flow cytomter.

Effector cell engineering with lentivirus

[00808] Pantropic VSV-G pseudotyped lentivirus was produced from Lenti-X 293T cells (Clontech Laboratories #632180) co-transfected with a pHR'SIN:CSW transgene expression vector, viral packaging plasmids pCMVdR8.91 and pMD2.G using Lipofectamine LTX (Life Technologies #15338). Infection medium supernatant was collected 48 hours after transfection and used directly for transduction.

[00809] Jurkat cells were split 1~2 days prior to transduction to ensure that the cell culture would be in log phase at the time of transduction. Transduced cells were cultured for at least 7 days and sorted for abundant CAR expression as needed before experiments were conducted. Expression levels of ON-switch CAR molecules were quantified by flow cytometry. Part 1 of the ON-switch CAR (featuring the scFv) was stained with a fluorophore-conjugated anti-myc antibody, which recognized a myc epitope upstream of the anti-CD19 scFv. Part 2 of the ON-switch CAR (featuring the CD3zeta ITAMs) was quantified using fluorescence of mCherry, which was downstream of the ITAMs.

EXAMPLE 1: PPARγ-BASED ON-SWITCH CAR CONSTRUCTS

[00810] PPARγ-based ON-switch CAR constructs were generated. FIG. 12 presents a schematic diagram of the overall structure of a generalized nuclear hormone ligand binding domain (LBD)/co-activator peptide ON-switch CAR. FIG. 13 presents a schematic diagram of the overall structure of the constructs. Constructs are listed in Table 3. ON-switch constructs (bCW197, bCW206, bCW207), with FKBP and FRB* domains, were used as positive and negative control CARs.

Table 3

construct ID #	encoded CAR molecule
bCW492	Part 1 (antigen binding) with SRC3 co-regulator peptide, short version
bCW493	Part 1 with SRC3 co-regulator peptide, short version, 3 tandem copies
bCW494	Part 1 with SRC3 co-regulator peptide, long version
bCW495	Part 2 with PPARgamma LBD

[00811] Amino acid sequences of the ligand-binding domain (LBD) of PPARγ included in the CAR constructs, and amino acid sequences of the co-regulator peptides used, are set forth below.

[00812] The amino acid sequence of the LBD of PPARγ included in the CAR construct bCW495 is as follows:

ESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKIKFKHITPLQ EQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVHEIIYTMLASLMNK DGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGDRPGLLN VKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQIVTEHVQLLQVIKKTETDMSLHP LLQEIYKDLY (SEQ ID NO:695).

[00813] The amino acid sequence of human SRC3 co-regulator peptide, short version (P729~A750) in construct bCW492 is as follows: PKKENNALLRYLLDRDDPSDA (SEQ ID NO:696).

[00814] Construct bCW493 contained three tandem copies, with a linker/spacer between the copies, of the shorter version (P729-A750) of human SRC3 co-regulator peptide, such that the amino acid sequence of the co-regulator peptide, with linkers/spacers, in bCW493 is as follows:

PKKENNALLRYLLDRDDPSDAGGGSGGGSPKKENNALLRYLLDRDDPSDAGGGSGGGSPKKE NNALLRYLLDRDDPSDA (SEQ ID NO:697), where the linker/spacer amino acid are underlined.

[00815] Construct bCW494 contained the long version (M673-A750) of human SRC3 co-regulator peptide; the amino acid sequence of the human SRC3 in bCW494 is:

MHGSLLQEKHRILHKLLQNGNSPAEVAKITAEATGKDTSSITSCGDGNVVKQEQLSPKKKENNA LLRYLLDRDDPSDA (SEQ ID NO:698).

[00816] The amino acid sequence of a co-regulator peptide-containing polypeptide chain of a PPARγ CAR construct p51 (p51 bCW 492 myc anti-CD19 ON-switch part 1 with 1x SRC3 short co-regulator peptide and 4-1BB) is as follows:

[00817] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSL*KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL*S RGSGSGSTSPKKENNALLRYLLDRDDPSDAGS* (SEQ ID NO:699), where the anti-CD19 sequence is in bold, the CD8α transmembrane (TM) domain is double underlined, the 4-1BB sequence is in italics and bold text, and the co-regulator peptide is underlined (single underline).

[00818] The amino acid sequence of the PPARγ CAR construct p52 (p52 bCW 494 myc aCD19 ON-switch part 1 with 1x SRC3 long co-regulator peptide and 4-1BB) is as follows:

MALPVTALLLPLALLLHAARPEQKLISEEDL**DIQMTQTTSSLSASLGDRVTISCRASQDISKYL NWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL PYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDY GVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIY YCAKHYYYGGSYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACD**IYIWAPLAGTCGVLLLSLVITLYCSL***KRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCEL*SRGSGSGSTSMHGSLLQEKHRILHKLLQNGNSPAEVAKITAEATGKDT SSITSCGDGNVVKQEQLSPKKKENNALLRYLLDRDDPSDAGS* (SEQ ID NO:700), where the anti-CD19 sequence is in bold, the CD8α transmembrane (TM) domain is double underlined, the 4-1BB sequence is in italics and bold text, and the co-regulator peptide is underlined (single underline).

[00819] The amino acid sequence of the PPARγ CAR construct p55 (p55 bCW493 dimeric myc aCD19 CD8a hinge TM 41BB linker 3x short SRC3 peptide in pHR) is as follows:

[00820] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSL*KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL*S RGSGSGSTSPKKENNALLRYLLDRDDPSDAGGGSGGGSPKKENNALLRYLLDRDDPSDAGGGS GGGSKKENNALLRYLLDRDDPSDAGS* (SEQ ID NO:701), where the anti-CD19 sequence is in bold, the CD8α transmembrane (TM) domain is double underlined, the 4-1BB sequence is in italics and bold text, and the co-regulator peptide is underlined (single underline).

[00821] The amino acid sequence of the PPARγ CAR construct P56 (p56 bCW495 Kozak dDAP10 CD8a TM-41BB-GSx8-PPARg LBD-GSx4-zeta-GSx4-mCherry in pHR) is as follows:

[00822] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL SLVITLYCSL**KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL**GSGSGSGSGS GSGSTSESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKIKFK HITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVHEIIYTMLA SLMNKDGVLISEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGDR PGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQIVTEHVQLLQVIKKTETD MSLHPLLQEIYKDLYGSGSGSGSSL***RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR***SRGSGSGSGSGSMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRP YEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFE DGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIK

QRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGG MDELYKCVTD* (SEQ ID NO:702). The LBD is underlined (single underline); the CD8α TM domain is double underlined; 4-1BB is in bold text; and zeta is in bold and italics.

Results

[00823] FIG. 14 depicts IL-2 cytokine production by ON-switch CAR+ Jurkat cells (transduced with the indicated lentiviral constructs) after 18 hours of co-culturing with K562 target cells (+/- CD19 Ag, as indicated), in the presence or absence of rosiglitazone dimerizer (10 micromolar).

EXAMPLE 2: ER-ALPHA-BASED ON-SWITCH CAR CONSTRUCTS

[00824] ERα-based ON-switch CAR constructs were generated. FIG. 15 presents a schematic diagram of the overall structure of the constructs. Constructs are listed in Table 4. ON-switch constructs (bCW197, bCW206, bCW207), with FKBP and FRB* domains, were used as positive and negative control CARs.

Table 4

construct ID #	encoded CAR molecule
bCW501	Part 1 myc aCD19 ON-switch part 1 with 3x AlphaBetaV peptide and 4-1BB
bCW502	Part 1 myc aCD19 ON-switch part 1 with 3x CoRNR peptide and 4-1BB
bCW503	ON-switch CAR part 2 with human ER alpha LBD
bCW504	ON-switch CAR part 2 with human ER alpha LBD w Y537F
bCW505	ON-switch CAR part 2 with human ER alpha LBD w Y537F G521R

[00825] Amino acid sequences of the various components of the ERα-based ON-switch CAR are as follows.

[00826] a. The LBD of human estrogen receptor alpha in construct bCW503:

[00827] DRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL LILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTS (SEQ ID NO:703);

[00828] b. The LBD of mutated human estrogen receptor alpha in construct bCW504:

[00829] DRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL LILSHIRHMSNKGMEHLYSMKCKNVVPLFDLLLEMLDAHRLHAPTS (SEQ ID NO:704);

[00830] c. The LBD of mutated human estrogen receptor alpha in construct bCW505:

[00831] DRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG

EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL LILSHIRHMSNKRMEHLYSMKCKNVVPLFDLLLEMLDAHRLHAPTS (SEQ ID NO:705);

[00832] d. The 3x AlphaBetaV co-activator peptide in bCW501:

[00833] SGSGPGSREWFKDMLGGGSGGGSSGSGPGSREWFKDMLGGGSGGGSSGSGPGSREWFKD M (SEQ ID NO:706);

[00834] e. The 3x CoRNR co-activator peptide in bCW502:

[00835] DAFQLRQLILRGLQDDGGGSGGGSDAFQLRQLILRGLQDDGGGSGGGSDAFQLRQLILRGL QDD (SEQ ID NO:707).

[00836] The amino acid sequence of the bCW501 construct (p25 bCW501 myc aCD19 ON-switch part 1 with 3x AlphaBetaV peptide and 4-1BB) is as follows:

[00837] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTSSGSGPGSREWFKDMLGGGSGGGSSGSGPGSREWFKDMLGGGSGGGSSGSGPGS REWFKDMLGS* (SEQ ID NO:708), where the co-regulator peptide αβV is underlined.

[00838] The amino acid sequence of the bCW502 construct (p26 bCW502 myc aCD19 ON-switch part 1 with 3x CoRNR peptide and 4-1BB) is as follows:

[00839] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTSDAFQLRQLILRGLQDDGGGSGGGSDAFQLRQLILRGLQDDGGGSGGGSDAFQLR QLILRGLQDDGS* (SEQ ID NO:709), where the 3x CoRNR co-regulator peptide is underlined.

[00840] The amino acid sequence of the bCW503 construct (p27 bCW503 ON-switch CAR part 2 with human ER alpha LBD) is as follows:

[00841] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL SLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSGSGSGSGSGS TSDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE

CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG
EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL
LILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSGSGSGSGSSLRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRSRGSGSGSGSMVSKGEEDN
MAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYG
SKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSD
GPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAY
NVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKCVTD* (SEQ ID NO:710), where the ER LBD is underlined.

[00842] The amino acid sequence of the bCW504 construct (p28 bCW504 ON-switch CAR part 2 with human ER alpha LBD w Y537F) is as follows:

[00843] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL
SLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSGSGSGSGSGS
TSDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV
SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE
CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG
EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL
LILSHIRHMSNKGMEHLYSMKCKNVVPL**F**DLLLEMLDAHRLHAPTSGSGSGSGSSLRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRSRGSGSGSGSMVSKGEEDN
MAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYG
SKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSD
GPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAY
NVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKCVTD* (SEQ ID NO:711), where the ERα LBD is underlined and the Y537F substitution is in bold.

[00844] The amino acid sequence of the bCW505 construct (p29 bCW505 ON-switch CAR part 2 with human ER alpha LBD w Y537F G521R) is as follows:

[00845] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL
SLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSGSGSGSGSGS
TSDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV
SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE
CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG
EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL
LILSHIRHMSNK**R**MEHLYSMKCKNVVPL**F**DLLLEMLDAHRLHAPTSGSGSGSGSSLRVKFSRSA

DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRSRGSGSGSGSMVSKGEEDN
MAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYG
SKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSD
GPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAY
NVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKCVTD* (SEQ ID NO:712), where the ERα LBD is underlined, and the Y536F and G521R substitutions are in bold.

[00846] The amino acid sequence of the bCW506 construct (p30 bCW506 ON-switch CAR part 2 with human ER alpha LBD w/o helix12) is as follows:

[00847] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL
SLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSGSGSGSGSGS
TSDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV
SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE
CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG
EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL
LILSHIRHMSNKGMEHLYSMKCKNVAGSGSGSGSSLRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
LYQGLSTATKDTYDALHMQALPPRSRGSGSGSGSMVSKGEEDNMAIIKEFMRFKVHMEGSVNG
HEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPE
GFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERM
YPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQ
YERAEGRHSTGGMDELYKCVTD* (SEQ ID NO:713), where the ERα LBD (without helix 12) is underlined.

[00848] The amino acid sequence of the bCW507 construct (p31 bCW507 ON-switch CAR part 2 with human ER alpha LBD w/o helix12 w G521R) is as follows:

[00849] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL
SLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSGSGSGSGSGS
TSDRRGGRMLKHKRQRDDGEGRGEVGSAGDMRAANLWPSPLMIKRSKKNSLALSLTADQMV
SALLDAEPPILYSEYDPTRPFSEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLE
CAWLEILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQG
EEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLAQLL
LILSHIRHMSNK**R**MEHLYSMKCKNVAGSGSGSGSSLRVKFSRSADAPAYQQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
LYQGLSTATKDTYDALHMQALPPRSRGSGSGSGSMVSKGEEDNMAIIKEFMRFKVHMEGSVNG
HEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPE

GFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERM YPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQ YERAEGRHSTGGMDELYKCVTD* (SEQ ID NO:714), where the ERα LBD (without helix 12) is underlined, and the G521R substitution is in bold.

[00850] The amino acid sequence of the construct referred to as "p40 dimeric myc aCD19 CD8a hinge TM 41BB linker 3x NR0B1 peptide in pHR" is as follows:

[00851] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTS<u>PRQGSILYSMLTSAKQTGGGSGGGSPRQGSILYSMLTSAKQTGGGSGGGSPRQGS ILYSMLTSAKQT</u>GS* (SEQ ID NO:715), where the 3x NR0B1 co-regulator peptide is underlined.

[00852] The amino acid sequence of the construct referred to as "p41 dimeric myc aCD19 CD8a hinge TM 41BB linker 3x NCOA1 peptide in pHR" is as follows:

[00853] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTS<u>LTERHKILHRLLQEGSPSDGGGSGGGSLTERHKILHRLLQEGSPSDGGGSGGGSL TERHKILHRLLQEGSPSD</u>GS (SEQ ID NO:716), where the 3x NCOA1 peptide is underlined.

[00854] The amino acid sequence of the construct referred to as "p42 dimeric myc aCD19 CD8a hinge TM 41BB linker 3x NCOA2 peptide in pHR" is as follows:

[00855] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTS<u>SKGQTKLLQLLTTKSDQGGGSGGGSSKGQTKLLQLLTTKSDQGGGSGGGSSKGQ TKLLQLLTTKSDQ</u>GS* (SEQ ID NO:717), where the 3x NCOA2 peptide is underlined.

[00856] The amino acid sequence of the construct referred to as "p43 dimeric myc aCD19 CD8a hinge TM 41BB linker 3x PGC-1 peptide in pHR" is as follows:

[00857] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTSAEEPSLLKKLLLAPANTGGGSGGGSAEEPSLLKKLLLAPANTGGGSGGGSAEEPS LLKKLLLAPANTGS* (SEQ ID NO:718), where the 3x PGC-1 peptide is underlined.

[00858] The amino acid sequence of the construct referred to as "p44 dimeric myc aCD19 CD8a hinge TM 41BB linker 3x NRIP1 peptide in pHR" is as follows:

[00859] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTSAANNSLLLHLLKSQTIPGGGSGGGSAANNSLLLHLLKSQTIPGGGSGGGSAANNS LLLHLLKSQTIPGS* (SEQ ID NO:719), where the 3x NRIP1 peptide is underlined.

[00860] The amino acid sequence of the construct referred to as "p45 Kozak dDAP10 CD8a TM-41BB-GSx8-ERb LBD-GSx4-zeta-GSx4-mCherry in pHR" is as follows:

[00861] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL SLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSGSGSGSGSGS TSVKCGSRRERCGYRLVRRQRSADEQLHCAGKAKRSGGHAPRVRELLLDALSPEQLVLTLLEA EPPHVLISRPSAPFTEASMMMSLTKLADKELVHMISWAKKIPGFVELSLFDQVRLLESCWMEVL MMGLMWRSIDHPGKLIFAPDLVLDRDEGKCVEGILEIFDMLLATTSRFRELKLQHKEYLCVKA MILLNSSMYPLVTATQDADSSRKLAHLLNAVTDALVWVIAKSGISSQQQSMRLANLLMLLSHV RHASNKGMEHLLNMKCKNVVPVYDLLLEMLNAHVLRGCKSSITGSECSPAEDSKSKEGSQNPQ SQGSGSGSGSSLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRS RGSGSGSGSMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVT KGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSS LQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYD

AEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKCVTD* (SEQ ID NO:720), where the ERβ LBD is underlined.

[00862] The amino acid sequence of the construct referred to as "p179 dimeric myc aCD19 CD8a hinge TM 41BB linker 3x SRC-1 NRIV peptide in pHR" is as follows:

[00863] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTSQAQQKSLLQQLLTEGGGSGGGSQAQQKSLLQQLLTEGGGSGGGSQAQQKSLLQ QLLTEGS* (SEQ ID NO:721), where the 3x NRIV1 peptide is underlined

Results

[00864] The data are depicted in FIG. 16A and FIG. 16B. Fig. 16A. CD69 upregulation by Jurkat cells expressing ER CAR+ (bCW502 and bCW505) after 24 hours of co-culturing with K562 target cells expressing CD19 or mesothelin, an irrelevant antigen ("meso"), in the presence or absence of a small-molecule dimerizer (4-hydroxytamoxifen or rapalog AP21967). CD69 expression in ER CAR+ Jurkat cells is strongly induced by 4-hydroxytamoxifen (EC50 ~100 nM) only in the presence of K562 cells expressing CD19. Rapamycin CAR and dead rapamycin CAR (with a mutation in ITAM that abolishes signalling) were tested as positive and negative controls, respectively. Fig. 16B. FACS data from the experiment described in Fig. 16A, showing CD69 upregulation in ER CAR+ Jurkat cells in a manner dependent on CD19 antigen and treatment with 4-hydroxytamoxifen.

EXAMPLE 3: DIMERIZER CONTROLED ACTIVATION OF PRIMARY HUMAN T CELLS

[00865] Control of cellular functions by induced heterodimerization of the ligand binding domain of human estrogen receptor beta with small peptides derived from transcriptional co-repressors in the presence of the drug 4-hydroxytamoxifen was evaluated. This method does not involve DNA-binding and/or transcriptional activation or any DNA-binding function or transcription activating function of the human estrogen receptor beta from which polypeptide components are derived.

[00866] A human estrogen receptor beta nuclear receptor-peptide system was expressed in primary human T cells and found to capable of modulating ON-switch CAR activity. A CD19 specific ON-switch CAR was constructed to include human estrogen receptor beta ligand binding domain and co-repressor peptide (CoRNR). A schematic representation of the general construct within the T cell membrane, and the associated CD19 antigen expressed on the surface of the target cell, is provided in FIG. 17. Upon introduction into primary CD8+ T cells, stable expression was observed and transduced cells displaed increased T cell activation upon addition of the drug 4-hyroxytamoxifen.

[00867] Nucleic acid sequences encoding the following constructs were employed: "p26 bCW 502 myc aCD19 ON-switch part 1 with 3x CoRNR peptide and 4-1BB" having the following sequence:

[00868] MALPVTALLLPLALLLHAARPEQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKY LNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYT FGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL SRGSGSGSTSDAFQLRQLILRGLQDDGGGSGGGSDAFQLRQLILRGLQDDGGGSGGGSDAFQLR QLILRGLQDDGS* (SEQ ID NO:722), where the 3x CoRNR co-regulator peptide is underlined, and "p45 Kozak dDAP10 CD8a TM-41BB-GSx8-ERb LBD-GSx4-zeta-GSx4-mCherry in pHR" having the following sequence:

[00869] MIHLGHILFLLLLPVAAAQTTPGERSSLPAFYPGTSGSCSGCGSLSLPIYIWAPLAGTCGVLLL SLVITLYCSLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSGSGSGSGSGS TSVKCGSRRERCGYRLVRRQRSADEQLHCAGKAKRSGGHAPRVRELLLDALSPEQLVLTLLEA EPPHVLISRPSAPFTEASMMMSLTKLADKELVHMISWAKKIPGFVELSLFDQVRLLESCWMEVL MMGLMWRSIDHPGKLIFAPDLVLDRDEGKCVEGILEIFDMLLATTSRFRELKLQHKEYLCVKA MILLNSSMYPLVTATQDADSSRKLAHLLNAVTDALVWVIAKSGISSQQQSMRLANLLMLLSHV RHASNKGMEHLLNMKCKNVVPVYDLLLEMLNAHVLRGCKSSITGSECSPAEDSKSKEGSQNPQ SQGSGSGSGSSLRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRS RGSGSGSGSMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVT KGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSS LQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYD AEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKCVTD* (SEQ ID NO:723), where the ERβ LBD is underlined.

[00870] Specifically, CD69 upregulation by primary human CD8+ T cells expressing a CD19 specific ON-switch CAR constructed to include human estrogen receptor beta ligand binding domain (ER-beta) and co-repressor peptide (CoRNR) was measured after 24 hours of co-culturing with K562 target cells expressing CD19 or mesothelin, an irrelevant antigen ("meso"), in the presence or absence of a small-molecule dimerizer (4-hydroxytamoxifen or rapalog "rapa"). As shown in FIG. 18A, CD19-antigen induced activation of the primary human CD8+ T cells expressing the ER-beta/CoRNR on-switch CAR construct was dependent on the dose of small-molecule dimerizer provided to the culture. Histograms derived from the flow cytometery data, showing the correlation between increasing 4-hydroxytamoxifen dose and increasing numbers of CD69 positive primary human CD8+ T cells, are provided in FIG. 18B.

[00871] Cell killing by primary human CD8+ T cells expressing the CD19 specific ER-beta/CoRNR ON-switch CAR was also assessed. Specifically, the percent of CD19-expressing K562 target cells remaining after 22 hours of co-culture with primary human CD8+ T cells expressing the CD19 specific ER-beta/CoRNR ON-switch CAR (ER-CAR) in the presence of various concentrations of drug (4-hydroxytamoxifen (4HT) or rapalog) was measured (FIG. 52). Cells expressing a CD19 specific single-chain second generation CAR ("2nd gen CAR") and cells expressing a CD19 speicific rapalog on-switch CAR, with administered rapalog dimerizer, were used as positive controls. Negative controls included cells expressing ER-CAR T cells in absence of 4HT (ethanol vehicle only, "ER-CAR + EtOH") and cells expressing only part 2 of the ER-CAR on-switch construct, i.e., without part 1, in the presence of 4HT. These results demonstrate drug (4HT) inducible target cell killing (i.e., inducible cytotoxic activity) by primary T cells expressing an ER-beta/CoRNR dimerizable ON-switch CAR.

[00872] Increased T cell activation was also observed in primary CD8+ T cells expressing a split ON-switch CAR construct in which the heterodimerization modules consisted of a portion of the human Vitamin D receptor ligand binding domain and a SRC2-3 coactivator peptide. In this system, the T cells showed increased activation in the presence of the vitamin D analog calcipotriol.

[00873] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A heterodimeric, conditionally active polypeptide comprising:

a) a first chimeric polypeptide comprising a first member of a dimerization pair and a first heterologous polypeptide; and

b) a second chimeric polypeptide comprising a second member of a dimerization pair and a second heterologous polypeptide,

wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or

wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and

wherein the first chimeric polypeptide and the second chimeric polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

2. The heterodimeric, conditionally active polypeptide of claim 1, wherein:

a) the first heterologous polypeptide is a T-cell receptor (TCR) alpha chain; and

b) the second heterologous polypeptide is a TCR beta chain.

3. The heterodimeric, conditionally active polypeptide of claim 1, wherein:

a) the first heterologous polypeptide is a first polypeptide of a chimeric antigen receptor (CAR) heterodimer; and

b) the second heterologous polypeptide is a second polypeptide of a CAR heterodimer.

4. The heterodimeric, conditionally active polypeptide of claim 1, wherein:

a) the first heterologous polypeptide is an N-terminal portion of an RNA-guided endonuclease; and

b) the second heterologous polypeptide is a C-terminal portion of the RNA-guided endonuclease,

wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide, mediated by the dimerization agent that induces binding of the LBD to the co-regulator, restores enzymatic function of the RNA-guided endonuclease.

5. The heterodimeric, conditionally active polypeptide of claim 4, wherein the RNA-guided endonuclease is a class 2 CRISPR/Cas endonuclease.

6. The heterodimeric, conditionally active polypeptide of claim 5, wherein the class 2 CRISPR/Cas endonuclease is a type II CRISPR/Cas protein, a type V CRISPR/Cas protein, or a type VI CRISPR/Cas protein.

7. The heterodimeric, conditionally active polypeptide of claim 1, wherein:

a) the first heterologous polypeptide is an N-terminal portion of an enzyme; and

b) the second heterologous polypeptide is a C-terminal portion of the enzyme,

wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide restore enzymatic activity of the enzyme.

8. The heterodimeric, conditionally active polypeptide of claim 7, wherein the enzyme is a kinase, a protease, a phosphatase, or a caspase.

9. The heterodimeric, conditionally active polypeptide of claim 1, wherein the first polypeptide and the second polypeptide exhibit an activity when brought into proximity upon dimerization mediated by the dimerization agent, but do not exhibit the activity individually.

10. The heterodimeric, conditionally active polypeptide of claim 1, wherein

a) the first heterologous polypeptide is an N-terminal portion of an antigen receptor; and

b) the second heterologous polypeptide is a C-terminal portion of the antigen receptor,

wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide restore signaling activity of the antigen receptor.

11. The heterodimeric, conditionally active polypeptide of claim 1, wherein:

a) the first heterologous polypeptide is an N-terminal portion of a receptor; and

b) the second heterologous polypeptide is a C-terminal portion of the antigen receptor,

wherein dimerization of the first chimeric polypeptide and the second chimeric polypeptide mediated by the dimerization agent restores signaling activity of the receptor.

12. The heterodimeric, conditionally active polypeptide of any one of claims 1-11, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from an estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid

receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

13. The heterodimeric, conditionally active polypeptide of any one of claims 1-12, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

14. The heterodimeric, conditionally active polypeptide of any one of claims 1-12, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);

ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);

eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);

fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);

ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);

hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);

iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);

jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);

kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);

lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);

mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

15. A heterodimeric, conditionally active receptor comprising:

a) a first chimeric polypeptide comprising:

i) a first member of a specific binding pair;

ii) a first modulatory domain;

iii) a first member of a dimerization pair; and

iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and

b) a second chimeric polypeptide comprising:

i) a transmembrane domain;

ii) a second modulatory domain;

iii) a second member of the dimerization pair; and

iv) an intracellular signaling domain;

or comprising:

a) a first chimeric polypeptide comprising:

i) a first member of a specific binding pair;

ii) a modulatory domain;

iii) a first member of a dimerization pair;

iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and

b) a second chimeric polypeptide comprising:

i) a second member of the dimerization pair; and

ii) an intracellular signaling domain.

wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or

wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor,

wherein the first chimeric polypeptide and the second chimeric polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

16. The heterodimeric, conditionally active receptor of claim 15, wherein the first polypeptide comprises a hinge region interposed between the first member of the specific binding pair and the transmembrane domain.

17. The heterodimeric, conditionally active receptor of claim 15, wherein the first member of the specific binding pair is an antibody or antibody fragment, a ligand, a receptor, or a non-antibody-based recognition scaffold.

18. The heterodimeric, conditionally active receptor of claim 17, wherein the hinge region is an immunoglobulin IgG hinge region or a hinge derived from CD8.

19. The heterodimeric, conditionally active receptor of claim 15, wherein the first and second modulatory domains are selected from 4-1BB (CD137), CD28, ICOS, BTLA, OX-40, CD27, CD30, GITR, HVEM, DAP10, DAP12, and CD28.

20. The heterodimeric, conditionally active receptor of claim 15, wherein the intracellular signaling domain is selected from ZAP70 and CD3-zeta.

21. The heterodimeric, conditionally active receptor of claim 15, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM).

22. The heterodimeric, conditionally active receptor of any one of claims 15-21, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X

receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

23. The heterodimeric, conditionally active receptor of any one of claims 15-22, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

24. The heterodimeric, conditionally active receptor of any one of claims 15-22, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);

ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);

eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);

fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);

ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);

hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);
iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);
jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);
kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);
lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);
mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);
nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);
ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);
ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and
qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

25. The heterodimeric, conditionally active receptor of claim 18, wherein:
i) the first and second modulatory domains are derived from 4-1BB;
ii) the first and second members of the dimerization pair are PPARγ and SRC3; and
ii) the signaling domain comprises an ITAM.

26. The heterodimeric, conditionally active receptor of claim 15, wherein the first member of the specific binding pair is a single-chain Fv.

27. The heterodimeric, conditionally active receptor of claim 15, wherein the first member of the specific binding pair binds an epitope present on a cell, on a solid surface, or a lipid bilayer.

28. The heterodimeric, conditionally active receptor of claim 27, wherein the cell is a cancer cell.

29. The heterodimeric, conditionally active receptor of claim 15, wherein the intracellular signaling domain is an intracellular inhibitory domain.

30. The heterodimeric, conditionally active receptor of claim 29, wherein the intracellular inhibitory domain is derived from a protein selected from the group consisting of: PD-1, CTLA4, HPK1, SHP1, SHP2, Sts1, and Csk.

31. A heterodimeric, conditionally repressible synthetic immune cell receptor (ICR) comprising:

a synthetic stimulatory ICR comprising a first member of a dimerization pair linked to the synthetic stimulatory ICR; and

a synthetic ICR repressor comprising a second member of the dimerization pair linked to an intracellular inhibitory domain,

wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or

wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor,

wherein the synthetic stimulatory ICR and the synthetic ICR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

32. The conditionally repressible synthetic ICR of Claim 31, wherein the synthetic stimulatory ICR comprises an intracellular co-stimulatory domain.

33. The conditionally repressible synthetic ICR of Claim 32, wherein the intracellular co-stimulatory domain is selected from the group consisting of: 4-1BB (CD137), CD28, ICOS, OX-40, BTLA, CD27, CD30, GITR, and HVEM.

34. The conditionally repressible synthetic ICR of any one of claims 31-33, wherein the first member of a dimerization pair is linked intracellularly to the synthetic stimulatory ICR and the second member of the dimerization pair is linked intracellularly to the intracellular inhibitory domain.

35. The conditionally repressible synthetic ICR of any one of claims 31-34, wherein the synthetic ICR repressor further comprises a transmembrane domain.

36. The conditionally repressible synthetic ICR of Claim 35, wherein the second member of the dimerization pair is linked intracellularly to the transmembrane domain.

37. The conditionally repressible synthetic ICR of Claim 35, wherein the second member of the dimerization pair is extracellular and linked to the intracellular inhibitory domain by way of the transmembrane domain.

38. The conditionally repressible synthetic ICR of any one of claims 31-37, wherein the stimulatory ICR binds a soluble antigen.

39. The conditionally repressible synthetic ICR of any one of claims 31-38, wherein the stimulatory ICR binds a cell surface antigen.

40. The conditionally repressible synthetic ICR of any one claims 31-39, wherein the intracellular inhibitory domain is an inhibitory domain derived from a protein selected from the group consisting of: PD-1, CTLA4, HPK1, SHP1, SHP2, Sts1 and Csk.

41. The conditionally repressible synthetic ICR of any one of claims 31-40, wherein the synthetic stimulatory ICR comprises an intracellular signaling domain selected from the group consisting of: a CD3-zeta signaling domain, a ZAP70 signaling domain and an immunoreceptor tyrosine-based activation motif (ITAM).

42. The conditionally repressible synthetic ICR of any one of claims 31-41, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

43. The conditionally repressible synthetic ICR of any one of claims 41-42, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

44. The conditionally repressible synthetic ICR of any one of claims 41-42, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);

ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);

eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);

fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);

ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);

hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);

iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);

jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);

kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);

lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);

mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);

nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);

ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);

ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and

qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

45. The conditionally repressible synthetic ICR of any one of claims 31-44, wherein the synthetic stimulatory ICR is a synthetic chimeric antigen receptor (CAR) or portion thereof.

46. The conditionally repressible synthetic ICR of any one of claims 31-45, wherein the synthetic stimulatory ICR is a synthetic T cell receptor (TCR) or portion thereof.

47. A heterodimeric, conditionally repressible synthetic chimeric antigen receptor (CAR) comprising:

a) a synthetic stimulatory CAR comprising:

i) a extracellular recognition domain;

ii) a transmembrane domain linked to the extracellular recognition domain;

iii) a first member of a dimerization pair linked to the transmembrane domain; and

iv) an intracellular stimulation domain; and

b) a synthetic CAR repressor comprising:

i) a second member of the dimerization pair; and

ii) an intracellular inhibitory domain linked to the second member of the dimerization pair,

wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or

wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and

wherein the synthetic stimulatory CAR and the synthetic CAR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

48. The heterodimeric, conditionally repressible synthetic CAR of Claim 47, wherein the synthetic CAR repressor further comprises a transmembrane domain linked to the second member of the dimerization pair, the intracellular inhibitory domain or both.

49. A heterodimeric, conditionally repressible synthetic T cell receptor (TCR) comprising:

a) a synthetic stimulatory TCR comprising:

i) a transmembrane domain;

ii) a first member of a dimerization pair linked to the transmembrane domain;

iii) an engineered TCR polypeptide comprising at least one TCR alpha or beta chain, wherein the at least one TCR alpha or beta chain is linked to the transmembrane domain or the first member of a dimerization pair; and

b) a synthetic TCR repressor comprising:

i) a second member of the dimerization pair; and

ii) an intracellular inhibitory domain linked to the second member of the dimerization pair,

wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or

wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and

wherein the synthetic stimulatory TCR and the synthetic TCR repressor are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

50. The heterodimeric, conditionally repressible synthetic TCR of Claim 49, wherein the synthetic TCR repressor further comprises a transmembrane domain linked to the second member of the dimerization pair, the intracellular inhibitory domain or both.

51. The heterodimeric, conditionally repressible synthetic TCR of Claims 49 or 50, wherein the engineered TCR polypeptide further comprises a TCR gamma chain.

52. A heterodimeric, conditionally active chimeric antigen receptor (CAR) comprising:

a) a first polypeptide comprising:

i) a first member of a specific binding pair;

ii) a first modulatory domain;

iii) a first member of a dimerization pair; and

iv) a transmembrane domain interposed between the first member of a specific binding pair and the first modulatory domain; and

b) a second polypeptide comprising:

i) a transmembrane domain;

ii) a second modulatory domain;

iii) a second member of the dimerization pair; and

iv) an intracellular signaling domain;

or comprising:

a) a first polypeptide comprising:

i) a first member of a specific binding pair;

ii) a modulatory domain;

iii) a first member of a dimerization pair;

iv) a transmembrane domain interposed between the first member of a specific binding pair and the modulatory domain; and

b) a second polypeptide comprising:

i) a second member of the dimerization pair; and

ii) an intracellular signaling domain,

wherein the first member of the dimerization pair comprises a ligand-binding domain (LBD) of a nuclear hormone receptor, and the second member of the dimerization pair comprises a co-regulator of the nuclear hormone receptor, or

wherein the first member of the dimerization pair is a co-regulator of a nuclear hormone receptor, and the second member of the dimerization pair comprises an LBD of the nuclear hormone receptor; and

wherein the first polypeptide and the second polypeptide are dimerized in the presence of a dimerization agent that induces binding of the LBD to the co-regulator.

53. The heterodimeric, conditionally active CAR of claim 52, wherein the first polypeptide comprises a hinge region interposed between the first member of the specific binding pair and the transmembrane domain.

54. The heterodimeric, conditionally active CAR of claim 52, wherein the first member of the specific binding pair is an antibody or antibody fragment, a ligand, or a receptor.

55. The heterodimeric, conditionally active CAR of claim 53, wherein the hinge region is an immunoglobulin IgG hinge region or a hinge derived from CD8.

56. The heterodimeric, conditionally active CAR of claim 52, wherein the first and second modulatory domains are selected from 4-1BB (CD137), CD28, ICOS, BTLA, OX-40, CD27, CD30, GITR, HVEM, DAP10, DAP12, and CD28.

57. The heterodimeric, conditionally active CAR of claim 52, wherein the intracellular signaling domain is selected from ZAP70 and CD3-zeta.

58. The heterodimeric, conditionally active CAR of claim 52, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM).

59. The heterodimeric, conditionally active CAR of any one of claims 52-58, wherein LBD of the nuclear hormone binding member of the dimerization pair is an LBD of a nuclear hormone receptor selected from estrogen receptor, an ecdysone receptor, a PPARγ receptor, a glucocorticoid receptor, an androgen receptor, a thyroid hormone receptor, a mineralocorticoid receptor, a progesterone receptor, a vitamin D receptor, a PPARβ receptor, a PPARα receptor, a pregnane X receptor, a liver X receptor, a farnesoid X receptor, a retinoid X receptor, a RAR-related orphan receptor, and a retinoic acid receptor.

60. The heterodimeric, conditionally active CAR of any one of claims 52-59, wherein the co-regulator of the nuclear hormone receptor is selected from SRC1, GRIP1, AIB1, PGC1a, PGC1b, PRC, TRAP220, ASC2, CBP, P300, CIA, ARA70, TIF1, NSD1, SMAP, Tip60, ERAP140, Nix1, LCoR, N-CoR, SMRT, RIP140, and PRIC285.

61. The heterodimeric, conditionally active CAR of any one of claims 52-59, wherein the co-regulator of the nuclear hormone receptor is selected from:

a) SRC1: CPSSHSSLTERHKILHRLLQEGSPS (SEQ ID NO:1);

b) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:2);

c) SRC3-1: ESKGHKKLLQLLTCSSDDR (SEQ ID NO:3);

d) SRC3: PKKENNALLRYLLDRDDPSDV (SEQ ID NO:4);

e) PGC-1: AEEPSLLKKLLLAPANT (SEQ ID NO:5);

f) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:6);

g) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:7);

h) NCoR (2051–2075): GHSFADPASNLGLEDIIRKALMGSF (SEQ ID NO:8);

i) NR0B1: PRQGSILYSMLTSAKQT (SEQ ID NO:9);

j) NRIP1: AANNSLLLHLLKSQTIP (SEQ ID NO:10);

k) TIF2: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:11);

l) CoRNR Box: DAFQLRQLILRGLQDD (SEQ ID NO:12);

m) αβV: SPGSREWFKDMLS (SEQ ID NO:13);

n) TRAP220-2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:14);

o) EA2: SSKGVLWRMLAEPVSR (SEQ ID NO:15);

p) TA1: SRTLQLDWGTLYWSR (SEQ ID NO:16);

q) EAB1: SSNHQSSRLIELLSR (SEQ ID NO:17);

r) SRC2: LKEKHKILHRLLQDSSSPV (SEQ ID NO:18);

s) SRC1-3: QAQQKSLLQQLLTE (SEQ ID NO:19);

t) SRC1–1: KYSQTSHKLVQLLTTTAEQQL (SEQ ID NO:20);

u) SRC1–2: SLTARHKILHRLLQEGSPSDI (SEQ ID NO:21);

v) SRC1–3: KESKDHQLLRYLLDKDEKDLR (SEQ ID NO:22);

w) SRC1–4a: PQAQQKSLLQQLLTE (SEQ ID NO:23);;

x) SRC1–4b: PQAQQKSLRQQLLTE (SEQ ID NO:24);

y) GRIP1–1: HDSKGQTKLLQLLTTKSDQME (SEQ ID NO:25);

z) GRIP1–2: SLKEKHKILHRLLQDSSSPVD (SEQ ID NO:26);

aa) GRIP1–3: PKKKENALLRYLLDKDDTKDI (SEQ ID NO:27);

bb) AIB1–1: LESKGHKKLLQLLTCSSDDRG (SEQ ID NO:28);

cc) AIB1–2: LLQEKHRILHKLLQNGNSPAE (SEQ ID NO:29);

dd) AIB1–3: KKKENNALLRYLLDRDDPSDA (SEQ ID NO:30);

ee) PGC1a: QEAEEPSLLKKLLLAPANTQL (SEQ ID NO:31);

ff) PGC1b: PEVDELSLLQKLLLATSYPTS (SEQ ID NO:32);

gg) PRC: VSPREGSSLHKLLTLSRTPPE (SEQ ID NO:33);

hh) TRAP220–1: SKVSQNPILTSLLQITGNGGS (SEQ ID NO:34);

ii) TRAP220–2: GNTKNHPMLMNLLKDNPAQDF (SEQ ID NO:35);

jj) ASC2–1: DVTLTSPLLVNLLQSDISAGH (SEQ ID NO:36);

kk) ASC2–2: AMREAPTSLSQLLDNSGAPNV (SEQ ID NO:37);

ll) CBP-1: DAASKHKQLSELLRGGSGSSI (SEQ ID NO:38);

mm) CBP-2: KRKLIQQQLVLLLHAHKCQRR (SEQ ID NO:39);

nn) P300: DAASKHKQLSELLRSGSSPNL (SEQ ID NO:40);

oo) CIA: GHPPAIQSLINLLADNRYLTA (SEQ ID NO:41);

pp) ARA70–1: TLQQQAQQLYSLLGQFNCLTH (SEQ ID NO:42);

qq) ARA70–2: GSRETSEKFKLLFQSYNVNDW (SEQ ID NO:43);

rr) TIF1: NANYPRSILTSLLLNSSQSST (SEQ ID NO:44);

ss) NSD1: IPIEPDYKFSTLLMMLKDMHD (SEQ ID NO:45);

tt) SMAP: ATPPPSPLLSELLKKGSLLPT (SEQ ID NO:46);

uu) Tip60: VDGHERAMLKRLLRIDSKCLH (SEQ ID NO:47);

vv) ERAP140: HEDLDKVKLIEYYLTKNKEGP (SEQ ID NO:48);

ww) Nix1: ESPEFCLGLQTLLSLKCCIDL (SEQ ID NO:49);

xx) LCoR: AATTQNPVLSKLLMADQDSPL (SEQ ID NO:50);

yy) CoRNR1 (N-CoR): MGQVPRTHRLITLADHICQIITQDFARNQV (SEQ ID NO:51);

zz) CoRNR2 (N-CoR): NLGLEDIIRKALMG (SEQ ID NO:52);

aaa) CoRNR1 (SMRT): APGVKGHQRVVTLAQHISEVITQDTYRHHPQQLSAPLPAP (SEQ ID NO:53);

bbb) CoRNR2 (SMRT): NMGLEAIIRKALMG (SEQ ID NO:54);

ccc) RIP140-C: RLTKTNPILYYMLQKGGNSVA (SEQ ID NO:55);
ddd) RIP140-1: QDSIVLTYLEGLLMHQAAGGS (SEQ ID NO:56);
eee) RIP140-2: KGKQDSTLLASLLQSFSSRLQ (SEQ ID NO:57);
fff) RIP140-3: CYGVASSHLKTLLKKSKVKDQ (SEQ ID NO:58);
ggg) RIP140-4: KPSVACSQLALLLSSEAHLQQ (SEQ ID NO:59);
hhh) RIP140-5: KQAANNSLLLHLLKSQTIPKP (SEQ ID NO:60);
iii) RIP140-6: NSHQKVTLLQLLLGHKNEENV (SEQ ID NO:61);
jjj) RIP140-7: NLLERRTVLQLLLGNPTKGRV (SEQ ID NO:62);
kkk) RIP140-8: FSFSKNGLLSRLLRQNQDSYL (SEQ ID NO:63);
lll) RIP140-9: RESKSFNVLKQLLLSENCVRD (SEQ ID NO:64);
mmm) PRIC285-1: ELNADDAILRELLDESQKVMV (SEQ ID NO:65);
nnn) PRIC285-2: YENLPPAALRKLLRAEPERYR (SEQ ID NO:66);
ooo) PRIC285-3: MAFAGDEVLVQLLSGDKAPEG (SEQ ID NO:67);
ppp) PRIC285-4: SCCYLCIRLEGLLAPTASPRP (SEQ ID NO:68); and
qqq) PRIC285-5: PSNKSVDVLAGLLLRRMELKP (SEQ ID NO:69).

63. The heterodimeric, conditionally active CAR of any one of claims 52-62, wherein the first member of the specific binding pair is a single-chain Fv.

64. The heterodimeric, conditionally active CAR of any one of claims 52-63, wherein the first member of the specific binding pair binds an epitope present on a cell, on a solid surface, or a lipid bilayer.

65. The heterodimeric, conditionally active CAR of claim 64, wherein the cell is a cancer cell.

66. A mammalian cell genetically modified to produce the heterodimeric, conditionally active polypeptide or receptor of any one of claims 1-65.

67. The cell of claim 66, wherein the cell is a stem cell, a progenitor cell, or a cell derived from a stem cell or a progenitor cell.

68. The cell of claim 66, wherein the cell is a T lymphocyte or an NK cell.

69. A nucleic acid comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide of any one of claims 1-65.

70. The nucleic acid of claim 69, wherein the nucleotide sequences are operably linked to a promoter.

71. The nucleic acid of claim 69, wherein the promoter is an inducible promoter.

72. The nucleic acid of claim 69, wherein the promoter is a cell type-specific or tissue-specific promoter.

73. The nucleic acid of claim 72, wherein the promoter is a T lymphocyte-specific promoter or an NK cell-specific promoter.

74. The nucleic acid of any one of claims 69-73, wherein the nucleic acid is *in vitro* transcribed RNA.

75. A recombinant expression vector comprising the nucleic acid of any one of claims 69-74.

76. A method of modulating an activity of a eukaryotic cell, the method comprising:

a) expressing the heterodimeric, conditionally active polypeptide or receptor of any one of claims 1-65 in the eukaryotic cell; and

b) contacting the cell with the ligand.

77. A method of modulating an activity of a T lymphocyte, the method comprising contacting the T lymphocyte with a dimerizing agent and a second member of a specific binding pair, wherein the T lymphocyte is genetically modified to produce a heterodimeric, conditionally active receptor of any one of claims 15-65, and wherein, in the presence of the dimerizing agent and the second member of a specific binding pair, the heterodimeric, conditionally active receptor dimerizes and modulates an activity of the T lymphocyte, thereby producing a modulated T lymphocyte.

78. The method of claim 77, wherein the second member of a specific binding pair is an antigen.

79. The method of claim 77, wherein said contacting occurs *in vivo*.

80. The method of claim 77, wherein the T lymphocyte is activated, thereby producing an activated T lymphocyte.

81. The method of claim 80, wherein the activated T lymphocyte mediates killing of a target cell.

82. The method of claim 80, wherein the activated T lymphocyte produces IL-2 and/or IFN-γ.

83. The method of claim 81, wherein the target cell is a cancer cell.

84. A method of making the cell of any one of claims 66-68, the method comprising genetically modifying a mammalian cell with an expression vector comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide of any one of claims 1-65, or genetically modifying a mammalian cell with an RNA comprising nucleotide sequences encoding the heterodimeric, conditionally active receptor or polypeptide of any one of claims 1-65.

85. The method of claim 84, wherein said genetic modification is carried out *ex vivo*.

86. The method of claim 84 wherein the cell is a T lymphocyte, a stem cell, an NK cell, a progenitor cell, a cell derived from a stem cell, or a cell derived from a progenitor cell.

87. A method of treating a cancer in an individual, the method comprising:

i) genetically modifying T lymphocytes obtained from the individual with an expression vector comprising nucleotide sequences encoding the heterodimeric, conditionally active chimeric antigen receptor (CAR) of any one of claims 49-65, wherein the antigen-binding domain of the heterodimeric, conditionally active CAR is specific for an epitope on a cancer cell in the individual, and wherein said genetic modification is carried out *ex vivo*;

ii) introducing the genetically modified T lymphocytes into the individual; and

iii) administering to the individual an effective amount of a dimerizing agent, wherein the dimerizing agent induces dimerization of the heterodimeric, conditionally active receptor, wherein said dimerization provides for activation of the genetically modified T lymphocytes and killing of the cancer cell, thereby treating the cancer.

88. The method of claim 87, wherein the dimerizing agent is a nuclear hormone that binds the LBD of the nuclear hormone receptor and the co-regulator.

89. A method of modulating the activity of a host cell, the method comprising contacting the host cell with a dimerizing agent and a second member of a specific binding pair, wherein the T lymphocyte is genetically modified to produce a heterodimeric, conditionally active receptor of any one of claims 49-65, and wherein, in the presence of the dimerizing agent and the second member of a specific binding pair, the heterodimeric, conditionally active receptor dimerizes and modulates at least one activity of the host cell.

90. The method of claim 89, wherein the activity is proliferation, cell survival, apoptosis, gene expression, or immune activation.

91. The method of claim 89, wherein the second member of a specific binding pair is an antigen.