



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2017/12/11	(51) Cl.Int./Int.Cl. <i>A61K 35/12</i> (2015.01), <i>A61K 35/28</i> (2015.01), <i>A61K 38/17</i> (2006.01)
(87) Date publication PCT/PCT Publication Date: 2018/06/21	
(85) Entrée phase nationale/National Entry: 2019/05/30	(71) Demandeur/Applicant: SEATTLE CHILDREN'S HOSPITAL (DBA SEATTLE CHILDREN'S RESEARCH INSTITUTE), US
(86) N° demande PCT/PCT Application No.: US 2017/065597	
(87) N° publication PCT/PCT Publication No.: 2018/111763	(72) Inventeurs/Inventors: JENSEN, MICHAEL C., US; OOI, TRACY, US; WEI, JIA, US...
(30) Priorité/Priority: 2016/12/12 (US62/433,183)	(74) Agent: AIRD & MCBURNEY LP

(54) Titre : VARIANTS DU FACTEUR DE TRANSCRIPTION CHIMERIQUE AYANT UNE SENSIBILITE ACCRUE A L'INDUCTION DE LIGAND DE MEDICAMENT D'EXPRESSION TRANSGENIQUE DANS DES CELLULES DE MAMMIFERE

(54) Title: CHIMERIC TRANSCRIPTION FACTOR VARIANTS WITH AUGMENTED SENSITIVITY TO DRUG LIGAND INDUCTION OF TRANSGENE EXPRESSION IN MAMMALIAN CELLS

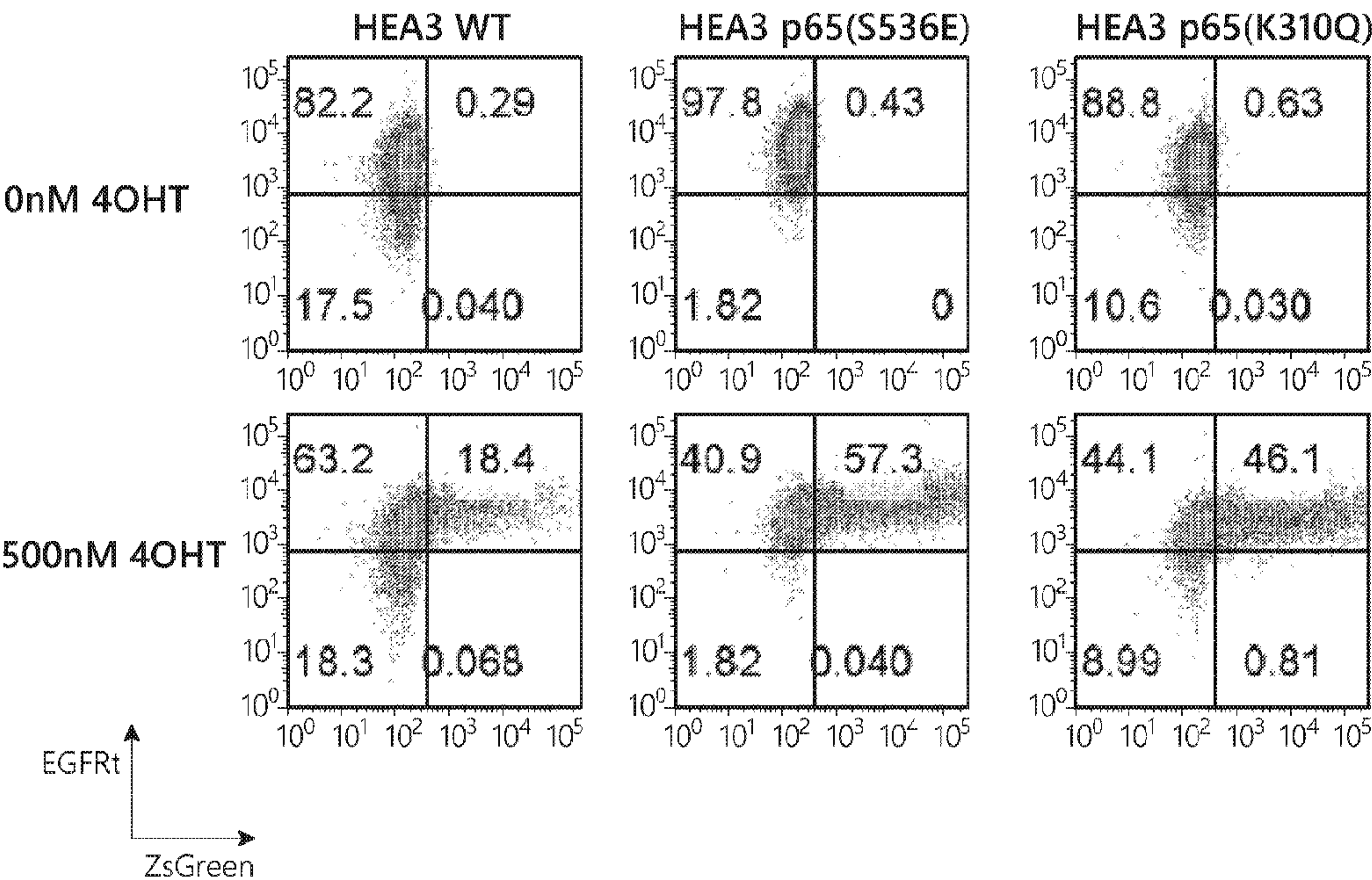


FIG. 1C

(57) Abrégé/Abstract:
Provided herein is a system for inducible expression of a chimeric antigen receptor in cells. The system comprises: a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that

(57) Abrégé(suite)/Abstract(continued):

encodes a chimeric antigen receptor, which comprises a ligand binding domain, a second polynucleotide, which encodes a spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug. Methods of making such cells and methods of treatment using these cells are also provided.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property
Organization
International Bureau



(10) International Publication Number

WO 2018/111763 A9

(43) International Publication Date
21 June 2018 (21.06.2018)

(51) International Patent Classification:

A61K 35/12 (2015.01) A61K 38/17 (2006.01)
A61K 35/28 (2015.01)

(21) International Application Number:

PCT/US2017/065597

(22) International Filing Date:

11 December 2017 (11.12.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/433,183 12 December 2016 (12.12.2016) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

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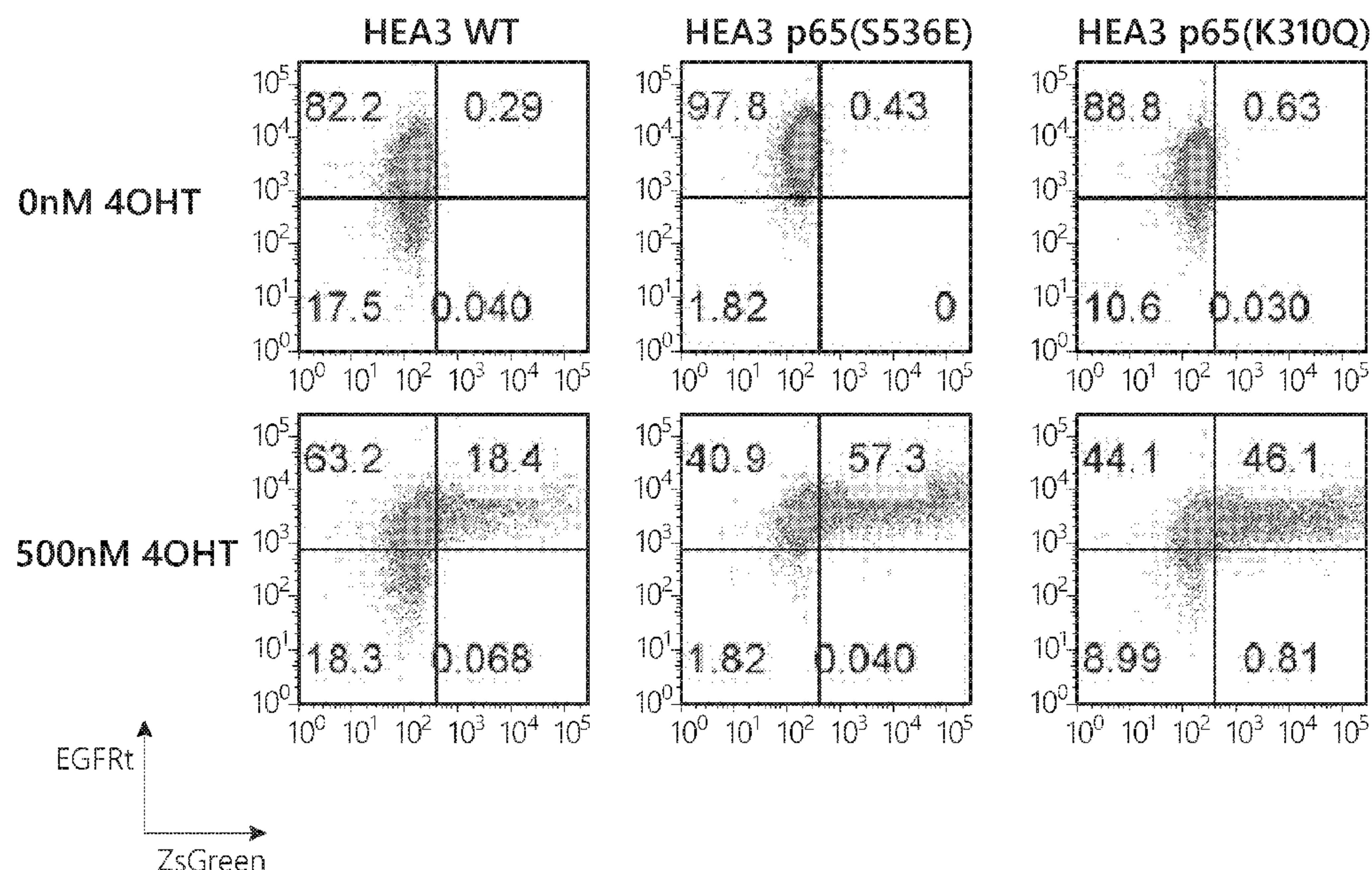


FIG. 1C

(57) Abstract: Provided herein is a system for inducible expression of a chimeric antigen receptor in cells. The system comprises: a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain, a second polynucleotide, which encodes a spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain, and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug. Methods of making such cells and methods of treatment using these cells are also provided.

[Continued on next page]

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DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

(48) Date of publication of this corrected version:

19 July 2018 (19.07.2018)

(15) Information about Correction:

see Notice of 19 July 2018 (19.07.2018)

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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CONTENANT LES PAGES 1 À 248

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

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THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 248

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CHIMERIC TRANSCRIPTION FACTOR VARIANTS WITH AUGMENTED
SENSITIVITY TO DRUG LIGAND INDUCTION OF TRANSGENE EXPRESSION IN
MAMMALIAN CELLS

INCORPORATION BY REFERENCE TO A PRIORITY APPLICATION

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 62/433,183, filed December 12, 2016. The entire disclosure of the aforementioned application is hereby expressly incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING, TABLE, OR COMPUTER PROGRAM LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled SCRI.118WO.txt, created December 6, 2017, which is 297 kb in size. The information provided is the electronic format of the Sequence Listing and is hereby expressly incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] Disclosed herein are embodiments relating to genetic systems that facilitate drug-regulated transgene expression in cells, for instance mammalian cells. The present disclosure also concerns the identification of variants of the wild-type HEA3 and wild-type HEA4 chimeric transcription factors, which are useful for the regulation of transgene expression in genetically modified cells including mammalian cells.

BACKGROUND

[0004] Human T lymphocytes can be engineered by gene transfer to express chimeric antigen receptors (CARs), which are specific for surface molecules expressed on tumor cells. Methods of gene transfer useful for this purpose include several types of viral transduction using viral vectors encoding the chimeric antigen receptors. Cells expressing the chimeric antigen receptors can then be adoptively transferred to patients to treat cancer and infectious diseases. Chimeric antigen receptors are synthetic receptors. These synthetic

receptors, can include an extracellular ligand binding domain, for example, a single chain variable fragment of a monoclonal antibody (scFv) linked to intracellular signaling components, such as CD3 ζ alone or combined with one or more costimulatory domains. Research in the design of chimeric receptors has focused on defining scFvs and other ligand binding elements that target malignant cells without causing severe toxicity to normal tissues, and on defining the optimal composition of intracellular signaling modules to activate T cell effector functions. Additional genetic systems that facilitate expression of chimeric antigen receptors are needed.

SUMMARY OF THE INVENTION

[0005] Aspects of the present invention concern genetic systems that facilitate drug-regulated transgene expression in cells. In one alternative, regulated transgene expression is targeted to cells, such as lymphocytes, preferably human lymphocytes, which are designed for use in adoptive immunotherapy. This system provides rigorous safety attributes to chimeric antigen receptor (CAR) redirected adoptive therapeutic strategies without sacrificing curative intent that permits real-time clinician control of CAR expression *in vivo*. By engineering vectors that enable drug responsive transcriptional control of CAR expression, the activity of CARs and other cell mediators can be turned “ON” and “OFF” *in vivo*, based on a clinician prescribed pharmaceutical drug input that exhibits clinically permissive pharmacokinetics, tissue distribution, and partitioning between the extracellular space and cytosol of lymphocytes. In addition to adenovirus and lentivirus genes, the vector platform can also include genes encoding minicircles or piggy bac transposons. The genetic systems described herein provide for drug regulated transgene expression to enforce a functional “OFF” state in the absence of the drug and a functional “ON” state transgene expression in the presence of the drug.

[0006] One alternative of such an approach utilizes the drug tamoxifen. Tamoxifen is an estrogen antagonist/partial agonist that is an FDA-approved and commercially available drug. It is taken orally and can be administered daily and over an extended period. Tamoxifen has a proven safety record, favorable pharmacokinetic profile, excellent tissue distribution and a low partition coefficient between the extracellular space and cytosol. Other drugs useful in the approaches described herein can be selected based on

safety record, favorable pharmacokinetic profile, and excellent tissue distribution, a low partition coefficient between the extracellular space and cytosol, and/or low toxicities.

[0007] Tamoxifen can have several side effects, however, such as cataracts, changes in menstrual period, constipation, diarrhea, edema (swelling), fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting and weight loss, for example. As such, it is necessary to turn on a gene for expressing the chimeric antigen receptor at a low plasma concentration of tamoxifen for some patients.

[0008] One aspect of the disclosure relates to changes that can be made to transcriptional activators so as to modulate the properties of the transcription factors including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain so as to increase the affinity of the factor for estrogen analogs and altering one or more amino acids in the p65 transactivating domain and/or the RelA (p65) transactivation domain, in which altering the amino acids in the p65 transactivating domain (RelA) can enhance the transcriptional activity in response to a drug. The RelA mutations enhance transcriptional activity in response to the drug, however, they do not affect drug-to-receptor binding. Only the mutations in the ER-LBD affect drug-to-receptor binding. In some alternatives, the system employs a synthetic transcriptional regulator, which, in the presence of tamoxifen, binds a synthetic promoter upstream of a transgene to induce expression. The tamoxifen regulated transcription factors ("TamR-tf", also can refer to HEA3/HEA4 transcription factors) is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1 α) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD), that is in turn fused to the p65 activation domain of NF- κ B (p65). HEA4 is a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1 α) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) containing three point mutations (G400V, M543A, L544A) that abolish estradiol binding but permit binding to tamoxifen metabolites and other estrogen analogues, that is in turn fused to the p65 activation domain of NF- κ B (p65). An exemplary amino acid sequence is set forth in SEQ ID NO: 1

(MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLSLALSLTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
 LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA
 QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
 ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
 FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
 SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
 KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
 VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
 DFSALLSQISS). SEQ ID NO: 1 comprises one point mutation in the ER-LBD that ablates
 binding to endogenous estrogen, but confers nanomolar specificity to tamoxifen metabolite
 4-OHT fulvestrant and other estrogen analogs. The mutant tamoxifen-specific ligand binding
 domain of the estrogen receptor ligand binding domain (ER-LBD) is found at amino acids
 282-595 of the TamR-tf and has a mutation at position 521 (G521R), and affects drug-to-
 receptor binding. The p65 activation domain of NF- κ B (p65) is found at amino acids 596-862
 of

SEQ	ID	NO:	1
(MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLSLALSLTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA			

QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
 ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
 FKSIMKKSPFSGPTDPRPPRRRIAVPSRSSASVPKPAPQYPYFTSSLSTINYDEFPTMVFP
 SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
 KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
 VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDEFSSIADM
 DFSALLSQISS).

[0009] In the absence of tamoxifen, TamR-tf is excluded from the nucleus by binding of cytosolic heat-shock protein 90 (HSP90) to the tamoxifen binding active site and transgene expression is in the “OFF” state. Nanomolar concentrations of cytosolic tamoxifen actively out compete HSP90 for ER-LBD binding, resulting in TamR-tf translocation to the nucleus. Upon nuclear translocation, TamR-tf is readily available to bind its restricted synthetic promoter (e.g. 7xHBD/mE1B as set forth in the nucleic acid sequence of SEQ ID NO: 23

(tagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaagagctcagggatatataatg). In the presence of tamoxifen, binding of TamR-tf to 7xHBD/mE1B promoter induces the “ON” state of transgene expression. In some alternatives, this transcriptional regulator can be modified to provide for a varying level of control of transgene expression. Amino acid substitutions in the LBD of TamR-tf permit selective responsiveness to tamoxifen and its metabolites, which affect the drug-to-receptor binding, where 4-hydroxy tamoxifen (4-OHT) is the most pharmacologically active metabolite, with respect to TamR-tf activity, while lacking interaction with endogenous estrogen.

[0010] In some alternatives, variants of wild-type HEA3 and wild-type HEA4 are identified with augmented sensitivity to a drug by virtue of substitutions of the human estrogen receptor ligand binding domain. Mutations in the RelA (p65) transactivation domain augment transcriptional activity in response to drug or drug ligand. In some alternatives, these high affinity forms are generated by amino acid substitutions within the human estrogen receptor that confer selective binding to 4-OHT and other estrogen analogs such as fulvestrant. These changes cooperatively enhance transgene expression in response to drug activators when combined with amino acid substitutions in the HEA3 and HEA4 RelA

domain that function as phosphomimetics and acetyl-mimetics, for example. Also described herein is a preferred version of the variant housing all three modifications that does not adversely affect the stringency of the "OFF" state in the absence of ligand, but dramatically improves the sensitivity and magnitude of transgene expression to activating estrogen analogue drug inputs. In another aspect, HEA3 is rendered responsive to the drug CMP8 due to mutations in the ER-LBD (L384M, M421G, G521R), not RelA mutations. The RelA mutations may not affect specificity to drug. The RelA mutations may enhance transcriptional activity. These enhanced HEA3 and HEA4 chimeric transcription factors make this regulatory system responsive to input drug levels that can easily be reached with low dose non-toxic dosing of tamoxifen, fulvestrant, or CMP8.

[0011] In a first aspect, a system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, is provided. The system comprises: a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from the group consisting of a tumor specific molecule, a viral specific molecule, and any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte; a second polynucleotide, which encodes a polypeptide spacer, preferably an optimized spacer; a third polynucleotide, which encodes a transmembrane domain; and a fourth polynucleotide, which encodes an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter, which is inducible by a drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug as compared to a wild type HEA3 chimeric transcription factor. In some alternatives, the transcriptional activator for the first promoter inducible by a drug, is HEA3 or HEA4. In some alternatives, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain

comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives, the at least one mutation affects the drug-to-receptor binding. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional expression at a given concentration compared to a system using a wild type HEA3. In some alternatives, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequence set forth in SEQ ID Nos: 1-3, 8-11 and 121. In some alternatives, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or at a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives, the non-toxic

dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 chimeric transcription factor. In some alternatives, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1 α p. In some alternatives, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives, the selectable marker is EGFRt and/or HER2t. In some alternatives, the selectable marker confers drug resistance. In some alternatives, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand, as compared to a reference chimeric receptor. In some alternatives, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives, the drug induces expression of the chimeric antigen receptor at a

non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is preferably optimized, a polynucleotide coding for a transmembrane domain and a polynucleotide encoding an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the second promoter. In some alternatives, the ligand binding domain is an antibody fragment, such as a binding fragment. In some alternatives, the ligand binding domain is a single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or any combinations thereof. In some alternatives, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, and/or Epstein-Barr virus early antigen protein. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in

between a range defined by any two aforementioned values. In some alternatives, the DNA spacer enhances gene expression of the chimeric antigen receptor.

[0012] In a second aspect, a system for inducible expression of a gene that regulates a T cell function is provided. The system comprises: a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity, and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter, which is inducible by a drug and, wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a wild type HEA3. In some alternatives, the transcriptional activator is HEA3 or HEA4. In some alternatives, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives, HEA3 or HEA4 chimeric transcription factor comprises p65

phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3 and/or 8-11 and/or 121. In some alternatives, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 and/or 122. In some alternatives, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is preferably optimized, a polynucleotide coding for a transmembrane domain; and a polynucleotide encoding an intracellular signaling domain. In some alternatives, the first promoter is in opposite orientation to the

second promoter. In some alternatives, the ligand binding domain is an antibody fragment, such as a binding fragment. In some alternatives, the ligand binding domain is a single chain variable fragment. In some alternatives, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or any combinations thereof. In some alternatives, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0013] In a third aspect, a chimeric receptor polypeptide coded for by the first second and/or second nucleic acid of the system of any one of the alternative systems described herein. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, the

system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a polypeptide spacer, preferably an optimized spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible

expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 and/or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 and/or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing

of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the

first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is a system for inducible expression of a gene that regulates a T cell function. In some alternatives, the system for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least

one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500

nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is preferably optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked

to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the systems described herein, both vectors are packaged in a viral vector. In some alternatives of the systems described herein, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0014] In a fourth aspect, a host cell comprising a system of any one of the alternative systems described herein is provided. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug

that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3 8-11 and/or. 121 In some alternatives of the system for inducible expression of a chimeric antigen

receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a

constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a

chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or

HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 and/or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a

polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the systems described herein, both vectors are packaged in a viral vector. In some alternatives of the systems described herein, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the host cell, the host cell is a mammalian cell, such as a precursor T cell. In some alternatives of the

host cell, the precursor T cell is a hematopoietic stem cell. In some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0015] In a fifth aspect, a composition comprising a host cell of any one or more of alternative host cells, such as mammalian cells, provided herein, in a pharmaceutically acceptable excipient, is provided. The host cell, such as a mammalian cell, comprises a system of any one of the alternative systems described herein is provided. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, , such as mammalian cells ,the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription

factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least

one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a

chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a

polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic

amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or a wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain,

which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the host cell, the host cell is a precursor T cell. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the systems described herein, both vectors are packaged in a viral vector. In some alternatives of the systems described herein, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the host cell, such as a mammalian cell, the cell is a precursor T cell, which can be a hematopoietic stem cell. In

some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells and a host cell, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a host cell wherein the host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0016] In a sixth aspect, an *in vitro* method for preparing a host cell of any one of the alternative host cells, such as mammalian cells, described herein is provided. The host cell, such as a mammalian cell, comprises a system of any one of the alternative systems described herein. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug,

wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells,

HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or

prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the

first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least

one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or a wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500

nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is preferably optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment, such as a binding fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the host cell, the host cell is a mammalian cell, such as a precursor T cell. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first

promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the systems described herein, both vectors are packaged in a viral vector. In some alternatives of the systems described herein, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the host cell, the precursor T cell is a hematopoietic stem cell. In some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells and a host cell, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a host cell wherein the host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or

VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the *in vitro* method for preparing a host cell of any one of the alternative host cells comprises a) providing a system of any one of the alternative systems provided herein and b) introducing the system into an isolated T lymphocyte population and expanding each T lymphocyte population *in vitro*. In some alternatives of the method, the T lymphocytes are expanded, and wherein the method further comprises culturing the cells in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine until the cells expand sufficiently for use as a cell infusion. In some alternatives of the method, the isolated T lymphocyte population comprises precursor T cells. In some alternatives of the method, the precursor T cells are hematopoietic stem cells. In some alternatives of the method, the lymphocyte is CD8+ or CD4+. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0017] In a seventh aspect, a method of treating, inhibiting, or ameliorating a disease in a subject in need thereof is provided. The method comprises administering to the subject the host cell of any one of the alternative host cells, such as mammalian cells, provided herein, or at least one composition or product combination of any one or more of the alternatives provided herein. The composition comprises a host cell of any one or more of alternative host cells, such as a mammalian cell, provided herein, in a pharmaceutically acceptable excipient. The host cell, such as a mammalian cell comprises a system of any one of the alternative systems described herein. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral specific

molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some

alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects

are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122.. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In

some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand, as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65)

transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or a wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than

10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment, such as a binding fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any

percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives of the host cell, such as a mammalian cell, the host cell is a precursor T cell. In some alternatives of the host cell, the precursor T cell is a hematopoietic stem cell. In some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells and a host cell, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell, wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a host cell wherein the host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives of the method, the disease is cancer or a viral disease. In some alternatives of the method, the subject is identified or selected to receive an anti-cancer therapy. In some alternatives of the method, the subject is identified or selected to receive an anti-viral therapy. In some alternatives of the method, the method further comprises measuring or evaluating an inhibition of the disease. In some alternatives of the method, the method further comprises providing said subject an additional anti-cancer therapy or anti-viral therapy before, during, or after administration of the cells of any one or more of any one of the alternatives provided herein or at least one composition or product combination of any one or more of the alternatives provided herein. In some alternatives of the method, the cells described above or

at least one composition or product combination described above are administered to said subject by adoptive cell transfer. In some alternatives of the method, the host cells described above or at least one composition or product combination described above are administered to said subject after said subject has received another form of anti-cancer therapy or anti-viral therapy. In some alternatives of the method, the method further comprises administering a drug that induces expression of a transgene in the host cell or composition for the treatment of cancer or a viral infection. In some alternatives of the method, the drug is tamoxifen and/or its metabolites, fulvestrant and/or other estrogen analogs or CMP8. In some alternatives of the method, tamoxifen is administered at a dose range of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mg/dose or within a range defined by any two of the aforementioned values. In some alternatives of the method, fulvestrant is administered at a dose range of 250, 300, 350, 400, 450 or 500 mg/dose mg/dose or within a range defined by any two of the aforementioned values. In some alternatives of the method, the CMP8 is administered at a dose range to provide 30, 40 or 50 nM of drug levels in serum, or within a range defined by any two of the aforementioned values. In some alternatives of the method, the cancer is a solid tumor or hematologic malignancy. In some alternatives of the method, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0018] In an eighth aspect, a method of treating, inhibiting, or ameliorating a disease in a subject in need thereof, is provided. The method comprises administering to the subject the host cell, such as a mammalian cell, of any one of the alternatives provided herein or at least one composition or product combination of any one or more of the alternative compositions provided herein. In some alternatives, the composition comprises a host cell of any one or more of alternative host cells, such as mammalian cells, provided herein, in a

pharmaceutically acceptable excipient. In some alternatives, the host cell, such as mammalian cells, comprises a system of any one of the alternative systems described herein. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral specific molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to

a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as

compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further

comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65)

transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems

described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or a wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the systems

described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives of the host cell, the host cell is a precursor T cell. In some alternatives of the host cell, such as a mammalian cell, the cell is a precursor T cell, which is a hematopoietic stem cell. In some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells and a host cell, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a host cell wherein the host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives of the method, the disease is cancer or a viral disease. In some alternatives of the method, the subject is identified or selected to

receive an anti-cancer therapy. In some alternatives of the method, the subject is identified or selected to receive an anti-viral therapy. In some alternatives of the method, the method further comprises measuring or evaluating an inhibition of the disease. In some alternatives of the method, the method further comprises providing said subject an additional anti-cancer therapy or anti-viral therapy before, during, or after administration of the cells of any one or more of the alternative cells described herein or at least one composition or product combination of any one or more of alternatives described herein. In some alternatives of the method, the cells of any one or more of the alternatives described herein or at least one composition or product combination of any one or more of the alternatives described herein are administered to said subject by adoptive cell transfer. In some alternatives of the method, the host cells of any one or more of the alternatives described herein or at least one composition or product combination of any one or more of the alternatives described herein are administered to said subject after said subject has received another form of anti-cancer therapy or anti-viral therapy. In some alternatives of the method, the method further comprises administering a drug that induces expression of a transgene in the host cell or composition for the treatment of cancer or a viral infection. In some alternatives of the method, the drug is tamoxifen and/or its metabolites, fulvestrant and/or other estrogen analogs or CMP8. In some alternatives of the method, tamoxifen is administered at a dose range of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mg/dose or within a range defined by any two of the aforementioned values. In some alternatives of the method, the fulvestrant is administered at a dose range of 250, 300, 350, 400, 450 or 500 mg/dose mg/dose or within a range defined by any two of the aforementioned values. In some alternatives of the method, the CMP8 is administered at a dose range to provide 30, 40 or 50 nM of drug levels in serum, or within a range defined by any two of the aforementioned values. In some alternatives of the method, the cancer is a solid tumor or hematologic malignancy. In some alternatives of the method, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the

polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0019] In a ninth aspect, a use of the host cell, such as a mammalian cell, of any one of or more of the alternatives described herein or at least one composition or product combination of any one or more of the alternatives described herein in combination with a drug that induces expression of a transgene in the host cell or composition for the treatment of cancer or a viral infection is provided. In some alternatives, the composition comprises a host cell, such as a mammalian cell, of any one or more of alternative host cells provided herein, in a pharmaceutically acceptable excipient. In some alternatives, the host cell, such as mammalian cell, comprises a system of any one of the alternative systems described herein. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for

inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set

forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for

inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug

that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid

sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or a wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter.

In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives of the host cell, the host cell, such as a mammalian cell, is a precursor T cell. In some alternatives of the host cell, the precursor T cell is a hematopoietic stem cell. In some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells and a host cell, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central

memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a host cell wherein the host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives of the use, the drug is tamoxifen and/or its metabolites, fulvestrant and/or other estrogen analogs or CMP8. In some alternatives of the use, tamoxifen is administered at a dose range of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mg/dose or within a range defined by any two of the aforementioned values. In some alternatives of the use, the fulvestrant is administered at a dose range of 250, 300, 350, 400, 450 or 500 mg/dose or within a range defined by any two of the aforementioned values. In some alternatives of the use, the CMP8 is administered at a dose range to provide 30, 40 or 50 nM of drug levels in serum, or within a range defined by any two of the aforementioned values. In some alternatives of the use, the cancer is a solid tumor or hematologic malignancy. In some alternatives of the use, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0020] In a tenth aspect, a method of performing cellular immunotherapy in a subject having cancer or a viral infection is provided. The method comprises administering the host cells, such as mammalian cells, of any one or more of alternatives provided herein or at least one composition or product combination of any one or more of the alternatives provided herein to the subject and administering a drug that induces expression of a transgene in the composition or the host cells. The composition comprises a host cell of any

one or more of alternative host cells, such as mammalian cells, provided herein, in a pharmaceutically acceptable excipient. In some alternatives, the host cell, such as a mammalian cell, comprises a system of any one of the alternative systems described herein. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation

domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a

concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for

inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function,

HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino

acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or a wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120

protein, or Epstein-Barr virus early antigen protein. In some alternatives of the host cell, such as a mammalian cell, the host cell is a precursor T cell. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives of the host cell, such as a mammalian cell, the cell is a precursor T cell, which is a hematopoietic stem cell. In some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells and a host cell, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a host cell wherein the host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives

of the method, the cancer is selected from a solid tumor or hematologic malignancy. In some alternatives of the method, the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

[0021] In an eleventh aspect, a use of the host cell of any one of or more of the alternatives described herein or at least one composition or product combination of any one or more of the alternatives described herein as a medicament, is provided. The composition comprises a host cell of any one or more of alternative host cells, such as mammalian cells, provided herein, in a pharmaceutically acceptable excipient. In some alternatives, the host cell, such as a mammalian cell, comprises a system of any one of the alternative systems described herein. In some alternatives, the system is for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4

chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth

in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is an inducible promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is a constitutive promoter. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second promoter is the EF1 α p. In some

alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator comprises at least one or more amino acid sequences set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the transcriptional activator is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, both vectors are packaged in a viral vector. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker is EGFRt and/or HER2t. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the selectable marker confers drug resistance. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system for inducible expression of a chimeric antigen receptor in cells, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function. In some alternatives, the system is for inducible expression of a gene that regulates a T cell function comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates

checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that augments transcriptional activity in response to a drug. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand.

In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the systems described herein, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the systems described herein, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the systems described herein, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or a wild type HEA4 chimeric transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the systems described herein, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the systems described herein, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 chimeric transcription factor. In some alternatives of the systems described herein, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the systems described herein, the second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population,

wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for a transmembrane domain, and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the systems described herein, the first promoter is in opposite orientation to the second promoter. In some alternatives of the systems described herein, the ligand binding domain is an antibody fragment. In some alternatives of the systems described herein, the ligand binding domain is a single chain variable fragment. In some alternatives of the systems described herein, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives of the systems described herein, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the systems described herein, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the systems described herein, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the systems described herein, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the systems described herein, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives of the host cell, such as a mammalian cell, the host cell is a precursor T cell. In some alternatives of the host cell, the precursor T cell is a hematopoietic stem cell. In some alternatives of the host cell, the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells. In some alternatives of the host cell, the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺

T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells and a host cell, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells. In some alternatives of the composition, the composition comprises a host cell wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells or wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells and a host cell wherein the host cell is a precursor T cell or a hematopoietic stem cell. In some alternatives, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC or VAR2CSA. In some alternatives, the spacer comprises IgG4, IgG4-CH2 (L235D, N297Q) or IgG4-CH3. In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] **Figures 1A to 1F** show HEA3 (low affinity) RelA single mutant variants and HEA4 (high affinity) RelA single mutant variants and their response when dosed with 4-OHT, when they are transduced into cells. Shown in the figures is a schematic of dual-packaged lentiviral constructs housing the constitutively expressed drug-inducible transcription factors, (Fig. 1A) HEA3 or (Fig. 1B) HEA4 single mutant RelA variants (S536E or K310Q positions in the RelA protein domain, which correspond to position S846E and K621Q in HEA3 and HEA4, respectively), and a ZsGreen fluorescent reporter. ZsGreen reporter expression in Jurkat cells expressing (Fig. 1C) HEA3 or (Fig. 1D) HEA4 RelA variants following 24 hours of culture with 500 nM 4-OHT. 4-OHT dose response:

Percentage of EGFRt+ZsGreen+ cells and ZsGreen median fluorescent intensity of (Fig. 1E) HEA3 or (Fig. 1F) HEA4 RelA variant expressing Jurkat cells.

[0023] **Figures 2A to 2E** show HEA4 (high affinity) RelA double mutant variants and CMP8-responsive HEA3 RelA variants and their response when dosed with 4-OHT and CMP8, respectively, when they are transduced into cells. Shown in the figures is a schematic of dual-packaged lentiviral constructs housing the constitutively expressed drug-inducible transcription factors, (Fig. 2A) HEA4 double mutant RelA variant (S536E/K310Q positions in the RelA protein domain, which correspond to position S846E and K621Q in HEA3 and HEA4, respectively) or (Fig. 2B) CMP8-responsive HEA3 single mutant RelA variant (S563E), and a ZsGreen fluorescent reporter. ZsGreen reporter expression in Jurkat cells expressing the (Fig. 2C) HEA4 double mutant RelA variant or the (Fig. 2D) CMP8-responsive HEA3 RelA variant, following 24 hours of culture with 500 nM 4-OHT and CMP8. 4-OHT and CMP8 dose response: Percentage of EGFRt+ZsGreen+ cells and ZsGreen median fluorescent intensity of Jurkat cells expressing the (Fig. 2E) HEA4 double mutant RelA variant or (Fig. 2F) CMP8-responsive HEA3 single mutant RelA variant.

[0024] **Figures 3A to 3C** shows the results of dual-packaged lentiviral constructs encoding drug-regulated transcription factor (HEA4 (p65/S536E/K310Q)) and inducible fluorescent reporter eGFP:ffluc, when they are transduced into cells. Shown in the Fig. 3A, CD4 and CD8 T cells were transduced with dual-packaged lentiviral constructs encoding drug-regulated transcription factor (HEA4(p65/S536E/K310Q)) and inducible fluorescent reporter eGFP:ffluc. Following purification and expansion of transduced CD4 and CD8 T cells, (Fig. 3B) Western blot was performed using anti-p65 antibody to confirm expression of chimeric transcription factor. (Fig. 3C) EGFRt expression was confirmed in sorted cells by flow cytometry, and leakiness of eGFP expression in the absence of inducing ligand was determined.

[0025] **Figures 4A to 4B** shows the dual-packaged lentiviral constructs encoding drug-regulated transcription factor (HEA4(p65/S536E/K310Q)) and inducible fluorescent reporter eGFP:ffluc and the results when the lentiviral constructs are transduced into cells. (Fig. 4A) CD4 and CD8 T cells were transduced with dual-packaged lentiviral constructs encoding drug-regulated transcription factor (HEA4(p65/S536E/K310Q)) and inducible fluorescent reporter eGFP:ffluc. Following purification and expansion of transduced CD4

and CD8 T cells, (Fig. 4B) T cells were incubated with 0 nM, 30 nM, or 500 nM of 4-OHT in the presence or absence of aCD3/aCD28 microbeads (2:1 bead:cell ratio) or PMA (2 uM)/Ionomycin (20 ng/mL) for 24 hours. eGFP expression was subsequently quantified by flow cytometry.

[0026] **Figures 5A to 5E** shows dual-packaged lentiviral constructs encoding drug-regulated transcription factor (HEA4(p65/S536E/K310Q)) and inducible fluorescent reporter eGFP:ffluc and the results when the lentiviral constructs are transduced into cells. (Fig. 5A) CD4 and CD8 T cells were transduced with dual-packaged lentiviral constructs encoding drug-regulated transcription factor (HEA4(p65/S536E/K310Q)) and inducible fluorescent reporter eGFP:ffluc. Following purification and expansion of transduced CD4 and CD8 T cells, T cells were incubated with increasing concentrations of 4-OHT in the presence or absence of aCD3/aCD28 microbeads (2:1 bead:cell ratio) or PMA (2 uM)/Ionomycin (20 ng/mL) for 24 hours. eGFP expression was quantified by flow cytometry. (Fig. 5B) %EGFRt+eGFP+ CD4 and (Fig. 5C) CD8 T cells and (Fig. 5D) MFI of eGFP expression in (Fig. 5D) CD4 and (Fig. 5E) CD8 T cells following 24 hours of treatment with 4-OHT.

[0027] **Figures 6A to 6D** shows results of cells transduced with lentiviral constructs. Jurkat cells were transduced with dual-packaged lentiviral constructs encoding drug-regulated transcription factor (HEA4(p65/S536E/K310Q)) and inducible (Fig. 6A) chimeric cytokine receptor (CCR(CD122)) or (Fig. 6B) Survivin linked to Her2tG via a cleavable 2A linker. Expression of (Fig. 6C) CCR(CD122) and (Fig. 6D) Survivin following 24 hours of treatment with 500 nM 4-OHT was determined by Her2tG upregulation.

Detailed Description

[0028] In the description that follows, the terms should be given their plain and ordinary meaning when read in light of the specification. One of skill in the art would understand the terms as used in view of the whole specification.

[0029] “About” has its plain and ordinary meaning when read in light of the specification, and may be used, for example, when referring to a measurable value and may be meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value.

[0030] “Antigen” or “Ag,” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a molecule that provokes an immune response. This immune response can involve either antibody production, or the activation of specific immunologically-competent cells, or both. It is readily apparent that an antigen can be generated synthesized, produced recombinantly or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid such, for example, blood, plasma or ascites fluid. In some alternatives of the systems provided herein, the ligand recognized by a chimeric antigen receptor is an antigen, capable of provoking an immune response.

[0031] “Anti-tumor effect” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a biological effect, which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or a decrease of various physiological symptoms associated with the cancerous condition. An “anti-tumor effect” can also be manifested by a decrease in recurrence or an increase in the time before recurrence.

[0032] “Chimeric receptor” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a synthetically designed receptor comprising a ligand binding domain of an antibody or other protein sequence that binds to a molecule associated with the disease or disorder and is linked via a spacer domain to one or more intracellular signaling domains of a T cell or other receptors, such as a costimulatory domain. Chimeric receptor can also be referred to as artificial T cell receptors, chimeric T cell receptors, chimeric immunoreceptors, and chimeric antigen receptors (CARs). These CARs are engineered receptors that can graft an arbitrary specificity onto an immune receptor cell. The term chimeric antigen receptors or “CARs” are also considered by some investigators to include the antibody or antibody fragment, the spacer, signaling domain, and transmembrane region. Due to the surprising effects of modifying the different components or domains of the CAR described herein, such as the epitope binding region (for example, antibody fragment, scFv, or portion thereof), spacer, transmembrane domain, and/ or signaling domain), the components of the CAR are

frequently distinguished throughout this disclosure in terms of independent elements. The variation of the different elements of the CAR can, for example, lead to stronger binding affinity for a specific epitope or antigen.

[0033] A “single-chain variable fragment (scFv)” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a fusion protein that can have variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. The scFv can be specific for an antigen. “Antigen” or “Ag” as used herein refers to a molecule that provokes an immune response. This immune response can involve either antibody production, or the activation of specific immunologically-competent cells, or both. An antigen can be generated, synthesized, produced recombinantly or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid such, for example, blood, plasma or ascites fluid. In some alternatives of the CARs provided herein, the CAR comprises an antibody fragment, scFv, or portion thereof e.g., a polypeptide comprising the CDR domains. In some alternatives, the scFv specifically binds to CD19. This particular scFv is referred to as huCD19 scFv (G01S) and is encoded by SEQ ID NO: 40 (atgcttctcttggtgacaagccttctgctctgtgagttaccacacccagcattctctgatcccagaggtgcagctggcgaatccggaggaggactgggtccagccccggccggagcctgagactgagctgtgccgctccggattcacttttgacgattacgcaatgcattgggtgaggcaggccccctggcaaggggctggaatgggtctccggaatctcttggacagtgggcgccattggatatgccattctgtgaagggccgattcactatctctcgggacaacgctaaaaatagtctgtttctgcagatgaattccctgcgcgccgaggataccgccgtgtactattgcgccccgagaccagggtaccactactatgatagcgccgaacatgcattcgacatttggggacagggaactgtggtcaccgtgagctcgggaggaggaggaagcggaggaggagggtccggaggcgggggatcacagagcgccactgacctgagccagccagcgagcgtgagcggatttctgggcagctctgtcaccattagttgcacaggcaccacatcagacgatgtgagctggtaccagcagcaccagggaaggctcaccagctgatgctgtatgacgtgtccaaaagaccttctggcgtccacataggtttagtgggaagccggagcggccgggcagccagctctgatcatttcagggtgcagacagaggacgaagctgattatttctgttctagttacgcaggcagatataactctgtgtgtttggcgggggaacaaagctgactgtcctg). In some alternatives, the CAR comprises an scFv. In some

alternatives, the scFv comprises an huCD19 scFv (G01S) amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 41 (MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGRIGYADSVKGRFTISRDNANKNSLFLQMNSLRAEDTAVYYCARDQGYHYYSAEHAFDIWGQGT VTVSSGGGGSGGGGSGGGGSQSALTQPRSVSGFPGQSVTISCTGTTSDDVSWYQQHPGKAPQLMLYDVSKRPSGVPHRFSGSRSGRAASLIISGLQTEDEADYFCSSYAGRYNSVLFGGGTKLTVL). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 41.

[0034] In some alternatives, the scFv is encoded by the muCD19 (FMC63) scFv nucleotide sequence. In some alternatives, the scFv specifically binds to CD19. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 42 (Atgctgctgctggtgaccagcctgctgctgtgcgagctgccccaccccgctttctgctgatccccgacatccagatgaccagacacctccagcctgagcgccagcctgggcgaccgggtgaccatcagctgccgggcccagccaggacatcagcaagtacctgaactgtatcagcagaagcccgacggcaccgtcaagctgctgatctaccacaccagccggctgcacagcggcgtgcccagccgggttagcggcagcggctccggcaccgactacagcctgaccatctccaacctggaacaggaagatatgccacctactttgccagcagggcaacacactgccctacacctttggcggcggaacaaagctggaaatcaccggcagcacctccggcagcggcaagcctggcagcggcgagggcagcaccaagggcgaggtgaagctgcaggaaagcgccctggcctggtggccccagccagagcctgagcgtgacctgcaacctgagcggcgtgagcctgcccgactacggcgtgagctggatccggcagccccccaggaagggcctggaatggctgggctgattctggggcagcgagaccactactacaacagcgccctgaagagccggctgaccatcatcaaggacaacagcaagagccaggtgttcctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcactactactacggcggcagctacgccatgactactggggccagggcaccagcgtgacctgagcagc). In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 43 (MLLLVTSLLLCELPHPAFLIPDIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIKD NSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 43.

[0035] In some alternatives, the scFv is encoded by a CD20 (CD20 (Leu16)) nucleic acid sequence. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 44 (atggagacagacacactcctgctatgggtgctgctgctctgggtccaggtccacaggtgacattgtgctgacccaatctccagctatcctgtctgcatctccaggggagaaggtcacaatgacttgcagggccagctcaagtgtaaattacatggactggtaccagaagaagccaggatcctccccc aaacctggatttatgccacatccaacctggcttctggagtcctgctcgttcagtggcagtggtctgggacctcttactctctcacaatcagcagagtgagggtgaagatgctgccacttattactgccagcagtgagggttttaatccaccacgttcggaggggggaccaagctggaaataaaaggcagtactagcgggtgggtccggggcggttccgggtggggcgggcagcagcgaggtgcagctgcagcagctctggggctgagctggtgaagcctggggcctcagtgaagatgtcctgcaaggcttctggctacacatttaccagttacaatatgcactgggtaaagcagacacctggacagggcctggaatggattggagctatttatccaggaaatggtgatacttcctacaatcagaagttcaaaggcaaggccacattgactgcagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgagga ctctgcggactattactgtgcaagatctaattattacggtagtagctactggttcttcgatgtctggggcgagggaccacggtcaccgtctcctca). In some alternatives, the CD20 (Leu 16) scFv comprises an amino acid sequence set forth in SEQ ID NO: 45 (METDTLLLWVLLLWVPGSTGDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWY QKKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSF NPPTFGGGTKLEIKGSTSGGGSGGGSGGGGSSEVQLQQSGAELVKPGASVKMSCKAS GYTFTSYNMHWVKQTPGQGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAY MQLSSLTSEDSADYYCARSNYYGSSYWFFDVWGAGTTVTVSS). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 45.

[0036] In some alternatives, the scFv is encoded by a CD22 (m971) scFv nucleotide sequence. In some alternatives, the scFv specifically binds to CD22. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 46 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacaggtacagctgcagcagtcagg tccaggactggtgaagccctcgcagacccctctcactcaccttgccatctccggggacagtgtctctagcaacagtgtgcttggaa cttgatcaggcagtcctccatcgagaggccttgagtggctgggaaggacatactacaggtccaagtgggtataatgattatgcagtatctgt gaaaagtcgaataaccatcaaccagacacatccaagaaccagttctcctgcagctgaactctgtgactcccaggacacggctgt gtattactgtgcaagagaagtgactggggatctcgaggatgcttttgatatctggggccaagggaacaatggtcaccgtctcctcaggc ggagggggctctggcggcgaggatctgggggagggggcagcgcacatccagatgaccagctctccatcgtccctgtctgcatctgt aggagacagagtcacatcacttgccgggcaaggcagaccatttgagctacttaattggtatcagcagagaccagggaagccc

ctaacctctgatctatgctgcatccagtttgcaaagtgggtcccatcaaggttcagtggcaggggatctgggacagatttcactctca
ccatcagcagctctgcaagctgaagattttgcaacttactactgtcaacagagttacagtatccctcagacttttggccaggggaccaagc
tgagatcaaacgaact). In some alternatives, the scFv comprises a CD22 (m971) scFv amino
acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in
SEQ ID NO: 47
(MLLLVTSLLLCELPHPAFLIPQVQLQQSGPGLVKPSQTLSTCAISGDSVSSNSAAW
NWIRQSPSRGLEWLGRYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPED
TAVYYCAREVTGDLEDAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS
LSASVGDRVTITCRASQTIWSYLNWYQQRPGKAPNLLIYAASSLQSGVPSRFSGRGS
GTDFTLTISLQAEDFATYYCQQSYSIPQTFGQGKLEIKRT). In some alternatives, the
scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
amino acid sequence of SEQ ID NO: 47.

[0037] In some alternatives, the scFv comprises a B7H3 (hBRCA84D) scFv
nucleotide sequence. In some alternatives, the scFv binds specifically to the B7-H3 receptor.
The B7-H3 receptor is known to be expressed on a variety of cancer cells. Without being
limiting, these cancer cells can included gastric, ovarian and/or non-small cell lung cancers.
In some alternatives, the CAR is used in the treatment or amelioration of cancer and/or
inflammation. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID
NO: 48

(atgctgcgtcggcggggcagccctggcatgggtgtgcatgtgggtgcagccctgggagcactgtggttctgcctcacaggagccc
tgagggtccaggtccctgaagaccagtggtggcactggtgggcaccgatgccaccctgtgctgctccttctccctgagcctggctt
cagcctggcacagctcaacctcatctggcagctgacagatacacaacagctggtgcacagctttgctgagggccaggaccagggca
gcgcctatgccaaaccgcagggccctctcccgacctgctggcacagggcaacgcacccctgaggctgcagcgcgtgcgtgtggc
ggacgagggcagcttcacctgctcgtgagcatccgggatttcggcagcgtgccgtcagcctgcaggtggccgctccctactcgaa
gcccagcatgaccctggagcccaacaaggacctgcgccaggggacacggtgacctcacgtgctccagctaccagggtaccct
gaggctgaggtgttctggcaggatgggcaggggtgtgcccctgactggcaacgtgaccacgtgcagatggccaacgagcagggct
tgtttgatgtgcacagcatcctgcgggtggtgctgggtgcaaatggcacctacagctgcctggtgcgcaaccccgctgctgcagcagg
atgcgcacagctctgtcaccatcacagggcagcctatgacattccccccagaggccctgtgggtgaccgtggggctgtctgtctgtct
cattgcactgctggtggccctggcttctgtgtgctggagaaagatcaaacagagctgtgaggaggagaatgcaggagctgaggacc
aggatggggagggagaaggctccaagacagccctgcagcctctgaaacactctgacagcaaagaagatgatggacaagaaatag
cc). In some alternatives, the scFv comprises a B7H3 (hBRCA84D) scFv amino acid

sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 49 (MLRRRGSPGMGVHVGAAALGALWFCLTGALEVQVPEDPVVALVGTDATLCCSFSPGPGFSLAQLNLIWQLTDTKQLVHSFAEGQDQGSAYANRTALFPDLLAQGNASRLQRVRVADEGSFTCFVSIRDFGSAAVSLQVAAPYSKPSMTLEPNKDLRPGDTVITITCSSYQGYPEAEVFWQDGQGVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTYSCLVRNPVLQQDAHSSVTITGQPMTFPPEALWVTVGLSVCLIALLLVALAFVCWRKIKQSCEENAGAEDQDGE GEGSKTALQPLKHSDSKEDDGQEIA). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 49.

[0038] In some alternatives, the scFv comprises the L1CAM (CE7) scFv nucleotide sequence. In some alternatives, the scFv is specific for the epitope, CE7 epitope, of L1CAM. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 50

(atgctgctgctggtgaccagcctgctgctgtgcgagctgccccacccgcctttctgctgatccccaggtgcagctgcagcagcctggcgccgagctggtgaagccaggcgccagcgtgaagctgtcctgcaaggccagcggctacaccttcaccggctactggatgcactgggtgaagcagagacccggccacggcctggaatggatcggcgagatcaacccagcaacggccggaccaactacaacgagcggttcaagagcaaggccaccctgaccgtggacaagagcagcaccaccgccttcagcagctgtccggcctgaccagcgaggacagcggccgtgtacttctgcgccagggactactacggcaccagctacaacttcgactactggggccaggggcaccacactgaccgtgagcagcggcgaggggggctctggcgggcggaggatctgggggagggggcagcgacatccagatgaccagagcagcagcagcttcagcgtgagcctggggcgaccgggtgaccatcacctgtaaggccaacgaggacatcaacaaccggctggcctggtatcagcagacccccggcaacagccccaggctgctgatcagcggcgccaccaacctggtgaccggcgtgccagccggttagcggcagcggctccggcaaggactacaccctgaccatcacaagcctgcaggccgaggacttcgccacctactactgccagcagtactggtccaccccccttcaccttcggcagcggcaccgagctggaaatcaaa). In some alternatives, the scFv comprises the L1CAM (CE7) scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 51 (MLLLVTSLLLCELPHPAFLIPQVQLQQPGAELVKPGASVKLSCKASGYTFTGYWMHWVKQRPGHGLEWIGEINPSNGRTNYNERFKSKATLTVDKSSTTAFMQLSGLTSEDSAVYFCARDYYGTSYNFDYWQGTTTLTVSSGGGGSGGGGSGGGGSDIQMTQSSSSFSVSLGDRVTITCKANEDINNRLAWYQQTPGNSPRLISGATNLVTGVPSRFSGSGSGKDYTLTITSLQAEDFATYYCQQYWSTPFTFGSGTELEIK). In some alternatives, the scFv

is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 51.

[0039] In some alternatives, the scFv is encoded by the EGFR (cetuximab) scFv nucleotide sequence. In some alternatives, the scFv is specific for EGFR. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 52 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacaggtgcagctgaaacagagcggcccggtggtgcagccgagccagagcctgagcattacctgcaccgtgagcggccttagcctgaccaactatggcgtgcattgggtgcgccagagcccgggcaaaggcctggaatggctgggcgtgatttgagcggcggcaacaccgattataacaccccgcttacca gccgcctgagcattaacaaagataacagcaaaagccaggtgttttttaaataaacagcctgcagagcaacgataccgcgatttattat tgcgcgcgcgcgctgacctattatgattatgaattgcgtattggggccagggcaccctggtgaccgtgagcggcgggcgggcggg cagcggcgggcgggcgggcagcggcgggcgggcgggcagcgatattctgctgaccagagcccggtgattctgagcgtgagcccgggcg aacgcgtgagcttagctgccgcgcgagccagagcattggcaccaacattcattggtatcagcagcgccaccaacggcagcccgcg cctgctgattaaatatgcgagcgaaagcattagcggcattccgagccgcttagcggcagcggcagcggcaccgattttaccctgagc attaacagcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactggccgaccacctttggcgcgggcaccaaa ctggaactgaaacgcacc). In some alternatives, the scFv comprises the EGFR scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 53

(MLLLVTSLLLCELPHPAFLIPQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHW VRQSPGKGLEWLGVWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIY YCARALTYDYEFAYWGQGLVTVSAGGGGSGGGGSGGGGSDILLTQSPVILSVSP GERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSI NSVESEDIADYYCQQNNNWPTTFGAGTKLELKRT). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 53.

[0040] In some alternatives, the scFv comprises the EGFRVIII (806) scFv nucleotide sequence. In some alternatives, the scFv is specific for EGFR. In some alternatives, the scFv is encoded by the nucleic acid sequence set forth in SEQ ID NO: 54 (atgctgctgctggtgacaagcctgctgctgtgcgagctgccacaccctgcttctcctgctgatccctgatgtgcagctgcaggaatcag gcccaagcctggtcaaaccctcccagctctctgagctgtacctgtacagtgactgggtactccatcacatctgatttcgcatggaactgg attaggcagtttccaggcaataagctggagtgatgggctacatctcatatagcgggaacactcgctataatcccagctctgaaatcacg gatcagcattactagagacaccagcaagaaccagttcttctgcagctgaattccgtgaccattgaggataccgccacatactattgcgt

cacagctggcagaggctttccatactggggacagggcacactggtgactgtcagcgccggctccacctctgggagtggaaaacctg
gctccgggggaaggatctacaaaggagacatcctgatgactcagtcaccaagctccatgtcagtgagcctggggcgacaccgtctcta
ttacatgtcactctagtcaggatatcaacagtaatatggctggctgcagcagcgacccggcaagtctttcaaagggtgatctatcatg
gaactaacctggacgatgaagtgcctagcagattttccggctctgggagtggagctgattacagtctgaccattcaagcctggagtca
gaagacttcgcagattactattgcgtccagtatgccagttcccctggacttttggcgggggaaccaagctggagatcaaacgg). In
some alternatives, the scFv comprises the EGFRVIII (806) scFv amino acid sequence. In
some alternatives, the scFv comprises the amino acid sequence set forth in SEQ ID NO: 55
(MLLLVTSLLLCELPHPAFLIPDVQLQESGPSLVKPSQSLSLTCTVTGYSITSDFAWN
WIRQFPGNKLEWMGYISYSGNTRYNPSLKSRSITRDTSKNQFFLQLNSVTIEDTATY
YCVTAGRGFPYWGQGLVTVSAGSTSGSGKPGSGEGSTKGDILMTQSPSSMSVSLG
DTVSI TCHSSQDINSNIGWLQQRPGKSFKGLIYHGTNLDDEVPSRFSGSGSGADYSLTI
SSLESEDFADYYCVQYAQFPWTFGGGTKLEIKR). In some alternatives, the scFv is
encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino
acid sequence of SEQ ID NO: 55.

[0041] In some alternatives, the scFv is encoded by the EphA2 (2A4) scFv
nucleotide sequence. In some alternatives, the scFv is encoded by the nucleic acid sequence
set forth in SEQ ID NO: 56
(atgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattcctcctgatccacaggtgcagctgttgagtctggg
ggaggcttggtacagcctgggggggtccctgagactctcctgtgcagcctctggattcacctttagcagctataccatgtcttgggtgcg
acagggccctggacaagcgcttgagtggatgggaaccattagtagtcgtggtacttacacctactatccagacagtgtgaagggccg
attcaccatctccagagacaacgccaagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggctgtgtattactgt
gcgagagaagctatctttactcactggggccgtggcacccctggtcaccgtctcctcaggtggtggtggttctggcggcgccggctcc
ggtggtggtggttctgacatccagttgacccagctctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgcaaggc
gagtcaggacattaataactatcacagctggtaccagcagaaacctggccaggctcccaggctcctcatctatcgtgcaaacagattg
tcgatgggggtcccagacaggttcagtggcagcgggtatggaacagattttaccctcacaattaataacatagaatctgaggatgctg
catattacttctgtctgaaatataatgtgtttccgtacacgttcggccaagggaagtgagatcaaa). In some
alternatives, the scFv comprises the EphA2 (2A4) scFv amino acid sequence. In some
alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 57
(MLLLVTSLLLCELPHPAFLIPQVQLLES GGGLVQPGGSLRLSCAASGFTFSSYTMS
WVRQAPGQALEWMGTISSRGTYTYYPDSVKGRFTISRDN AKNSLYLQMNSLRAEDT
AVYYCAREAI FTHWGRGTLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDR

VTITCKASQDINNYHSWYQQKPGQAPRLLIYRANRLVDGVPDRFSGSGYGTDFTLTI
NNIESEDAAYYFCLKYNVFPYTFGQGKVEIK). In some alternatives, the scFv is
encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino
acid sequence of SEQ ID NO: 57.

[0042] In some alternatives, the scFv is encoded by the EpHA2 (4H5) scFv
nucleotide sequence. In some alternatives, the scFv is encoded by the nucleic acid sequence
set forth in SEQ ID NO: 58
(atgcttctcctggtagacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacaggtgcagctgttgagctctggg
ggaggcttggtacagcctgggggggtccctgagactctcctgtgcagcctctggattcaccttttagcagctataccatgtcttgggtgcg
acaggcccctggacaagcgcttgagtggatgggaaccattagtagtggtggtacttacacctactatccagacagtgtgaagggccg
attcacctctccagagacaacgccaagaactcactgtatctgcaaatgaacagcctgagagccgaggacacggctgtgtattactgt
gcgagagaagctatctttacttactggggccgtggcaccctggtcaccgtctcctcaggtggtggtggttctggcggcggcggctccg
gtggtggtggttctgacatccagttgacctcctcctgtctgcatctgtaggagacagagtcaccatcacttgcaaggcg
agtcaggacattaataactatttaagctggtaccagcagaaacctggccaggctcccaggctcctcatctatcgtgcaaacagattggt
agatgggggtcccagacaggttcagtggcagcgggtatggaacagattttaccctcacaattaataacatagaatctgaggatgctgcat
attacttctgtctgaaatatgatgtgttccgtacacgttcggccaagggaaggtggagatcaaa). In some alternatives,
the scFv comprises the EpHA2 (4H5) scFv amino acid sequence. In some alternatives, the
scFv comprises an amino acid sequence set forth in SEQ ID NO: 59
(MLLLVTSLLLCELPHPAFLIPQVQLLESGGGLVQPGGSLRLSCAASGFTFSSYTMS
WVRQAPGQALEWMGTISSGGTYTYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDT
AVYYCAREAIPTYWGRGTLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDR
VTITCKASQDINNYLSWYQQKPGQAPRLLIYRANRLVDGVPDRFSGSGYGTDFTLTI
NNIESEDAAYYFCLKYDVFPYTFGQGKVEIK). In some alternatives, the scFv is
encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino
acid sequence of SEQ ID NO: 59.

[0043] In some alternatives, the scFv is encoded by a FITC (E2) scFv nucleotide
sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in
SEQ ID NO: 60
(atgcttctcctggtagacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccaagcgtgctgacacagcctagctc
cgtgtctgccgcccctggccagaaagtgacctcagctgtagcggcagcaccagcaacatcggcaacaactacgtgtcctggtatca
gcagcaccgccgcaaggcccccaagctgatgatctacgacgtgtccaagcggcccagcggcgtgcccgatagattttccggcagc

aagagcggcaacagcgccagcctggatatcagcggcctgcagtctgaggacgaggccgactactattgcgccgctgggacgata
gcctgagcgcagttcctgtttggcaccggcaccaagctgacagtgcctgggcggaggcgaggatctggcggcgaggaggaagtggcg
gagggggatctcaggtgcagctggtggaaagcggcggaacctggtgcagcctggcggatctctgagactgagctgtgccgccag
cggcttcaccttcggcagcttcagcatgagctgggtgcgccaggctcctgggggaggactggaatgggtggcaggactgagcgcc
agaagcagcctgaccactacgccgatagcgtgaagggccggttcaccatcagccgggacaacgccaagaacagcgtgtacctgc
agatgaacagcctgcgggtggaagataccgccgtgtactactgcgccagacggctctacgacagcagcggctactggggccacttc
tacagctacatggacgtgtggggccagggcaccctcgtgacagtgtct). In some alternatives, the scFv
comprises the FITC (E2) scFv amino acid sequence. In some alternatives, the scFv comprises
an amino acid sequences set forth in SEQ ID NO: 61
(MLLLVTSLLLCELPHPAFLIPSVLTQPSSVSAAPGQKVTISCSGSTSNIGNNYVSWY
QQHPGKAPKLMYDVSKRPSGVPDRFSGSKSGNSASLDISGLQSEDEADYYCAAWD
DSLSEFLFGTGTKLTVLGGGGGSGGGGSGGGGSQVQLVESGGNLVQPGGSLRLSCA
ASGFTFGSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVKGRFTISRDNKNSV
YLQMNSLRVEDTAVYYCARRSYDSSGYWGHFYSDMDVWGQGTLVTVS). In some
alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid
sequence encoding the amino acid sequence of SEQ ID NO: 61.

[0044] In some alternatives, the scFv is encoded by the GD2 (hu3F8) scFv
nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set
forth in SEQ ID NO: 62
(atgcttctctggtgacaagccttctgctctgtgagttaccacacccagcattctctctgatcccaagcatcgtgatgaccagacccc
caagttcctgctggtgagcgccggcgacagggtgaccatcacctgcaaggccagccagagcgtgagcaacgacgtgacctggtac
cagcagaaggccggccagagccccaagctgctgatctacagcgccagcaacaggtacagcggcgtgcccagcagggttcaccggc
agcggctacggcaccgccttcaccttcaccatcagcaccgtgcaggccgaggacctggccgtgtacttctgccagcaggactacag
cagcttcggcgggcgccaccaagctggagatcaagagg). In some alternatives, the scFv comprises the GD2
(hu3F8) scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid
sequence set forth in SEQ ID NO: 63
(MLLLVTSLLLCELPHPAFLIPSIIVMTQTPKFLLVSAGDRVITCKASQSVSNDVTWY
QQKAGQSPKLLIYSASNRYSGVPDRFTGSGYGTAFTFTISTVQAEDLAVYFCQQDYS
SFGGGTKLEIKR). In some alternatives, the scFv is encoded by a nucleic acid sequence
comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 63.

[0045] In some alternatives, the scFv is encoded by the Her2 (Herceptin) scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 64 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagatatccagatgacccagtccccg agctccctgtccgcctctgtggcgatagggtcaccatcacctgccgtgccagtcaggatgtgaatactgctgtagcctgggtatcaaca gaaaccaggaaaagctccgaaactactgatttactcggcatccttctactctggagtccttctcgttctctggtccagatctggg acggatttactctgaccatcagcagctctgcagccggaagacttcgcaacttattactgtcagcaacattatactactcctcccacgttcg gacagggtaccaaggtggagatcaaaggcagtagcggcggtggctccggggggcgatccgggtggggggcggcagcagcga ggttcagctgggtggagctctggcggtggcctgggtgcagccaggggggctcactccgtttgtcctgtgcagcttctggcttcaacattaaag acacctatatactgggtgcgtcaggccccgggtaagggcctggaatgggttgcaaggatttatcctacgaatggttatactagatat gccgatagcgtcaagggccgtttactataagcgcagacacatccaaaaacacagcctacctgcagatgaacagcctgcgtgctga ggacactgccgtctattattgttctagatggggaggggacggcttctatgctatggactactggggtaaggaaccctggtcaccgtct cgagt). In some alternatives, the scFv comprises the Her2 (Herceptin) scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 65 (MLLLVTSLLLCELPHPAFLIPDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAW YQQKPGKAPKLLIYSASFLYSGVPSRFSGRSGTDFTLTISLQPEDFATYYCQQHYTT PPTFGQGTKVEIKGSTSGGGSGGGSGGGGSSEVQLVESGGGLVQPGGSLRLSCAASG FNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQ MNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 65.

[0046] In some alternatives, the scFv is encoded by the IL13R2a (hu08) V1Vh scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 66 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagacatccagatgacccagtcccc ctcttctctgtctgcctctgtggcgacagagtgaccatcacctgtaaggccagtcaggatgtaggtactgctgtagcctgggtatcagca gaagcctggcaaggctccaagctgctgatctactcggcatcctaccgggtccactggcgtgccttccagattctccggctctggctctg gcaccgatttaccctgaccatctcctccctccagcctgaggatttcgccacctactactgccagcaccattatagtgtccgtggacgt ttggcggcggaacaaaggtggagatcaaagggtggtggtggttctggcgcgcgcggtccgggtggtggtggttctgaggtgcagctg gtggagctctggcgcgcgactggtgcagcctggcggtctctgagactgtcttgtgccgcctccgggttcaccttcagtaggaatggca

tgtcttgggtgaggcagggccctggcaagggcctggagtgggtggccaccgttagtagtggtggttagttacatctactatgcagaca
gtgtgaagggcggttcaccatctccagggacaacgccagaactccctgtacctccagatgaactccctgagggccgaggataacc
gccgtgtactactgtgc). In some alternatives, the scFv comprises the IL13Ra2 (hu08) V1Vh scFv
amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set
forth in SEQ ID NO: 67
(MLLLVTSLLLCELPHPAFLIPDIQMTQSPSSLSASVGDRVTITCKASQDVGTAFAW
YQQKPGKAPKLLIYSASYRSTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHHYS
APWTFGGGKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGF
TFSRNGMSWVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNKNSLYLQ
MNSLRAEDTAVYYC). In some alternatives, the scFv is encoded by a nucleic acid
sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID
NO: 67.

[0047] In some alternatives, the scFv is encoded by an IL13Ra2 (hu08) VhV1
scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid
sequence set forth in SEQ ID NO: 68
(atgcttctctggtgacaagccttctgctctgtgagttaccacaccagcattcctctgatcccagaggtgcagctggtggagtctgg
cggcggactggtgcagcctggcggctctctgagactgtcttgtgccgcctccggcttcaccttcagtaggaatggcatgtcttgggtga
ggcagggccctggcaagggcctggagtgggtggccaccgttagtagtggtggttagttacatctactatgcagacagtgtgaagggg
cggttcaccatctccagggacaacgccagaactccctgtacctccagatgaactccctgagggccgaggataaccgccgtgtactac
tgtgccagacaagggactacggcactagctacgaggttcttcgatgtctggggccagggcacctggtgaccgtgtcctctggtggt
ggtggttctggcggcgggctccggtggtggtggttctgacatccagatgaccagtcaccttctctgtctgcctctgtgggcga
cagagtgaccatcacctgtaaggccagtcaggatgtaggtactgctgtagcctggtatcagcagaagcctggcaaggctcccaagct
gctgatctactcggcatcctaccggctccactggcgtgccttccagattctccggctctggctctggcaccgatttcacctgaccatctc
ctccctccagcctgaggatttcgccacctactactgccagcaccattatagtgtccgtggacgtttggcggcggaacaaaggtggag
atcaaggaatctaagta). In some alternatives, the scFv comprises an IL13Ra2 hu08 VhV1 scFv
amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set
forth in SEQ ID NO: 69
(MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMS
WVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDT
AVYYCARQGTTALATRFDDVWGQGLTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS
LSASVGDRVTITCKASQDVGTAFAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGS

GTDFTLTISSLQPEDFATYYCQHHYSAPWTFGGGTKVEIKESK). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 69.

[0048] In some alternatives, the scFv is encoded by an IL13Ra2 (hu07) V1Vh scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 70 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagatattcagatgacccagagccc gagcagcctgagcgcgagcgtggcgatcgctgaccattacctgcaccgcgagcctgagcgtgagcagcacctatctgcattggt atcagcagaaaccgggcaaagcgccgaaactgctgatttatagcaccagcaacctggcgagcggcgtgccgagccgcttttagcgg cagcggcagcggcaccgattttaccctgaccattagcagcctgcagccggaagattttgcgacctattattgccatcagtatcatcgca gcccgtgacctttggcggcgccaccaaagtggaaattaaagtggtggtggttctggcggcgccgctccggtggtggtggttctg aagtgcagctggtggaaagcggcggcgccctggtgcagccggcgccagcctgcgcctgagctgcgcggcgagcggcctttacct ttaccaaataatggcgtgcattgggtgcgccaggcgccggggcaaaggcctggaatgggtggcggtgaaatgggcggcgccgagca ccgattataacagcgcgctgatgagccgctttaccattagccgcgataacgcgaaaaacagcctgtatctgcagatgaacagcctgc gcgcggaagataccgcggtgtattattgcgcgcgcgatcatcgcgatgcgatggattattggggccagggcaccctggtgaccgtg agcagcgaatctaagta). In some alternatives, the scFv comprises an IL13Ra2 (hu07) VhV1 scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 71 (MLLLVTSLLLCELPHPAFLIPDIQMTQSPSSLSASVGDRVITITCTASLSVSSTYLHWY QQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQYHRSP LTFGGGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFT KYGVHWVRQAPGKGLEWVAVKWAGGSTDYNALMSRFTISRDNALNSLYLQMNS LRAEDTAVYYCARDHRDAMDYWGQGLVTVSSESK). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 71.

[0049] In some alternatives, the scFv is encoded by the IL13Ra2 (hu07) VhV1 scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 72 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagaagtgcagctggtggaaagcg gcggcgccctggtgcagccggcgccagcctgcgcctgagctgcgcggcgagcggcctttacctttaccaaataatggcgtgcattgg gtgcgccaggcgccgggcaaaggcctggaatgggtggcggtgaaatgggcggcgccgagcaccgattataacagcgcgctgat

gagccgctttaccattagccgcgataacgcgaaaaacagcctgtatctgcagatgaacagcctgcgcgcggaagataccgcggtgt
attattgcgcgcgcgatcatcgcgatgcgatggattattggggccagggcaccctggtgaccgtgagcagcgggtggtggtggtctg
gcggcggcggctccggtggtggtggttctgatattcagatgacctagagcccgagcagcctgagcgcgagcgtgggcgatcgcgt
gaccattacctgcaccgcgagcctgagcgtgagcagcacctatctgcattggtatcagcagaaaccgggcaaagcgccgaaactgc
tgatttatagcaccagcaacctggcgagcggcgtgccgagccgcttagcggcagcggcagcggcaccgattttacctgaccatta
gcagcctgcagccggaagattttgcgacctattattgccatcagtatcatcgcagcccgtgacctttggcggcggcaccaaagtga
aattaaagaatctaagta). In some alternatives, the scFv comprises an IL13Ra2 (hu07) VhV1 scFv
amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set
forth in SEQ ID NO: 73
(MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFTKYGVH
WVRQAPGKGLEWVAVKWAGGSTDYNSALMSRFTISRDNKNSLYLQMNSLRAED
TAVYYCARDHRDAMDYWGQGTLLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSA
SVGDRVITITCTASLSVSSTYLHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTD
FTLTISSLQPEDFATYYCHQYHRSPLTFGGGTKVEIKESK). In some alternatives, the
scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
amino acid sequence of SEQ ID NO: 73.

[0050] In some alternatives, the antibody or portion thereof or scFv is encoded by
the Anti-IL13Ra2 (IL13 zetakine) nucleotide sequence. In some alternatives, the antibody or
portion thereof or scFv is encoded by a sequence set forth in SEQ ID NO: 74
(atgcttctctggtgacaagccttctgctctgtgagttaccacaccagcattcctctgatcccaggccctgtgcctccctctacagcc
ctcaggtacctcattgaggagctggtcaacatcaccagaaccagaaggetccgctctgcaatggcagcatggtatggagcatcaac
ctgacagctggcatgtactgtgcagccctggaatccctgatcaacgtgtcaggctgcagtgccatcgagaagaccagaggatgctg
agcggattctgcccgcacaaggtctcagctgggcagttttccagcttgcattgctccgagacaccaaaatcgaggtggcccagtttgtaa
aggacctgctcttacatttaaagaaacttttcgcgagggacggttcaa). In some alternatives, the antibody or
portion thereof or scFv comprises an Anti-IL13Ra2 (IL13 zetakine) amino acid sequence. In
some alternatives, the antibody or portion thereof or scFv comprises an amino acid sequence
set forth in SEQ ID NO: 75
(MLLLVTSLLLCELPHPAFLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSI
NLTAGMYCAALESINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTEKIEVAQ
FVKDLLLHLKKLFREGRF). In some alternatives, the scFv is encoded by a nucleic acid

sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 75.

[0051] In some alternatives, the scFv is encoded by an oaGD2 (8B6) VIVh scFv nucleotide sequence. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 76

(atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagatgttgtgatgacccaaactcca
ctctccctgcctgtcagtcttggagatcaagcctcaatctcttgcagatctagtcagagccttctaaaaataatggaaacacctttttaca
ttggtacctgcagaagtcaggccagtctcaaagctccttatctacaaagttccaaccgacttctgggggtcccagacaggttcagtgg
cagtggatcagggacatatttcacactcaagatcagcagagtgagggtgaggatctgggagtttattctgctctcaaagtacacatat
tccgtacacattcggaggggggaccaagctcgagctgaaacgaggcggagggggctctggcgggaggatctgggggagggg
gcagcgaggtgaaactggtggagtctggaggaggcttgggtgctgcctggggattctctgagactctcctgtgcaacttctgagttcac
cttcactgattactacatgacttgggtccgccagcctccaagaaaggcacttgagtgggtgggtttattagaaacagagctaattggttac
acaacagagtacaatccatctgtgaagggtcggttcaccatttcagagataattcccaaagcattcctctatcttcaaataaacacccctg
agaactgaggacagtgccacttattactgtgcaagagtcttaactgggcctttgactactggggccaaggcaccactctcacagtctc
ctca). In some alternatives, the scFv comprises the oaGD2 (8B6) VIVh scFv amino acid
sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ
ID NO: 77

(MLLLVTSLLLCELPHPAFLIPDVVMTQTPLSLPVSLGDQASISCRSSQSLLKNNGNT
FLHWYLQKSGQSPKLLIYKVS NRLSGVPDRFSGSGSGTYFTLKISRVEAEDLGVYFCS
QSTHIPYTFGGGTKLELKRGGGGSGGGGSGGGGSEVKLVESGGGLVLPGDSLRLSCA
TSEFTFTDYYMTWVRQPPRKALEWLGFIRNRANGYTTTEYNPSVKGRFTISRDNQSIL
YLQMNTLRTEDSATYYCARVSNWAFDYWGQGTTTLTVSS). In some alternatives, the
scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
amino acid sequence of SEQ ID NO: 77.

[0052] In some alternatives, the scFv is encoded by an ROR1 (R12) scFv nucleotide sequence. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 78

(atgctgctgctggtgacaagcctgctgctgtgcgagctgccccccccgccttctgctgatccccaggaacagctcgtcgaaagc
ggcggcagactggtgacacctggcggcagcctgaccctgagctgcaaggccagcggcttcgacttcagcgcctactacatgagctg
ggtccgccaggcccctggcaagggactggaatggatgccaccatctaccccagcagcggcaagacctactacgccacctgggtg
aacggacgggttcaccatctccagcgacaacgcccagaacaccgtggacctgcagatgaacagcctgacagccgcccagccgggcc

acctacttttgcgccagagacagctacgccgacgacggcgccctgttcaacatctggggccctggcaccctggtgacaatctctagc
ggcggaggcggtatctggtggcggaggaagtggcggcgaggatctgagctggtgctgacctagagcccctctgtgtctgctgccc
tggaagccctgccaagatcacctgtacctgagcagcgcccaagaccgacaccatcgactggtatcagcagctgcagggcga
ggccccagatacctgatgcaggtgcagagcgacggcagctacaccaagaggccaggcgtgcccgaccggttcagcggatctag
ctctggcgccgaccgctacctgatcatccccagcgtgcaggccgatgacgaggccgattactactgtggcgccgactacatcggcg
gctacgtgttcggcggaggcaccagctgaccgtgaccggc). In some alternatives, the scFv comprises an
amino acid sequence set forth in SEQ ID NO: 79 (ROR1 (R12) scFv amino acid sequence;
MLLLVTSLLLCELPHPAFLIPQEQLVESGGRLVTPGGSLTSLCKASGFDFSAYYMSW
VRQAPGKGLEWIA TIYPSSGKTYATWVNGRFTISSDNAQNTVDLQMNSLTAAADRA
TYFCARDSYADDGALFNIWGPGLVTISSGGGGSGGGGSGGGGSELVLTQSPSVSAA
LGSPAKITCTLSSAHKTDITDWYQQQLQGEAPRYLMQVQSDGSYTKRPGVPDRFSGSS
SGADRYLIIPSVQADDEADYYCGADYIGGYVFGGGTQLTVTG). In some alternatives,
the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding
the amino acid sequence of SEQ ID NO: 79.

[0053] In some alternatives, the scFv comprises an amino acid sequence set forth
in SEQ ID NO: 80 (CD33 (h2H12) VhV1 scFv nucleotide sequence
(atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacaggttcagctggtgcagctctgga
gctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttctggttacacctttaccaattatgatataaattgggtgag
acagggccctggacaagggccttgagtggattggatggatttatcctggagatggtagtagtaccataatgagaaattcaaggccaag
gctaccctgacagctgacacatccaccagcacagcctacatggagctgaggagcctgagatctgatgacacagctgtgtattactgtg
cttctggatatgaagatgctatggactactgggggcaagggaccacagtcacagtctcctca). In some alternatives, the
scFv is specific for CD33. In some alternatives, the scFv comprises an amino acid sequence
set forth in SEQ ID NO: 81 (CD33 (h2H12) VhV1 scFv amino acid sequence;
MLLLVTSLLLCELPHPAFLIPQVQLVQSGAEVKKPGASVKVSCKASGYTFTNYDIN
WVRQAPGQGLEWIGWIYPGDGSTKYNEKFKAKATLTADTSTSTAYMELRSLRSDDT
AVYYCASGYEDAMDYWGQGTTVTVSS). In some alternatives, the scFv is encoded by a
nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence
of SEQ ID NO: 81.

[0054] In some alternatives, the scFv is encoded by a sequence set forth in SEQ
ID NO: 82 (CD33 (h2H12) VhVh scFv nucleotide sequence:
atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacagatccagatgaccagctctccat

cctcactgtctgcatctgtaggagacagagtcacatcaattgtaaggctagtcaggacattaatagctatttgagctggtttcagcaga
aaccagggaaagcccctaagaccctgatctatagagcaaataagattggtagatgggggtcccatcaagggtctctggcagtggtatctgg
gcaagattatactctcaccatcagcagcctgcagcctgaagattttgcaacttattactgcttgagtgatgatgagtttctctcacatttg
aggagggaccaaggtggagatcaaa). In some alternatives, the scFv comprises an amino acid
sequence set forth in SEQ ID NO: 83 (CD33 (h2H12) VIVh scFv amino acid sequence:
MLLLVTSLLLCELPHPAFLIPDIQMTQSPSSLSASVGDRVTINCKASQDINSYLSWFQ
QKPGKAPKTLIYRANRLVDGVPSRFSGSGSGQDYTLTISSLQPEDFATYYCLQYDEFP
LTFGGGKVEIK). In some alternatives, the scFv is encoded by a nucleic acid sequence
comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 83.

[0055] In some alternatives, the scFv is encoded by a nucleic acid sequence set
forth in SEQ ID NO: 84 (Mesothelin (P4) scFv nucleotide sequence:
atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacaggtacagctgcagcagtcaggt
ccaggactcgtgacgccctcgcagaccctctcactcacctgtgccatctccggggacagtggtctctagcaacagtgctacttggaact
ggatcaggcagtcacccatcgagaggccttgagtggctgggaaggacatactacaggtccaagtggataacgactatgcagtatctg
tgaaaagtcgaatgagcatcaaccagacacatccaagaaccagttctccctgcagctgaactctgtgactcccaggagacacggctg
tgtattactgtgcaagaggaatgatgacttactattacgggtatggacgtctggggccaaggaccacggtcaccgtctcctcaggaatt
ctaggatcc). In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ
ID NO: 85 (Mesothelin (P4) scFv amino acid sequence:
MLLLVTSLLLCELPHPAFLIPQVQLQQSGPGLVTPSQTLSTCAISGDSVSSNSATWN
WIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRMSINPDTSKNQFSLQLNSVTPED
TAVYYCARGMMTTYYYGMDVWGQGTTVTVSSGILGS). In some alternatives, the scFv
is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
amino acid sequence of SEQ ID NO: 85.

[0056] In some alternatives, the scFv is encoded by a nucleic acid sequence set
forth in SEQ ID NO: 86 (VAR2CSA (ID1-DBL2Xb) scFv nucleotide sequence:
atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccaagtctcacgaatgggtataagtgcg
acaaatgcaaatcaggcacatcccgtccaaaaaaaatggatctggaaaaagagcagcggaatgaggagggcctgcaggaag
aatacgctaacacaatcggactgccccctgaactcaatctctgtaccttgggaacttgctaaactggaaaacgtctgtgaagacgtc
aaggacataaacttcgatacgaaagagaagttcctggctggatgccttatcgtgagctttcacgagggaaagaatctgaagaaaaggt
accacagaataaaaaactctggcaataaagagaatttgtgcaaggccctggagtactcttttgcggactatggcgatctcataaagggc
acaagcatctgggataatgagtataccaaggatctggagctgaatctgcagaataacttgggaaattgttgggaagtatattaaaaaa

aataataccgccgagcaggacacatcctactcttccctcgacgagctgcgggagtcaggtggaacaccaacaaaaagtatatctgg
acggcaatgaaacatggcgcggaatgaatatcaccacttgtaatgcagacggctctgtcaccgggtccgggttcttcttgcatgatat
accaaccatcgatttgattccgcagtatctgagatttctccaagagtgggtggaaaattttgcgagcagaggcaagctaaggtcaagg
acgtgatcaccaattgtaaaagttgcaaagaatcaggggaacaagtgcaaaaccgagtgaagacgaagtgaaggacgaatgcgag
aatataaaaaattcatcgaggcttggtggaacagccgggggtgggattgggaccgcaggcagcccatggagcaagcgggtgggacc
aatctataaacgatacagcaagcacatcgaagatgccaaagcgggaaccgcaaagctggcacaaaaaactgtgggactagcagcac
gactaatgccgcggcaagcactgatgaaaataaatgcgtgcaaagtgcacatcgactctttcttcaaacacctgatcgatatcgggtcttac
tacgccaagcagctacctcagtaatgttctggatgataatatttggggcggaacaaagcaccttgactacctacaccacctatacca
caacagagaagtgaataaagagagagacaaatcaaagtctcagagctctgatacactggctggttgtaatgtgccaagcccttggg
gaatacgccctacagatataaatac). In some alternatives, the scFv comprises an amino acid sequence
set forth in SEQ ID NO: 87 (VAR2CSA (ID1-DBL2Xb) scFv amino acid sequence:
MLLLVTSLLLCELPHPAFLIPSLTNGYKCDKCKSGTSRSKKKWIWKKSSGNEEGLQ
EEYANTIGLPPRTQSLYLGNLPKLENCEDVKDINFDTKEKFLAGCLIVSFHEGKNLK
KRYPQNKNNSGNKENLCKALEYSFADYGDLIKGTSIWDNEYTKDLELNLQNNFGKLF
GKYIKKNNTAEQDTSYSSLDELRESWWNTNKKYIWTAMKHGAEMNITTCNADGSV
TGSGSSCDDIPTIDLIPQYLRFLQEWFENFCEQRQAKVKDVITNCKSCKESGNKCKTE
CKTKCKDECEKYKKFIEACGTAGGGIGTAGSPWSKRWDQIYKRYSKHIEDAKRNRK
AGTKNCGTSSTTNAASTDENKCVQSDIDSFFKHLIDIGLTPSSYLSNVLDDNICGA
DKAPWTTYTTYTTTEKCNKERDKSKSQSSDTLVVVNVPSPLGNTTPYRYKY). In some
alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid
sequence encoding the amino acid sequence of SEQ ID NO: 87.

[0057] “Spacer”, can also be referred to as a “linker,” and has its plain and
ordinary meaning when read in light of the specification, and may include but is not limited
to, for example, residues which resides between the light and the heavy chains. The spacer
can be 10 to 20 amino acids in length (*e.g.*, at least, equal to 10, 11, 12, 13, 14, 15, 16, 17,
19, or 20 amino acids or a length within a range defined by any two of the aforementioned
lengths). The flexible spacer allows the scFv domain to orient in multiple directions in order
to enable an optimized antigen binding specificity. The scFv domain can be preceded by a
signal peptide so as to direct the nascent protein to the endoplasmic reticulum and subsequent
surface expression, whereby cleavage can occur. A flexible spacer can also allow the scFv
domain to orient in different directions to facilitate antigen binding. A good spacer for

allowing specific binding of the scFv domain can be determined empirically and is dependent on the scFv domain antigen recognition domain. In some alternatives, the linker comprises optimized spacer lengths so as to improve binding of scFv domain to the target cell, which may increase cytotoxic efficacy. In some alternatives, the linker or spacer between the scFv domain and the transmembrane can be 25 to 55 amino acids in length (*e.g.*, at least, equal to 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55 amino acids or a length within a range defined by any two of the aforementioned lengths. In some alternatives, the spacer is encoded by the nucleic acid sequence set forth in SEQ ID NO: 102 (IgG4 hinge nucleotide sequence: gagagcaagtacggaccgccctgcccccttgccct). In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 103 (IgG4 hinge amino acid sequence: ESKYGPPCPPCP). In some alternatives, the spacer is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 103. In some alternatives, the spacer is encoded by a nucleic acid sequence set forth in SEQ ID NO: 104 (IgG4-CH2(L235D, N297Q) nucleotide sequence; gcccccgagttcgacggcggacccagcgtgttctgttccccccaagccaaggacaccctgatgatcagccggacccccgagg tgacctgctgtgtgtgtggacgtgagccaggaagatcccaggtccagttcaattggtacgtggacggcgtggaagtgcacaacgcc aagaccaagcccagagaggaacagttccagagcacctaccgggtgtgtgtgtgtgaccgtgctgcaccaggactggctgaacg gcaaagaataacaagtgaaggtgtccaacaagggcctgccagcagcatcgaaaagaccatcagcaaggccaag). In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 105 (IgG4-CH2(L235D, N297Q) amino acid sequence; APEFDGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN AKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK). In some alternatives, the spacer is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 105. In some alternatives, the spacer is encoded by a nucleic acid sequence set forth in SEQ ID NO: 106 (IgG4-CH3 nucleotide sequence: ggccagcctcgcgagccccaggtgtacaccctgcctccctcccaggaagagatgaccaagaaccaggtgtccctgacctgctggt gaagggcttctaccccagcgacatcgccgtggagtgaggagagcaacggccagcctgagaacaactacaagaccacccctcccggtg ctggacagcgacggcagcttcttctgtacagccggctgaccgtggacaagagccggtggcaggaaggcaacgtctttagctgcag cgtgatgcacgaggccctgcacaaccactacaccagaagagcctgagcctgtccctgggcaag). In some alternatives,

the spacer comprises an amino acid sequence set forth in SEQ ID NO: 107 (IgG4 CH3 amino acid sequence:

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK). In some alternatives, the spacer is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 107. In some alternatives, the spacer is encoded by a nucleic acid sequence set forth in SEQ ID NO: 108 (CD8 hinge/Transmembrane domain nucleotide sequence:

gaatctaagtacggaccggccaagcctaccaccacccctgcccctagacctccaacacccgcccccaacaatcgccagccagcctctgtctctgaggcccgaggcttagaccagctgctggcggagccgtgcacaccagaggactggattcgctgcgacatctacatctggcccctctggccggcacatgtggcgtgctgctgctgagcctcgatgacc). In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 109 (CD8 hinge/Transmembrane domain amino acid sequence:

ESKYGPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVIT. In some alternatives, the spacer is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 109.

[0058] “Transmembrane domain” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a transmembrane region of a protein. This may be a single alpha helix, a transmembrane beta barrel, a beta-helix, or any other structure. In some alternatives herein, the transmembrane domain comprises CD28tm. In some alternatives, the transmembrane domain is encoded by a sequence set forth in SEQ ID NO: 110 (CD28 transmembrane domain nucleotide sequence: atgttctgggtgctggtggtggtcggaggcgtgctggcctgctacagcctgctggtcaccgtggccttcacatcttttgggtg). In some alternatives, the transmembrane domain comprises an amino acid sequence set forth in SEQ ID NO: 111 (CD28 transmembrane domain amino acid sequence: MFWVLVVVGVLACYSLLVTVAFIIFWV). In some alternatives, the transmembrane domain is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 111. In some alternatives, the transmembrane domain is encoded by a sequence set forth in SEQ ID NO: 112 (CD8 hinge/Transmembrane domain nucleotide sequence:

gaatctaagtacggaccggccaagcctaccaccacccctgccctagacctccaacacccgccccacaatcgccagccagcctctgtctctgaggccccgaggctttagaccagctgctggcggagccgtgcacaccagaggactggattcgctgcgacatctacatctggccccctctggccggcacatgtggcgtgctgctgctgagcctcgtgatcacc). In some alternatives, the transmembrane domain comprises an amino acid sequence set forth in SEQ ID NO: 113 (CD8 hinge/Transmembrane domain amino acid sequence: ESKYGPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW APLAGTCGVLLLSLVIT). In some alternatives, the transmembrane domain is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 113.

[0059] A “chimeric cytokine receptor,” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a protein that has an amino acid sequence of a chemokine or portion thereof and is a fusion protein with another protein.

[0060] “Intracellular signaling domain,” or “Co-stimulatory domain,” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a signaling moiety that provides to T cells a signal which, in addition to the primary signal provided by for instance the CD3 zeta chain of the TCR/CD3 complex, mediates a T cell response, including, but not limited to, activation, proliferation, differentiation, cytokine secretion, and the like. A co-stimulatory domain can include all or a portion of, but is not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83. In some alternatives, the co-stimulatory domain is an intracellular signaling domain that interacts with other intracellular mediators to mediate a cell response including activation, proliferation, differentiation and cytokine secretion, and the like. In some alternatives of the signaling domain described herein, the signaling domain comprises CD3ζ, CD28 cytoplasmic domain, and/or 4-1BB. In some alternatives, the intracellular signaling domain is encoded by a nucleic acid sequence set forth in SEQ ID NO: 114 (CD3 Zeta nucleotide sequence:

cgggtgaagttcagcagaagcgccgacgcccctgcctaccagcagggccagaatcagctgtacaacgagctgaacctgggcagaagggaagagtacgacgtcctggataagcggagaggccgggaccctgagatgggcggcaagcctcggcggaagaacccccagg aaggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgagatcggcataagggcgagcggaggcggggc

aagggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacgacgccctgcacatgcaggccctgcccccaa gg). In some alternatives, the intracellular signaling domain comprises an amino acid sequence set forth in SEQ ID NO: 115 (CD3 Zeta amino acid sequence: RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR). In some alternatives, the intracellular signaling domain is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 115. In some alternatives, the intracellular signaling domain is encoded by a nucleic acid sequence set forth in SEQ ID NO: 116 (4-1BB nucleotide sequence: aaacggggcagaaagaaactcctgtatatattcaaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtagct gccgatttccagaagaagaagaaggaggatgtgaactg). In some alternatives, the intracellular signaling domain comprises an amino acid sequence set forth in SEQ ID NO: 117 (4-1BB amino acid sequence: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL). In some alternatives, the intracellular signaling domain is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 117. In some alternatives, the intracellular signaling domain is encoded by a sequence set forth in SEQ ID NO: 118 (CD28 cytoplasmic domain nucleotide sequence: cgcagcaagcggagcagaggcggccacagcgactacatgaacatgaccctagacggcctggccccaccagaaagcactacca gccctacggccctccccgggactttgccgcctacagaagc). In some alternatives, the intracellular signaling domain comprises an amino acid sequence set forth in SEQ ID NO: 119 (CD28 cytoplasmic domain amino acid sequence: RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS). In some alternatives, the intracellular signaling domain is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 119.

[0061] As used herein, “nucleic acid” or “nucleic acid molecule” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of

naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term “nucleic acid molecule” also includes so-called “peptide nucleic acids,” which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

[0062] “DNA spacer” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a spacer comprising nucleotides. A DNA spacer may reside in between a promoter and a gene that is to be expressed that is under control of the promoter, for example. In some alternatives, a DNA spacer is between the inducible promoter and the transgene. In some alternatives, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120 (SEQ ID NO: 120; gctagcggtttaaacttaagcttggtaccgagctcggatccgccacc). In some alternatives, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values.

“Coding for” and “encoding” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other macromolecules such as a defined sequence of amino acids. Thus, a gene codes for a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. A

"nucleic acid sequence coding for a polypeptide" includes all nucleotide sequences that are degenerate versions of each other and that code for the same amino acid sequence.

[0063] “Survivin,” is also known as baculoviral inhibitor of apoptosis repeat-containing 5 or BIRC5, and has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, a protein that is a member of the inhibitor of apoptosis family. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124 (AtgggtgccccgacgttgccccctgcctggcagccctttctcaaggaccaccgcatctctacattcaagaactggcccttcttgagggtgctgcgcctgcacccccggagcggatggccgaggctggcttcatccactgccccactgagaacgagccagacttgccccagtggttcttctgcttcaaggagctggaaggctgggagccagatgacgaccccatagaggaacataaaaagcattcgtccggttgcgctttccttctgtcaagaagcagtttgaagaattaacccttggtgaatTTTTGAAactggacagagaaagagccaagaacaaaattgcaaaggaaacaacaataagaagaagaatttgaggaaactgtgaagaaagtgcgccgtgccatcgagcagctggctgcaatggat). In some alternatives, Survivin comprises the amino acid sequence set forth in SEQ ID NO: 123 (MGAPTLPPAWQPFLKDHRISTFKNWPFLGCACTPERMAEAGFIHCPTENEPDLAQCFFCFKELEGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDREERAKNKKIAKETNNKKKEFEETVKKVRRRAIEQLAAMD).

[0064] “Conditional” or “Inducible” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, nucleic acid construct that includes a promoter that provides for gene expression in the presence of an inducer and does not substantially provide for gene expression in the absence of the inducer.

[0065] “Constitutive” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, the nucleic acid construct that includes a promoter that is constitutive providing for expression of a polypeptide that is continuously produced.

[0066] “Specific” or “Specificity” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, the characteristic of a ligand for the binding partner or alternatively, the binding partner for the ligand, and can include complementary shape, charge and hydrophobic specificity for binding. Specificity for binding can include stereospecificity, regioselectivity and chemoselectivity. In some alternatives, a method of making a nucleic acid encoding a

chimeric antigen receptor is provided such that a nucleic acid encoding a chimeric antigen receptor is generated, wherein the chimeric antigen receptor binds specifically to a desired or selected ligand.

[0067] “TamR-tf” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, tamoxifen regulated transcription factors. Without being limiting, tamoxifen regulated transcription factors can include wild-type HEA3, wild-type HEA4, mutant HEA3, mutant HEA4 and/or RelA variants.

[0068] “HEA3” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1 α) fused in frame to the mutant tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) which contains 1 amino acid substitution (G521R), which ablates binding of estradiol but confers nanomolar estrogen analogues, that is in turn fused to the p65 activation domain of NF- κ B (p65).

[0069] HEA-3 has been described by Roscilli *et al.* (Molecular Therapy, Vol. 6, Issue 5, pp. 653-663 (2002)) as a novel 4-hydroxytamoxifen (4-OHT) dependent regulator which was shown to have long term control of gene expression in mouse skeletal muscle (Roscilli *et al.*; included by reference in its entirety herein.)

[0070] “HEA4” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a chimeric transcription factor composed of human subunits including the N-terminal DNA binding domain of Hepatocyte Nuclear Factor 1-alpha (HNF-1 α) fused in frame to the tamoxifen-specific ligand binding domain of the estrogen receptor ligand binding domain (ER-LBD) which contains 3 amino acid substitutions (G400V, M543A, L544A) that ablate binding of estradiol but confers nanomolar sensitivity to estrogen analogues, that is in turn fused to the p65 activation domain of NF- κ B (p65).

[0071] “Regulate” or “modulate” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, the act of

controlling a biological process, or to exert a modifying or controlling influence on a biological or cellular process or pathway.

[0072] “Cytotoxic T lymphocyte “(CTL) has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a T lymphocyte that expresses CD8 on the surface thereof (e.g., a CD8⁺ T cell). In some alternatives, such cells are preferably "memory" T cells (T_M cells) that are antigen-experienced. In some alternatives, the host cells comprising the alternative systems provided herein are CTL cells.

[0073] "Central memory" T cell (or "T_{CM}") has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, an antigen experienced CTL that expresses CD62L, CCR-7 and/or CD45RO on the surface thereof, and does not express or has decreased expression of CD45RA, as compared to naïve cells. In some alternatives, central memory cells are positive for expression of CD62L, CCR7, CD28, CD127, CD45RO, and/or CD95, and may have decreased expression of CD54RA, as compared to naïve cells. "Effector memory" T cell (or "T_{EM}") as used herein refers to an antigen experienced T cell that does not express or has decreased expression of CD62L on the surface thereof, as compared to central memory cells, and does not express or has a decreased expression of CD45RA, as compared to naïve cell. In some alternatives, effector memory cells are negative for expression of CD62L and/or CCR7, as compared to naïve cells or central memory cells, and may have variable expression of CD28 and/or CD45RA. In some alternatives, the host cells comprising the alternative systems provided herein are T_{CM} cells. In some alternatives of the host cells provided herein, the host cell is a central memory T cell.

[0074] “Naïve “ T cells has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a non-antigen experienced T lymphocyte that expresses CD62L and/or CD45RA, and does not express CD45RO-, as compared to central or effector memory cells. In some alternatives, naïve CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naïve T cells including CD62L, CCR7, CD28, CD127, and/or CD45RA. In some alternatives, the host cells comprising the alternative systems provided herein are naïve T cells.

[0075] “Effector “ “T_E” T cells has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, antigen experienced cytotoxic T lymphocyte cells that do not express or have decreased expression of CD62L, CCR7, and/or CD28, and are positive for granzyme B and/or perforin, as compared to central memory or naïve T cells. In some alternatives, the host cells comprising the alternative systems provided herein are effector T cells.

[0076] "Enriched" and "depleted" has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, amounts of cell types in a mixture and subjecting of the mixture of the cells to a process or step, which results in an increase in the number of the "enriched" type and a decrease in the number of the "depleted" cells. Thus, depending upon the source of the original population of cells subjected to the enriching process, a mixture or composition may contain 60, 70, 80, 90, 95, or 99 percent or more (in number or count) of the "enriched" cells and/or 40, 30, 20, 10, 5 or 1 percent or less (in number or count) of the "depleted" cells. In some alternatives, an *in vitro* method for preparing a host cell of any one of any one of the alternatives is provided herein. The *in vitro* method comprises a) providing a system of any one of the alternatives described herein, and b) introducing the system into an isolated T lymphocyte population and expanding each T lymphocyte population in vitro. The process of expanding each T lymphocyte population is a process of enriching the T lymphocyte population.

[0077] “Epitope” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a part of an antigen or molecule that is recognized by the immune system including antibodies, T cells, and/or B cells. Epitopes usually have at least 7 amino acids and can be linear or conformational. In some alternatives herein, an antibody or ligand binding domain can be optimized to bind a specific epitope. Techniques for optimizing binding can be appreciated by those skilled in the art. For example, a ligand binding motif can be optimized by point mutation in order to increase binding affinity and/or epitope recognition.

[0078] "Isolated," has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, various polypeptides or nucleic acids that have been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide or nucleic acid is free of

association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide or nucleic acid, and can include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Isolated can also be used to describe isolation of a subpopulation of cells, such as, for example isolation of subpopulations of CD4+ and/or CD8+ T lymphocytes during methods of manufacturing cells for use.

[0079] “Intracellular signaling domain” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, all or a portion of one or more domains of a molecule (here the chimeric receptor molecule) that provides for activation of a lymphocyte. Intracellular domains of such molecules mediate a signal by interacting with cellular mediators to result in proliferation, differentiation, activation and other effector functions. In some alternatives, such molecules include all or portions of CD28, CD3, or 4-1BB, or combinations thereof.

[0080] “Ligand” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a substance that binds specifically to another substance to form a complex. Examples of ligands include epitopes on antigens, molecules that bind to receptors, substrates, inhibitors, hormones, and/or activators. “Ligand binding domain” as used herein refers to substance or portion of a substance that binds to a ligand. Examples of ligand binding domains include antigen binding portions of antibodies, extracellular domains of receptors, and/or active sites of enzymes. “Operably linked” as used herein refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two proteins coding regions, in the same reading frame.

[0081] “Tumor specific molecule,” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, tumor-typical

antigens, proteins or molecules expressed by a tumor. In some of the alternatives described herein, the tumor specific molecule is CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or MAGE A3 TCR or combinations thereof.

[0082] A “viral molecule” or “viral specific molecule” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a protein encoded by a viral RNA or DNA, or a molecule that is expressed by a cell in reaction to a viral infection. In some alternatives described herein the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein.

[0083] "Percent (%) amino acid sequence identity" with respect to the chimeric receptor polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference sequence for each of the ligand binding domain, spacer, transmembrane domain, and/or the lymphocyte activating domain, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, % amino acid sequence identity values generated using the WU-BLAST-2 computer program [Altschul et al., Methods in Enzymology, 266:460-480 (1996); herein incorporated by reference] uses several search parameters, most of which are set to the default values. Those that are not set to default values (i.e., the adjustable parameters) are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T) =11 and scoring matrix=BLOSUM62. A % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between each or all of the polypeptide amino acid sequence of the reference chimeric receptor sequence and the

comparison amino acid sequence of interest as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the polypeptide of interest. In some alternatives, the percent sequence identity of amino acids or nucleic acids are determined by computer software.

[0084] "Substantially purified" refers to a molecule that has 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% or less other molecule types or other cell types. A substantially purified cell also refers to a cell, which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells.

[0085] "Not substantially found" when used in reference the presence of a tumor antigen or other molecules on normal cells refers to the percentage of a normal cell type that has the antigen or molecule, and/or the density of the antigen on the cells. In some alternatives, not substantially found means that the antigen or molecule is found on less than 50% of normal cell type and/or at a 50% less density as compared to the amount of cells or antigen found on a tumor cell or other diseased cell.

[0086] "T cells" or "T lymphocytes" has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, can be T-cells from any mammal, preferably a primate, species, including monkeys, dogs, and/or humans. In some alternatives of the host cells provided herein, the host cells are T cells, wherein the T cells are allogeneic (from the same species but different donor) as the recipient subject; in some alternatives the T cells are autologous (the donor and the recipient are the same); in some alternatives the T cells are syngeneic (the donor and the recipients are different but are identical twins).

[0087] "Vector" or "construct" has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, nucleic acid used to introduce heterologous nucleic acids into a cell that has regulatory elements to provide expression of the heterologous nucleic acids in the cell. Vectors include but are not limited to plasmid, minicircles, yeast, transposons, and viral genomes. In some alternatives, the vectors are plasmid, minicircles, yeast, transposons, or viral genomes. In some alternatives, the transposons are piggy bac transposons. In some alternatives of the systems

provided herein, the first and/or second nucleic acid reside on a vector. In some alternatives, the vector is a viral vector.

[0088] “Transgene” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, a gene or genetic material that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another. In some alternatives described herein, a method is provided for inducible expression of a transgene in cells, the system comprising: a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes the transgene; and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by a drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives, the transgene encodes a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity. In some alternatives, the transgene encodes a protein that modulates T cells.

[0089] A viral vector can be used to deliver genetic material into a cell. Delivery of the vector can be performed by transduction and the technique is known to those skilled in the art. In some alternatives herein, a vector for introducing the nucleic acid into a cell is an adenovirus viral vector or a lentivirus viral vector.

[0090] “Minicircles,” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, small circular plasmid derivatives that have been freed or liberated from prokaryotic vector components. Minicircles can serve as an expression vector, where they have been applied as transgene carriers for the genetic modification of mammalian cells, with the advantage that, since they contain no bacterial DNA sequences, they are less likely to be perceived as foreign and

destroyed. As such, typical transgene delivery methods involve plasmids, which contain foreign DNA. The smaller size of minicircles also extends their cloning capacity and facilitates their delivery into cells. Without being limiting, the preparation of minicircles can follow a two-step procedure, which can involve production of a parental plasmid (bacterial plasmid with eukaryotic inserts) in *E. coli* and induction of a site-specific recombinase at the end of this process but still in bacteria. These steps can be followed by the excision of prokaryotic vector parts via two recombinase-target sequences at both ends of the insert and recovery of the resulting minicircle (vehicle for the highly efficient modification of the recipient cell) and the miniplasmid by capillary gel electrophoresis (CGE).

[0091] The purified minicircle can be transferred into the recipient cell by transfection, by electroporation, or by other methods known to those skilled in the art. Conventional minicircles can lack an origin of replication, so they cannot replicate within the target cells and the encoded genes will disappear as the cell divides (which can be either an advantage or disadvantage depending on whether the application demands persistent or transient expression). Some alternatives utilize a gene delivery polynucleotide for stable insertion of a nucleic acid into a gene, wherein the nucleic acid for insertion is flanked by inverted terminal repeat gene sequences in the gene delivery polynucleotide, and wherein the gene delivery polynucleotide is selectable. In some alternatives, the gene delivery polynucleotide is a minicircle.

[0092] Plasmids containing transposon systems such as *Sleeping Beauty* (SB) or *piggyBac* offer a non-viral approach for stably introducing genes into T-cells. Recently, the *piggyBac* system was used to produce stably-transfected mammalian cells expressing multiple transgenes of interest by delivery of multiple transposons. The SB system, first reactivated for mammalian cell use by Ivics and coworkers, has been used as the gene delivery modality in clinical trials of T-cell immunotherapy. Gene integration by SB has weaker preference for transcriptional units and their regulatory sequences compared to the γ -retroviral and lentiviral vectors and is therefore considered to be safer. In some alternatives described herein, genetic modification by minicircles comprising the Sleeping Beauty system are contemplated. In some alternatives described herein, genetic modification by minicircles comprising the *piggyBac* system are contemplated. In some alternatives described herein,

genetic modification by minicircles comprising the Sleeping Beauty system are contemplated.

[0093] “Apoptosis” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, the process of programmed cell death (PCD) that can occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. In apoptosis, a cell initiates intracellular apoptotic signaling in response to stress, which can bring about cell suicide. The binding of nuclear receptors by glucocorticoids, heat, radiation, nutrient deprivation, viral infection, hypoxia and increased intracellular calcium concentration, for example, by damage to the membrane, can all trigger the release of intracellular apoptotic signals by a damaged cell. A number of cellular components, such as poly ADP ribose polymerase, can also help regulate apoptosis.

[0094] Before the actual process of cell death is precipitated by enzymes, apoptotic signals must cause regulatory proteins to initiate the apoptosis pathway. This step allows apoptotic signals to cause cell death, or the process to be stopped, should the cell no longer need to die. Several proteins are involved, but two main methods of regulation have been identified: targeting mitochondria functionality, or directly transducing the signal via adaptor proteins to the apoptotic mechanisms. Another extrinsic pathway for initiation identified in several toxin studies is an increase in calcium concentration within a cell caused by drug activity, which also can cause apoptosis via a calcium binding protease calpain.

[0095] Apoptosis can be regulated by many factors. These factors can include but are not limited to genes that can express IL-2, IL-7, IL-15, Chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. IL-15 regulates T and natural killer cell activation and proliferation. In rodent lymphocytes, IL-15 was shown to prevent apoptosis by inducing an apoptosis inhibitor, BCL2L1/BCL-X(L). In humans with celiac disease, IL-15 similarly suppresses apoptosis in T-lymphocytes by inducing Bcl-2 and/or BCL-xL. Bcl-2 (B-cell lymphoma 2), encoded in humans by the BCL2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) it or inhibiting it (anti-apoptotic). Bcl-2 is specifically considered as an important anti-apoptotic protein and is, thus classified as an oncogene.

Protein kinase B (PKB), also known as Akt, is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. In some alternatives, a system for inducible expression of a gene that regulates a T cell function is provided, wherein the system comprises: a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives, the polypeptide that regulates apoptosis or modulates checkpoint signaling comprises IL-2, IL-7, IL-15, chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2 or PD-1CD28 chimeras.

[0096] “Checkpoint signaling” has its plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, blocking the cell cycle at specific transition points, and/or checkpoints to ensure that the events of the cell cycle take place in the correct order. Checkpoint signaling can also be activated. By way of example and not of limitation, checkpoint signaling can occur by damage to the DNA so that the cell cycle does not have to proceed until the damage is repaired. “Cell cycle checkpoints” are control mechanisms in eukaryotic cells which ensure proper division of the cell. Each checkpoint serves as a potential halting point along the cell cycle, during which the conditions of the cell are assessed, with progression through the various phases of the cell cycle occurring when favorable conditions are met. Currently, there are three known checkpoints: the G1 checkpoint, also known as the restriction or start checkpoint; the G2/M checkpoint; and the metaphase checkpoint, also known as the spindle checkpoint. The

biochemical pathways that restrain cell cycle transition and/or induce cell death after stress are known as cell cycle checkpoints. These checkpoints maintain the fidelity of DNA replication, repair, and division. Polypeptides that can regulate checkpoint signaling can include but are not limited to p53, p107, p130, and transcriptional repressor Rb. In some alternatives of the systems provided herein, the polypeptide that modulates checkpoint signaling comprises p53, p107, p130 or transcriptional repressor Rb.

[0097] “Negative checkpoint regulators” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, factors that can restrict the ability of T-cell responses to effectively attack tumors. They are also referred to as negative checkpoint signaling. In some alternatives, a system for inducible expression of a transgene is provided. In some alternatives, the transgene encodes a chimeric antigen receptor. In some alternatives, a system for inducible expression of chimeric antigen receptor in cells is provided. In some alternatives of the systems for inducible expression of a gene that regulates a T cell function, the polypeptide that modulates checkpoint signaling is a negative checkpoint regulator comprising PD-1, VISTA, LAG-3 or TIM3. In some alternatives, the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3.

[0098] In another example, cell cycle inhibitors mediating the growth inhibitory cues of upstream signaling pathways, the cyclin-CDK inhibitors of the Cip/Kip family p21Cip1, p27Kip1, and p57Kip2 have emerged as multifaceted proteins with functions beyond cell cycle regulation. In addition to regulating the cell cycle, Cip/Kip proteins can also play important roles in apoptosis, transcriptional regulation, cell fate determination, cell migration and cytoskeletal dynamics. A complex phosphorylation network modulates Cip/Kip protein functions by altering their subcellular localization, protein-protein interactions, and stability. These functions are essential for the maintenance of normal cell and tissue homeostasis, in processes ranging from embryonic development to tumor suppression. In some alternatives, a system for inducible expression of a transgene is provided. In some alternatives, the transgene encodes a chimeric antigen receptor. In some alternatives, a system for inducible expression of a transgene is provided. In some

alternatives, the transgene encodes a chimeric antigen receptor. In some alternatives, a system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, is provided. In some alternatives of the system for inducible expression of a gene that regulates a T cell function, the polypeptide modulates checkpoint signaling. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3.

[0099] “T cell precursors” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, lymphoid precursor cells that can migrate to the thymus and become T cell precursors, which do not express a T cell receptor. All T cells originate from hematopoietic stem cells in the bone marrow. Hematopoietic progenitors (lymphoid progenitor cells) from hematopoietic stem cells populate the thymus and expand by cell division to generate a large population of immature thymocytes. The earliest thymocytes express neither CD4 nor CD8, and are therefore classed as double-negative ($CD4^-CD8^-$) cells. As they progress through their development, they become double-positive thymocytes ($CD4^+CD8^+$), and finally mature to single-positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) thymocytes that are then released from the thymus to peripheral tissues.

[0100] About 98% of thymocytes die during the development processes in the thymus by failing either positive selection or negative selection, whereas the other 2% survive and leave the thymus to become mature immunocompetent T cells.

[0101] The double negative (DN) stage of the precursor T cell is focused on producing a functional β -chain whereas the double positive (DP) stage is focused on producing a functional α -chain, ultimately producing a functional $\alpha\beta$ T cell receptor. As the developing thymocyte progresses through the four DN stages (DN1, DN2, DN3, and DN4), the T cell expresses an invariant α -chain but rearranges the β -chain locus. If the rearranged β -chain successfully pairs with the invariant α -chain, signals are produced which cease rearrangement of the β -chain (and silence the alternate allele) and result in proliferation of the cell. Although these signals require this pre-TCR at the cell surface, they are dependent on ligand binding to the pre-TCR. These thymocytes will then express both CD4 and CD8 and progresses to the double positive (DP) stage where selection of the α -chain takes place.

If a rearranged β -chain does not lead to any signaling (e.g. as a result of an inability to pair with the invariant α -chain), the cell may die by neglect (lack of signaling).

[0102] “Hematopoietic stem cells” or “HSC” have their plain and ordinary meaning when read in light of the specification, and may include but is not limited to, for example, precursor cells that can give rise to myeloid cells such as, for example, macrophages, monocytes, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells and lymphoid lineages (such as, for example, T-cells, B-cells, NK-cells). HSCs have a heterogeneous population in which three classes of stem cells exist, which are distinguished by their ratio of lymphoid to myeloid progeny in the blood (L/M). In some alternatives described herein a host cell comprising a system of any one of the alternatives described herein is provided. In some alternatives of the host cells provided herein, the host cells are hematopoietic stem cells.

[0103] This disclosure provides for a system that has an inducible component for expression of transgenes and a constitutive component for expression of transgenes. The system can be tailored to provide for regulated expression of one or more transgenes to provide for functional characteristics in the transduced cells.

[0104] Although, T cell (CAR-T) adoptive therapy clinical trials (chimeric antigen receptor expressing T cells) can demonstrate potent anti-tumor activity, significant toxicities can arise, for example, by engraftment-induced cytokine storm (cytokine release syndrome), tumor lysis syndromes (TLS) and ongoing B cell cytopenias, each of which are attributable to unregulated functional outputs of constitutively expressed CARs. Such toxicities can threaten to limit the applicability of CAR-T cell adoptive therapy, in some patients. Clinical trials using transgene-modified adoptive T cell immunotherapies have tested T cells that can constitutively express the transgene, or are always in the “ON” state, contributing in large part to transgene associated side-effects. Methods to eliminate CAR-T cells such as suicide gene-mediated elimination of CAR-T cells, for example, can ameliorate such toxicities; however, this approach risks premature attenuation of anti-tumor activity and can significantly impact curative potential.

[0105] Current small molecule-regulated transgene expression technologies rely on a variety of drug inputs, which include, for example, macrolides, ecdysones and rapamycin analogs. However, use of these drugs may be limited due to the toxic off target

effects, unfavorable biodistribution and pharmacodynamics profiles, limited output dynamic range, and/or limited availability as FDA-approved commercially available pharmaceuticals. Furthermore, many of these systems use chimeric transcriptional regulators built from xenogeneic components, thus introducing the complication of immunogenicity when applying these systems to human therapeutics.

[0106] Small molecule regulated transgene expression is a highly sought technology in biomedicine, particularly in CAR T cell immunotherapy. Competing systems typically use chimeric transcription factors composed of xenogeneic domains and drug inducers that are not currently FDA approved.

[0107] As such, there is a need to identify methods for controlling chimeric receptor expression that are important for therapeutic activity and cell populations that will provide enhanced survival and efficacy *in vivo* while minimizing adverse side effects of the drugs that induce chimeric antigen receptor expression. There is also a need for expression systems and methods for modulating cells for use in cell therapy, such as for modulating expression of recombinant antigen receptors such as CARs and/or other molecules expressed by such cells, such as to improve therapeutic activity, enhance survival and/or efficacy *in vivo* and/or minimize adverse side effects. Described herein, are methods that surprisingly led to enhanced survival and efficacy of the treatments *in vivo*, with minimized adverse side effects.

[0108] In some alternatives, a transgene under the control of the inducible promoter, is provided. In some alternatives, the transgene encodes a chimeric antigen receptor (CAR). The inducible promoter provides for the capacity to terminate CAR expression in cells while providing for reactivation of the cells at a later date (e.g. in the case of relapse). In addition, the cycling of CAR T cells through on and off periods can minimize exhaustion and/or anergy due to chronic stimulation of the T cell receptors.

[0109] In some alternatives, a gene is under the control of the inducible promoter. In some alternatives, the gene encodes a chimeric cytokine receptor.

[0110] The design of the vectors also provides for additional transgenes that can enhance one or more functional characteristics of transduced cells, such as enhanced tumor potency, survival and proliferation of transduced cells. In some alternatives, these transgenes are under the control of an inducible promoter. Such transgenes include, without limitation,

genes that promote survival and proliferation, genes that prevent apoptosis, and genes that regulate checkpoint signaling. Such genes include genes encoding IL-2, IL-15, chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the transgenes are genes encoding IL-2, IL-7, IL-15, chemokine receptors, Bcl2, chimeric cytokine receptors, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the gene that modulates checkpoint signaling, encodes a polypeptide that inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3. In some alternatives, the gene is under a control of an inducible promoter. In some alternatives, the gene under control of an inducible promoter comprises a nucleic acid sequence set forth in SEQ ID NO:

88 (CCR(CD122) nucleotide sequence:
atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagattgtgatattgaaggtaaagatg
gcaaacaatatgagagtggttctaattggcagcatcgatcaattattggacagcatgaaagaaattggttagcaattgcctgaataatgaatt
taacttttttaaagacatatctgtgatgctaataaggaaggtatgttttattccgtgctgctcgcaagttgaggcaatttcttaaataaata
gcactggtgattttgatctccacttattaaaagtttcagaaggcacaacaatactgttgaactgcactggccagggttaaaggaagaaaac
cagctgccctgggtgaagcccaaccaacaagagtttgaagaaaataaatctttaaggaacagaaaaactgaatgacttgtgttt
cctaaagagactattacaagagataaaaaacttgttgaataaaaattttgatgggcactaaagaacacggaggcggtgggagcggagg
cgggtgggagcatgacaattctaggtacaacttttggcatggtttttcttactcaagtcgtttctggagaaagtggctatgctcaaaatgg
agacttgaagatgcagaactggatgactactcattctcatgctatagccagttggaagtgaatggatcgagcactcactgacctgtg
cttttgaggaccagatgtcaacatcaccaatctggaatttgaaatatgtggggccctcgtggaggtaaagtgcctgaatttcaggaaa
ctacaagagatatattcatcgagacaaagaaattcttactgattggaaagagcaatatatgtgtgaagggttgagaaaagagtctaacc
tgcaaaaaaataagacctaaccactatagttaaacctgaggctcctttgacctgagtgtcatctatcggaaggagccaatgactttgtg
gtgacatttaatacatcacacttgcaaaagaagtatgtaaaagtttaatgcacgatgtagcttaccgccaggaaaaggatgaaaaca
atggacgcatgtgaatttatccagcacaactgacactcctgcagagaaagctccaaccggcagcaatgtatgagattaaagttcga
tccatccctgatcactattttaaggcttctggagtgaatggagtccaagtattacttcagaactccagagatcaataatagctcagggg
agatggatcctatcttactaaccatcagcattttgagtttttctctgtcgtctgttggtcatcttggcctgtgtgttatggaactgcaggaac
accgggccatggctgaagaaggtcctgaagtgtaacaccccagacccctcgaagtcttttccagctgagctcagagcatggagga
gacgtccagaagtggctctcttcgcccttcccctcatcgtccttcagccctggcggcctggcacctgagatctcgccactagaagtgt
ggagaggggacaaggtgacgcagctgctcctgcagcaggacaaggtgcctgagcccgcatccttaagcagcaaccactcgctgacc
agctgcttcaccaaccagggttacttcttccacctcccggatgccttgagatagaggcctgccaggtgtactttacttacgaccct
actcagaggaagaccctgatgagggtgtggccggggcacccacagggtcttcccccaaccctgcagcctctgtcaggggagga

cgacgcctactgcaccttccccctccaggatgacctgctgcttctccccagtcctcctcggtggccccagcccccaagcactgcc
 cctggggggcagtgggggccggtgaagagaggatgcccccttctttgcaagaaagagtccccagagactgggacccccagccccctgg
 ggctcccacccccaggagtcccagacctggtggattttcagccacccccctgagctggtgctgcgagaggctggggaggaggtccct
 gacgctggccccagggaggagtcagtttccccctggtccaggcctcctgggcagggggagttcagggcccttaatgctcgctgcc
 cctgaacactgatgcctacttgcctccaagaactccagggtcaggaccaactcacttggtg). In some alternatives,
 the sequence under control of an inducible promoter encodes for an amino acid sequence set
 forth in SEQ ID NO: 89 (CCR(CD122) amino acid sequence:
 MLLVTSLLLCELPHPAFLIPDCDIEGKDQKQYESVLMVSIQQLDSMKEIGSNCLN
 NEFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCT
 GQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLQEIKTCWNKILMGT
 KEHGGGGSGGGGSMITLGTTFGMVFSLLQVVSAGESGYAQNGDLEDAELDDYSFSCY
 SQLEVNGSQHSLTCAFEDPDVNITNLEFEICGALVEVKCLNFRKLQEIYFIETKKFLLI
 GKSNIQVKVGEKSLTCKKIDLTIVKPEAPFDLSVIYREGANDFVVTFTNTSHLQKKYV
 KVLMDHVAIRQEKDENKWTHVNLSSTKLTLLQRKLQPAAMYEIQVRSIPDHYFKGF
 WSEWSPSYFRTPPEINNSSGEMDPILLTISLSFFSVALLVILACVLWNCRNTGPWLKK
 VLKCNTPDPSKFFSQLSSEHGGDVQKWLSPPFPSSSFSPGGLAPEISPLEVLERDKVTQ
 LLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPD
 EGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGE
 ERMPPSLQERVPRDWDPPQLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREGV
 SFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV). In some alternatives,
 the sequence under control of an inducible promoter is encoded by a nucleic acid sequence
 comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 89.

[0111] In some alternatives, the gene under control of an inducible promoter
 comprises the nucleic acid sequence set forth in SEQ ID NO: 90 (PD1ECD-IFNalpha
 nucleotide sequence nucleotide sequence:
 atgcagatccctcaggccccctggcctgtcgtgtgggctgtgctgcagctgggatggcggcctggctggttctggacagccccgac
 agacctggaacccccctacatttccccctgcctgctggtcgtgaccgagggcgacaatgccaccttcacctgtagcttcagcaaca
 ccagcgagagcttcgtgctgaactggtacagaatgagccccagcaaccagaccgacaagctggccgccttccccgaggatagatct
 cagccccggccaggactgccggttcagagtgaccagctgccaacggccgggactccacatgtctgtcgtgcgggcccagacgga
 acgacagcggcacatatctgtgcggcgccatcagcctggcccccaaggcccagatcaaagagagcctgagagccgagctgagag
 tgaccgagagaagggccgaagtgcctaccgccccaccctagcccatctccaagacctgccggccagttccagacactcgtgggcgg

aggatgcgacctgcctcagacacacagcctgggcagcagacggacctgatgctgctggcccagatgcggaagatcagcctgttc
agctgcctgaaggaccggcagcacttcggcttccctcaggaagagttcggcaaccagtttcagaaggccgagacaatccccgtgct
gcacgagatgatccagcagatcttcaacctgttctccaccaaggacagcagcgccgctgggacgagacactgctggacaagttcta
caccgagctgtaccagcagctgaatgacctggaagcctgcgtgatccagggcgtgggctgacagagacaccctgatgaaggaa
gatagcatcctggcctgcgcaagtacttccagcggatcacctgtacctgaaagagaagaagtacagcccctgcgcctgggaggt
cgtgcgcgcccagatcatgagaagcttcagcctgagcaccaacctgcaggaaagcctgcggagcaaagaa). In some
alternatives, the sequence under control of an inducible promoter encodes the amino acid
sequence set forth in SEQ ID NO: 91 (PD1ECD-IFNalpha amino acid sequence:
MQIPQAPWPVWVAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSF
SNTSESFVLNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVR
ARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSRPAGQFQTLV
GGGCDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGFPQEEFGNQFQKAETIP
VLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQQLNDLEACVIQGVGVVTETPLM
KEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE). In
some alternatives, the sequence under control of an inducible promoter is encoded by a
nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence
of SEQ ID NO: 91.

[0112] In some alternatives, the gene under control of an inducible promoter
comprises the nucleic acid sequence set forth in SEQ ID NO: 92 (STAT5a nucleotide
sequence:

atggcgggctggatccaggcccagcagctgcaggagacgcgctgcgccagatgcaggtgctgtacggccagcacttccccatcg
aggtccggcactacttggcccagtggattgagagccagccatgggatgccattgacttgacaatccccaggacagagcccaagcc
accagctcctggagggcctggtgcaggagctgcagaagaaggcggagcaccaggtgggggaagatgggtttttactgaagatca
agctggggcactacgccacgcagctccagaaaacatatgaccgctgccccctggagctggtccgctgcatccggcacattctgtaca
atgaacagaggctggtccgagaagccaacaattgcagctctccggctgggatcctggttgacgccatgtcccagaagcaccttcaga
tcaaccagacatttgaggagctgcgactggtcacgcaggacacagagaatgagctgaagaaactgcagcagactcaggagtacttc
atcatccagtaccaggagagcctgaggatccaagctcagtttggccagctggcccagctgagccccaggagcgtctgagccggg
agacggccctccagcagaagcaggtgtctctggaggcctggttcagcgtgaggcacagacactgcagcagtagccgctggagct
ggccgagaagcaccagaagacctgcagctgctgcggaagcagcagaccatcatcctggatgacgagctgatccagtggaagcg
gcggcagcagctggccgggaacggcgggccccccgagggcagcctggacgtgctacagtctggtgtgagaagttggccgagat
catctggcagaaccggcagcagatccgcagggtgagcacctctgccagcagctgcccatccccggcccagtgaggagatgctg

gccgaggtcaacgccaccatcacggacattatctcagccctggtagaccagcacattcatcattgagaagcagcctcctcaggtcctga
agaccagaccaagtttgagccaccgtacgcctgctgggtggcgagggaagctgaacgtgcacatgaatccccccaggtgaaggc
caccatcatcagtgagcagcaggccaagtctctgcttaaaaatgagaacacccgcaacgagtgagtggtgagatcctgaacaactg
ctgcgtgatggagtaccaccaagccacgggcaccctcagtgcccacttcaggaacatgtcactgaagaggatcaagcgtgctgacc
ggcgggggtgcagagtccgtgacagaggagaagttcacagtcctgtttgagtctcagttcagtggtggcagcaatgagcttgtgtcca
gggtgaagactctgtccctacctgtgggtgtcatcgtccacggcagccaggaccacaatgccacgggctactgtgctgtgggacaatgcc
tttctgagccgggcagggtgccatttgccgtgcctgacaaagtgtgtggccgcagctgtgtgaggcgctcaacatgaaattcaag
gccgaagtgcagagcaaccgggggcctgaccaaggagaacctcgtgttcctggcgcagaaactgttcaacaacagcagcagccacc
tgaggactacagtggcctgtccgtgtcctgggtcccagttcaacagggagaacttgccgggctggaactacaccttctggcagtggtt
tgacgggggtgatggaggtgtgaagaagcaccacaagccccactggaatgatggggccatcctaggtttgtgaataagcaacagg
cccacgacctgctcatcaacaagcccgacgggaccttcttgttgcgcttagtgactcagaaatcgggggcatcaccatcgccctggaa
gtttgattccccggaacgcaacctgtggaacctgaaaccattcaccacgcgggatttctccatcaggtccctggctgaccggctgggg
gacctgagctatctcatctatgtgttctgaccgccccaaaggatgaggtcttctccaagtactacactcctgtgctggctaaagctgttg
atggatatgtgaaaccacagatcaagcaagtgggtccctgagtttgtaatgcatctgcagatgtgggggcagcagcgccacgtacat
ggaccaggccccctccccagctgtgtgccccaggctccctataacatgtaccacagaacctgacctgtactcgatcaggatgg
agaattcgacctggatgagacctggatgtggccaggcacgtggaggaaactcttacgccgaccaatggacagtcttgactcccgccct
ctcgccccctgccggtctttcacctctgccagaggctccctctca).

[0113] In some alternatives, the sequence under control of an inducible promoter encodes for the amino acid sequence set forth in SEQ ID NO: 93 (STAT5a amino acid sequence:

MAGWIIQAQQLQGDALRQMQLVLYGQHFPIEVRHYLAQWIESQPWDAIDLDPQDRA
QATQLLEGLVQELQKKAELHGVGEDGFLKIKLGHYATQLQKTYDRCPLELVR CIRHI
LYNEQRLVREANNCSSPAGILVDAMSQKHLQINQTFEELRLVTQDTENELKKLQQTQ
EYFIIQYQESLRIQAQFAQLAQLSPQERLSRETALQQKQVSLEAWLQREAQTLQQYR
VELAEKHQKTLQLLRKQQTHLDDELIQWKRRQQLAGNGGPPEGS LDVLQSWCEKL
AEIIWQNRQQIRRAEHL CQQLP IGPVEEMLAEVNAITDIISALVTSTFIIEKQPPQVL
KTQTKFAATVRLLVGGKLVHNMNPPQVKATIIEQQAKSLLKNENTRNECSGEILNN
CCVMEYHQATGTLSAHFRNMSLKRIKRADRRGAESVTEEKFTVLFESQFSVGSNELV
FQVKTLSPVVVIVHGSQDHNATATVLWDNAFAEPGRVPFAVPDKVLWPQLCEALN
MKFKAQVQSNRGLTKENLVFLAQKLFNNSSSHLEDYSGLSVSWSQFNRENLPGWNY
TFWQWFDGVMEVLKKHHKPHWNDGAILGFVNKQQAHDLLINKPDGTFLLRFS DSEI

GGITIAWKFDSPERNLWNLKPFTTRDFSIRSLADRLGDLSYLIYVFPDRPKDEVFSKY
 YTPVLAKAVDGYVKPQIKQVVPEFVNASADAGGSSATYMDQAPSPAVCPQAPYNM
 YPQNPDHVLQDGEFDLDETMDVARHVEELLRRPMDSLDSRLSPPAGLFTSARGSL
). In some alternatives, the sequence under control of an inducible promoter is encoded by a
 nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence
 of SEQ ID NO: 93.

[0114] In some alternatives, the gene under control of an inducible promoter
 comprises the nucleic acid sequence set forth in SEQ ID NO: 94 (Constitutively active-
 STAT5a nucleotide sequence:
 atggcgggctggatccaggcccagcagctgcaggagacgcgctgcgccagatgcaggtgctgtacggccagcacttccccatcg
 aggtccggcactacttggcccagtggattgagagccagccatgggatgccattgacttgacaatccccaggacagagcccaagcc
 acccagctcctggagggcctggtgcaggagctgcagaagaaggcggagcaccaggtgggggaagatgggttttactgaagatca
 agctggggcactacgccacgcagctccagaaaacatatgaccgctgccccctggagctggtccgctgcatccggcacattctgtaca
 atgaacagaggctggtccgagaagccaacaattgcagctctccggctgggatcctggttgacgccatgtcccagaagcaccttcaga
 tcaaccagacatttgaggagctgcgactggtcacgcaggacacagagaatgagctgaagaaactgcagcagactcaggagtacttc
 atcatccagtaccaggagagcctgaggatccaagctcagtttcccagctggcccagctgagccccaggagcgtctgagccggg
 agacggccctccagcagaagcaggtgtctctggaggcctggttgacgcgtgaggcacagacactgcagcagtagccgctggagct
 ggccgagaagcaccagaagaccctgcagctgctgcggaagcagcagaccatcatcctggatgacgagctgatccagtgaagcg
 gcggcagcagctggccgggaacggcgggccccccgagggcagcctggacgtgctacagtctgtgtgagaagtggccgagat
 catctggcagaaccggcagcagatccgcagggtgagcgcctctgccagcagctgccatccccggcccagtgaggagatgct
 ggccgaggtcaacgccaccatcacggacattatctcagccctggtgaccagcacattcatcattgagaagcagcctcctcaggtcctg
 aagaccagaccaagtttgagccaccgtacgcctgctggtgggcgggaagctgaacgtgcacatgaatccccccaggtgaagg
 ccaccatcatcagtgagcagcaggccaagtctctgcttaaaaatgagaacacccgcaacgagtgcagtggtgagatcctgaacaact
 gctgcgtgatggagtaccaccaagccacgggcaccctcagtgcccacttcaggaaatgtcactgaagaggatcaagcgtgctgac
 cggcgggggtgcagagtccgtgacagaggagaagttcacagtctgtttgagtctcagttcagtggtggcagcaatgagcttggtcca
 ggtgaagactctgtccctacctgtggtgtcatcgtccacggcagccaggaccacaatgccacggctactgtgctgtgggacaatgcc
 tttgctgagccgggcagggtgccatttgccgtgcctgacaaagtgtgtggccgcagctgtgtgaggcgctcaacatgaaattcaag
 gccgaagtgcagagcaaccggggcctgaccaaggagaacctcgtgttcctggcgcagaaactgttcaacaacagcagcagccacc
 tggaggactacagtggcctgtccgtgtcctggtcccagttcaacagggagaacttgccgggctggaactacaccttctggcagtggtt
 tgacgggggtgatggaggtgtgaagaagcaccacaagccccactggaatgatggggccatcctaggtttgtgaataagcaacagg
 cccacgacctgctcatcaacaagcccgacgggaccttctgtgtgcgttagtgactcagaaatcgggggcatcaccatcgccctggaa

gtttgattccccggaacgcaacctgtggaacctgaaaccattcaccacgcgggatttctccatcaggctccctggctgaccggctgggg
gacctgagctatctcatctatgtgtttcctgaccgcccccaaggatgaggtcttctccaagtactacactcctgtgctggctaaagctgttg
atggatatgtgaaaccacagatcaagcaagtgggtccctgagtttgtgaatgcatttgcatatgctgggggcagcagcgccacgtacat
ggaccaggccccctccccagctgtgtgccccaggctccctataacatgtaccacagaacctgacctgtactcgatcaggatgg
agaattcgacctggatgagaccatggatgtggccaggcacgtggaggaactcttacgccgaccaatggacagtcttgactcccgct
ctcgccccctgccggtcttttcacctctgccagaggctccctctca).

[0115] In some alternatives, the sequence under control of an inducible promoter encodes for the amino acid sequence set forth in SEQ ID NO: 95 (Constitutively active-STAT5a amino acid sequence: MAGWIIQAQQLQGDALRQMQLVLYGQHFPIEVRHYLAQWIESQPWDAIDLNDNPQDRA QATQLLEGLVQELQKKAQEHQVGEDGFLKIKLGHYATQLQKTYDRCPLELVR CIRHI LYNEQRLVREANNCSSPAGILVDAMSQKHLQINQTFEELRLVTQDTENELKKLQQTQ EYFIIQYQESLRIQAQFAQLAQLSPQERLSRETALQQKQVSLEAWLQREAQTLQQYR VELAEKHQKTLQLLRKQQTIILDDELIQWKRRQQLAGNGGPPEGSLDVLQSWCEKL AEIIWQNRQQIRRAERLCQQLPPIGPVEEMLAEVNATITDIISALVTSTFIEKQPPQVL KTQTKFAATVRLLVGGKLVHMHMNPQVKATIISEQQAKSLLKNENTRNECSGEILNN CCVMEYHQATGTLSAHFRNMSLKRIKRADRRGAESVTEEEKFTVLFESQFSVGSNELV FQVKTLSPVVVIVHGSQDHNATATVLWDNAFAEPGRVPFAVPDKVLWPQLCEALN MKFKAQEVQSNRGLTKENLVFLAQKLFNNSSSHLEDYSGLSVSWSQFNRENLPGWNY TFWQWFDGVMELKKHHKPHWNDGAILGFVNKQQAHDLLINKPDGTFLLRFS DSEI GGITIAWKFDSPERNLWNLKPFTTRDFSIRSLADRLGDLSYLIYVFPDRPKDEVFSKY YTPVLAKAVDGYVKPQIKQVVPEFVNAFADAGGSSATYMDQAPSPAVCPQAPYNM YPQNPDHVL DQDGEFDLDETMDVARHVEELLRRPMDSLDSRLSPPAGLFTSARGSL S). In some alternatives, the sequence under control of an inducible promoter is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 95.

[0116] In some alternatives, the gene under control of an inducible promoter comprises the nucleic acid sequence set forth in SEQ ID NO: 96 (Dominant negative TGFbRII nucleotide sequence: atgggtcgggggctgctcaggggcctgtggccgctgcacatcgctctgtggacgcgtatgccagcacgatcccaccgcacgttca gaagtcggttaataacgacatgatatgactgacaacaacgggtgcagtcaagttccacaactgtgtaaatttgtgatgtgagattttcc

acctgtgacaaccagaaatcctgcatgagcaactgcagcatcacctccatctgtgagaagccacaggaagtctgtgtggctgtatgga
 gaaagaatgacgagaacataacactagagacagtttgccatgaccccaagctcccctaccatgactttattctggaagatgctgcttct
 ccaaagtgcattatgaaggaaaaaaaaaagcctggtgagactttctcatgtgttcctgtagctctgatgagtgcaatgacaacatcatct
 tctcagaagaatataacaccagcaatcctgacttggtgctagtcatattcaagtgcagggcatcagcctcctgccaccactgggagttg
 ccatactgtcatcatcatcttctactgctaccgcgttaaccggcagcagaagctgagttcaacctgggaaaccggcaagacgcggaa
 gctcatggagttcagcgagcactgtgccatcatcctggaagatgaccgctctgacatcagctccacgtgtgccaacaacatcaaccac
 aacacagagctgctgcccattgagctggacaccctggtggggaaaggctcgctttgctgaggtctataaggccaagctgaagcagaa
 cacttcagagcagtttgagacagtggcagtcaagatctt).

[0117] In some alternatives, the sequence under control of an inducible promoter encodes for the amino acid sequence set forth in SEQ ID NO: 97 (Dominant negative TGFbRII amino acid sequence: MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDV RFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILE DAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLL VIFQVTGISLLP PLGVAISVIII FYCYRVNRQQKLSSTWETGKTRKLMEFSEHCAIILED RSDISSTCAN NINHNTPELLPIELDTLVGKGRFAEVYKAKLKQNTSEQFETVAVKIF). In some alternatives, the sequence under control of an inducible promoter is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 97.

[0118] In some alternatives, the gene under control of an inducible promoter comprises the nucleic acid sequence set forth in SEQ ID NO: 98 (Dominant negative SHP1 nucleotide sequence: atggtgaggtggtttcaccgagacctcagtgggctggatgcagagaccctgctcaagggccgaggtgtccacggtagcttcctggct cggcccagtcgcaagaaccaggggtgacttctcgctctccgtcaggggtgggggatcaggtgacccatattcgatccagaactcagg ggatttctatgacctgtatggaggggagaagtttgcgactctgacagagctggtggagtactacactcagcagcaggggtgtcctgcag gaccgcgacggcaccatcatccacctcaagtacccgctgaactgctccgatcccactagtgcagaggtgtaccatggccacatgtct ggcgggagcaggcagagacgctgctgcaggccaagggcgagccctggacgtttctgtgcgtgagagcctcagccagcctggagact tcgtgctttctgtgctcagtgaccagcccaaggctggcccaggctccccgctcagggtcacccacatcaaggtcatgtgcgaggggtg gacgctacacagtgggtggtttggagaccttcgacagcctcacggacctggtggagcatttcaagaagacggggattgaggaggcc tcaggcgcctttgtctacctgcggcagccgtactatgccacgaggggtgaatgcggctgacattgagaaccgagtggttgaactgaac aagaagcaggagtcgaggatacagccaaggctggcttctgggaggagtttgagagtttgagaagcaggaggtgaagaactgc

accagcgtctggaagggcagcggccagagaacaagggcaagaaccgctacaagaacattctccccttgaccacagccgagtgat
cctgcagggacgggacagtaacatccccgggtccgactacatcaatgccaaactacatcaagaaccagctgctaggccctgatgaga
acgctaagacctacatcgccagccagggctgtctggaggccacgggtcaatgacttctggcagatggcgtggcaggagaacagccgt
gtcatcgtcatgaccacccgagaggtggagaaaggccggaacaaatgcgtcccatactggcccaggtgggcatgcagcgtgctta
tgggccctactctgtgaccaactgcggggagcatgacacaaccgaatacaaactccgtaccttacaggtctccccgctggacaatgg
agacctgattcgggagatctggcattaccagtagctggcccagcatgggggtccccagtgagcctgggggtgtcctcagctt
cctggaccagatcaaccagcggcaggaaagtctgcctcacgcagggcccatcatcgtgcactccagcgcggcatcgggcgca
ggcaccatcattgtcatcgacatgctcatggagaacatctccaccaagggcctggactgtgacattgacatccagaagaccatccaga
tggtgcggggcgagcgtcgggcatggtgcagacggaggcgagtagacaagttcatctacgtggccatcgcccagttcattgaaacc
actaagaagaagctggaggtcctgcagtcgcagaagggccaggagtcggagtaggggaacatcacctatccccagccatgaaga
atgcccattgccaaggcctcccgacctcgtccaaacacaaggaggatgtgtatgagaacctgcacactaagaacaagagggagga
gaaagtgaagaagcagcggtcagcagacaaggagaagagcaaggggtccctcaagaggaagtga).

[0119] In some alternatives, the sequence under control of an inducible promoter encodes for the amino acid sequence set forth in SEQ ID NO: 99: Dominant negative SHP1 amino acid sequence: MVRWFHRDLSGLDAETLLKGRGVHGSFLARPSRKNQGDFSLSVRVGDQVTHIRIQN SGDFYDLYGGEKFATLTELYEYYTQQQGV LQDRDGTIIHLKYPLNCSDPTSERWYH GHMSGGQAETLLQAKGEPWTF LVRRESLSQPGDFVLSVLSDQPKAGPGSPLRVTHIKV MCEGGRYTVGGLET FDSLTDLVEHF KKTGIEEASGAFVYLRQPYATRVNAADIEN RVLELNKKQESED TAKAGFWEEFESLQKQEVKNLHQRLEGQRPENKGKNRYKNILP FDHSRVILQGRDSNIPGSDYINANYIKNQ L L GPDENAKTYIASQGCLEATVNDFWQM AWQENSRVIVMTTREV EKG R N K C V P Y W P E V G M Q R A Y G P Y S V T N C G E H D T T E Y K L R T L Q V S P L D N G D L I R E I W H Y Q Y L S W P D H G V P S E P G G V L S F L D Q I N Q R Q E S L P H A G P I I V H S S A G I G R T G T I I V I D M L M E N I S T K G L D C D I D I Q K T I Q M V R A Q R S G M V Q T E A Q Y K F I Y V A I A Q F I E T T K K K L E V L Q S Q K G Q E S E Y G N I T Y P P A M K N A H A K A S R T S S K H K E D V Y E N L H T K N K R E E K V K K Q R S A D K E K S K G S L K R K). In some alternatives, the sequence under control of an inducible promoter is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 99.

[0120] In some alternatives, the gene under control of an inducible promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 100 (PD1TM-MyD88 nucleotide sequence:

atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccaggcggaggagtgggagtcgtggg
 cggactgctgggatctctggtgctgctcgtgtgggtgctggccgtgattggcggaggaatggctgctggcggacctggcgctggatc
 tgctgctcctgtgtctagcaccagcagcctgcctctggccgccctgaatatgagagtgcggcggagactgagcctgttctgaacgtg
 cggacacaggtggccgccgattggacagctctggccgaggaaatggacttcgagtacctggaaatccggcagctggaaaccagg
 ccgaccctacaggacgcctgctggatgcttggcagggcagaccaggcgcttctgtggggagactgctggaactgctgaccaagctg
 ggccgggacgacgtgctgctggaactgggccctagcatcgaagaggactgccagaagtacatcctgaagcagcagcaggaagag
 gccgagaagcctctgcaggtggcagccgtggatagcagcgtgccaagaacagctgagctggccggaatcaccacctggacgat
 cctctgggccacatgcccagagattcgacgccttcatctgctactgccccagcgacatccagttcgtgcaggaaatgatcagacag
 ctggaacagaccaactaccggctgaagctgtgctgtccgaccgggatgtgctgcctggcacctgtgtgtggtctatgccagcgag
 ctgatcgagaagcgggtgcagacggatggtcgtggtggtgtccgacgactacctgcagtccaaagagtgcgacttccagaccaagttc
 gccctgagcctgagccctggcgcccaccagaagagactgatcccatcaagtacaaggccatgaagaaagagttccccagcatcct
 gcggttcatcacctgtgctgactacaccaaccctgcaccaagtctggttctggaccagactggccaaggccctgtctctgcct).

In some alternatives, the gene under control of an inducible promoter encodes for an amino acid sequence set forth in SEQ ID NO: 101 (PD1TM-MyD88 amino acid sequence: MLLLVTSLLLCELPHPAFLIPGGGVGVVGGLLGSLVLLVWVLAVIGGGMAAGGPG AGSAAPVSSTSSLPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYLEIRQL ETQADPTGRLLDAWQGRPGASVGRLLLELLTKLGRDDVLELGPSIEEDCQKYILKQQ QEEAEKPLQVA AVDSSVPRTAELAGITTLDDPLGHMPERFDAFICYCPSDIQFVQEMI RQLEQTNYRLKLCVSDRDVLPGTCVWSIASSELIEKRCRRMVVVVSDDYLQSKECDF QTKFALSLSPGAHQKRLIPIKYKAMKKEFPSILRFITVCDYTNPCTKSWFWTRLAKAL SL). In some alternatives, the sequence under control of an inducible promoter is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 101.

[0121] The disclosure provides for a system comprising first and second nucleic acids, and vectors and host cells including such nucleic acids. Each of the first and second nucleic acids comprise a number of modular components that can be excised and replaced with other components in order to customize the system for a specific target cell. In some alternatives, the first nucleic acid includes an inducible promoter for control of the expression of the genes (e.g., polynucleotide coding for a chimeric antigen receptor) in an on and off manner as needed, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a

wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In other alternatives, the second nucleic acid comprises a constitutive promoter that provides for expression of a transcriptional activator. In some alternatives, the gene encodes for a chimeric antigen receptor. In some alternatives, the gene encodes for a chimeric antigen receptor AND a transcriptional activator (encoded by one plasmid).

Inducible System.

[0122] The disclosure provides a system useful for providing regulated expression of transgenes in cells, such as mammalian cells. Such transgenes include, without limitation, T cell receptors, affinity matured T cell receptors, chimeric antigen receptors, chemokine receptors, chimeric chemokine receptors, cytokines, genes that inhibit apoptosis, and/or genes that modulate checkpoint signaling. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3. In some alternatives, the system contains a number of modular components that provide for easy substitution of elements of the nucleic acid. In some alternatives of the system, the system provides regulation of expression of transgenes in cells, such as mammalian cells, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives, the transgenes code for T cell receptors, affinity matured T cell receptors, chimeric antigen receptors, chemokine receptors, chimeric chemokine receptors, cytokines, genes that regulate apoptosis, and/or genes that modulate checkpoint signaling. In some alternatives, the gene that modulates checkpoint signaling encodes a polypeptide inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3.

[0123] In some alternatives, a system for inducible expression of a transgene is provided. In some alternatives, the transgene encodes a chimeric antigen receptor. In some alternatives, a system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, is provided. In some alternatives, a system for inducible expression of a

chimeric antigen receptor in cells, such as mammalian cells, comprises: a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain, and a fourth polynucleotide, which encodes an intracellular signaling domain, and a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives, the second promoter is constitutive or inducible.

[0124] In some alternatives, a polynucleotide coding for a chimeric antigen receptor comprises a polynucleotide coding for a ligand binding domain, wherein the target molecule is a tumor specific antigen, a polynucleotide coding for a polypeptide spacer wherein the spacer is optionally optimized; a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for an intracellular signaling domain. In some alternatives, an expression vector comprises a first and/or second nucleic acid, as described herein. Polypeptides encoded by all of or a portion of the chimeric receptor nucleic acids are also included herein.

[0125] In other alternatives, a first nucleic acid comprises a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for a gene that promotes cell survival and proliferation, a gene that regulates apoptosis, and/or a gene that modulates checkpoint signaling. Such genes include genes encoding IL-2, IL-7, IL-15, chemokine receptors, chimeric chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, and/or PD-1CD28 chimeras. In some alternatives, the gene that modulates checkpoint signaling encodes a polypeptide that inhibits negative

checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3.

[0126] In some alternatives, the system is for inducible expression of a gene that regulates a T cell function.

Inducible Promoters.

[0127] A system comprises a first nucleic acid comprising a first promoter inducible by a drug. By utilizing an inducible promoter, transgene expression can be turned on and off in order to avoid toxic side effects and/or to allow the cells to rest during remission. Another purpose of the inducible promoter system is to improve anti-tumor efficacy by turning on genes that enhance T cell function. Although several inducible promoter systems are known, clinical applicability of these systems is limited due to toxic off target effects, unfavorable biodistribution and pharmacodynamics profiles, limited output dynamic range, and/or limited availability as FDA-approved commercially available pharmaceuticals. Furthermore, many of these systems use chimeric transcriptional regulators built from xenogeneic components, thus introducing the complication of immunogenicity when applying these systems to human therapeutics. In some alternatives, the first nucleic acid comprises a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from the group consisting of a tumor specific molecule, a viral specific molecule, and other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain.

[0128] In some alternatives, a first promoter is inducible by a drug. The drug is selected based on safety record, favorable pharmacokinetic profile, tissue distribution, a low partition coefficient between the extracellular space and cytosol, low immunogenicity, low toxicities, and/or high expression in lymphocytes. In a specific alternative, a drug is selected that is FDA approved, provides for transgene expression in lymphocytes, does not activate other undesirable gene expression, and induces a promoter that does not contain any xenogeneic components. In some alternatives, the inducible promoter is activated by a

transcriptional activator that interacts with a drug. The transcriptional activator is activated or able to bind to and activate the inducible promoter in the presence of the drug.

[0129] A specific alternative of a drug is a drug that binds to an estrogen receptor ligand binding domain of a transcriptional activator. In some alternatives, the drug includes tamoxifen, its metabolites, analogs, and pharmaceutically acceptable salts and/or hydrates or solvates thereof. In some alternatives, the drug includes tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8.

[0130] Tamoxifen, CAS RN: 10540-29-1, useful in some approaches described herein, is also known as 2-(4-((1Z)-1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethylethanamine, or (Z)-2-(para-(1,2-Diphenyl-1-butenyl)phenoxy)-N,N-dimethylamine (IUPAC), and has a molecular formula of C₂₆H₂₉NO and a molecular weight (M.W.) of 371.52 g/mol. Tamoxifen is a Selective Estrogen Receptor Modulator with tissue-specific activities. Tamoxifen acts as an anti-estrogen (inhibiting agent) agent in the mammary tissue, but as an estrogen (stimulating agent) in cholesterol metabolism, bone density, and cell proliferation in the endometrium. Tamoxifen is frequently administered orally as a pharmaceutically acceptable salt. For example, Tamoxifen citrate (RN 54965-24-1, M.W. 563.643) is indicated for treatment of metastatic breast cancer, and as an adjuvant for the treatment of breast cancer in women following mastectomy axillary dissection, and breast irradiation. Tamoxifen citrate is also indicated to reduce incidence of breast cancer in women at high risk for breast cancer.

[0131] Metabolites of tamoxifen in rat, mouse and human breast cancer patients, useful in some approaches described herein, include major metabolites N-desmethyldtamoxifen (RN 31750-48-8, M.W. 357.494) and 4-hydroxytamoxifen (4-OHT) (RN 68392-35-8, M.W. 387.52, Afimoxifene), and are disclosed in Robinson *et al.* (Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Metab Dispos January 1991 19:36-43; incorporated by reference in its entirety herein). Additional cytochrome P-450 metabolites, useful in some approaches described herein, include cis-4-hydroxytamoxifen (RN 174592, M.W. 387.52; Afimoxifene, E-isomer), and 4'-hydroxytamoxifen ((Z)-4-(1-(4-(2-(dimethylamino)ethoxy)phenyl)-1-phenylbut-1-en-2-yl)phenol) as disclosed in Crewe *et al.* (Metabolism of Tamoxifen by recombinant human cytochrome P-450 enzymes: Formation of

the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen, Drug Metab Dispos, 30(8): 869-874, 2002, FIG. 1; incorporated by reference in its entirety herein).

[0132] Compounds with structural similarity to tamoxifen, useful in some approaches described herein, include, but are not limited to, cis-tamoxifen (RN 13002-65-8, M.W. 371.521), 4-methyltamoxifen (RN 73717-95-5, M.W. 385.548), N-desmethyltamoxifen (RN 31750-48-8, M.W. 357.494), (Z)-desethyl methyl tamoxifen (RN 15917-50-7, M.W. 357.494), (E)-desethyl methyl tamoxifen (RN 31750-45-5, M.W. 357.494), trans-4-hydroxytamoxifen (RN 68047-06-3, M.W. 387.52), Afimoxifene (RN 68392-35-8, M.W. 387.52, 4-hydroxytamoxifen), Afimoxifene, E-isomer (RN 174592-47-3, M.W. 387.52), 4-chlorotamoxifen (RN 77588-46-6, M.W. 405.966), 4-fluorotamoxifen (RN 73617-96-6, M.W. 389.511), Toremifene (RN 89778-26-7, M.W. 405.966), desethyl tamoxifen (RN 19957-51-8, M.W. 343.47), (E)-desethyl tamoxifen (RN 97151-10-5, M.W. 343.47), (Z)-desethyl tamoxifen (RN 97151-11-6, M.W. 343.47), Miproxifene (RN 129612-87-9, M.W. 429.6), 2-(p-(beta-ethyl-alpha-phenylstyryl)phenoxy)triethylamine (RN 749-86-0, M.W. 399.575), Droloxifene (RN 82413-20-5, M.W. 387.52), 4-iodo-tamoxifen (RN 116057-68-2, M.W. 497.413), dihydrotamoxifen (RN 109640-20-2, M.W. 373.537), (E)-N,N-dimethyl-2-(4-(1-(2-methylphenyl)-2-phenyl-1-butenyl)phenoxy)ethanamine (RN 97150-96-4, M.W. 385.548), or 4-hydroxytoremifene (RN 110503-62-3, M.W. 421.965); and/or pharmaceutically acceptable salts and/or hydrates or solvates thereof.

[0133] For example, citrate salts of tamoxifen, or citrate salts of compounds with structural similarity to tamoxifen, useful in some approaches described herein, include, but are not limited to tamoxifen citrate (RN 54965-24-1, M.W. 563.64), 2-(p-(1,2-diphenyl-1-butenyl)phenoxy)-N,N-dimethylethylamine citrate (RN 7244-97-5, 563.64), (E)-tamoxifen citrate (RN 76487-65-5, M.W. 563.64), Toremifene citrate (RN 89778-27-8, M.W. 598.088), Droloxifene citrate (RN 97752-20-0, M.W. 579.64), 2-(p-(1,2-bis(p-methoxyphenyl)-1-butenyl)phenoxy)triethylamine citrate (RN 42920-39-8, M.W. 651.748), 2-(4-(1,2-diphenylethenyl)phenoxy)-N,N-diethyl-ethanamine 2-hydroxy-1,2,3-propanetricarboxylate (RN 40297-42-5, M.W. 563.643), 2-(p-(alpha-phenylstyryl)phenoxy)triethylamine citrate (RN 102433-95-4, M.W. 563.64), 2-(p-(2-(p-methoxyphenyl)-1-phenyl-1-butenyl)phenoxy)triethylamine citrate (1:1) (RN 42824-34-0, M.W. 637.72), 2-(p-(1-(p-

methoxyphenyl)-2-phenylpropenyl)phenoxy)triethylamine citrate (RN 13554-24-0, M.W. 607.696), 2-(p-(alpha-(p-methoxyphenyl)styryl)phenoxy)triethylamine citrate monohydrate (RN 13542-71-7, M.W. 593.669), 2-(p-(p-methoxy-alpha-phenylphenethyl)phenoxy)triethylamine citrate (RN 16421-72-0, M.W. 595.685), alpha-(p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-p-methoxy-alpha-phenylphenethyl alcohol citrate (1:1) (RN 35263-93-5, M.W. 639.737), 1-(p-(2-(diethylamino)ethoxy)phenyl)-2-(p-methoxyphenyl)-1-phenylethanol citrate (M.W. 611.68), alpha-p-(2-(diethylamino)ethoxy)phenyl)-beta-ethyl-alpha-(p-hydroxyphenyl)-p-methoxyphenethyl alcohol citrate (RN 35263-96-8, M.W. 655.737), and/or 2-(p-(p-methoxy-alpha-methylphenethyl)phenoxy)-triethylamine citrate (RN 15624-34-7, M.W. 533.614).

[0134] In some alternatives, an effective amount of the drug for inducing expression is an amount that provides for an increase in transgene expression over uninduced and/or basal level of expression. In some alternatives, this amount can be readily determined using known dosages and pharmacokinetic profile of the drug. In the systems provided herein, wherein the systems are inducible by the drug, the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor.

[0135] In some alternatives, the inducible promoter has a low level of basal activity. When a lentiviral vector is used, the level of basal activity in uninduced cells is 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% or less, or any level of basal activity in between a range defined by any two aforementioned values, as compared to when cells are induced to express the gene. The level of basal activity can be determined by measuring the amount of the expression of the transgene (e.g. marker gene) in the absence of the inducer (e.g. drug) using flow cytometry.

[0136] In some alternatives, the inducible promoter provides for a high level of induced activity, as compared to uninduced or basal activity. In some alternatives, the level of activity in the induced state is 2, 4, 6, 8, or 10 fold or greater than the activity level in the uninduced state. In some alternatives, transgene expression under control of the inducible promoter is turned off in the absence of a transactivator in less than 10, 8, 6, 4, 2, or 1 days excluding 0 days.

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KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
DFSALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid
sequence that encodes SEQ ID NO: 1. The mutant tamoxifen-specific ligand binding domain
of the estrogen receptor ligand binding domain (ER-LBD) is found at amino acids 282-595
of the TamR-tf and has a mutation at position 521. The p65 activation domain of NF- κ B (p65
or TAD) is found at amino acids 596 to 862. SEQ ID NO: 1 comprises one point mutation in
the ER-LBD that ablates binding to endogenous estrogen, but confers nanomolar specificity
to 4-OHT, fulvestrant and other estrogen analogs.

[0139] In some alternatives, the HEA3 transcription factor comprises at least one
point mutation that ablates binding to the endogenous estrogen but confers nanomolar
specificity tamoxifen metabolite, 4-hydroxytamoxifen, fulvestrant, and other estrogen
analogues. In some alternatives, the one point mutation is at position 521 of the sequence of the
wild-type HEA3. In some alternatives, the amino acid sequence comprises a sequence set
forth in SEQ ID NO: 1
(MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLDQVHLLECAWLEILMIGLV
WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLRA
QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
DFSALLSQISS). SEQ ID NO: 1 comprises one point mutation in the ER-LBD that ablates

binding to endogenous estrogen, but confers nanomolar specificity to 4-OHT, fulvestrant and other estrogen analogs.

[0140] In some alternatives, the HEA3 transcription factor comprises at least one point mutation in the p65 domain that enhances transcriptional activity. In some alternatives, the point mutation is at position 846 of the sequence of the wild-type HEA3 (position 536 in the RelA protein, which correspond to position 846 in HEA3). In some alternatives, the amino acid sequence comprises a sequence set forth in SEQ ID NO: 2 (SEQ ID NO: 2; MVSKLSQLQTELLAALLESGLSKEALIQUALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLV WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM DFSALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 2. SEQ ID NO: 2 comprises a point mutation in HEA3, in the p65 domain which enhances transcriptional activity and is referred to as HEA3 (p65/S536E). The p65/S536E position in the RelA protein corresponds to position 846 in wild type HEA3 and wild type HEA4.

[0141] In some alternatives, the HEA3 transcription factor comprises at least one point mutation in the p65 domain that enhances transcriptional activity. In some alternatives, the point mutation is at position 621 of the sequence of the wild-type HEA3 (This is position 310 of the WT RelA protein). In some alternatives, the amino acid sequence comprises a

sequence set forth in SEQ ID NO: 3 (SEQ ID NO: 3; MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLEMLDAHRLHAPTSRGGASVE ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET FQSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM DFSALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 3. SEQ ID NO: 3 comprises a sequence of HEA3 containing one point mutation (K310Q positions in the RelA protein) in the p65 domain that enhances transcriptional activity. This is referred to as HEA3 (p65/K310Q). K310Q refers to position 310 of RelA. This position thus corresponds to position 621 of WT HEA3 AND WT HEA4.

[0142] In some alternatives, the HEA3 transcription factor comprises at least three point mutations in the estrogen receptor ligand binding domain that ablates binding to endogenous estrogen, and confer nanomolar sensitivity to CMP8 and 4-hydroxytamoxifen. In some alternatives, the point mutations are at positions 384, 421 and 521 of the sequence of the wild-type HEA3. In some alternatives, the amino acid sequence comprises a sequence set forth in SEQ ID NO: 8 (SEQ ID NO: 8; MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR

KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWMEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCVEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRQAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYETF
 KSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPTSSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK
 PTQAGEGTLSEALLQLQFDDDELGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIAD MDF
 SALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence
 that encodes SEQ ID NO: 8. SEQ ID No: 8 comprises the sequence of HEA3 containing
 three point mutations (L384M, M421G, G521R) in the ER-LBD that ablate binding to
 endogenous estrogen, and confer nanomolar sensitivity to CMP8 and 4-hydroxytamoxifen.
 This is referred to as CMP8-HEA3.

[0143] In some alternatives, the HEA3 transcription factor is a CMP8-responsive
 HEA3 transcription factor. In some alternatives, the CMP8-responsive HEA3 transcription
 factor comprises at least one point mutation in the p65 domain that enhances transcriptional
 activity. In some alternatives, the point mutation is at position 846 of the sequence of the
 CMP8-responsive HEA3 (position 536 in the RelA protein, which correspond to position 846
 in HEA3). In some alternatives, the amino acid sequence comprises a sequence set forth in
 SEQ ID NO: 9 (SEQ ID NO: 9;
 MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWMEILMIGLV

WRSMEHPGKLLFAPNLLDRNQGKCVEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRRLAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRRKRTYETF
 KSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPTSSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK
 PTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADMDF
 SALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence
 that encodes SEQ ID NO: 9. SEQ ID NO: 9 is the sequence of CMP8-HEA3 containing one
 point mutation (S536E) in the p65 domain that enhances transcriptional activity. This is
 referred to as CMP8-HEA3 (p65/S536E positions in the RelA protein, which correspond to
 position 846 in HEA3).

[0144] In some alternatives, the HEA3 transcription factor is a CMP8-responsive
 HEA3 transcription factor. In some alternatives, the CMP8-responsive HEA3 transcription
 factor comprises at least one point mutation in the p65 domain that enhances transcriptional
 activity. In some alternatives, the point mutation is at position 310 of the sequence of the
 RelA protein which corresponds to position 621 of the wild-type HEA3 protein, and the
 wild-type HEA4 protein. In some alternatives, the amino acid sequence comprises a
 sequence set forth in SEQ ID NO: 10 (SEQ ID NO: 10;
 MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHNLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWMEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCVEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRRLAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRRKRTYETF

QSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQYPFTSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK
 PTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIAD MDF
 SALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence
 that encodes SEQ ID NO: 10. SEQ ID NO: 10 is the sequence of CMP8-HEA3 containing
 one point mutation (K310Q positions in the RelA protein) in the p65 domain that enhances
 transcriptional activity. This is referred to as CMP8-HEA3 (p65/K310Q). Position 310 of the
 RelA protein is position 621 of wild-type HEA3 and wild-type HEA4.

[0145] In some alternatives, the HEA3 transcription factor is a CMP8-responsive
 HEA3 transcription factor. In some alternatives, the CMP8-responsive HEA3 transcription
 factor comprises at least two point mutations in the p65 domain that enhances transcriptional
 activity. In some alternatives, the point mutations are at positions 621 of the sequence of the
 wild-type HEA3 (The 310 position in the RelA protein corresponds to position 621 in wild
 type HEA3). In some alternatives, the amino acid sequence comprises a sequence set forth in
 SEQ ID NO: 11 (SEQ ID NO: 11;
 MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLDQVHLLCAWMEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCVEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQR LAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRRTYETF
 QSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQYPFTSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK
 PTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIAD MDF

SALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 11. SEQ ID NO: 11 comprises the sequence of CMP8-HEA3 containing two point mutations (K310Q and S536E are positions in the RelA protein, which correspond to positions 621 (K310A of the RelA protein) and 846 (S536E of the RelA protein) in both HEA3 and HEA4) in the p65 domain that enhance transcriptional activity. This is referred to as CMP8-HEA3 (p65/S536E/K310Q positions in the RelA protein, which correspond to position 621 and 846 in both HEA3 and HEA4).

[0146] In some alternatives, the system employs a synthetic transcriptional activator which, in the presence of the drug, binds a synthetic promoter upstream of a transgene to induce expression. In some alternatives, the transcriptional activator is HEA4. HEA4 comprises an estrogen receptor ligand binding domain ((ER-LBD) and a p65 domain. In some alternatives, the HEA4 further comprises an HNF1alpha DNA binding domain. In some alternatives, the HEA4 comprises at least three point mutations in the ER-LBD that ablate binding to endogenous estrogen, and confer higher sensitivity to 4-hydroxytamoxifen, fulvestrant, and estrogen analogs compared to wild-type HEA3. In some alternatives, the positions of the three point mutations are at positions 400, 543 and 544 in HEA4 transcription factor. In some alternatives, the amino acid sequence of the HEA4 comprising the at least three mutations comprise an amino acid sequence set forth in SEQ ID NO: 4 (SEQ ID NO: 4;

MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLV
WRSMEHPVKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSIIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQR
LAQLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEADAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP

SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAAPAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
DFSALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid
sequence that encodes SEQ ID NO: 4. SEQ ID NO: 4 comprises the sequence of wild-type
HEA4 containing three point mutations (G400V, M543A, L544A) in the ER-LBD that ablate
binding to endogenous estrogen, and confer higher sensitivity to 4-hydroxytamoxifen,
fulvestrant, and estrogen analogs compared to wild-type HEA3.

[0147] In some alternatives, the HEA4 transcription factor comprises at least one
point mutation. In some alternatives, the at least one mutation is in the p65 domain and
enhances transcriptional activity. In some alternatives, the at least one mutation is at position
846 of the wild type HEA4 transcription factor. In some alternatives, the amino acid
sequence of the HEA4 comprising the at least one mutation comprises an amino acid
sequence set forth in SEQ ID NO: 5 (SEQ ID NO: 5;
MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
WRSMEHPVKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRILA
QLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEAADAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRITYET
FKSIMKKSPFSGPTDPRPPPRRIA VPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAAPAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM
DFSALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid
sequence that encodes SEQ ID NO: 5. SEQ ID NO: 5 comprises the sequence of HEA4

containing one point mutation (S536E positions in the RelA protein) in the p65 domain that enhances transcriptional activity. This is referred to as HEA4 (p65/S536E). The mutation S536E refers to the mutation at position 536 of WT RelA. This position translates to position 846 of HEA3. The mutation K310Q refers to the mutation at position 310 of WT RelA. This position translates to position 621 of HEA3.

[0148] In some alternatives, the HEA4 transcription factor comprises at least one point mutation. In some alternatives, the at least one mutation is in the p65 domain and enhances transcriptional activity. In some alternatives, the at least one mutation is at position 621 of the HEA4 transcription factor. In some alternatives, the amino acid sequence of the HEA4 comprising the at least one mutation comprises an amino acid sequence set forth in SEQ ID NO: 6 (SEQ ID NO: 6; MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLDQVHLLECAWLEILMIGLV WRSMEHPVKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLRA QLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEAADAHRLHAPTSRGGASVE ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET FQSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPTSSLSTINYDEFPTMVFP SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM DFSALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 6. SEQ ID NO: 6 comprises the sequence of HEA4 containing one point mutation (K310Q positions in the RelA protein) in the p65 domain that enhances transcriptional activity. This is referred to as HEA4 (p65/K310Q). K310Q refers to the position in RelA, which corresponds to position 621 in WT HEA4.

[0149] In some alternatives, the HEA4 transcription factor comprises at least two point mutations. In some alternatives, the at least two mutations are in the p65 domain and enhances transcriptional activity. In some alternatives, at least two mutations are at positions 846 and 621 of the HEA4 transcription factor. In some alternatives, the amino acid sequence of the HEA4 comprising the at least two mutations comprise an amino acid sequence set forth in SEQ ID NO: 7 (SEQ ID NO: 7; MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLDQVHLLECAWLEILMIGLV WRSMEHPVKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQR LA QLLLILSHIRHMSNKGMEHLYSMKCKNVPLYDLLLEAADAHRLHAPTSRGGASVE ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKR TYET FQSIMKKSPFSGPTDPRPPPRRIA VPSRSSASV PKPAPQPY PFTSSLSTINYDEFPTMVFP SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM DFSALLSQISS). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 7. SEQ ID NO: 7 comprises the sequence of HEA4 containing two point mutations (K310Q and S536E, positions in the RelA protein) in the p65 domain that enhance transcriptional activity. This is referred to as HEA4 (p65/S536E/K310Q positions in the RelA protein). K310Q in RelA corresponds to position 621 in HEA4; S536E corresponds to position 846 in HEA4.

[0150] Additional chimeric transcription factors are also contemplated herein. In some alternatives, the HEA4 transcription factor comprises HNF1alpha, an ER-LBD that is the same as HEA4, and a VP64 activation domain instead of the p65 activation domain nucleotide sequence (referred herein as HEA4(VP64), and is encoded by SEQ ID NO: 24

(atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcagg
 cactcggcgaacctggacattatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagct
 ggccgagctgcctaacggcctgggcgagacaagaggcagcagaggacgagacagacgacgacggcgaggacttcaccccccc
 atcctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccc
 tggcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaac
 cagagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgccctgtacacttggtacgtgcgg
 aagcagagagaggtggcccagcagtttacacacgcccggccaggcgccctgatcgaggaacctaccggcgacgagctgccac
 caagaagggcagacggaaccgggttaagtggggccctgcatctcagcagatcctgttcaggccctacgagcggcagaagaacccc
 agcaaagaggaacgggagacactggtggaagagtgaaccggggcagtgcatccagagaggcgtgagcccttctcaggctcag
 ggcctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagct
 gtctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagc
 ctgaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccaccagacccttcagc
 gaggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggc
 ttcgtggacctgacctgcacgaccaggtccacctgctggaatgtgctggctggaaatcctgatgatcggcctcgtgtggagaagc
 atggaacaccccgctgaagctgctgttcgcccccaacctgctcctggaccggaaccagggaagtgctggaggggcatggtggaga
 tcttcgacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtccatcatcct
 gctgaacagcggcgtgtacaccttctgtcatccacctgaagtcctggaagagaaggaccacatccaccgggtgctggacaaga
 tcaccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatc
 ctgagccacatccggcacatgagcaacaagggaatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacct
 gctgctcgaggctgccgatgcccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctca
 cctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcacccggcgaggccgagggattccctgccacct
 ggacgctctggacgacttcgaccttgacatgttgggtccgacgcgctggatgattttgatttgacatgctgggaagcgacgcactgg
 atgactttgatctcgatatgctcggtctgacgcattggacgacttcgacttggatatgctgggttct; HEA4 (VP64)). In
 some alternatives, HEA4(VP64) comprises an amino acid sequence set forth in SEQ ID NO:

25

(MVSKLSQLQTELLAALLESGLSKEALIQUALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHNLKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF

[0151] In some alternatives, HEA4 comprises HNF1alpha, an ER-LBD that is the same as HEA4, and an HSF1 activation domain instead of a p65 activation domain. HEA4 (HSF-1) comprises HNF1alpha, ER-LBD that is the same as HEA4, and HSF1 activation domain instead of a p65 activation domain, and is encoded by SEQ ID NO: 26 (atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggcactcggcgaaacctggaccttatctgctcgctggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctggccgagctgcctaacggcctgggcgagacaagaggcagcggaggacgagacagacgacgacggcgaggacttcacccccccatcctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaaagccgtggtggagacactgctgcaggaagatccctggcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaaccagagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgccctgtacacttggtacgtgcggaagcagagagaggtggcccagcagtttacacacgccggccaggggcggcctgatcgaggaacctaccggcgacgagctgcccacaagaagggcagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccagcaaagaggaaacgggagacactggtggaagagtgcaccggggccgagtgcatccagagaggcgtgagcccttctcaggctcaggcctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagagggaagccttcggcacaagctgtctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcctgaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcaggtacgacccccaccagacccttcaggaggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggttcgtggacctgaccctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagcatggaacaccccgtgaagctgctgttcgcccccaacctgctcctggaccggaaccagggaagtgcgtggagggcagtggtggagatcttcgacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgagggaattcgtgtgcctgaagtccatcatcctgctgaacagcggcgtgtacaccttcctgtcatccaccctgaagtccctggaagagaaggaccacatccaccgggtgctggacaaga

tcaccgacaccctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgac
ctgagccacatccggcacatgagcaacaagggaatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacct
gctgctcgaggctgccgatgccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctca
cctggccaccgccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgt
ggaaaagtgcctcagcgtagcctgcctggacaagaatgagctcagtgaccacttgatgctatggactccaacctggataacctga
gacctgctgagcagccacggcttcagcgtggacaccagtgcctgctggacctgttcagcccctcggtgacctgcccacatga
gcctgcctgacctgacagcagcctggccagtatccaagagctcctgtctccccaggagccccccaggcctcccaggcagagaaac
agcagcccggattcagggaagcagctggtgcactacacagcgcagccgctgttcctgctggaccccggctccgtggacaccggga
gcaacgacctgccggtgctgtttgagctgggagagggctcctacttctccgaaggggacggcttcgcccaggacccccaccatctcc
ctgctgacaggctcggagcctcccaaagccaaggaccccactgtctcc). In some alternatives, herein the
chimeric transcription factor is HEA4(HSF-1) and is encoded by SEQ ID NO: 26. In some
alternatives, herein the chimeric transcription factor is HEA4(HSF-1) and comprises an
amino acid sequence set forth in SEQ ID NO: 27
(MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
WRSMEHPVKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA
QLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEADAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEKCLSVACLDKNELSDHLDAMDS
NLDNLQTMLSSHGFSVDTSALLDLFSPSVTVPDMSLPDL DSSLASIQELLSPQEPPRPP
EAENSSPD SGKQLVHYTAQPLFLLDPGSVD TGSNDLPVLFELGEGSYFSEGDGFAEDP
TISLLTGSEPPKAKDPTVS). Some alternatives include a nucleic acid comprising a nucleic
acid sequence that encodes SEQ ID NO: 27.

[0152] In some alternatives herein the chimeric transcription factor is HEA4(Arnt). HEA4(Arnt) comprises HNF1alpha, ER-LBD that is the same as HEA4, and an Arnt activation domain instead of an p65 activation domain amino acid sequence. In some

alternatives, HEA4(Arnt) is encoded by a sequence set forth in SEQ ID NO: 28 (atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcagg cactcggcgaacctggaccttatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagct ggccgagctgcctaacggcctgggcgagacaagaggcagcgaggacgagacagacgacgacggcgaggacttcaccccccc atcctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccc tggcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaac cagagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacacttggtacgtgcgg aagcagagagaggtggcccagcagtttacacacgcccggccaggggcgccctgatcgaggaacctaccggcgacgagctgccac caagaaggcgagacggaaccgggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcgagaagaacccc agcaaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcag ggccctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcgaaagaggaagccttcggcacaagct gtctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagc ctgaccgcccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccccaccagacccttcagc gaggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggc ttcgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagc atggaacaccccgtgaagctgctgttcgcccccaacctgctcctggaccggaaccagggaagtgctggaggggcatggtggaga tcttcgacatgctgctggccacctccagccggttcgggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtccatcatcct gctgaacagcggcgtgtacaccttctgtcatccacctgaagtcctggaagagaaggaccacatccaccgggtgctggacaaga tcaccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatc ctgagccacatccggcacatgagcaacaagggaatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacct gctgctcgaggctgccgatgcccacagactgcacgcccctacaagcagaggcgagccagcgtggagggaaccgaccagtctca cctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcacccggcgaggccgagggattccctgccacct gcctgaggtcttccaggagatgctgtccatgctgggagatcagagcaacagctacaacaatgaagaattccctgatctaactatgttc ccccttttcagaa). In some alternatives, HEA4(Arnt) comprises an amino acid sequence set forth

in	SEQ	ID	NO:	29
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(MVS KLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF

SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLV
 WRSMEHPVKLLFAPNLLLDNRNQGKCEGMVEIFDMLLATSSRFRMMNLQGEEFVC
 LKSIILLNSGVYTFLLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQR
 LAQLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEAADAHRLHAPTSRGGASVE
 ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVPEVFQEMLSMGLGDQSNSYNNEEF
 PDLTMFPPFSE). Some alternatives include a nucleic acid comprising a nucleic acid
 sequence that encodes SEQ ID NO: 29.

[0153] "Selectable marker cassette," has its plain and ordinary meaning when read in light of the specification, and may be used, for example, to describe a gene that encodes a "selectable marker" and is introduced into a vector or a cell that confers a trait for artificial selection. A selectable marker cassette can be a screenable marker to allow a researcher to distinguish between wanted and unwanted cells, or to enrich for a specific cell type. In some alternatives, a gene delivery polynucleotide is provided. In some alternatives, the gene delivery polynucleotide comprises a selectable marker cassette. The selectable marker cassette as described herein, encodes a protein (the selectable marker) that is used for selecting or tracking a protein or cell that has a protein of interest. In the alternatives described herein, the fusion protein provided herein can comprise a marker sequence that can be selected in experiments, such as flow cytometry. In some alternatives, the marker is the protein Her2tG.

[0154] In some alternatives, the selectable marker is encoded by an EGFRt nucleotide sequence, wherein the selectable marker is encoded by a sequence set forth in SEQ ID NO: 30
 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacgcaaagtgtgtaacggaataggt
 attggtgaatttaaagactcactctccataaatgctacgaatattaacacttcaaaaactgcacctccatcagtggcgatctccacatcct
 gccggtggcatttaggggtgactccttcacacatactcctcctctggatccacaggaactggatattctgaaaaccgtaaaggaaatca
 cagggttttctgctgattcaggcttggcctgaaaacaggacggacctccatgcctttgagaacctagaaatcatacgcggcaggaccaa
 gcaacatggtcagttttcttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtgatggagatgt
 gataatttcaggaaacaaaatttgctatgcaaatacaataaactggaaaaactgttgggacctccggtcagaaaacccaaaattat
 aagcaacagaggtgaaaacagctgcaaggccacaggccaggctctgcatgccttgtgctccccgagggctgctggggcccgga
 gccagggactgcgtctcttgcggaatgtcagccgaggcagggaatgcgtggacaagtgaaccttctggagggtgagccaagg
 gagtttgggagaactctgagtgcatcagtgccacccagagtgccctgcctcaggccatgaacatcacctgcacaggacggggacc

agacaactgtatccagtgtgccactacattgacggccccactgCGTcaagacctgcccggcaggagtcattgggagaaaacaacacccctggtctggaagtacgcagacgcccggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggcccaggtcttg aaggctgtccaacgaatgggcctaagatcccgtccatcgccactgggatgggtggggccctcctcttgctgctggtggtggccctgg ggatcggcctctcatgtga). In some alternatives, the selectable marker comprises an EGFRt amino acid sequence set forth in SEQ ID NO: 31 (MLLLVTSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTISISGDLHI LPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRT KQHGGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKT KIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGE PREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGE NNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMVGALLLLL VVALGIGLFM). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 31.

[0155] In some alternatives, the selectable marker is encoded by a Her2t nucleotide sequence, set forth in SEQ ID NO: 32 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccatgccaccctgagtgtcagcccca gaatggctcagtgcactgttttgaccggagggtgaccagtgtgtggcctgtgccactataaggaccctcccttctgCGTggcccgtg ccccagcgggtgtgaaacctgacctctcctacatgccatctggaagttccagatgaggagggcgcatgccagccttgccccatca actgcacccactcctgtgtggacctggatgacaagggctgccccgccgagcagagagccagccccttgacgtccatcatctctgcg gtggttggcattctgctggctcgtggtcttgggggtggtctttgggatcctcatctga). In some alternatives, the selectable marker comprises the Her2t amino acid sequence set forth in SEQ ID NO: 33 (MLLLVTSLLLCELPHPAFLIPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCV ARCP SGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTSII SAVVGILLVVVLGVVFGILI). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 33.

[0156] In some alternatives, the selectable marker is encoded by theHer2tG nucleotide sequence set forth in SEQ ID NO: 34 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccatgccaccctgagtgtcagccccag aatggctcagtgcactgttttgaccggagggtgaccagtgtgtggcctgtgccactataaggaccctcccttctgCGTggcccgtg ccccagcgggtgtgaaacctgacctctcctacatgccatctggaagttccagatgaggagggcgcatgccagccttgccccatcaa ctgcacccactcctgtgtggacctggatgacaagggctgccccgccgagcagagagccagcccgttaacgggtggaggcagcgg

aggtggctccatcatctctgcggtggtggcattctgctggctcgtggcttgggggtggtcttgggatcctcatctga). In some alternatives, the selectable marker comprises the Her2tG amino acid sequence, set forth in SEQ ID NO: 35 (MLLLVTSLLLCELPHPAFLIPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCV ARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTGG GSGGGSIIISAVVGILLVVVLGVVFGILI). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 35.

[0157] In some alternatives, the selectable marker is encoded by the CD19t nucleotide sequence, set forth in SEQ ID NO: 36 (atgccacctcctcgcctcctcttcttctcctcctcctcaccatgggaagtcaggcccgaggaaacctctagtgggtgaaggtggaag agggagataacgctgtgctgcagtgcccaaggggacctcagatggccccactcagcagctgacctgggtctcgggagtcctcgctt aaaccttcttaaaactcagcctggggctgccaggcctgggaatccacatgaggccctggccatctggctttcatcttcaacgtctt caacagatggggggcttctacctgtgccagccggggccccctctgagaaggcctggcagcctggctggacagtcagtgtggagg gcagcggggagctgttccgggtggaatgtttcggacctaggtggcctgggctgtggcctgaagaacaggtcctcagaggggccccagc tccccctccgggaagctcatgagccccaagctgtatgtgtgggccaagaccgacctgagatctgggagggagagcctccgtgtgtc ccaccgagggacagcctgaaccagagcctcagccaggacctcaccatggccccctggctccacactctggctgtcctgtgggtacc ccctgactctgtgtccagggggccccctctcctggacctatgtgcacccaaggggcctaagtcattgctgagcctagagctgaagga cgatcgccggccagagatatgtgggtaatggagacgggtctgttgttggccggccacagctcaagacgctggaaagtattattgt caccgtggcaacctgacctgtcattccacctggagatcactgctcgccagctactatggcactggctgctgaggactgggtggctgga aggtctcagctgtgactttggcttatctgatcttctgcctgtgttccctgtgggcattcttcatcttcaaagagccctggctcctgaggagga aaagataa). In some alternatives, the selectable marker comprises the CD19t amino acid sequence, set forth in SEQ ID NO: 37 (MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESP LKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVN VEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGE PPCVPPRDSL NQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLS LELKDDRPARDMWVMETGLLLPRATAQDAGKYYCHRGNL TMSFHLEITARPVLWH WLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRKR). Some alternatives include a nucleic acid comprising a nucleic acid sequence that encodes SEQ ID NO: 37.

[0158] In some alternatives, the selectable marker is encoded by the DHFRdm nucleotide sequence set forth in SEQ ID NO: 38

(atggttggttcgctaaactgcatcgctcgctgtgtcccagaacatgggcatcggcaagaacggggacttcccctggccaccgctcag
 gaatgaatccagatatttccagagaatgaccacaacctcttcagtagaaggtaaacagaatctggtgattatgggtaagaagacctggt
 tctccattcctgagaagaatcgacctttaaagggtagaattaatttagttctcagcagagaactcaaggaacctccacaaggagctcattt
 tcttccagaagtctagatgatgccttaaaacttactgaacaaccagaattagcaaataaagtagacatggtctggatagttggtggcagt
 tctgtttataaggaagccatgaatcaccaggccatcttaactatttggacaaggatcatgcaagactttgaaagtgacacgtttttcc
 agaaattgatttggagaaatataaacttctgccagaataccagggtgttctctctgatgtccaggaggagaaaggcattaagtacaaattt
 gaagtatatgagaagaatgat). In some alternatives, the selectable marker comprises the DHFRdm
 amino acid sequence, set forth in SEQ ID NO: 39
 (VGSLNCIVAVSQNMGIGKNGDFWPPLRNESRYFQRM TTTSSVEGKQNLVIMGKKT
 WFSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW
 IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQE
 EKGIKYKFEVYEKND). Some alternatives include a nucleic acid comprising a nucleic acid
 sequence that encodes SEQ ID NO: 39.

[0159] In some alternatives, a system for inducible expression of a chimeric antigen receptor is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor comprising a ligand binding domain specific for a ligand selected from a tumor specific molecule, viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is preferably optimized; a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter operably linked to nucleic acid coding for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives, the drug is tamoxifen and/or its metabolites. In some alternatives, the second promoter is constitutive or inducible. In some alternatives, the first promoter comprises a nucleic acid sequence of SEQ ID NO: 23 (SEQ ID NO: 23;

tagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaatagttaataatctacaagagctcagggtatataatg). In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1 α p. In some alternatives, the transcriptional activator comprises an amino acid sequence set forth in SEQ ID NO's: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is a lentivirus. In some alternatives, the first and second nucleic acid comprise a vector. In some alternatives, the first nucleic acid further comprises a nucleic acid sequence coding for a selectable marker. In some alternatives, the second nucleic acid further comprises a nucleic acid coding for a selectable marker. In some alternatives, a system for inducible expression is provided, wherein the system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor. In some alternatives, the transcriptional activator is HEA3 or HEA4. In some alternatives, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises mutations that allow selective binding to the drug ligand. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some

alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least three mutations that allow selective binding to the drug ligand, wherein HEA4 comprises an estrogen receptor ligand binding domain. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to the drug ligand. Mutations in the ER-LBD affect drug to receptor binding. In some alternatives, the HEA3 further comprises an HNF1alpha DNA binding domain. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand, wherein HEA4 comprises an estrogen receptor ligand binding domain, wherein the estrogen receptor ligand binding domain comprises mutations that allow selective binding to the drug ligand. In some alternatives, the HEA4 further comprises an HNF1alpha DNA binding domain. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives, HEA4 comprises three mutations in the ER-LBD that enhance sensitivity to drug compared to HEA3. In some alternatives, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives, the variant of a wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives, the wild type HEA3 transcription factor comprises an amino acid sequence set forth

in	SEQ	ID	No:	1
	(MVS	KLS	Q	TELLA
	A	ALLES	G	LSKEA
	L	I	Q	ALGEP
	G	P	Y	LLAGE
	G	P	L	D
	K	G	E	S
	C	G	G	G
	R	G	E	L
	A	E	L	P
	N	G	L	G
	E	T	R	G
	S	E	D	E
	T	D	D	D
	G	E	D	F
	T	P	P	I
	L	K	E	L
	N	L	S	P
	E	E	A	A
	H	Q	K	A
	V	V	E	T
	L	L	Q	E
	D	P	W	R
	V	A	K	M
	V	K	S	Y
	L	Q	Q	H
	N	I	P	Q
	R	E	V	V
	D	T	T	G
	L	N	Q	S
	H	L	S	Q
	H	L	N	K
	G	T	P	M
	K	T	Q	K
	R	A	A	L
	Y	T	W	Y
	V	R		
	K	Q	R	E
	V	A	Q	Q
	F	T	H	A
	G	Q	G	L
	I	E	E	P
	T	G	D	E
	L	P	T	K
	K	G	R	R
	N	R	F	K
	W	G	P	A
	S	Q	Q	I
	L	F	Q	A
	Y	E	R	Q
	K	N	P	
	S	K	E	E
	R	E	T	L
	V	E	E	C
	N	R	A	E
	C	I	Q	R
	G	V	S	P
	S	Q	A	Q
	G	L	G	S
	N	L	V	T
	E	V	R	V
	Y	N	W	F
	A	N	R	R
	K	E	E	A
	F	R	H	K

LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQR
LAQLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
DFSALLSQISS). SEQ ID NO: 1 comprises one point mutation in the ER-LBD that ablates
binding to endogenous estrogen, but confers nanomolar specificity to 4-OHT, fulvestrant and
other estrogen analogs. In some alternatives, the variant of a wild type HEA3 transcription
factor comprises an amino acid sequence set forth in SEQ ID No: 2 (SEQ ID NO: 2;
MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHNLKGTMPKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQR
LAQLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM
DFSALLSQISS). SEQ ID NO: 2 comprises a point mutation in HEA3, in the p65 domain
which enhances transcriptional activity and is referred to as HEA3 (p65/S536E positions in

the RelA protein). In some alternatives, the variant of a wild type HEA3 transcription factor comprises an amino acid sequence set forth in SEQ ID NO: 3 (SEQ ID NO: 3; MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRITYET FQSIMKKSPFSGPTDPRPPPRRIA VPSRSSASVPKPAPQPYPTSSLSTINYDEFPTMVFP SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM DFSALLSQISS). SEQ ID NO: 3 comprises a sequence of HEA3 containing one point mutation (K310Q positions in the RelA protein) in the p65 domain that enhances transcriptional activity. This is referred to as HEA3(p65/K310Q). In some alternatives, the variant of the wild type HEA3 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives, the variant of a wild type HEA4 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 4-7. In some alternatives, the variant of the wild type HEA4 transcription factor is encoded by at least one or more nucleic acid sequences set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives, the variant of a wild type HEA3 transcription factor comprises an amino acid sequence set forth in SEQ ID NO: 8 (SEQ ID NO: 8; MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR

KQREVAQQFTHAGQGGLIEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWMEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCVEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRQAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYETF
 KSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPTSSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK
 PTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIAD MDF
 SALLSQISS). SEQ ID NO: 8 comprises the sequence of HEA3 containing three point
 mutations (L384M, M421G, G521R) in the ER-LBD that ablate binding to endogenous
 estrogen, and confer nanomolar sensitivity to CMP8 and 4-hydroxytamoxifen. This is
 referred to as CMP8-HEA3. In some alternatives, the variant of a wild type HEA3
 transcription factor comprises an amino acid sequence set forth in SEQ ID NO: 9 (SEQ ID
 NO: 9;

MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHNLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWMEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCVEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRQAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYETF
 KSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPTSSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK

PTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADMDF
 SALLSQISS). SEQ ID NO: 9 is the sequence of CMP8-HEA3 containing one point mutation
 (S536E positions in the RelA protein) in the p65 domain that enhances transcriptional
 activity. This is referred to as CMP8-HEA3(p65/S536E positions in the RelA protein). In
 some alternatives, the variant of a wild type HEA3 transcription factor comprises an amino
 acid sequence set forth in SEQ ID NO: 10 (SEQ ID NO: 10;
 MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWMEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLLAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYETF
 QSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK
 PTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDF
 SALLSQISS). SEQ ID NO: 10 is the sequence of CMP8-HEA3 containing one point
 mutation (K310Q positions in the RelA protein) in the p65 domain that enhances
 transcriptional activity. This is referred to as CMP8-HEA3(p65/K310Q positions in the RelA
 protein). In some alternatives, the variant of a wild type HEA3 transcription factor comprises
 an amino acid sequence set forth in SEQ ID NO: 11 (SEQ ID NO: 11;
 MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP

SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWMEILMIGLV
 WRSMEHPGKLLFAPNLLDRNQGKCVEGGVEIFDMLLATSSRFRMMNLQGEEFVCL
 KSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLLAQ
 LLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVEE
 TDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRRKRTYETF
 QSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPS
 GQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPK
 PTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPV
 APHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADMDF
 SALLSQISS). SEQ ID NO: 11 comprises the sequence of CMP8-HEA3 containing two
 point mutations (K310Q and S536E positions in the RelA protein) in the p65 domain that
 enhance transcriptional activity. This is referred to as CMP8-HEA3(p65/S536E/K310Q
 positions in the RelA protein). In some alternatives, the polypeptide that modulates
 checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the
 negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3. In some
 alternatives, the second promoter is constitutive or inducible. In some alternatives, the
 second nucleic acid further comprises a polynucleotide encoding a chimeric antigen receptor
 comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or
 another molecule expressed on a target cell population, wherein the ligand elicits
 recognition, modulation, inhibition, and/or elimination by a lymphocyte, a polynucleotide
 coding for a polypeptide spacer, wherein the spacer is optimized, a polynucleotide coding for
 a transmembrane domain and a polynucleotide encoding an intracellular signaling domain. In
 some alternatives, the first promoter is in opposite orientation to the second promoter. In
 some alternatives, the ligand binding domain is an antibody fragment, preferably a binding
 fragment thereof. In some alternatives, the ligand binding domain is a single chain variable
 fragment. In some alternatives, the tumor specific molecule is CD19, CD20, CD22, CD23,
 CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, or
 MAGE A3 TCR or combinations thereof. In some alternatives, the system employs a
 synthetic transcriptional activator which, in the presence of a drug binds a synthetic promoter

upstream of a transgene to induce expression. In some alternatives, the transcriptional activator is HEA3. In some alternatives, the drug is tamoxifen. In some alternatives, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein.

[0160] Additional changes can be made to the transcriptional activator to increase the properties of the transcription factor including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain and/or altering one or more amino acids in the p65 transactivating domain. Altering amino acids in the estrogen receptor binding domain can provide for more specific and higher sensitivity binding of the drug to the transcriptional activator. In some alternatives, HEA4 comprises at least three point mutations. For example, mutations can be made at amino acid position 400, 543, and 544. In some alternatives, the wild type HEA4 comprises the sequence set forth in SEQ ID NO: 4 (SEQ ID NO: 4; MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV WRSMEHPVKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRLA QLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEADAHRLHAPTSRGGASVE ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET FKSIMKKSPFSGPTDPRPPPRRIA VPSRSSASVPKPAPQPYPTSSLSTINYDEFPTMVFP SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM DFSALLSQISS). SEQ ID NO: 4 comprises the sequence of wild-type HEA4 containing three point mutations (G400V, M543A, L544A) in the ER-LBD that ablate binding to

endogenous estrogen, and confer higher sensitivity to 4-hydroxytamoxifen, fulvestrant, and estrogen analogs compared to wild-type HEA3. The transcriptional activator with altered sequence has increased affinity for tamoxifen or 4-hydroxytamoxifen, fulvestrant, and estrogen analogs compared to wild-type HEA3. Altering amino acids in the p65 transactivating domain can provide for increased expression of the transgene in the absence of activation of the transduced cells (SEQ ID NO: 5 and SEQ ID NO: 6).

[0161] In the absence of tamoxifen, TamR-tf is excluded from the nucleus by binding of cytosolic heat-shock protein 90 (HSP90) to the tamoxifen binding active site and transgene expression is in the “OFF” state. Nanomolar concentrations of cytosolic tamoxifen actively outcompete HSP90 for ER-LBD binding, resulting in TamR-tf translocation to the nucleus. Upon nuclear translocation, TamR-tf is readily available to bind its restricted synthetic promoter. In the presence of tamoxifen, binding of TamR-tf to 7xHBD/mE1B promoter induces the “ON” state of transgene expression. In some alternatives, this transcriptional regulator can be modified to provide for varying level of control of transgene expression. Amino acid substitutions in the LBD of TamR-tf (HEA3) permit selective responsiveness to tamoxifen and its metabolites, where 4-hydroxy tamoxifen (4-OHT) is the most pharmacologically active metabolite, with respect to TamR-tf (HEA3) activity, while lacking interaction with endogenous estrogen.

[0162] In some alternatives, a system for inducible expression of a transgene is provided. In some alternatives, the transgene encodes a chimeric antigen receptor. In some alternatives, a system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, is provided. In some alternatives, a system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, is provided, the system comprises: a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral specific molecule, or other molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b)

a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives, the transcriptional activator is HEA3 or HEA4. In some alternatives, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective and high sensitivity binding to the drug ligand. In some alternatives, the HEA3 further comprises an HNF1alpha DNA binding domain. Mutations in the ER-LBD affect drug-to-receptor binding. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective and high sensitivity binding to the drug ligand. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives, the HEA4 further comprises an HNF1alpha DNA binding domain. In some alternatives, HEA4 comprises three mutations in the ER-LBD that enhance sensitivity to drug compared to HEA3. Mutations in the ER-LBD affect drug-to-receptor binding. In some alternatives, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives, the variant of the wild type HEA3 transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives, the HEA3 transcription factor is encoded by a nucleic acid sequence set forth in

SEQ ID NO: 12 (SEQ ID NO: 12;

atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
actcggcgaacctggaccttatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
gccgagctgcctaacggcctgggcgagacaagaggcagcaggacgagacagacgacgacggcgaggacttcacccccccat
cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
gcggtgcgaagatggtcaagagctacctgcagcagcacaacatccccagcgggagggtggtggacaccaccggcctgaacca
gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccgtgtacacttggtacgtgcggaa
gcagagagaggtggcccagcagtttacacacgccggccaggcgccctgatcgaggaaacctaccggcgacgagctgccacca
agaaggcgagacggaaccggtttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccag
caaagaggaaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcagg
cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaaagccttcggcacaagctgt
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cgtggacctgacctgcacgaccagggtccacctgctggaatgtgctggctggaaatcctgatgatcgccctcgtgtggagaagcat
ggaacaccccggaagctgctgttcgcccccaacctgctcctggaccggaaccagggaagtgctggaggggcatggtggagatc
ttcgacatgctgctggccacctccagccggttcgggatgatgaacctgcagggcgaggaattcgtgtgctgaagtccatcatcctgc
tgaacagcggcgtgtacaccttctgtcatccacctgaagtccctggaagagaaggaccacatccaccgggtgctggacaagatca
ccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcctg
agccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctgct
gctcgagatgctggatgcccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctcacct
ggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtgga
gttcagtagctgcccagaccgacgaccggcaccggatcgaggaaaagcggaaagcggacctacgagacattcaagagcatcatg
aagaagtcccccttcagcggccccaccgatcccagacccccccctagaagaatcgccgtgccagcagatctagcggcagcgtgc
ccaagcctgccccccagccctaccctttaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccagcg
gccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgctcaggccccctgctccagccccagccccctgcatggt
gtctgcactggcccagggtccagctcctgtgctgtgctggccccctggacctcctcaggctgtggccccctcctgcccctaaacctacc
caggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatagca
ccgacccccgcgtgtttaccgacctggcctccgtggacaacagcaggttccagcagctcctcaaccaggggcatccctgtcgcccca
cacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagcac
cagctccactgggagccccctggcctgcctaattgggctgctgtctggcgacgaggacttctccagcattgccgacatggacttcagcg

ccctgtgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA3 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 13 (SEQ ID NO: 13;

atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
actcggcgaaacctggaccttatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
gccgagctgcctaacggcctgggcgagacaagaggcagcaggacgagacagacgacgacggcgaggacttcacccccccat
cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaacca
gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgacctgtacacttggtacgtgcggaa
gcagagagaggtggcccagcagtttacacacgccggccaggcgccctgatcgaggaaacctaccggcgacgagctgcccacca
agaagggcagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccag
caaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcaggg
cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcggcacaagctgt
ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct
gaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccccaccagacccttcagcg
aggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggctt
cgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcgccctcgtgtggagaagcat
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agccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctgct
gctcgagatgctggatgccacagactgcacgccccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctcacct
ggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtgga
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aagaagtcccccttcagcggccccaccgatcccagacccccctagaagaatcgccgtgcccagcagatctagcggcagcgtgc
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gccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgctcaggccccctgctccagccccagccccctgcatggt
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caggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatagca
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cacaccaccgagcccatgctgatggaatacccccaggccatcaccagactgggtcacaggcgcccagaggcctccagatccagcac
cagctccactgggagcccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttcagcg
ccctgctgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA3
transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 14 (SEQ
ID NO: 14;
atgggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
actcggcgaaacctggaccttatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
gccgagctgcctaaccggcctgggcgagacaagaggcagcgaggacgagacagacgacgacggcgaggacttcacccccccat
cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
gcggtgcgaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaacca
gagccacctgagccagcacctgaacaagggcaccatgaaaaccagaagagagccgacctgtacacttggtacgtgcggaa
gcagagagaggtggcccagcagtttacacacgcccggccaggcgccctgatcgaggaacctaccggcgacgagctgccacca
agaaggcgagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccag
caaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcaggg
cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcggcacaagctgt
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gaccgccgatcagatggtgtccgctctgctggacgcccagccccctatcctgtacagcgagtacgacccccaccagacccttcagcg
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gctcgagatgctggatgcccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctcacct
ggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtgga
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caggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatagca
 ccgacccccgccgtgtttaccgacctggcctccgtggacaacagcgagttccagcagctcctcaaccagggcatccctgtcgcccca
 cacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagcac
 cagctccactgggagcccctggcctgcctaattgggctgctgtctggcgacgaggacttctccagcattgccgacatggacttcagcg
 ccctgtgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA3
 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 19 (SEQ
 ID NO: 19;
 atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
 actcggcgaaacctggaccttatctgctcgctggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
 gccgagctgcctaacggcctgggcgagacaagaggcagcgaggacgagacagacgacgacggcgaggacttcacccccccat
 cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
 gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaacca
 gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgccctgtacacttggtacgtgcggaa
 gcagagagaggtggcccagcagtttacacacgccggccaggcgccctgatcgaggaacctaccggcgacgagctgcccacca
 agaaggcgagacggaaccggttaagtggggccctgcatctcagcagatcctgttccaggcctacgagcggcagaagaacccag
 caaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcaggg
 cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagctgt
 ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct
 gaccgccgatcagatggtgtccgtctgctggacgccgagccccctatcctgtacagcagtacgacccccaccagacccttcagcg
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cggccagatctctcaggcctctgctctggcacctgtccacctcaggtgctgcctcaggccccctgtccagccccagccccctgcatg
 gtgtctgactggcccagggtccagctcctgtgcctgtgctggccccctggacctcctcaggctgtggccccctcctgcccctaaaccta
 cccaggccggggagggaacactgtctgaggccctgtgcagctccagttcgacgacgaggatctgggagcactgctgggcaatag
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 accagctccactgggagccccctggcctgcctaattgggctgctgtctggcgacgaggacttctccagcattgccgacatggacttcag
 cgccctgctgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA3
 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 20 (SEQ
 ID NO: 20;
 atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
 actcggcgaaacctggaccttatctgctcgctggcgaaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
 gccgagctgcctaaccggcctggcgagacaagaggcagcgaggacgagacagacgacgacgagcaggacttcccccccccat
 cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
 gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaacca
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 caaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcaggg
 cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttccggcacaagctgt
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 accgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcct
 gagccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctg
 ctgctcgagatgctggatgcccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctcac
 ctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggttccctgccaccgtg
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atgaagaagtccttcagcggccccaccgatcccagacccccccctagaagaatcgccgtgccagcagatctagcgccagcgt
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 cggccagatctctcaggcctctgctctggcacctgtccacctcaggtgctgcctcaggccccctgtccagccccagccccctgcatg
 gtgtctgcactggcccagggtccagctcctgtgcctgtgctggccccctggacctcctcaggctgtggccccctcctgcccctaaaccta
 cccaggccggggagggaacactgtctgaggccctgtgcagctccagttcgacgacgaggatctgggagcactgctgggcaatag
 caccgaccccgccgtgtttaccgacctggcctccgtggacaacagcgagttccagcagctcctcaaccaggggcatccctgtcgccc
 cacacaccaccgagcccatgctgatggaataccccgaggccatcaccagactgtcacaggcgcccagaggcctccagatccagc
 accagctccactgggagccccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttcag
 cgccctgctgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA3

ID NO: 21;

atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
 actcggcgaaacctggaccttatctgctcgtggcgaaaggccctctggataagggcgagagctgtggcggagggaagaggagagctg
 gccgagctgcctaaccggcctggcgagacaagaggcagcaggacgagacagacgacgacggcgaggacttcacccccccat
 cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
 gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaacca
 gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacacttggtacgtgcggaa
 gcagagagaggtggcccagcagtttacacacgccggccaggcgccctgatcgaggaaacctaccggcgacgagctgcccacca
 agaagggcagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccag
 caaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcaggg
 cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagctgt
 ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct
 gaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcaggtacgacccccaccagacccttcagcg
 aggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggctt
 cgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggatggaaatcctgatgateggcctcgtgtggagaagcat
 ggaacaccccggaagctgctgttcgcccccaacctgctcctggaccggaaccagggaagtgcgtggagggcggcgtggagat
 cttegacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgagggaattcgtgtgcctgaagtccatcatcctg
 ctgaacagcggcgtgtacaccttctgtcatccacctgaagtcctggaagagaaggaccacatccaccgggtgctggacaagatc
 accgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcct
 gagccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctg
 ctgctcgagatgctggatgcccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctcac

ctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtg
gagttccagtagctgcccgcacccgacgaccggcaccggatcgaggaaaagcggaagcggaacctacgagacattccagagcatc
atgaagaagtcccccttcagcggccccaccgatcccagacccccccctagaagaatcgccgtgccagcagatctagcgccagcgt
gcccagcctgccccccagccctaccctttcaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccag
cgccagatctctcaggcctctgctctggcacctgtccacctcaggtgctgcctcaggccccctgctccagccccagccccctgcatg
gtgtctgactggcccagggtccagctcctgtgcctgtgctggccccctggacctcctcaggctgtggccccctcctgcccctaaaccta
cccaggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatag
caccgaccccgccgtgtttaccgacctggcctccgtggacaacagcgagttccagcagctcctcaaccaggggcatccctgtcgccc
cacacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagc
accagctccactgggagccccctggcctgcctaatgggctgctgtctggcgacgaggacttctccagcattgccgacatggacttcag
cgccctgctgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA3
transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 22 (SEQ
ID NO: 22;
atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
actcggcgaacctggaccttatctgctcgctggcgaaggccctctggataagggcgagagctgtggcggagggaagaggagagctg
gccgagctgcctaacggcctgggcgagacaagaggcagcaggacgagacagacgacgacgagcaggacttcccccccccat
cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggagggtggtggacaccaccggcctgaacca
gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacacttggtacgtgcggaa
gcagagagaggtggcccagcagtttacacacgccggccaggcgccctgatcgaggaaacctaccggcgacgagctgccacca
agaagggcagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccag
caaagaggaaacgggagacactggtggaagagtgaaccgggcccagtgatccagagaggcgtgagcccttctcaggctcaggg
cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaaagccttccggcacaagctgt
ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct
gaccgcccgatcagatggtgtccgctctgctggacgcccagccccctatcctgtacagcagtagcagccccaccagacccttcagcg
aggccagcatgatgggctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggtt
cgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggatggaaatcctgatgateggcctcgtgtggagaagcat
ggaacaccccggaagctgctgttcgcccccaacctgctcctggaccggaaccagggaagtgctggagggcggcgtggagat
cttcgacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgaggaaatcgtgtgcctgaagtccatcatcctg
ctgaacagcggcgtgtacaccttctgtcatccacctgaagtcctggaagagaaggaccacatccaccgggtgctggacaagatc
accgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcct

gagccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctg
ctgctcgagatgctggatgcccacagactgcacgcccctacaagcagaggcggagccagcgtggaggaaaccgaccagtctcac
ctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcacggcgaggccgagggattccctgccaccgtg
gagttccagtacctgcccgcacccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattccagagcatc
atgaagaagtcccccttcagcggccccaccgatcccagacccccccctagaagaatcgccgtgcccagcagatctagcgccagcgt
gcccagcctgccccccagccctaccctttcaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccag
cggccagatctctcaggcctctgctctggcacctgtccacctcaggtgctgcctcaggccccctgtccagccccagcccctgccatg
gtgtctgactggcccagggtccagctcctgtgcctgtgctggccccctggacctcctcaggctgtggccccctcctgcccctaaacct
cccaggccggggagggaacactgtctgaggccctgtgcagctccagttcgacgacgaggatctgggagcactgctgggcaatag
caccgaccccgccgtgtttaccgacctggcctccgtggacaacagcgagttccagcagctcctcaaccaggggcatccctgtcgccc
cacacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagc
accagctccactgggagccccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttcag
cgccctgtgtcccagatcagcagc). In some alternatives, the variant of a wild type HEA4
transcription factor comprises at least one or more amino acid sequences set forth in SEQ ID
Nos: 4-7. In some alternatives, the variant of the wild type HEA4 transcription factor is
encoded by a nucleic acid sequence set forth in SEQ ID NO: 15 (SEQ ID NO: 15;
atggtgtccaagctgtcccagctgcagacagaactgtggcagcactgctggaaagcggcctgagcaaaggccctgattcaggc
actcggcgaacctggaccttatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
gccgagctgcctaaccggcctgggcgagacaagaggcagcagggacgagacagacgacgacggcgaggacttcccccccccat
cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
gcggtgcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaacca
gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacattggtacgtgcggaa
gcagagagaggtggcccagcagtttacacacgccggccaggcgccctgatcgaggaacctaccggcgacgagctgccacca
agaagggcagacggaaccggtttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccag
caaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcaggg
cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagctgt
ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct
gaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccaccagacccttcagcg
aggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggctt
cgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagcat
ggaacaccccgtaagctgctgttcgcccccaacctgctcctggaccggaaccagggaagtgctgaggggcatggtggagatc

ttcgacatgctgctggccacctccagccgggtccggatgatgaacctgcagggcgaggaattcgtgtgctgaagtccatcatcctgc
tgaacagcggcgtgtacaccttctgtcatccacctgaagtccctggaagagaaggaccacatccaccgggtgctggacaagatca
ccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcctg
agccacatccggcacatgagcaacaagggaatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctgct
gctcgaggctgccgatgccacagactgcacgcccctacaagcagaggcggagccagcgtggaggaaaccgaccagtctcacct
ggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtgga
gttcagtagctgcccagaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattcaagagcatcatg
aagaagtcccccttcagcggccccaccgatcccagacccccccctagaagaatcgccgtgccagcagatctagcggcagcgtgc
ccaagcctgccccccagccctaccctttaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccagcg
gccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgcctcaggcccctgctccagccccagcccctgcatggt
gtctgactggcccaggctccagctcctgtgctgtgctggcccctggacctcctcaggctgtggcccctcctgcccctaaacctacc
caggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatagca
ccgacccccgcgtgtttaccgacctggcctccgtggacaacagcaggttccagcagctcctcaaccagggcatccctgtcgcccca
cacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagcac
cagctccactgggagcccctggcctgcctaattgggctgctgtctggcgacgaggacttctccagcattgccgacatggacttcagcg
ccctgctgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA4
transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 16 (SEQ
ID NO: 16;

atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
actcggcgaacctggaccttatctgctcgctggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
gccgagctgcctaacggcctgggcgagacaagaggcagcaggacgagacagacgacgacggcgaggacttcccccccat
cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggagggtggtggacaccaccggcctgaacca
gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacattggtacgtgcggaa
gcagagagaggtggcccagcagtttacacacgccggccaggcgggcctgatcgaggaaacctaccggcgacgagctgccacca
agaagggcagacggaaccggtttaagtggggccctgcatctcagcagatcctgttccaggcctacgagcggcagaagaacccag
caaagagggaacgggagacactggtggaagagtgaaccggggccgagtgcattccagagaggcgtgagcccttctcaggctcaggg
cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagctgt
ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct
gaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccaccagacccttcagcg
aggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggctt

cgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagcat
 ggaacaccccgtaagctgctgttcgccccaacctgctcctggaccggaaccagggaaagtgcgtggagggcatggtggagatc
 ttcgacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgaggaattcgtgtgctgaagtcacatcctgc
 tgaacagcggcgtgtacaccttcctgtcatccacctgaagtcctggaagagaaggaccacatccaccgggtgctggacaagatca
 ccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcctg
 agccacatccggcacatgagcaacaagggaatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctgct
 gctcgaggctgccgatgccacagactgcacgcccctacaagcagagggcgagccagcgtggaggaaaccgaccagtctcacct
 ggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtgga
 gttccagtacctgcccagaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattcaagagcatcatg
 aagaagtccttcagcggccccaccgatccagacccccctagaagaatcgccgtgccagcagatctagcggcagcgtgc
 ccaagcctgccccccagccctacccttcaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccagcg
 gccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgcctcaggccccctgctccagccccagccccctgcatggt
 gtctgactggcccaggctccagctcctgtgctgtgctggcccctggacctcctcaggctgtggcccctcctgcccctaaacctacc
 caggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatagca
 ccgacccccgctgtttaccgacctggcctccgtggacaacagcagagttccagcagctcctcaaccagggcatccctgtcgcccca
 cacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagcac
 cagctccactgggagcccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttcagcg
 ccctgctgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA4
 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 17 (SEQ

ID NO: 17;
 atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
 actcggcgaacctggaccttatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
 gccgagctgcctaaccggcctgggcgagacaagaggcagcagggacgagacagacgacgacggcgaggacttcacccccccat
 cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
 gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggagggtggtggacaccaccggcctgaacca
 gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacattggtacgtgcggaa
 gcagagagaggtggcccagcagtttacacacgcccggccaggcggcctgatcgaggaaacctaccggcgacgagctgcccacca
 agaagggcagacggaaccggtttaagtggggccctgcatctcagcagatcctgttccaggcctacgagcggcagaagaacccag
 caaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcaggg
 cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagctgt
 ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct

gaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccaccagacccttcagcg
 aggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggctt
 cgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagcat
 ggaacaccccgtgaagctgctgttcgcccccaacctgctcctggaccggaaccagggaaagtgcgtggagggcatggtggagatc
 ttcgacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtccatcatcctgc
 tgaacagcggcgtgtacaccttcctgtcatccacctgaagtccctggaagagaaggaccacatccaccgggtgctggacaagatca
 ccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcctg
 agccacatccggcacatgagcaacaagggaatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctgct
 gctcgaggctgccgatgccacagactgcacgcccctacaagcagaggcggagccagcgtggaggaaaccgaccagtctcacct
 ggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtgga
 gtccagtagctgcccagaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattccagagcatcatg
 aagaagtcccccttcagcggccccaccgatcccagacccccccctagaagaatcgccgtgccagcagatctagcggcagcgtgc
 ccaagcctgccccccagccctaccctttaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccagcg
 gccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgcctcaggcccctgctccagccccagcccctgcatggt
 gtctgcactggcccaggctccagctcctgtgcctgtgctggcccctggacctcctcaggctgtggcccctcctgcccctaaacctacc
 caggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatagca
 ccgacccccgcgtgtttaccgacctggcctccgtggacaacagcaggttcagcagctcctcaaccagggcatccctgtcgcccca
 cacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagcac
 cagctccactgggagcccctggcctgcctaattgggctgctgtctggcgacgaggacttctccagcattgccgacatggacttcagcg
 ccctgctgtcccagatcagcagc). In some alternatives, the variant of the wild type HEA4

transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 18 (SEQ
 ID NO: 18;

atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcaggc
 actcggcgaaacctggaccttatctgctcgctggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagctg
 gccgagctgcctaaccggcctgggcgagacaagaggcagcagggacgagacagacgacgacggcgaggacttcccccccat
 cctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccctg
 gcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggagggtggtggacaccaccggcctgaacca
 gagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacacttggtacgtgcggaa
 gcagagagaggtggcccagcagtttacacacgccggccaggcggcctgatcgaggaacctaccggcgacgagctgccacca
 agaagggcagacggaaccggtttaagtggggccctgcatctcagcagatcctgttccaggcctacgagcggcagaagaacccag
 caaagagggaacgggagacactggtggaagagtgaaccggggccgagtgcatccagagaggcgtgagcccttctcaggctcaggg

cctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcggcacaagctgt
ctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagcct
gaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccccaccagacccttcagcg
aggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggtt
cgtggacctgacctgcacgaccagggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagcat
ggaacaccccgtagagctgctgttcgcccccaacctgctcctggaccggaaccagggaaagtgcgtggagggcatggtggagatc
ttcgacatgctgctggccacctccagccgggtccggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtcacatcctgc
tgaacagcggcgtgtacaccttcctgtcatccacctgaagtccctggaagagaaggaccacatccaccgggtgctggacaagatca
ccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatcctg
agccacatccggcacatgagcaacaagggaatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacctgct
gctcgaggctgccgatgccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctcacct
ggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgtgga
gttcagtagctgcccagaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattccagagcatcatg
aagaagtcccccttcagcggccccaccgatccagacccccccctagaagaatcgccgtgccagcagatctagcggcagcgtgc
ccaagcctgccccccagccctaccctttaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccccagcg
gccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgcctcaggccccctgctccagccccagccccctgcatggt
gtctgcactggcccagggtccagctcctgtgcctgtgctggccccctggacctcctcaggctgtggccccctcctgcccctaaacctacc
caggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaatagca
ccgacccccgccgtgtttaccgacctggcctccgtggacaacagcagagttccagcagctcctcaaccagggcatccctgtcgcccca
cacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccagcac
cagctccactgggagcccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttcagcg
ccctgctgtcccagatcagcagc). In some alternatives, the drug is tamoxifen and/or its metabolites,
4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8.

[0163] In some alternatives, the inducible promoter functions in a lentiviral construct and/or in lymphocytes.

Chimeric Antigen Receptors.

[0164] A system with an enhanced sensitivity to a drug ligand for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, comprises: a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from a tumor specific molecule, a viral specific

molecule, or any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In other alternatives, another polynucleotide coding for a chimeric antigen receptor is under the control of a constitutive promoter. In some alternatives, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8.

Ligand binding domain.

[0165] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for a ligand binding domain. In some alternatives, the ligand binding domain specifically binds to a tumor a viral molecule, or other molecule expressed on a target or selected cell population, and it may be humanized. In some alternatives, a ligand binding domain, includes without limitation, receptors or portions thereof, small peptides, peptidomimetics, substrates, cytokines, and the like. In some alternatives, the ligand binding domain is an antibody or fragment thereof, preferably a binding fragment thereof, any of which may be humanized. A nucleic acid sequence coding for an antibody or antibody fragment can readily be determined. In a specific alternative, the polynucleotide codes for a scFv that specifically binds CD19. In other specific alternatives, the polynucleotide codes for a single chain Fv that specifically binds HER2, CE7, hB7H3, or EGFR and, optionally, said polynucleotide encodes a humanized version thereof. The sequences of these antibodies and binding domains thereof are known to or can readily be determined by those of skill in the art. In some alternatives, the polynucleotide codes for a single scFv that specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC or VAR2CSA.

[0166] Tumor antigens are proteins that are produced by tumor cells that elicit an immune response. The selection of the ligand binding domain of the invention will depend on the type of cancer to be treated, and can target tumor antigens or other tumor cell surface molecules. A tumor sample from a subject can be characterized for the presence of certain biomarkers or cell surface markers. For example, breast cancer cells from a subject can be positive or negative for each of Her2Neu, Estrogen receptor, and/or the Progesterone receptor. A tumor antigen or cell surface molecule that is found on the individual subject's tumor cells is selected. Tumor antigens and cell surface molecules are well known in the art and include, for example, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD19, CD20, CD22, CD23, CD123, CS-1, CE7, hB7H3, ROR1, mesothelin, c-Met, GD-2, and/or MAGE A3 TCR. In some alternatives, the tumor antigen or tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR and/or any combinations thereof. In some alternatives, a target molecule is a cell surface molecule that is found on tumor cells and is not substantially found on normal tissues, or restricted in its expression to non-vital normal tissues.

[0167] In some alternatives, the target is CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, GD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC, and/or VAR2CSA.

[0168] In one alternative, the target molecule on the tumor comprises one or more epitopes associated with a malignant tumor. Malignant tumors express a number of proteins that can serve as target antigens for T cell receptor or chimeric receptor mediated recognition. Other target molecules belong to the group of cell transformation-related molecules such as the oncogene HER-2/Neu/ErbB2. In some alternatives, the tumor antigen is selectively expressed or overexpressed on the tumor cells as compared to control cells of the same tissue type. In other alternatives, the tumor antigen is a cell surface polypeptide.

[0169] Once a tumor cell surface molecule that may be targeted with a chimeric receptor is identified, an epitope of the target molecule is selected and characterized. Antibodies that specifically bind a tumor cell surface molecule can be prepared using methods of obtaining monoclonal antibodies, methods of phage display, methods to generate

human or humanized antibodies, or methods using a transgenic animal or plant engineered to produce human antibodies. Phage display libraries of partially or fully synthetic antibodies are available and can be screened for an antibody or fragment thereof that can bind to the target molecule. Phage display libraries of human antibodies are also available. In some alternatives, antibodies specifically bind to a tumor cell surface molecule and do not cross react with nonspecific components such as bovine serum albumin or other unrelated antigens. Once identified, the amino acid sequence or polynucleotide sequence coding for the antibody can be isolated and/or determined.

[0170] Antibodies or antigen binding fragments include all or a portion of polyclonal antibodies, a monoclonal antibody, a human antibody, a humanized antibody, a synthetic antibody, a chimeric antibody, a bispecific antibody, a minibody, and a linear antibody. "Antibody fragments" has its plain and ordinary meaning when read in light of the specification, and may be used, for example, to described a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody and can readily be prepared. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. In some alternatives, the antibody fragments are Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; or multispecific antibodies formed from antibody fragments. Any of such aforementioned antibodies or antibody fragments can be humanized and used with the compositions and methods described herein.

[0171] In some alternatives, a number of different antibodies that bind to a particular tumor cell surface molecule can be isolated and characterized. In some alternatives, the antibodies are characterized based on epitope specificity of the targeted molecule. In addition, in some cases, antibodies that bind to the same epitope can be selected based on the affinity of the antibody for that epitope. In some alternatives, an antibody has an affinity of at least 1 mM, and preferably <50 nM. In some alternatives, the antibody has an affinity of 50 nM, 100 nM, 200 nM, 300nM 400 nM, 500 nM, 1uM, 100uM, 200uM, 300uM, 400uM, 500uM, 600uM, 700 uM, 800uM, 900uM or 1mM or an affinity within a range defined by any two of the aforementioned values. In some alternatives, an antibody is selected that has a higher affinity for the epitope, as compared to other antibodies. For

example, an antibody is selected that has at least a 2 fold, at least a 5 fold, at least a 10 fold, at least a 20 fold, at least a 30 fold, at least a 40 fold, or at least a 50 fold greater affinity than a reference antibody that binds to the same epitope or an affinity that is greater than a reference antibody within a range defined by any two of the aforementioned values.

[0172] In some alternatives, target molecules are CD19, CD20, CD22, CD23, CE7, hB7H3, EGFR, CD123, CS-1, ROR1, mesothelin, Her2, c-Met, PSMA, GD-2, or MAGE A3 TCR or any combination thereof. In some alternatives, the antibody or binding fragment thereof specific for these target molecules is humanized.

[0173] In specific alternatives, the target antigen is CD19, CD20, CD22, EGFRvIII, EphA2, IL13Ra2, oaGD2, GD2, CD33, Mesothelin, ROR-1, and/or VAR2CSA. A number of antibodies specific for CD19, CD20, CD22, EGFRvIII, EphA2, IL13Ra2, oaGD2, GD2, CD33, Mesothelin, ROR-1 and/or VAR2CSA are known to those of skill in the art and can be readily characterized for sequence, epitope binding, and affinity. The disclosure also contemplates variable regions that have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to that of the scFv for CD19, CD20, CD22, EGFRvIII, EphA2, IL13Ra2, oaGD2, GD2, CD33, Mesothelin, ROR-1 and/or VAR2CSA.

[0174] "Humanized antibodies," has its plain and ordinary meaning when read in light of the specification, and may be used, for example, and may refer to antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans. The process of "humanization" can be applied to monoclonal antibodies developed for administration to humans (for example, antibodies developed as anti-cancer drugs). Humanization can be desirable when the process of developing a specific antibody involves utilization of a non-human immune system (such as that in mice). The protein sequences of antibodies produced in this way are partially distinct from homologous antibodies occurring naturally in humans, and are therefore potentially immunogenic when administered to human patients. Humanized antibodies are distinct from chimeric antibodies, in that they have the protein sequences made more similar to human antibodies but can carry a larger stretch of non-human protein. A derivative of a humanized antibody can refer to a segment of an antibody or sequence that is derived from a humanized antibody. In some alternatives, the ligand binding domain comprises a humanized

antibody or portion thereof. In some alternatives, the ligand binding domain comprises a scFv. In some alternatives, the scFv is a humanized scFv.

[0175] In some alternatives, the scFv binds specifically to CD19, CD22, CD20, B7H3 receptor, L1CAM, EGFR, EGFRVIII, EphA2, FITC(E2), GD2, Her2, IL13R2a (hu08) VIVh, IL13Ra2, IL13Ra2, oaGD2, ROR1, CD33, Mesothelin, and/or VAR2CSA.

[0176] Humanization can be desirable in some alternatives for reducing the immunogenicity of monoclonal antibodies that are derived from xenogeneic sources, such as, for example, rodents. Humanization is also desirable in some alternatives so as to improve the interaction of the antibody or a fragment thereof with the human immune system. Due to the development of hybridoma technology, a large number of xenogeneic antibodies are highly immunogenic in humans, which can ultimately limit their clinical applications especially when administration may need to be repeated. Additionally, they can be rapidly removed from the circulation and can cause systemic inflammatory effects as well. Therefore, humanization strategies are desirable in some alternatives to circumvent these situations. Techniques for antibody humanization are known to those skilled in the art.

[0177] In some alternatives, a polynucleotide coding for a ligand binding domain is operably linked to a polynucleotide coding for a spacer region. In some alternatives, the polynucleotide coding for a ligand binding domain can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide with another polynucleotide coding for a ligand binding domain coding for a different antigen or that has different binding characteristics. For example, a restriction site, NheI, is encoded upstream of the leader sequence; and a 3' RsrII located within the hinge region allows subcloning of any desirable scFv into a chimeric receptor vector. In some alternatives, the polynucleotide is codon optimized for expression in mammalian cells, such as humans. Subcloning into a restriction site of a desired vector is a technique that is appreciated by those skilled in the art.

[0178] In some alternatives, the polynucleotide coding for a ligand binding domain is operably linked to a signal peptide. In some alternatives, the signal peptide is a signal peptide for granulocyte colony stimulating factor. Polynucleotides coding for other signal peptides such as CD8 alpha can be utilized. In some alternatives, the polynucleotide codes for CD8 alpha.

[0179] In some alternatives, the polynucleotide coding for a ligand binding domain is operably linked to a promoter. A promoter is selected that provides for expression of the chimeric antigen receptor in a mammalian cell, such as a human cell. In a specific alternative, the promoter is an inducible promoter.

Spacer

[0180] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for a spacer region. Typically, a spacer region is found between the ligand binding domain and the transmembrane domain of the chimeric receptor. In some alternatives, a spacer region provides for flexibility of the ligand binding domain and allows for high expression levels in lymphocytes. For example, a CD19-specific chimeric receptor having a spacer domain of 229 amino acids had less antitumor activity than a CD19-specific chimeric receptor with a short spacer region comprised of the modified IgG4 hinge only. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0181] In some alternatives, a spacer region has at least 10 to 229 amino acids, 10 to 200 amino acids, 10 to 175 amino acids, 10 to 150 amino acids, 10 to 125 amino acids, 10 to 100 amino acids, 10 to 75 amino acids, 10 to 50 amino acids, 10 to 40 amino acids, 10 to 30 amino acids, 10 to 20 amino acids, or 10 to 15 amino acids, or a length within a range defined by any two of the aforementioned lengths. In some alternatives, a spacer region has 12 amino acids or less, 119 amino acids or less, or 229 amino acids or less but greater than 1 or 2 amino acids. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0182] In some alternatives, the spacer region is derived from a hinge region of an immunoglobulin molecule. In some alternatives, a spacer region comprises all or a portion of the hinge region from a human IgG1, human IgG2, a human IgG3, or a human IgG4, and can contain one or more amino acid substitutions. In some alternatives, a portion of the hinge region includes the upper hinge amino acids found between the variable heavy chain and the core, and the core hinge amino acids including a polyproline region. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

[0183] In some alternatives, a short spacer region has 12 amino acids or less and comprises all or a portion of a IgG4 hinge region sequence or variant thereof, an intermediate spacer region has 119 amino acids or less and comprises all or a portion of a IgG4 hinge region sequence and a CH3 region or variant thereof, and a long spacer has 229 amino acids or less but not zero and comprises all or a portion of a IgG4 hinge region sequence, a CH2 region, and a CH3 region or variant thereof. In some alternatives, a short spacer region has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acids or a size within a range defined by any two of the aforementioned amino acid lengths. In some alternatives, a medium spacer region has 13, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 or 119 amino acids or a size within a range defined by any two of the aforementioned amino acid lengths. In some alternatives, a spacer region has 120, 130, 140, 150, 160, 170, 180, 190, 200, 210 or 219 amino acids or a size within a range defined by any two of the aforementioned amino acid lengths.

[0184] A polynucleotide coding for a spacer region can be readily prepared by synthetic or recombinant methods from the amino acid sequence. In some alternatives, a polynucleotide coding for a spacer region is operably linked to a polynucleotide coding for a transmembrane region. In some alternatives, the polynucleotide coding for the spacer region can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide with another polynucleotide coding for a different spacer region. In some alternatives, the polynucleotide coding for the spacer region is codon optimized for expression in mammalian cells, such as human cells.

[0185] In an alternative, the spacer region is a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 or a portion thereof, a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 in combination with all or a portion of a CH2 region or variant thereof, a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 in combination with all or a portion of a CH3 region or variant thereof, and a hinge region sequence from IgG1, IgG2, IgG3, or IgG4 in combination with all or a portion of a CH2 region or variant thereof, and/or a CH3 region or variant thereof. In some alternatives, a short spacer region is a modified IgG4 hinge sequence having 12 amino acids or less but greater than one or two amino acids, an intermediate sequence is a IgG4 hinge sequence with a CH3 sequence having 119 amino acids or less but

greater than one or two amino acids; or a IgG4 hinge sequence with a CH2 and CH3 region having 229 amino acids or less but greater than one or two amino acids.

Transmembrane domain.

[0186] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for a transmembrane domain. The transmembrane domain provides for anchoring of the chimeric receptor in the membrane.

[0187] In an alternative, the transmembrane domain that naturally is associated with one of the domains in the chimeric receptor is used. In some cases, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0188] The transmembrane domain can be derived either from a natural or a synthetic source. When the source is natural, the domain can be derived from any membrane-bound or transmembrane protein.

[0189] Transmembrane regions in some alternatives comprise at least the transmembrane region(s) of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3, CD45, CD4, CD8, CD9, CD16, CD22; CD33, CD37, CD64, CD80, CD86, CD134, CD137 and/or CD154.

[0190] A transmembrane domain can be synthetic or a variant of a naturally occurring transmembrane domain. In some alternatives, synthetic or variant transmembrane domains comprise predominantly hydrophobic residues such as leucine and valine, for example. In some alternatives, a transmembrane domain can have at least 80%, 85%, 90%, 95%, or 100% amino acid sequence identity with a transmembrane domain as set forth in SEQ ID NO: 110-113 or an amino acid sequence identity that is within a range defined by any two of the aforementioned values. Variant transmembrane domains preferably have a hydrophobic score of at least 50 as calculated by Kyte Doolittle.

[0191] A polynucleotide coding for a transmembrane domain can be readily prepared by synthetic or recombinant methods. In some alternatives, a polynucleotide coding for a transmembrane domain is operably linked to a polynucleotide coding for an intracellular signaling region. In some alternatives, the polynucleotide coding for a

transmembrane domain can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide coding for a transmembrane domain with another polynucleotide coding for a different transmembrane domain. In some alternatives, the polynucleotide coding for a transmembrane domain is codon optimized for expression in mammalian cells, such as human cells.

Intracellular signaling domain.

[0192] In some alternatives, the chimeric receptor nucleic acid comprises a polynucleotide coding for an intracellular signaling domain. The intracellular signaling domain provides for activation of one function of the transduced cell expressing the chimeric receptor upon binding to the ligand expressed on tumor cells. In some alternatives, the intracellular signaling domain contains one or more intracellular signaling domains. In some alternatives, the intracellular signaling domain is a portion of and/or a variant of an intracellular signaling domain that provides for activation of at least one function of the transduced cell.

[0193] Examples of intracellular signaling domains for use in a chimeric receptor of the disclosure include the cytoplasmic sequences of the CD3 zeta chain, and/or co-receptors that act in concert to initiate signal transduction following chimeric receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation and provide a T cell receptor like signal (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). Primary cytoplasmic signaling sequences that act in a stimulatory manner can contain signaling motifs, which are known as receptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences suitable for use with alternatives described herein include those derived from CD3 zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and/or CD66d.

[0194] In a preferred alternative, the intracellular signaling domain of the chimeric receptor can be designed to comprise the CD3-zeta signaling domain by itself or

combined with any other desired cytoplasmic domain(s). For example, the intracellular signaling domain of the chimeric receptor can comprise a CD3zeta chain and a costimulatory signaling region.

[0195] The costimulatory signaling region refers to a portion of the chimeric receptor comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for a response of lymphocytes to an antigen. Examples of such molecules suitable for use in the alternatives described herein include CD27, CD28, 4-1BB (CD 137), OX40, CD30, CD40, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, zeta chain associated protein kinase (ZAP70), and/or a ligand that specifically binds with CD83.

[0196] The intracellular signaling sequences of the chimeric receptor can be linked to each other in a random or specified order. In some alternatives, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length, such as 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length, can form the linkage. In one alternative, the intracellular signaling domains comprises all or a portion of the signaling domain of CD3-zeta or variant thereof and all or a portion of the signaling domain of CD28 or a variant thereof. In another alternative, the intracellular signaling domain comprises all or a portion of the signaling domain of CD3-zeta or variant thereof and all or a portion of the signaling domain of 4-1BB or variant thereof. In yet another alternative, the intracellular signaling domain comprises all or a portion of the signaling domain of CD3-zeta or variant thereof, all or a portion of the signaling domain of CD28 or variant thereof, and all or a portion of the signaling domain of 4-1BB or variant thereof. In a specific alternative, the amino acid sequence of the intracellular signaling domain comprises a variant of CD3zeta and a portion of the 4-1BB intracellular signaling domain.

[0197] In an alternative, a polynucleotide coding for an intracellular signaling domain comprises a 4-1BB intracellular domain linked to a portion of a CD3zeta domain. In other alternatives, a 4-1BB intracellular domain and a CD28 intracellular domain are linked to a portion of a CD3 zeta domain.

[0198] In some alternatives, the intracellular domain is encoded by a sequence set forth in SEQ ID NO: 114. In some alternatives, the intracellular domain is encoded by a

sequence set forth in SEQ ID NO: 116. In some alternatives, the intracellular domain is encoded by a sequence set forth in SEQ ID NO: 118. In some alternatives, the intracellular domain comprises an amino acid sequence set forth in SEQ ID NO: 115. In some alternatives, the intracellular domain comprises an amino acid sequence set forth in SEQ ID NO: 117. In some alternatives, the intracellular domain comprises an amino acid sequence set forth in SEQ ID NO: 119.

[0199] A polynucleotide coding for an intracellular signaling domain can be readily prepared by synthetic or recombinant methods from the amino acid sequence. In some alternatives, the polynucleotide coding for an intracellular signaling domain can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide coding for an intracellular signaling domain with another polynucleotide coding for a different intracellular signaling domain. In some alternatives, the polynucleotide coding for an intracellular signaling domain is codon optimized for expression in mammalian cells. In some alternatives, the mammalian cells are human cells.

Marker sequences.

[0200] In some alternatives, the system further comprises one or more marker sequences under the control of an inducible promoter. A marker sequence can provide for selection of transduced cells, and/or identification of transduced cells. In some alternatives, the marker sequence is for a selection of transduced cells and/or identification of transduced cells. In some alternatives, the marker sequence is operably linked to a polynucleotide sequence coding for a linker sequence. In some alternatives, the linker sequence is a cleavable linker sequence. In some alternatives, the linker is 2A linker such as, for example, T2A, P2A or F2A. In some alternatives, the linker is a cleavable T2A linker.

[0201] A number of different marker sequences can be employed. Typically, a marker sequence has a functional characteristic that allows for selection of transduced cells and/or detection of transduced cells. In some alternatives, the marker sequence is compatible with transduction of human lymphocytes. In some alternatives, the marker sequence allows for selection of transduced cells and/or detection of transduced cells.

[0202] The positive selectable marker can be a gene, which upon being introduced into the host cell, expresses a dominant phenotype permitting positive selection of

cells carrying the gene. Genes of this type are known in the art, and include, inter alia, hygromycin-B phosphotransferase gene (hph) which confers resistance to hygromycin B, the amino glycoside phosphotransferase gene (neo or aph) from Tn5, which codes for resistance to the antibiotic G418, the double mutant of the dihydrofolate reductase (DHFR) gene, which provides resistance to methotrexate, DHFR dm. Transduced cells cultured in the presence of these agents will survive and be selected.

[0203] In an alternative, a first nucleic acid further comprises a polynucleotide coding for a marker sequence. In an alternative, the marker sequence is a truncated epidermal growth factor receptor (EGFRt). In some alternatives, the marker sequence is a truncated Her2 sequence (Her2t). Selectable marker sequences that can be used in the alternatives set forth in this disclosure are provided below.

SELECTABLE MARKER SEQUENCES

EGFRt nucleotide sequence:

Atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacgcaaagtgtgtaacggaataggt attggtgaattaaagactcactctccataaatgctacgaatattaaacacttcaaaaactgcacctccatcagtggcgatctccacatcct gccggtggcatttaggggtgactccttcacacatactcctcctctggatccacaggaactggatattctgaaaaccgtaaaggaaatca cagggttttgctgattcaggcttggcctgaaaacaggacggacctccatgccttgagaacctagaaatcatacgcggcaggaccaa gcaacatggtcagttttcttgcagtcgtcagcctgaacataacatccttgggattacgctccctcaaggagataagtgatggagatgt gataattcaggaaacaaaatttgctatgcaaatacaataaactggaaaaaactgttgggacctccggtcagaaaaccaaattat aagcaacagaggtgaaaacagctgcaaggccacaggccaggctgcatgccttgtgctccccgagggctgctggggcccgga gccagggactgcgtctcttgcgggaatgtcagccgaggcagggaatgcgtggacaagtgaaccttctggagggtgagccaagg gagtttgggagaactctgagtgcatacagtgccaccagagtgctgctcaggccatgaacatcacctgcacaggacggggacc agacaactgtatccagtgtgccactacattgacggccccactgcgtcaagacctgcccggcaggagtcattgggagaaaacaaca ccctggtctggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggccaggctctg aaggctgtccaacgaatgggcctaagatcccgtccatcgccactgggatggtgggggcccctccttctgctgctggtggtggccctgg ggatcggcctctcatgtga (SEQ ID NO: 30);

EGFRt amino acid sequence:

MLLLVTSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSSISGDLHIL PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGR TK QHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKI ISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEP REFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCAPAGVMGEN NTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMVGALLLLLV VALGIGLFM (SEQ ID NO: 31);

Her2t nucleotide sequence:

Atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccatgccaccctgagtgtcagccccag aatggctcagtgcacctgttttgaccggaggctgaccagtgtgtggcctgtgccactataaggacctcccttctgcgtggcccgctg cccagcgggtgtgaaacctgacctctctacatgccatctggaagttccagatgaggagggcgcatgccagccttggcccatcaa ctgcacctcctgtgtggacctggatgacaagggtgccccgccgagcagagagccagccctctgacgtccatcatctctgcggt gggtggcattctgctggtcgtggtcttgggggtggtcttgggatcctcatctga (SEQ ID NO: 32);

Her2t amino acid sequence:

MLLLVTSLLLCELPHPAFLIPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVA
RCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTSIIS
AVVGILLVVVLGVVFGILI (SEQ ID NO: 33);

Her2tG nucleotide sequence:

Atgcttctctggtgacaagccttctgctctgtgagttaccacacccagcattcctctgatcccatgccaccctgagtgtcagccccag
aatggctcagtgacctgttttgaccggaggctgaccagtgtgtggcctgtgccactataaggacctcccttctgctggcccgctg
ccccagcgggtgtgaaacctgacctctctacatgccatctggaagtttcagatgaggaggcgcatgccagccttgccccatcaa
ctgacccactcctgtgtggacctggatgacaagggctgccccgccgagcagagagccagcccgtaacgggtggaggcagcgg
aggtggctccatcatctctgcggtggttgccattctgctggctggtcttgggggtggtctttgggatcctcatctga (SEQ ID
NO: 34);

Her2tG amino acid sequence:

MLLLVTSLLLCELPHPAFLIPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVA
RCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTGGG
SGGGSIIISAVVGILLVVVLGVVFGILI (SEQ ID NO: 35);

CD19t nucleotide sequence:

Atgccacctctcgctcctcttcttctcctctcctcaccatgggaagtcaggcccgaggaaacctctagtgggtgaaggtggaaga
gggagataacgctgtgctgcagtgctcaaggggacctcagatggccacctcagcagctgacctggtctcgggagtcctccgctta
aaccttcttaaaaactcagcctggggctgccaggcctgggaatccacatgaggccctggccatctggctttcatcttcaacgtctctc
aacagatggggggcttctacctgtgccagccggggccccctctgagaaggcctggcagcctggctggacagtcaatgtggaggg
cagcggggagctgttccgggtggaatgttcggacctaggtggcctgggctgtggcctgaagaacaggtcctcagaggggccccagct
ccccttccgggaagctcatgagcccaagctgtatgtgtgggccaaagaccgacctgagatctgggagggagagcctccgtgtgtc
ccaccgagggacagcctgaaccagagcctcagccaggacctcaccatggccctggctccacactctggctgtcctgtgggggtacc
ccctgactctgtgtccagggggccccctctcctggacctgtgcaccccaaggggcctaagtcattgctgagcctagagctgaagga
cgatgccccggccagagatatgtgggtaatggagacgggtctgttgttggccggccacagctcaagacgctggaaagtattattgt
cacctggcaacctgacctgtcattccacctggagatcactgctcggccagtactatggcactggctgctgaggactggtggctgga
aggtctcagctgtgactttggcttatctgatcttctgcctgtgttccctgtgggcattcttcatcttcaaagagccctggtcctgaggagga
aaagataa (SEQ ID NO: 36);

CD19t amino acid sequence:

MPPPRLLFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESP
LKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVN
VEGSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGE
PPCVPPRDSL NQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLS
LELKDDRPARDMWVMETGLLLPRATAQDAGKYYCHRGNL TMSFHLEITARPVLWH
WLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRRKR (SEQ ID NO: 37)

DHFRdm nucleotide sequence:

Atggttggttcgctaaactgcategtcgtgtgtccagaacatgggcatcggaagaacggggacttcccctggccaccgctcagg
aatgaatccagatatttccagagaatgaccacaacctcttcagtagaaggtaaacagaatctggtgattatgggtaagaagacctggtt
ctccattcctgagaagaatcgacctttaaagggtagaattaatttagttctcagcagagaactcaaggaaacctccacaaggagctcattt
ctttccagaagtctagatgatgccttaaaacttactgaacaaccagaattagcaataaagtagacatggctctggatagttggtggcagtt
ctgtttataaggaagccatgaatcaccagggccatcttaaaactatttgtgacaaggatcatgcaagactttgaaagtgacacgttttcca
gaaattgatttgagaaatataaactctgccagaataccaggtgttctctctgatgtccaggaggagaaaggcattaagtacaaatt
gaagtatatgagaagaatgat (SEQ ID NO: 38); and

DHFRdm amino acid sequence:

VGSLNCIVAVSQNMGIGKNGDFWPPLRNESRYFQRM TTTSSVEGKQNLVIMGKKT
WFSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVW

IVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQE
EKGIKYKFEVYEKND (SEQ ID NO: 39).

DNA spacer sequence:

Gctagcgtttaaacttaagcttggtaccgagctcgatccgccacc (SEQ ID NO: 120).

The DNA spacer sequence may be described also as the 7xHBD/mE1B_DNA spacer.

[0204] In some alternatives herein, the DNA spacer, a 46 base pair sequence, may reside in between the 7xHBD/mE1B promoter and inducible transgene. The DNA spacer which comprises SEQ ID NO: 120, allows enhanced gene expression of the transgene. In some alternatives herein, a vector construct comprising a DNA spacer between a promoter and a transgene showed an increase in transcription of the transgene as compared to a vector that had the promoter and the transgene without a DNA spacer. In some alternatives herein, the DNA spacer enhanced transgene expression as compared to a vector that did not have a DNA spacer between the promoter and the transgene. In some alternatives, a DNA spacer is between the mE1B promoter and the transgene.

[0205] In some alternatives, the polynucleotide coding for the marker sequence is operably linked to a polynucleotide coding for a linker sequence. In some alternatives, the linker is a 2A linker, such as, for example, T2A, P2A, E2A or F2A.

[0206] A polynucleotide coding for marker sequence can be readily prepared by synthetic or recombinant methods from the amino acid sequence. In some alternatives, a polynucleotide coding for a marker sequence is operably linked to a polynucleotide coding for an intracellular signaling domain. In some alternatives, the polynucleotide coding for a marker sequence can also have one or more restriction enzyme sites at the 5' and/or 3' ends of the coding sequence in order to provide for easy excision and replacement of the polynucleotide coding for a marker sequence with another polynucleotide coding for a different marker sequence. In some alternatives, the polynucleotide coding for a marker sequence is codon optimized for expression in mammalian cells, preferably humans.

[0207] In some alternatives, two or more marker sequences can be employed. In some alternatives, a first marker sequence is under control of a constitutive promoter and provides for an indication that the transduced cell is expressing the transgene. In other alternatives, a second marker sequence is under the control of the inducible promoter and provides an indication that the transgene expression has been induced. In some alternatives, the marker under the control of the inducible promoter can be used to select for cells in

which noninduced or basal expression is much lower than in other cells by selecting cells that have a lower expression of the marker sequence under the control of the inducible promoter and expand those cells for further applications. In some alternatives, the transgene encodes a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity. In some alternatives, the transgene encodes a protein that modulates T cells. In some alternatives, the transgene encodes a chimeric antigen receptor.

Other Genetic Components under control of Inducible Promoter.

[0208] In some alternatives, the first nucleic acid comprises a polynucleotide sequence coding for genes that promote survival and proliferation, genes that prevent apoptosis, and/or genes that inhibit negative checkpoint signaling under the control of an inducible promoter. Such genes include genes encoding IL-2, IL-7, IL-15, chemokine receptors, Bcl2, CA-Akt, dn-TGFbetaRIII, dn-SHP1/2, PD-1-IFNalpha and/or PD-1CD28 chimeras. These genes are also placed under the control of an inducible promoter as described herein. In some alternatives, the genes encode IL-2, IL-7, IL-15, chemokine receptors, chimeric cytokine receptors, Bcl2, CA-Akt, dn-TGFbetaRII, dn-SHP1/2, PD-1-IFNalpha, and/or PD-1CD28 chimeras. In some alternatives, the gene that modulates checkpoint signaling encodes a polypeptide that inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3.

[0209] In some alternatives, a first nucleic acid comprises a first inducible promoter linked to a polynucleotide coding for a cytokine, chemokine receptor, or chimeric cytokine receptor. chemokines, also referred to as chemotactic cytokines, are a group of structurally related proteins that regulate cell trafficking of lymphocytes. In some alternatives, the chemokines are homeostatic or inflammatory. Chemokine receptors include CCR2, CCR7, or CCR15. Cytokines include interleukins such as IL2, IL-12, IL-7, and/or IL-15, interferons, such as interferon δ , tumor necrosis factor, and a TLR4 agonist. Chimeric cytokine receptors can also include CCR(CD122). In some alternatives, the chemokine receptors comprise CCR2, CCR7, and/or CCR15. In some alternatives, the chemokine receptors include CCR2, CCR7, or CCR15. In some alternatives, the cytokines include

interleukins, wherein the interleukins are IL2, IL-12, IL-7 and/or Il-15 or interferons, wherein the interferons comprise interferon δ , tumor necrosis factor, or a TLR4 agonist.

[0210] In some alternatives, a first nucleic acid comprises a first inducible promoter linked to a polynucleotide coding for a polypeptide that regulates apoptosis. In some alternatives, genes that inhibit apoptosis include, for example, Bcl2, and/or CA-Akt. In some alternatives, the polypeptide is Bcl2 or CA-Akt. In some alternatives, genes that inhibit apoptosis include, for example, Survivin, Bcl2, CA-Akt, and/or dnCaspase3. In some alternatives, the polypeptide is Survivin, Bcl2, or CA-Akt and/or dnCaspase 3. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124 (Atgggtgccccgacgttgccccctgcctggcagccctttctcaaggaccaccgcatctctacattcaagaactggcccttcttgagggtgcgcctgcaccccgagcggtatggccgaggctggcttcactgccccactgagaacgagccagactggcccagtggttcttctgcttcaaggagctggaaggctgggagccagatgacgaccccatagaggaacataaaaagcattcgtccggttgcgcttctcttctgtcaagaagcagtttgaagaattaacccttggtgaattttgaaactggacagagaaagagccaagaacaaaattgcaaaggaaacaacaataagaagaagaatttgaggaaactgtgaagaaagtgcgccgtgccatcgagcagctggctgcaatggat). In some alternatives, Survivin comprises the amino acid sequence set forth in SEQ ID NO: 123 (MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTERMAEAGFIHCPTENEPDLAQCFFCFKELEGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDREERAKNKIAKETNNKKKEFEETVKKVRRRAIEQLAAMD).

[0211] In some alternatives, a first nucleic acid comprises a first inducible promoter linked to a polynucleotide coding for a polypeptide that modulates checkpoint signaling. Such genes include dn-TGFbetaR2, dn-SHP1/2, PD-1-IFNalpha, and/or PD-1CD28 chimeras. In some alternatives, the polypeptide is dn-TGFbetaR2, dn-SHP1/2, PD-1-IFNalpha, and/or PD-1CD28 chimeras. In some alternatives, the polypeptide that modulates checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3.

[0212] Any number of nucleic acids can be placed under the control of an inducible promoter including those coding for chimeric antigen receptor, a marker sequences, a cytokine, a chemokine, an inhibitor of apoptosis, and/ or an inhibitor of negative checkpoint signaling. In some alternatives, one or more inducible promoters can be utilized to provide for an adequate expression level of each of the nucleic acids. In some alternatives, constructs can be prepared with a gene such as a cytokine under the control of an inducible

promoter and a construct comprising a chimeric antigen receptor under the control of a constitutive promoter. Such constructs are useful to provide for cell survival and proliferation of transduced cells, for example lymphocytes expressing a chimeric antigen receptor.

[0213] In the systems provided herein, the systems are inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor.

[0214] In some alternatives, HEA3 comprises two amino acid mutations, wherein the sequence is encoded by a sequence set forth in SEQ ID NO: 122 (atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcagg cactcggcgaacctggacattatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagct ggccgagctgcctaacggcctgggcgagacaagaggcagcgaggacgagacagacgacgacggcgaggacttcaccccccc atcctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggaggagacactgctgcaggaagatccc tggcgggtcgccaagatgggtcaagagctacctgcagcagcacaacatccccagcgggaggtgggtggacaccacggcctgaac cagagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgccctgtacacttggtacgtgcgg aagcagagagaggtggcccagcagtttacacacgcccggccaggcgccctgatcgaggaacctaccggcgacgagctgcccac caagaaggcgagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccc agcaaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcag ggcctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagct gtctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagc ctgaccgcccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtagacccccaccagacccttcagc gaggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggc ttcgtggacctgacctgcacgaccaggtccacctgctggaatgtgctggctggaaatcctgatgatcggcctcgtgtggagaagc atggaacacccccggcaagctgctgttcgcccccaacctgctcctggaccggaaccagggaaagtgcgtggaggggcatggtggaga tcttcgacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtccatcatcct gctgaacagcggcgtgtacaccttctgtcatccacctgaagtccctggaagagaaggaccacatccaccgggtgctggacaaga tcaccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatc ctgagccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtgggtggccctgtacgacct gctgctcgagatgctggatggccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaaccgaccagtctca cctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggattccctgccaccgt ggagttccagtacctgcccgaaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctaagagacattccagagcat catgaagaagtcccccttcagcggccccaccgatccagacccccccctagaagaatcgccgtgcccagcagatctagcgccagc

gtgccaagcctgccccccagccctaccctttcaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttcccca
 gcggccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgcctcaggccccctgctccagccccagccccctgcat
 ggtgtctgcactggcccaggctccagctcctgtgcctgtgctggccccctggacctcctcaggctgtggccccctcctgccctaaacct
 acccaggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaata
 gcaccgacccccgccgtgtttaccgacctggcctccgtggacaacagcgagttccagcagctcctcaaccaggggcatccctgtcgcc
 ccacacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccag
 caccagctccactgggagccccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttca
 gcgccctgctgtcccagatcagcagc). In some alternatives, the HEA3 comprises an amino acid
 sequence set forth in SEQ ID NO: 121
 (MVSQLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
 AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
 VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
 WRSMEHPGKLLFAPNLLLDNRNQGKCEVGMVEIFDMLLATSSRFRMMNLQGEEFVC
 LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA
 QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
 ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
 FQSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
 SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
 KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
 VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM
 DFSALLSQISS). This sequence comprises the sequence of HEA3 containing two point
 mutations (K310Q and S536E, positions in the RelA protein) in the p65 domain that enhance
 transcriptional activity. This is referred to as HEA3(p65/S536E/K310Q). K310Q in RelA
 corresponds to position 621 in HEA3; S536E corresponds to position 846 in HEA3.

Constitutive Promoter Systems.

[0215] In other alternatives, a system comprises a second nucleic acid that comprises a constitutive promoter or a second inducible promoter linked to a transcriptional

activator. In other alternatives, a system comprises a second nucleic acid that comprises a promoter linked to a transcriptional activator. In some alternatives, the promoter is a constitutive promoter or an inducible promoter. In some alternatives, a constitutive promoter includes EF1 α promoter, actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. In some alternatives, viral promoters such as the CMV promoter are excluded. In some alternatives, the constitutive promoter can be linked to one or more of a polynucleotide coding for marker, or a chimeric antigen receptor as described herein.

Constitutive Promoters.

[0216] A constitutive promoter provides for continuous gene expression of the gene under the control of the promoter. In some alternatives, the constitutive promoter is a promoter that provides for gene expression in a lentiviral construct and/or in lymphocytes. In some alternatives, the promoter is not derived from a xenogenic source such as a plant or a virus.

[0217] In a specific alternative, the constitutive promoter comprises EF1 α promoter, actin promoter, the myosin promoter, the hemoglobin promoter, and/or the creatine kinase promoter. In some alternatives, viral promoters such as the CMV promoter are excluded.

Transcriptional Activators.

[0218] In some alternatives, the constitutive promoter is operably linked to a transcriptional activator. In some alternatives, the transcriptional activator activates an inducible promoter in the presence of the inducer (e.g. drug).

[0219] In some alternatives, an inducible promoter is induced in the presence of a transcriptional activator. In some alternatives, the transcriptional activator preferentially binds to the promoter in the presence of the drug. In some alternatives, the transcriptional activator is TamR-tf (HEA3 or HEA4). Modification of the transcriptional activator can be made in the amino acid sequence that can affect the ability of an activator to bind to the drug, the promoter, or both. For example, binding in the ER ligand binding domain would affect the binding of the drug to the transcriptional activator. In some alternatives, HEA3 comprises two amino acid mutations, wherein the sequence is encoded by a sequence set forth in SEQ

(atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcagg cactcggcgaacctggacattatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagct ggccgagctgcctaacggcctgggcgagacaagaggcagcagggacgagacagacgacgacggcgaggacttcaccccccc atcctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccc tggcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaac cagagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgcccctgtacacttggtacgtgcgg aagcagagagaggtggcccagcagtttacacacgcccggccaggcgccctgatcgaggaacctaccggcgacgagctgcccac caagaagggcagacggaaccgggttaagtggggccctgcatctcagcagatcctgttcaggccctacgagcggcagaagaacccc agcaaagaggaacgggagacactggtggaagagtgaaccggggcagtgcatccagagaggcgtgagcccttctcaggctcag ggcctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcgggcacaagct gtctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagc ctgaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccaccagacccttcagc gaggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggc ttcgtggacctgacctgcacgaccagggtccacctgctggaatgtgcctggctggaaatcctgatgatcgccctcgtgtggagaagc atggaacacccccggcaagctgctgttcgcccccaacctgctcctggaccggaaccagggaaaagtgcgtggagggcatggtggaga tcttcgacatgctgctggccacctccagccggttcggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtccatcatcct gctgaacagcggcgtgtacaccttctgtcatccacctgaagtccctggaagagaaggaccacatccaccgggtgctggacaaga tcaccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatc ctgagccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacct gctgctcgagatgctggatggccacagactgcacgcccctacaagcagagggcggagccagcgtggaggaaaccgaccagtctca cctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcacccggcgaggccgagggattccctgccacct ggagttccagtacctgcccagaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattccagagcat catgaagaagtcccccttcagcggccccaccgatccagacccccctagaagaatcgccgtgcccagcagatctagcggcagc gtgccaagcctgccccccagccctaccctttaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccca gggccagatctctcaggcctctgctctggcacctgctccacctcagggtgctgcctcaggccccctgctccagccccagccccctgcat ggtgtctgcactggcccaggctccagctcctgtgcctgtgctggcccctggacctcctcaggctgtggcccctcctgcccctaaacct acccaggccggggagggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaata gcaccgacccccgccgtgtttaccgacctggcctccgtggacaacagcgagttccagcagctcctcaaccagggcacccctgtcgcc ccacacaccaccgagcccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggccctccagatccag caccagctccactgggagccccctggcctgcctaatgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttca gcgccctgctgtcccagatcagcagc). In some alternatives, the HEA3 comprises an amino acid

sequence set forth in SEQ ID NO: 121

(MVS KLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHNLKGTMPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLSLALTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
WRSMEHPGKLLFAPNLLLDNRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA
QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLEMLDAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
FQSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM
DFSALLSQISS). This sequence comprises the sequence of HEA3 containing two point
mutations (K310Q and S536E, positions in the RelA protein) in the p65 domain that enhance
transcriptional activity. This is referred to as HEA3(p65/S536E/K310Q). K310Q in RelA
corresponds to position 621 in HEA3; S536E corresponds to position 846 in HEA3.

[0220] In some alternatives, the system employs a synthetic transcriptional
activator which, in the presence of the drug (e.g. tamoxifen), binds a synthetic promoter
upstream of a transgene to induce expression. In some alternatives, it is a variant of a wild
type “TamR-tf” HEA3 transcription factor. In some alternatives, it is a variant of a wild type
“TamR-tf” HEA4 transcription factor. An exemplary amino acid sequence is provided in
SEQ ID NO: 1

(MVS KLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHNLKGTMPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK

LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA
QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
DFSALLSQISS). The mutant tamoxifen-specific ligand binding domain of the estrogen
receptor ligand binding domain (ER-LBD) is found at amino acids 282-595 of the HEA3 and
has a mutation at position 521 (SEQ ID NO: 1;
MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHNLNKGTPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLCAWLEILMIGLV
WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC
LKSILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA
QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
FKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP
SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
DFSALLSQISS). The p65 activation domain of NF- κ B (p65 or TAD) is found at amino
acids 596 to 862. SEQ ID NO: 1 comprises one point mutation in the ER-LBD that ablates
binding to endogenous estrogen, but confers nanomolar specificity to 4-OHT, fulvestrant and

other estrogen analogs. In some alternatives, HEA3 comprises two amino acid mutations, wherein the sequence is encoded by a sequence set forth in SEQ ID NO: 122 (atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcagg cactcggcgaacctggaccttatctgctcgctggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagct ggccgagctgcctaacggcctgggcgagacaagaggcagcgaggacgagacagacgacgacggcgaggacttcaccccccc atcctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccc tggcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaac cagagccacctgagccagcacctgaacaagggcacccccatgaaaaccagaagagagccgccctgtacacttggtacgtgcgg aagcagagagaggtggcccagcagtttacacacgcccggccaggcgccctgatcgaggaacctaccggcgacgagctgcccac caagaagggcagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggccctacgagcggcagaagaacccc agcaaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcag ggccctcggcagcaatctggtcacccaagtgcgggtgtacaattggttcgccaaccggcggaagaggaagccttcggcacaagct gtctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagc ctgaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgacccaccagacccttcagc gaggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggc ttctgtagacctgacctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagc atggaacacccccggcaagctgctgttcgcccccaacctgctcctggaccggaaccagggaaagtgcgtggagggcatggtggaga tcttcgacatgctgctggccacctccagccggttcgggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtccatcatcct gctgaacagcggcgtgtacaccttcctgtcatccacctgaagtccttggaaagagaaggaccacatccaccgggtgctggacaaga tcaccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatc ctgagccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacct gctgctcgagatgctggatgcccacagactgcacgcccctacaagcagaggcgagccagcgtggaggaaccgaccagtctca cctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcacccggcgaggccgagggattccctgccaccgt ggagttccagtacctgcccagaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattccagagcat catgaagaagtcccccttcagcggccccaccgatcccagacccccctagaagaatcgccgtgcccagcagatctagcggcagc gtgccaagcctgccccccagccctacccttcaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttcccca gggccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgcctcaggccccctgctccagccccagccccctgcat ggtgtctgcactggcccaggctccagctcctgtgcctgtgctggcccctggacctcctcaggctgtggcccctcctgcccctaaacct acccaggccgggggaggggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaata gcaccgacccccgccgtgtttaccgacctggcctccgtggacaacagcagttccagcagctcctcaaccagggcacccctgtcgcc ccacacaccaccgagccccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggcctccagatccag

caccagctccactgggagcccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttcagcgccctgctgtcccagatcagcagc). In some alternatives, the HEA3 comprises an amino acid sequence set forth in SEQ ID NO: 121 (MVS KLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLV WRSMEHPGKLLFAPNLLDRNQGKCVEGMVEIFDMLLATSSRFRMMNLQGEEFVC LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQLA QLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLEMLDAHRLHAPTSRGGASVE ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET FQSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFP SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM DFSALLSQISS). This sequence comprises the sequence of HEA3 containing two point mutations (K310Q and S536E, positions in the RelA protein) in the p65 domain that enhance transcriptional activity. This is referred to as HEA3(p65/S536E/K310Q). K310Q in RelA corresponds to position 621 in HEA3; S536E corresponds to position 846 in HEA3.

[0221] Additional changes can be made to the transcriptional activator to increase the properties of the transcription factor including, without limitation, altering one or more amino acids in the estrogen receptor ligand binding domain and/or altering one or more amino acids in the p65 transactivating domain. Altering amino acids in the estrogen receptor binding domain can provide for more specific and sensitive binding of the drug to the transcriptional activator. For example, mutations are made at amino acid position 400, 543, and 544 of SEQ ID NO: 4 (SEQ ID NO: 4; MVS KLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR

VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
 KQREVAQQFTHAGQGGLIEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
 SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
 LSAGDMRAANLWPSPLMIKRSKKNSLALSALTADQMVSALLDAEPPILYSEYDPTRPF
 SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHLLECAWLEILMIGLV
 WRSMEHPVKLLFAPNLLDRNQGKCEGMVEIFDMLLATSSRFRMMNLQGEEFVC
 LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQRRLA
 QLLLILSHIRHMSNKGMEHLYSMKCKNVVPLYDLLLEADAHRLHAPTSRGGASVE
 ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
 FKSIMKKSPFSGPTDPRPPPRRIA VPSRSSASVPKPAPQPYPTSSSLSTINYDEFPTMVFP
 SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
 KPTQAGEGTLSEALLQLQFDDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
 VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADM
 DFSALLSQISS). SEQ ID NO: 4 comprises the sequence of wild-type HEA4 containing
 three point mutations (G400V, M543A, L544A) in the ER-LBD that ablate binding to
 endogenous estrogen, and confer higher sensitivity to 4-hydroxytamoxifen, fulvestrant, and
 estrogen analogs compared to wild-type HEA3. The transcriptional activator with altered
 sequence has increased affinity for tamoxifen or 4-OHT. Altering amino acids in the p65
 transactivating domain can provide for increased expression of the transgene in the absence
 of activation of the transduced cells. In some alternatives, the transgene encodes a
 polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular
 environment, a polypeptide that modulates checkpoint signaling, a microRNA product that
 regulates growth, a microRNA product that regulates survival, or a microRNA product that
 regulates cytolytic capacity. In some alternatives, the transgene encodes a protein that
 modulates T cells. In some alternatives, the transgene encodes a chimeric antigen receptor.

Marker

[0222] In some alternatives, the constitutive promoter is operably linked to a polynucleotide coding for a marker polypeptide. Such marker polypeptides are described herein, and include EGFRt, Her2t, and/or DHFRdm.

[0223] In some alternatives, the marker is encoded by a sequence set forth in SEQ ID NO's: 30, 32, 34, 36 or 38. In some alternatives, the marker comprises an amino acid sequence set forth in SEQ ID NO's: 31, 33, 35, 37 or 39.

Chimeric Antigen Receptor

[0224] In some alternatives, the constitutive promoter is operably linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and a polynucleotide coding for an intracellular signaling domain. In some alternatives, the spacer is optimized to provide for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.

Vectors

[0225] A variety of vector combinations can be constructed to provide for efficiency of transduction and transgene expression. In some alternatives, the vector is a dual packaged or single (all in one) viral vector. In other alternatives, the vectors can include a combination of viral vectors and plasmid vectors. Other viral vectors include foamy virus, adenoviral vectors, retroviral vectors, and lentiviral vectors. In some alternatives, the vector is a lentiviral vector. In some alternatives, the vector is a foamy viral vector, adenoviral vectors, retroviral vectors or lentiviral vectors.

[0226] In some alternatives, a plasmid vector or a viral vector comprises a first nucleic acid comprising an inducible promoter linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, a plasmid vector or viral vector comprises a first nucleic acid sequence comprising a polynucleotide coding for a gene that enhances cell survival or proliferation, a gene that regulates apoptosis, and/or a gene that modulates checkpoint signaling. In some alternatives, the modulation of checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3. Such polynucleotides code for a cytokine, a

chimeric cytokine receptor, or a chemokine receptor. In some alternatives, a plasmid vector or a viral vector comprises a first nucleic acid comprising an inducible promoter linked to a polynucleotide coding for a marker sequence. In some alternatives, the marker sequence is compatible with transduction of human lymphocytes. In some alternatives, the marker sequence allows for selection of transduced cells and/or detection of transduced cells. In some alternatives, the marker is a gene that can include inter alia, hygromycin-B phosphotransferase gene (hph), which confers resistance to hygromycin B, the amino glycoside phosphotransferase gene (neo or aph) from Tn5, which codes for resistance to the antibiotic G418, the mutated dihydrofolate reductase (DHFR) gene, which provides resistance to methotrexate, DHFRdm, the pac gene that provides resistance to puromycin, Sh ble gene, which inactivates zeocin, the adenosine deaminase gene (ADA), and/or the multi-drug resistance (MDR) gene. A first nucleic acid can include a number of different polynucleotide sequences all under the control of the inducible promoter. For example, a polynucleotide coding for a chimeric antigenic receptor can be linked to a polynucleotide coding for a marker polypeptide and/or a polynucleotide coding for cytokine or chemokine receptor. In some alternatives, a gene is under the control of the inducible promoter. In some alternatives, the gene encodes a chimeric cytokine receptor.

[0227] In some alternatives, a lentiviral vector comprises a second nucleic acid comprising a constitutive promoter linked to a nucleic acid sequence coding for transcriptional activator that binds to drug and activates expression of an inducible promoter. In some alternatives, a lentiviral vector with a constitutive promoter can also include a nucleic acid sequence including a marker gene, piggybac transposase, and/or a polynucleotide coding for a chimeric antigen receptor. Each element of the nucleic acid can be separated from one another with a sequence such as a T2A self-cleaving sequence. In some alternatives, the elements of the nucleic acid are separated from one another with a sequence self-cleaving sequence. In some alternatives, the self-cleaving sequence is a 2A linker. In some alternatives, the linker is T2A, P2A, E2A or F2A.

[0228] In other alternatives, the heterogeneous (heterogeneous to the vector, e.g, lentiviral vector) nucleic acid sequence is limited by the amount of additional genetic components that can be packaged in the vector. In some alternatives, a construct contains at least two genes heterogenous to the viral vector. In some alternatives, the construct contains

no more than 4 genes heterogenous to the viral vector. The number of genes heterogenous to the viral vector that can be packaged in the vector can be determined by detecting the expression of one or more transgenes, and selecting vector constructs that provide for transduction of at least 10% of the cells and/or detectable expression levels of the transgene in at least 10% of the cells. In some alternatives, the transgene encodes a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity. In some alternatives, the transgene encodes a protein that modulates T cells. In some alternatives, the transgene encodes a chimeric antigen receptor.

[0229] In some alternatives, a lentivirus is a dual packaged virus. A dual packaged virus contains at least one conditional construct comprising an inducible promoter operably linked to a polynucleotide coding for a chimeric antigen receptor. Optionally the conditional construct comprises a marker gene, a nucleic acid for a cytokine, a nucleic acid for a chimeric cytokine receptor, a nucleic acid for a chemokine receptor. In some alternatives, a dual packaged lentivirus contains a constitutive construct comprising a constitutive promoter. In an alternative, the constitutive construct comprises a constitutive promoter linked to a transcriptional activator for the inducible promoter. In some alternatives, the constitutive construct also includes a marker gene and/or a polynucleotide encoding a cytokine or chemokine. In some alternatives of a system with two constructs, each construct can be packaged in a separate viral vector and the viral vectors can be mixed together for transduction in a cell population.

[0230] When the constitutive and conditional constructs both contain a marker gene, the marker gene on each construct is the same or different from one another. In some alternatives, when the constitutive and conditional constructs both contain a polynucleotide coding for a chimeric antigen receptor, the chimeric antigen receptor can be targeted to the same antigen but have different ligand binding domains, can be targeted to the same antigen but different epitopes, or can be targeted to different antigens.

[0231] In some alternatives, the vector is a minicircle. Minicircles are episomal DNA vectors that are produced as circular expression cassettes devoid of any bacterial plasmid DNA backbone. Their smaller molecular size enables more efficient transfections

and offers sustained expression over a period of weeks as compared to standard plasmid vectors that only work for a few days. In some alternatives, a minicircle contains a drug inducible promoter linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, the inducible promoter can be linked to chemokine receptor, a marker gene, and/or a cytokine. One or more minicircles can be employed. In some alternatives, a minicircle comprises an inducible promoter linked to a polynucleotide coding for a first chimeric antigen receptor, another minicircle comprises an inducible promoter linked to a polynucleotide coding for a second and different chimeric antigen receptor, and/ or a minicircle comprises an inducible promoter linked to a polynucleotide coding for a chemokine receptor, a chimeric antigen receptor, and a marker gene. Each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives, each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives, each minicircle differs from one another in the chimeric antigen receptor including but not limited to the spacer length and sequence, the intracellular signaling domain, and/or the marker sequence. The minicircle vector can be used with a constitutive lentivirus vector coding for a transcriptional activator for the inducible promoter. In some alternatives, the minicircle vector is used with a constitutive lentivirus vector coding for a transcriptional activator for the inducible promoter. In the systems provided herein, the systems are inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor.

[0232] In some alternatives, the vector is a piggy bac transposon. The PiggyBac (PB) transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition, the PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and efficiently moves the contents from the original sites and efficiently integrates them into TTAA chromosomal sites. The powerful activity of the PiggyBac transposon system enables genes of interest between the two ITRs in the PB vector to be easily mobilized into target genomes.

[0233] In some alternatives, a PB contains a drug inducible promoter linked to a polynucleotide coding for a chimeric antigen receptor. In some alternatives, the inducible

promoter can be linked to chemokine receptor, a marker gene, and/or a cytokine. One or more PB transposons can be employed. In some alternatives, a PB comprises an inducible promoter linked to a polynucleotide coding for a first chimeric antigen receptor, another PB comprises an inducible promoter linked to a polynucleotide coding for a second and different chimeric antigen receptor, and/ or a PB comprises an inducible promoter linked to a polynucleotide coding for a chemokine receptor, a chimeric antigen receptor, and a marker gene. Each element of the constructs is separated by a nucleic acid, such as that coding for a self-cleaving T2A sequence. In some alternatives, each PB differs from one another in the chimeric antigen receptor including but not limited to the spacer length and sequence, the intracellular signaling domain, and/or the marker sequence. The PB vector can be used with a constitutive lentivirus vector coding for a transcriptional activator for the inducible promoter and constitutive vector comprising the piggyback transposase linked to a constitutive promoter.

[0234] Some alternatives concern a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a polynucleotide coding for chimeric antigen receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; and a polynucleotide coding for an intracellular signaling domain; and a second nucleic acid comprising a second constitutive or inducible promoter operably linked to nucleic acid coding for a transcriptional activator for the inducible promoter. In some alternatives, the first and second nucleic acid are in a single lentivirus vector. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first nucleic acid further comprises a marker gene. In some alternatives, the second nucleic acid further comprises polynucleotide coding for a second and different chimeric antigen receptor. The first and second chimeric antigen receptor can differ from one another in the ligand binding domain, the target antigen, an epitope of the target antigen, the spacer domain in length and sequence (short medium or long), and in the intracellular signaling domains. In some

alternatives, in a single lentivirus construct the first and second nucleic acids can be separated by a genomic insulator nucleic acid such as the sea urchin insulator chromatin domain. In other alternatives, the inducible promoter of the first nucleic acid and the constitutive promoter of the second nucleic acid are in opposite orientation. One or more of these vectors can be used in conjunction with one another to transduce target cells and provide for inducible expression of a chimeric antigen receptor.

Host Cells and Compositions: T lymphocyte populations.

[0235] The compositions described herein provide for genetically modified host cells with the vectors and/or constructs as described herein. In some alternatives, the host cells are CD4+ and/or CD8+ T lymphocytes.

[0236] T lymphocytes can be collected in accordance with known techniques and enriched or depleted by known techniques such as affinity binding to antibodies such as flow cytometry and/or immunomagnetic selection. After enrichment and/or depletion steps, *in vitro* expansion of the desired T lymphocytes can be carried out in accordance with known techniques or variations thereof that will be apparent to those skilled in the art. In some alternatives, the T cells are autologous T cells obtained from the patient.

[0237] For example, the desired T cell population or subpopulation can be expanded by adding an initial T lymphocyte population to a culture medium *in vitro*, and then adding to the culture medium feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g. for a time sufficient to expand the numbers of T cells). The non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some alternatives, the PBMC are irradiated with gamma rays in the range of 3000 to 3600 rads to prevent cell division. In some alternatives, the PBMC are irradiated with gamma rays of 3000, 3100, 3200, 3300, 3400, 3500 or 3600 rads or any value of rads between any two endpoints of any of the listed values to prevent cell division. The order of addition of the T cells and feeder cells to the culture media can be reversed if desired. The culture can typically be incubated under conditions of temperature and the like that are suitable for the growth of T lymphocytes. For the growth of human T lymphocytes, for example, the temperature will generally be at least 25 degrees Celsius, preferably at least 30 degrees, more

preferably 37 degrees. In some alternatives, the temperature for the growth of human T lymphocytes is 22, 24, 26, 28, 30, 32, 34, 36, 37 degrees Celsius or any other temperature between a range defined by any two endpoints of any of the listed values.

[0238] The T lymphocytes expanded include CD8⁺ cytotoxic T lymphocytes (CTL) and CD4⁺ helper T lymphocytes that can be specific for an antigen present on a human tumor or a pathogen. In some alternatives, the cells include precursor T cells. In some alternatives, the cells are hematopoietic stem cells.

[0239] In some alternatives, the expansion method can further comprise adding non-dividing EBV-transformed lymphoblastoid cells (LCL) as feeder cells. LCL can be irradiated with gamma rays in the range of 6000 to 10,000 rads. In some alternatives, the LCL are irradiated with gamma rays in of 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500 or 10,000 rads or any amount of rads in between a range defined by any two endpoints of any of the listed values. The LCL feeder cells can be provided in any suitable amount, such as a ratio of LCL feeder cells to initial T lymphocytes of at least 10:1.

[0240] In some alternatives, the expansion method can further comprise adding anti-CD3 and/or anti CD28 antibody to the culture medium (*e.g.*, at a concentration of at least 0.5 ng/ml). In some alternatives, the expansion method can further comprise adding IL-2 and/or IL-7 and/or IL-15 to the culture medium (*e.g.*, wherein the concentration of IL-2 is at least 10 units/ml).

[0241] After isolation of T lymphocytes both cytotoxic and helper T lymphocytes can be sorted into naïve, memory, and effector T cell subpopulations either before or after expansion.

[0242] CD8⁺ cells can be obtained by using standard methods. In some alternatives, CD8⁺ cells are further sorted into naïve, central memory, and effector memory cells by identifying cell surface antigens that are associated with each of those types of CD8⁺ cells. In some alternatives, memory T cells are present in both CD62L⁺ and CD62L⁻ subsets of CD8⁺ peripheral blood lymphocytes. PBMC are sorted into CD62L⁻CD8⁺ and CD62L⁺CD8⁺ fractions after staining with anti-CD8 and anti-CD62L antibodies. In some alternatives, the expression of phenotypic markers of central memory T_{CM} include CD45RO, CD62L, CCR7, CD28, CD3, and/or CD127 and are negative or low for granzyme B. In some alternatives, central memory T cells are CD45RO⁺, CD62L⁺, and/or CD8⁺ T cells. In some

alternatives, effector T_E are negative for CD62L, CCR7, CD28, and/or CD127, and positive for granzyme B and/or perforin. In some alternatives, naïve CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naïve T cells including CD62L, CCR7, CD28, CD3, CD127, and/or CD45RA.

[0243] CD4⁺ T helper cells are sorted into naïve, central memory, and effector cells by identifying cell populations that have cell surface antigens. CD4⁺ lymphocytes can be obtained by standard methods. In some alternatives, naïve CD4⁺ T lymphocytes are CD45RO⁻, CD45RA⁺, CD62L⁺, and/or CD4⁺ T cells. In some alternatives, central memory CD4⁺ cells are CD62L⁺ and/or CD45RO⁺. In some alternatives, effector CD4⁺ cells are CD62L⁻ and/or CD45RO⁻.

[0244] Whether a cell or cell population is positive for a particular cell surface marker can be determined by flow cytometry using staining with a specific antibody for the surface marker and an isotype matched control antibody. A cell population negative for a marker refers to the absence of significant staining of the cell population with the specific antibody above the isotype control, positive refers to uniform staining of the cell population above the isotype control. In some alternatives, a decrease in expression of one or markers refers to loss of 1 log₁₀ in the mean fluorescence intensity and/or decrease of percentage of cells that exhibit the marker of at least 20% of the cells, 25% of the cells, 30% of the cells, 35% of the cells, 40% of the cells, 45% of the cells, 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, and 100% of the cells or any % between 20 and 100% when compared to a reference cell population. In some alternatives, a cell population positive for one or markers refers to a percentage of cells that exhibit the marker of at least 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, or 100% of the cells or any % between 50 and 100% when compared to a reference cell population.

[0245] Whether a cell or cell population is positive for a particular cell surface marker can be determined by flow cytometry using staining with a specific antibody for the surface marker and an isotype matched control antibody. A cell population negative for a marker refers to the absence of significant staining of the cell population with the specific antibody above the isotype control, positive refers to uniform staining of the cell population

above the isotype control. In some alternatives, a decrease in expression of one or markers refers to loss of 1 log₁₀ in the mean fluorescence intensity and/or decrease of percentage of cells that exhibit the marker of at least 20% of the cells, 25% of the cells, 30% of the cells, 35% of the cells, 40% of the cells, 45% of the cells, 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, and 100% of the cells or any % between 20 and 100% when compared to a reference cell population. In some alternatives, an increase refers to an increase in mean or median fluorescence intensity and/or to an increase in the number of cells in a cell population that are positive for one or a given marker, such as a population in which s refers to a percentage of cells that exhibit the marker of at least 50% of the cells, 55% of the cells, 60% of the cells, 65% of the cells, 70% of the cells, 75% of the cells, 80% of the cells, 85% of the cells, 90% of the cell, 95% of the cells, or 100% of the cells or any % between 50 and 100% exhibit the marker, e.g., when compared to a reference cell population.

[0246] In some alternatives, populations of CD4⁺ and CD8⁺ that are antigen specific can be obtained by stimulating naïve or antigen specific T lymphocytes with antigen. For example, antigen-specific T cell lines or clones can be generated to Cytomegalovirus antigens by isolating T cells from infected subjects and stimulating the cells in vitro with the same antigen. Naïve T cells can also be used. Any number of antigens from tumor cells can be utilized as targets to elicit T cell responses. In some alternatives, the adoptive cellular immunotherapy compositions are useful in the treatment of a disease or disorder including a solid tumor, hematologic malignancy, breast cancer or melanoma.

Modification of T lymphocyte populations.

[0247] In some alternatives, it can be desired to introduce functional genes into the T cells to be used in immunotherapy in accordance with the present disclosure. For example, the introduced gene or genes can improve the efficacy of therapy by promoting the viability and/or function of transferred T cells; or they can provide a genetic marker to permit selection and/or evaluation of *in vivo* survival or migration; or they can incorporate functions that improve the safety of immunotherapy, for example, by making the cell susceptible to controlled expression of the transgene. This can be carried out in accordance with known techniques that will be apparent to those skilled in the art based upon the present disclosure.

[0248] In some alternatives, T cells are modified with a vector coding for drug inducible chimeric receptors as described herein. In some alternatives, the T cells are modified with a vector coding for drug inducible transgenes. In some alternatives, the transgene encodes a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity. In some alternatives, the transgene encodes a protein that modulates T cells. In some alternatives, the transgene encodes a chimeric antigen receptor. In some alternatives, cells are modified with a vector comprising a polynucleotide coding for a chimeric antigen receptor under control of an inducible promoter. In other alternatives, cells are modified with a vector comprising a polynucleotide coding for a cytokine, a chimeric cytokine receptor, chemokine receptor, a gene that regulates apoptosis, or a gene that modulates checkpoint signaling under the control of an inducible promoter. In some alternatives, the T cells are obtained from the subject to be treated, in other alternatives the lymphocytes are obtained from allogeneic human donors, preferably healthy human donors. In some alternatives, the modulation of checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3.

[0249] Chimeric receptors can be constructed with a specificity for any cell surface marker by utilizing antigen binding fragments or antibody variable domains of, for example, antibody molecules. The antigen binding molecules can be linked to one or more cell signaling modules. In some alternatives, cell signaling modules include CD3 transmembrane domains, CD3 intracellular signaling domains, and/or CD28 transmembrane domains. In some alternatives, the intracellular signaling domain comprises a CD28 transmembrane and signaling domain linked to a CD3 zeta intracellular domain. In some alternatives, a chimeric receptor can also include a transduction marker such as EGFRt.

[0250] In some alternatives, the same or a different chimeric receptor can be introduced into each of population of CD4⁺ and/or CD8⁺ T lymphocytes. In some alternatives, the chimeric receptor in each of these populations has a ligand binding domain that specifically binds to the same ligand on the tumor or infected cell or a different antigen or epitope. The cellular signaling modules can differ. In some alternatives, the intracellular

signaling domain of the CD8⁺ cytotoxic T cells is the same as the intracellular signaling domain of the CD4⁺ helper T cells. In other alternatives, the intracellular signaling domain of the CD8⁺ cytotoxic T cells is different than the intracellular signaling domain of the CD4⁺ helper T cells.

[0251] In some alternatives, each of the CD4 or CD8 T lymphocytes can be sorted into naïve, central memory, effector memory or effector cells prior to transduction, as described herein. In some alternatives, each of the CD4 or CD8 T lymphocytes can be sorted into naïve, central memory, effector memory, or effector cells after transduction.

[0252] As described herein, in some alternatives, naïve CD4⁺ cells are CD45RO⁻, CD45RA⁺, CD62L⁺, and/or CD4⁺ positive T cells. In some alternatives, central memory CD4⁺ cells are CD62L positive and/or CD45RO positive. In some alternatives, effector CD4⁺ cells are CD62L negative and/or CD45RO positive. Each of these populations can be independently modified with a chimeric receptor.

[0253] As described, in some alternatives, memory T cells are present in both CD62L⁺ and CD62L⁻ subsets of CD8⁺ peripheral blood lymphocytes. PBMC are sorted into CD62L⁻CD8⁺ and CD62L⁺CD8⁺ fractions after staining with anti-CD8 and anti-CD62L antibodies. In some alternatives, the expression of phenotypic markers of central memory T cells (TCM) include CD62L, CCR7, CD28, CD3, and/or CD127 and are negative or low for granzyme B. In some alternatives, central memory T cells are CD45RO⁺, CD62L⁺, and/or CD8⁺ T cells. In some alternatives, effector T cells (T_E) are negative for CD62L, CCR7, CD28, and/or CD127, and positive for granzyme B and/or perforin. In some alternatives, naïve CD8⁺ T lymphocytes are characterized by CD8⁺, CD62L⁺, CD45RO⁺, CCR7⁺, CD28⁺ CD127⁺, and/or CD45RO⁺. Each of these populations can be independently modified with a chimeric receptor.

[0254] Various transduction techniques have been developed, which utilize recombinant infectious virus particles for gene delivery. This represents a currently preferred approach to the transduction of T lymphocytes of the present invention. The viral vectors, which have been used in this way include virus vectors derived from simian virus 40, adenoviruses, adeno-associated virus (AAV), lentiviral vectors, and/or retroviruses. Thus, gene transfer and expression methods are numerous but essentially function to introduce and express genetic material in mammalian cells. Several of the above techniques have been used

to transduce hematopoietic or lymphoid cells, including calcium phosphate transfection, protoplast fusion, electroporation, and infection with recombinant adenovirus, adeno-associated virus and retrovirus vectors. Primary T lymphocytes have been successfully transduced by electroporation and by retroviral or lentiviral infection.

[0255] Retroviral and lentiviral vectors provide a highly efficient method for gene transfer into eukaryotic cells. Moreover, retroviral or lentiviral integration takes place in a controlled fashion and results in the stable integration of one or a few copies of the new genetic information per cell.

[0256] It is contemplated that overexpression of a stimulatory factor (for example, a lymphokine or a cytokine) can be toxic to the treated individual. Therefore, it is within the scope of the invention to include gene segments that cause the T cells of the invention to be susceptible to negative selection *in vivo*. By "negative selection" has its plain and ordinary meaning when read in light of the specification, and may be used, for example, when an infused cell is eliminated as a result of a change in the *in vivo* condition of the individual. The negative selectable phenotype can result from the insertion of a gene that confers sensitivity to an administered agent, for example, a compound. Negative selectable genes are known in the art, and include, *inter alia* the following: the Herpes simplex virus type I thymidine kinase (HSV-I TK) gene, which confers ganciclovir sensitivity; the cellular hypoxanthine phosphoribosyltransferase (HPRT) gene, the cellular adenine phosphoribosyltransferase (APRT) gene, and bacterial cytosine deaminase.

[0257] In some alternatives, it can be useful to include in the T cells a positive marker that enables the selection of cells of the negative selectable phenotype *in vitro*. The positive selectable marker can be a gene that upon being introduced into the host cell expresses a dominant phenotype permitting positive selection of cells carrying the gene. Genes of this type are known in the art, and include, *inter alia*, hygromycin-B phosphotransferase gene (hph), which confers resistance to hygromycin B, the amino glycoside phosphotransferase gene (neo or aph) from Tn5, which codes for resistance to the antibiotic G418, the double mutant of the dihydrofolate reductase (DHFR_{dm}) gene, the adenosine deaminase gene (ADA), and/or the multi-drug resistance (MDR) gene.

[0258] A variety of methods can be employed for transducing T lymphocytes, as is well known in the art. In some alternatives, transduction is carried out using lentiviral vectors.

[0259] In some alternatives, CD4⁺ and CD8⁺ cells each can separately be modified with an expression vector encoding a chimeric receptor to form defined populations. In some alternatives, cells can be separately modified with a vector comprising a polynucleotide under the control of a constitutive promoter and a vector comprising a polynucleotide coding for a cytokine or chemokine receptor under control of an inducible promoter.

[0260] In some alternatives, these cells are further sorted into subpopulations of naïve, central memory and effector cells as described above, by sorting for cell surface antigens unique to each of those cell populations. In addition, CD4⁺ or CD8⁺ cell populations can be selected by their cytokine profile or proliferative activities. For example, CD4⁺ T lymphocytes that have enhanced production of cytokines such as IL-2, IL-4, IL-10, TNF α , and/or IFN γ , as compared to sham transduced cells or transduced CD8⁺ cells when stimulated with antigen can be selected. In other alternatives, naïve or central memory CD4⁺ T cells that have enhanced production of IL-2 and/or TNF α are selected. Likewise, CD8⁺ cells that have enhanced IFN γ production are selected, as compared to sham transduced CD8⁺ cells.

[0261] In some alternatives, CD4⁺ and CD8⁺ cells are selected that are cytotoxic for antigen bearing cells. In some alternatives, CD4⁺ are expected to be weakly cytotoxic as compared to CD8⁺ cells. In a preferred alternative, transduced lymphocytes, such as CD8⁺ central memory cells, are selected that provide for tumor cell killing *in vivo* using an animal model established for the particular type of cancer.

[0262] In yet other alternatives, transduced chimeric receptor expressing T cells are selected that can persist *in vivo* using an animal model established for the particular type of cancer. In some alternatives, transduced chimeric receptor CD8⁺ central memory cells with a short spacer region have been shown to persist *in vivo* after introduction into the animal for 3 days or more, 10 days or more, 20 days or more, 30 days or more, 40 days or more, or 50 days or more.

[0263] The disclosure contemplates that combinations of CD4⁺ and CD8⁺ T cells will be utilized in the compositions. In one alternative, combinations of chimeric receptor transduced CD4⁺ cells can be combined with chimeric receptor transduced CD8⁺ cells of the same ligand specificity or combined with CD8⁺ T cells that are specific for a distinct tumor ligand. In other alternatives, chimeric receptor transduced CD8⁺ cells are combined with chimeric receptor transduced CD4⁺ cells specific for a different ligand expressed on the tumor. In yet another alternative, chimeric receptor modified CD4⁺ and CD8⁺ cells are combined. In some alternatives, CD8⁺ and CD4⁺ cells can be combined in different ratios for example, a 1:1 ratio of CD8⁺ and CD4⁺, a ratio of 10:1 of CD8⁺ to CD4⁺, or a ratio of 100:1 of CD8⁺ to CD4⁺, or any other ratio of CD8⁺ to CD4⁺ that is between a range defined by any of the listed ratios. In some alternatives, the combined population is tested for cell proliferation in vitro and/or in vivo, and the ratio of cells that provides for proliferation of cells is selected.

[0264] After transduction and/or selection for chimeric receptor bearing cells, the cell populations are preferably expanded in vitro until a sufficient number of cells are obtained to provide for at least one infusion into a human subject, typically around 10⁴ cells/kg to 10⁹ cells/kg. In some alternatives, the transduced cells are cultured in the presence of antigen bearing cells, anti CD3, anti CD28, and IL 2, IL-7, IL 15, or IL-21 or any combination thereof.

[0265] In some alternatives, CD4⁺ and CD8⁺ cells that proliferate in response to cytokine stimulation, antigen or tumor targets in vitro or in vivo are selected. For example, CD4⁺ or CD8⁺ transduced cells that proliferate vigorously when stimulated with antiCD3 and/ or anti-CD28 are selected. In some alternatives, stimulation of transduced cells provides for enhanced transgene expression in the presence of an inducer (e.g. drug) of those trans genes under the control of an inducible promoter. In some alternatives, the transgene encodes a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity. In some alternatives, the transgene encodes a protein that modulates T cells. In some alternatives, the transgene encodes a chimeric antigen receptor.

[0266] Each of the subpopulations of CD4⁺ and CD8⁺ cells can be combined with one another. In a specific alternative, modified naïve or central memory CD4⁺ cells are combined with modified central memory CD8⁺ T cells to provide a synergistic cytotoxic effect on antigen bearing cells, such as tumor cells.

Compositions.

[0267] The disclosure provides for an adoptive cellular immunotherapy composition comprising a genetically modified T lymphocyte cell preparation as described herein. In some alternatives, the T lymphocyte cell preparation comprises CD4⁺ T cells that have a chimeric receptor comprising an extracellular antibody variable domain specific for a ligand associated with the disease or disorder, a spacer region, a transmembrane domain, and an intracellular signaling domain of a T cell receptor or other receptors under the control of a drug inducible promoter as described herein. In other alternatives, an adoptive cellular immunotherapy composition further comprises a chimeric receptor modified tumor-specific CD8⁺ cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8⁺ T cells that have a chimeric receptor comprising an extracellular single chain antibody specific for a ligand associated with the disease or disorder, a spacer region, a transmembrane domain, and an intracellular signaling domain of a T cell receptor under the control of a drug inducible promoter as described herein. In some alternatives, the chimeric receptor modified T cell population of the disclosure can persist *in vivo* for at least 3 days or longer. In an alternative, each of these populations can be combined with one another or other cell types to provide a composition. In some alternatives, the T cell population can express genes that modulate the T cell function. In some alternatives, the genes encode cytokines, chemokine receptors and proteins that can modulate T cell function.

[0268] In some alternatives, the CD4⁺ T helper lymphocyte cell is naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, or bulk CD4⁺ T cells. In some alternatives, CD4⁺ helper lymphocyte cell is a naïve CD4⁺ T cell, wherein the naïve CD4⁺ T cell comprises a CD45RO⁻, CD45RA⁺, and/or is a CD62L⁺ CD4⁺ T cell.

[0269] In some alternatives, the CD8⁺ T cytotoxic lymphocyte cell is a naïve CD8⁺ T cell, central memory CD8⁺ T cell, effector memory CD8⁺ T cell and/or bulk CD8⁺ T cell. In some alternatives, the CD8⁺ cytotoxic T lymphocyte cell is a central memory T

cell, wherein the central memory T cell comprises a CD45RO+, CD62L+, and/or CD8+ T cell. In yet other alternatives, the CD8+ cytotoxic T lymphocyte cell is a central memory T cell and the CD4+ helper T lymphocyte cell is a naïve or central memory CD4+ T cell.

[0270] In some alternatives, the compositions comprise T cell precursors. In some alternatives, the compositions comprise hematopoietic stem cells. In some alternatives, the composition comprises a host cell wherein the host cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells and bulk CD8+ T cells or a CD4+ T helper lymphocyte cell that is selected from the group consisting of naïve CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells and a second host cell, wherein the second host cell is a precursor T cell. In some alternatives, the precursor T cell is a hematopoietic stem cell.

Methods

[0271] The disclosure provides methods of making adoptive immunotherapy compositions and uses or methods of using these compositions for performing cellular immunotherapy in a subject having a disease or disorder. In some alternatives, a method of manufacturing the compositions comprises obtaining a modified naïve or central memory CD4+ T helper cell, wherein the modified helper T lymphocyte cell preparation comprises CD4+ T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of an inducible promoter as described herein. In other alternatives, CD4+ cells have a cytokine or chemokine receptor under the control of an inducible promoter. CD4+ cells can also have other genes under control of the inducible promoter (genes that control apoptosis, alter extracellular environment etc).

[0272] In another alternative, a method further comprises obtaining a modified CD8+ central memory T cell, wherein the modified central memory CD8 T lymphocyte cell preparation comprises CD8+ cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a spacer domain, a transmembrane domain, and an

intracellular signaling domain under control of the inducible promoter as described herein. In other alternatives, CD8⁺ cells have a cytokine or chemokine receptor under the control of an inducible promoter.

[0273] The drug inducible promoter in both modified CD4⁺ T cells and modified CD8⁺ cytotoxic T cells can be the same or different. In some alternatives, in one population of cells the promoter linked to the chimeric antigen receptor is a constitutive promoter and in the other population it is an inducible promoter. For example, modified CD4⁺ T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of a constitutive promoter, while the CD8⁺ cytotoxic T cell comprises CD8⁺ cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of the inducible promoter. In some alternatives, the genes being controlled by the constitutive or inducible promoter are genes encoding cytokines, chemokine receptors and apoptosis regulators.

[0274] In some alternatives, the polynucleotide can code for a chimeric antigen receptor that differs in the CD4⁺ versus the CD8⁺ cell population. The difference between the two constructs can include the specificity or affinity of the ligand binding domain for an antigen or epitope, the length and sequence of the spacer region, and the intracellular signaling components.

[0275] The preparation of the CD4⁺ and CD8⁺ cells that are modified with a chimeric receptor is described throughout this disclosure. Antigen specific T lymphocytes can be obtained from a patient having the disease or disorder or can be prepared by in vitro stimulation of T lymphocytes in the presence of antigen. Subpopulations of CD4⁺ and/or CD8⁺ T lymphocytes that are not selected for antigen specificity can also be isolated as described herein and combined in the methods of manufacturing.

[0276] In some alternatives, the combination of cell populations can be evaluated for uniformity of cell surface makers, the ability to proliferate through at least two generations, to have a uniform cell differentiation status. Quality control can be performed by co-culturing a cell line expressing the target ligand with chimeric receptor modified T cells and the drug that induces expression of the chimeric antigen receptor to determine if the

chimeric receptor modified T cells recognize the cell line using cytotoxicity, proliferation, or cytokine production assays in the presence of the inducer that are known in the field. Cell differentiation status and cell surface markers on the chimeric receptor modified T cells can be determined by flow cytometry. In some alternatives, the markers and cell differentiation status on the CD8⁺ cells include CD3, CD8, CD62L, CD28, CD27, CD69, CD25, PD-1, CTLA-4, CD45RO, and/or CD45RA. In some alternatives, the markers and the cell differentiation status on the CD4⁺ cells include CD3, CD4, CD62L, CD28, CD27, CD69, CD25, PD-1, CTLA-4 CD45RO, and/or CD45RA.

[0277] In some alternatives, the chimeric receptor modified T cells as described herein are able to persist *in vivo* for at least 3 days, or at least 10 days. In some alternatives, the chimeric receptor modified T cells as described herein, can proliferate *in vivo* through at least 2, or at least 3 generations as determined by CFSE dye dilution. Proliferation and persistence of the chimeric receptor modified T cells can be determined by using an animal model of the disease or disorder and administering the cells and determining persistence and/or proliferative capacity of the transferred cells. In other alternatives, proliferation and activation can be tested *in vitro* by going through multiple cycles of activation with antigen bearing cells.

[0278] The disclosure also provides methods of performing cellular immunotherapy in a subject having a disease or disorder comprising: administering a composition of lymphocytes expressing a chimeric receptor under the control of a drug inducible promoter as described herein, and administering the drug.

[0279] In some alternatives, the drug is tamoxifen, variants, derivatives, pharmaceutical salts, solvates, and hydrates thereof as described herein. In some alternatives, the drug is delivered prior to, at the same time as the composition, or at later time points after the composition has been administered.

[0280] In some alternatives, the drug is administered with the composition, and if a toxic effect of the composition is observed the drug is withdrawn until the toxic effects diminish. After the symptoms of toxicity diminish, the drug is administered again. In some alternatives, a drug can be administered again once symptoms of toxicity diminish.

[0281] In some alternatives, the drug is administered with the composition but once the subject has a decrease in the tumor load or cancer cells, the drug is withdrawn for a

period of time to allow the modified cells to rest and if there is no activity of the modified cells, the modified cells are not needed because of remission of the cancer.

[0282] In other alternatives, a method comprises administering to the subject a genetically modified cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8 + T cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a spacer domain, a transmembrane domain, and an intracellular signaling domain under the control of a drug inducible promoter as described herein, and/or a genetically modified helper T lymphocyte cell preparation that elicits direct tumor recognition and enhances the genetically modified cytotoxic T lymphocyte cell preparations ability to mediate a cellular immune response, wherein the helper T lymphocyte cell preparation comprises CD4+ T cells that have a chimeric receptor comprising a ligand binding domain, wherein the ligand binding domain is specific for a ligand, wherein the ligand is a tumor specific molecule, viral specific molecule, or any other molecule expressed on a target cell population, wherein the ligand can elicit recognition, modulation, inhibition, and/or elimination by a lymphocyte, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of a constitutive or drug inducible promoter as described herein and administering the drug that induces the inducible promoter. In some alternatives, the administering of the drug is performed after administering of the composition or host cells, wherein administering is performed 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 4 weeks or two months, or any time in between any two values of time listed. In some alternatives of the methods, the systems are inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor.

[0283] In other alternatives, a method comprises administering to the subject a genetically modified cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8 + T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor

cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under the control of a constitutive promoter as described herein, and/or a genetically modified helper T lymphocyte cell preparation that elicits direct tumor recognition and enhances the genetically modified cytotoxic T lymphocyte cell preparations ability to mediate a cellular immune response, wherein the helper T lymphocyte cell preparation comprises CD4⁺ T cells that have a chimeric receptor comprising a ligand binding domain specific for a tumor cell surface molecule, a spacer domain, a transmembrane domain, and an intracellular signaling domain under control of a constitutive or drug inducible promoter as described herein and administering the drug that induces the inducible promoter. In some alternatives, the tumor specific molecule is a tumor surface molecule.

[0284] In other alternatives, a method comprises administering to the subject a genetically modified cytotoxic T lymphocyte cell preparation that provides a cellular immune response, wherein the cytotoxic T lymphocyte cell preparation comprises CD8⁺ T cells that express a cytokine, chemokine receptor, a polypeptide that regulates apoptosis, and/or a polypeptide that modulates checkpoint signaling under the control of an inducible promoter as described herein, and/or a genetically modified helper T lymphocyte cell preparation that elicits direct tumor recognition and enhances the genetically modified cytotoxic T lymphocyte cell preparations ability to mediate a cellular immune response, wherein the helper T lymphocyte cell preparation comprises CD4⁺ T cells that express a cytokine, chemokine receptor, a polypeptide that regulates apoptosis, and/or a polypeptide that modulates checkpoint signaling under control of a constitutive or drug inducible promoter as described herein and administering the drug that induces the inducible promoter. In some alternatives, one or more of the cell populations expresses a chimeric antigen receptor under the control of a constitutive promoter. In some alternatives, the modulation of checkpoint signaling inhibits negative checkpoint regulators. In some alternatives, the negative checkpoint regulator comprises PD-1, VISTA, LAG-3 and/or TIM3.

[0285] An effective amount of the drug for induction is an amount of the drug that provides for induction of the chimeric antigen receptor in at least 5 %, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100% or any number in between a range defined by any of the listed

percent values of the transduced cells as described herein. In the methods provided herein, the systems are inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor.

[0286] Another alternative describes a method of performing cellular immunotherapy in a subject having a disease or disorder comprising: analyzing a biological sample of the subject for the presence of a target molecule associated with the disease or disorder and administering the adoptive immunotherapy compositions described herein and administering the drug that induces the inducible promoter, wherein the chimeric receptor specifically binds to the target molecule.

[0287] Subjects that can be treated in accordance with alternatives described herein are, in general, human and other primate subjects, such as monkeys and apes for veterinary medicine purposes. The subjects can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects.

[0288] The methods are useful in the treatment or inhibition of, for example, hematologic malignancy, melanoma, breast cancer, brain cancer, and other epithelial malignancies or solid tumors. In some alternatives, the molecule associated with the disease or disorder is an orphan tyrosine kinase receptor ROR1, Her2, EGFR, CE7, hB7H3, CD19, CD20, CD22, mesothelin, CEA, or a hepatitis B surface antigen.

[0289] Subjects that can be addressed using the methods described herein include subjects identified or selected as having cancer, including but not limited to colon, lung, liver, breast, renal, prostate, ovarian, skin (including melanoma), bone, and brain cancer, etc. Such identification and/or selection can be made by clinical or diagnostic evaluation. In some alternatives, the tumor associated antigens or molecules to which alternatives are directed are antigens associated with melanoma, breast cancer, brain cancer, squamous cell carcinoma, colon cancer, leukemia, myeloma, and/or prostate cancer. In other alternatives the tumor associated molecules can be targeted with genetically modified T cells expressing an engineered chimeric receptor. Examples include but are not limited to B cell lymphoma, breast cancer, brain cancer, prostate cancer, and/or leukemia.

[0290] Cells prepared as described above can be utilized in methods and compositions for adoptive immunotherapy in accordance with known techniques, or

variations thereof that will be apparent to those skilled in the art based on the instant disclosure.

[0291] In some alternatives, the cells are formulated by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system suitable for administration (a "pharmaceutically acceptable" carrier) in a treatment-effective amount. Suitable infusion medium can be any isotonic medium formulation, typically normal saline, Normosol R (Abbott) or Plasma-Lyte A (Baxter), but also 5% dextrose in water or Ringer's lactate can be utilized. The infusion medium can be supplemented with human serum albumin, fetal bovine serum or other human serum components.

[0292] In some alternatives, a treatment or inhibitory effective amount of cells in the composition is a transduced CD4 or CD8 cell or at least 2 cell subsets (for example, 1 CD8⁺ central memory T cell subset and 1 CD4⁺ helper T cell subset) or is more typically greater than 10^2 cells, and up to 10^6 , up to and including 10^8 or 10^9 cells and can be more than 10^{10} cells. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. For example, if cells that are specific for a particular antigen are desired, then the population will contain greater than 70%, generally greater than 80%, 85% and 90-95% of such cells. For uses provided herein, the cells are generally in a volume of a liter or less, can be 500 mls or less, even 250 mls or 100 mls or less or a volume in between a range defined by any two listed volume values. Hence the density of the desired cells is typically greater than 10^4 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^6 , 10^7 , 10^8 , 10^8 , 10^9 , 10^{10} or 10^{11} cells or any amount of cells in between a range defined by any two endpoints of any of the listed values.

[0293] In some alternatives, the lymphocytes of the invention can be used to confer immunity to individuals. "Immunity" has its plain and ordinary meaning when read in light of the specification, and may be used, for example, to mean a lessening of one or more physical symptoms associated with a response to infection by a pathogen, or to a tumor, to which the lymphocyte response is directed. The amount of cells administered is usually in the range present in normal individuals with immunity to the pathogen. Thus, the cells are

usually administered by infusion, with each infusion in a range of from 2 cells, up to at least 10^6 to 3×10^{10} cells, preferably in the range of at least 10^7 to 10^9 cells. The T cells can be administered by a single infusion, or by multiple infusions over a range of time. However, since different individuals are expected to vary in responsiveness, the type and amount of cells infused, as well as the number of infusions and the time range over which multiple infusions are given are determined by the attending physician, and can be determined by routine examination. The generation of sufficient levels of T lymphocytes (including cytotoxic T lymphocytes and/or helper T lymphocytes) is readily achievable using the rapid expansion method of the present invention, as exemplified herein.

[0294] In some alternatives, a composition as described herein is administered to an identified or selected subject, such as a subject identified or selected as having melanoma, breast cancer, brain cancer, squamous cell carcinoma, colon cancer, leukemia, myeloma, and/or prostate cancer, intravenously, intraperitoneally, intratumorally, into the bone marrow, into the lymph node, and /or into cerebrospinal fluid. In some alternatives, the chimeric receptor engineered compositions are delivered to the site of the tumor. Alternatively, the compositions as described herein can be combined with a compound that targets the cells to the tumor or the immune system compartments and avoid sites such as the lung.

[0295] In some alternatives, the compositions as described herein are administered with chemotherapeutic agents and/or immunosuppressants. In an alternative, a patient is first administered a chemotherapeutic agent that inhibits or destroys other immune cells followed by the compositions described herein. In some cases, chemotherapy can be avoided entirely.

[0296] In some alternatives, a method comprising administering the modified T cells as described herein in combination with the inducer (e.g. inducible drug) until the tumor burden is diminished. Once the tumor burden is diminished, the inducer drug can be withdrawn in order to switch the expression of the chimeric antigen receptor off and decrease the number of T cells expressing the receptor. In other alternatives, the inducer drug can be administered at a different time in order to switch the expression of the chimeric antigen receptor on in the event of a relapse or increase in tumor growth.

[0297] In other alternatives, the inducer drug can be given for a period of time of days, weeks, or months, and then withdrawn for days, weeks or months, followed by re-

administration of the inducer drug for days, weeks or months to allow for cycling of the expression of the chimeric antigen receptor to avoid anergy or nonresponsiveness due to chronic stimulation of the cells.

More Alternatives.

[0298] In some alternatives, a system for inducible expression of a transgene is provided. In some alternatives, the transgene encodes a chimeric antigen receptor. In some alternatives, a system for inducible expression of a chimeric antigen receptor in cells, such as mammalian cells, is provided. In some alternatives, the transgene encodes a polypeptide that regulates apoptosis. In some alternatives, genes that inhibit apoptosis include, for example, Bcl2, and/or CA-Akt. In some alternatives, the polypeptide is Bcl2 or CA-Akt. In some alternatives, genes that inhibit apoptosis include, for example, Survivin, Bcl2, CA-Akt, and/or dnCaspase3. In some alternatives, the polypeptide is Survivin, Bcl2, or CA-Akt and/or dnCaspase 3. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124

(Atgggtgccccgacgttgccccctgcctggcagccctttctcaaggaccaccgcatctctacattcaagaactggcccttcttgagggtgcgctgcacccccggagcggatggccgaggctggcttcactgccccactgagaacgagccagactggcccagtggttcttctgcttcaaggagctggaaggctgggagccagatgacgaccccatagaggaaacataaaaagcattcgtccggttgcgctttccttctgtcaagaagcagtttgaagaattaacccttggtgaatttttaaactggacagagaaagagccaagaacaaaattgcaaaggaaacaacaataagaagaagaatttgaggaaactgtgaagaaagtgcgccgtgccatcgagcagctggctgcaatggat). In some alternatives, Survivin comprises the amino acid sequence set forth in SEQ ID NO: 123 (MGAPTLPPAWQPFLKDHRISTFKNWPFLGCACTPERMAEAGFIHCPTENEPDLAQCFFCFKELEGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDREERAKNKIAKETNNKKKEFEETVKKVRRRAIEQLAAMD). In some alternatives, the polypeptide that regulates apoptosis is Survivin. In some alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124. In some alternatives, Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123. The system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a transgene, which comprises a ligand binding domain that is specific for a ligand selected from the group consisting of a tumor specific molecule, a viral specific molecule, and any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a

lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by a drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. The system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from the group consisting of a tumor specific molecule, a viral specific molecule, and any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte, a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer, a third polynucleotide, which encodes a transmembrane domain and a fourth polynucleotide, which encodes an intracellular signaling domain and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by a drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system, the transcriptional activator is HEA3 or HEA4. In some alternatives of the system, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that

allows selective binding to the drug ligand. In some alternatives of the system, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives of the system, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives of the system, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system, the variant of the wild type HEA3 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system, the variant of the wild type HEA3 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system, the variant of a wild type HEA4 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 4-7. In some alternatives of the system, the variant of the wild type HEA4 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives of the system, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives of the system, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug. In some alternatives of the system, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with

a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the system, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives of the system, the second promoter is an inducible promoter. In some alternatives of the system, the second promoter is a constitutive promoter. In some alternatives of the system, the second promoter is the EF1 α p. In some alternatives of the system, the transcriptional activator comprises at least one amino acid sequence set forth in SEQ ID NO: 1-11 or 121. In some alternatives of the system, the transcriptional activator is encoded by at least one nucleic acid sequence set forth in SEQ ID NO: 12-22 or 122. In some alternatives of the system, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system, both vectors are packaged in a viral vector. In some alternatives of the system, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives of the system, the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker. In some alternatives of the system, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives of the system, selectable marker is EGFRt and/or HER2t. In some alternatives of the system, the selectable marker confers drug resistance. In some alternatives of the system, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives of the system, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives of the system, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives of the system, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the system, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the system, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the system, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence

identity in between a range defined by any two aforementioned values. In some alternatives of the system, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives, the chimeric antigen receptor comprises a scFv. In some alternatives, the scFv specifically binds to CD19. In some alternatives, the scFv comprises an huCD19 scFv (G01S) amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 41 (MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGRIGYADSVKGRFTISRDNKNSLFLQMNSLRAEDTAVYYCARDQGYHYYDSAEHAFDIWGQGT VVTVSSGGGGSGGGGSGGGGSSQSALTQPRSVSGFPGQSVTISCTGTTSDDVSWYQQHPGKAPQLMLYDVSKRPSGVPHRFSGSRSGRAASLIISGLQTEDEADYFCSSYAGRYNSVLFGGGTKLTVL). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 41. In some alternatives, the scFv is encoded by the muCD19 (FMC63) scFv nucleotide sequence. In some alternatives, the scFv specifically binds to CD19. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 42

(Atgctgctgctggtgaccagcctgctgctgtgcgagctgccccaccccgctttctgctgatccccgacatccagatgaccagacacactccagcctgagcgccagcctggcgaccgggtgaccatcagctgccgggcccagccaggacatcagcaagtacctaactggtatcagcagaagccccgacggcaccgtcaagctgctgatctaccacaccagccggctgcacagcggcgtgcccagccgggttagcggcagcggctccggcaccgactacagcctgaccatctccaacctggaacaggaagatatgccacctaactttgccagcagggcaacacactgccctacacctttggcgggcgaacaaagctggaaatcaccggcagcacctccggcagcggcaagcctggcagcggcgagggcagcaccaagggcgaggtgaagctgcaggaaagcggccctggcctggtggccccagccagagcctgagcgtgacctgaccgtgagcggcgtgagcctgcccgactacggcgtgagctggatccggcagccccccaggaaggcctggaatggctgggcgtgatctggggcagcgagaccactactacaacagcgccctgaagagccggctgaccatcatcaaggacaacagcaagagccaggtgttcctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcactactactacggcggcagctacgcatgactactggggccagggcaccagcgtgacctgagcagc). In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 43 (MLLLVTSLLLCELPHPAFLLIPIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIKD NSKSQVFLKMNSL

QTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 43. In some alternatives, the scFv comprises a B7H3 (hBRCA84D) scFv nucleotide sequence. In some alternatives, the scFv binds specifically to the B7-H3 receptor. In some alternatives, the scFv comprises the L1CAM (CE7) scFv nucleotide sequence. In some alternatives, the scFv is specific for the epitope, CE7 epitope, of L1CAM. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 50

(atgctgctgctggtgaccagcctgctgctgtgcgagctgccccaccccgctttctgctgatccccaggtgcagctgcagcagcctggcgccgagctggtgaagccaggcgccagcgtgaagctgtcctgcaaggccagcggctacaccttcaccggctactggatgcactgggtgaagcagagacccggccacggcctggaatggatcggcgagatcaacccagcaacggccggaccaactacaacgagcggttcaagagcaaggccaccctgaccgtggacaagagcagcaccaccgccttcctgagctgtccggcctgaccagcaggacagcggccgtgtacttctgcgccagggactactacggcaccagctacaacttcgactactggggccagggcaccacactgaccgtgagcagcggcgagggggctctggcgggcgaggatctgggggagggggcagcgacatccagatgaccagagcagcagcagcttcagcgtgagcctgggcgaccgggtgaccatcacctgtaaggccaacgaggacatcaacaaccggctggcctggtatcagcagacccccggcaacagccccaggctgctgatcagcggcgccaccaacctggtgaccggcgtgccagccgggttagcggcagcggctccggcaaggactacaccctgaccatcacaagcctgcaggccgaggacttcgccacctactactgccagcagtactggtccaccccttcaccttcggcagcggcaccgagctggaaatcaaa). In some alternatives, the scFv comprises the L1CAM (CE7) scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence

set forth in SEQ ID NO: 51

(MLLLVTSLLLCELPHPAFLIPQVQLQQPGAELVKPGASVKLSCKASGYTFTGYWMHWVKQRPGHGLEWIGEINPSNGRTNYNERFKSKATLTVDKSSTTAFMQLSGLTSEDSAVYFCARDYYGTSYNFDYWGQGTTTLTVSSGGGGSGGGGSGGGGSDIQMTQSSSSFSVSLGDRVTITCKANEDINNRLAWYQQTPGNSPRLISGATNLVTGVPSRFSGSGSGKDYTLTITSLQAEDFATYYCQQYWSTPFTFGSGTELEIK). In some alternatives, the scFv

is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 51. In some alternatives, the scFv is encoded by the EGFR (cetuximab) scFv nucleotide sequence. In some alternatives, the scFv is specific for EGFR. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 52

(atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatccacaggtgcagctgaaacagagcgg

gcccgggcctggtgcagccgagccagagcctgagcattacctgcaccgtgagcggccttagcctgaccaactatggcgtgcattgg
gtgcgccagagccccgggcaaaggcctggaatggctgggcgtgatttgagcggcggaacaccgattataacaccccggttacca
gccgcctgagcattaacaaagataacagcaaaagccaggtgtttttaaaatgaacagcctgcagagcaacgataccgcgatttattat
tgcgcgcgcgcgctgacctattatgattatgaatttgctattggggccagggcaccctggtgaccgtgagcgcggcgcgcgcg
cagcggcgcgcgcgcgagcggcgcgcgcgcgagcgatattctgctgaccagagcccgggtgattctgagcgtgagccccggcg
aacgcgtgagcttagctgccgcgcgagccagagcattggcaccaacattcattggtatcagcagcgcaccaacggcagccccgcgc
ctgctgattaaatatgcgagcgaaagcattagcggcattccgagccgcttagcggcagcggcagcggcaccgattttaccctgagc
attaacagcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactggccgaccaccttggcgcgggcaccaaa
ctggaactgaaacgcacc). In some alternatives, the scFv comprises the EGFR scFv amino acid

ID NO: 53

(MLLLVTSLLLCELPHPAFLIPQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHW
VRQSPGKGLEWLGVIWSSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIY
YCARALTYDYEFAYWGQGLVTVSAGGGGSGGGGSGGGGSDILLTQSPVILSVSP
GERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTL
NSVESEDIADYYCQQNNNWPTTFGAGTKLELKRT). In some alternatives, the scFv is
encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino
acid sequence of SEQ ID NO: 53. In some alternatives, the scFv comprises the EGFRVIII
(806) scFv nucleotide sequence. In some alternatives, the scFv is specific for EGFR. In some
alternatives, the scFv is encoded by the EphA2 (2A4) scFv nucleotide sequence. In some
alternatives, the scFv is encoded by the nucleic acid sequence set forth in SEQ ID NO: 56. In
some alternatives, the scFv is encoded by the EpHA2 (4H5) scFv nucleotide sequence. In
some alternatives, the scFv is encoded by the nucleic acid sequence set forth in SEQ ID NO:
58. In some alternatives, the scFv is encoded by a FITC (E2) scFv nucleotide sequence. In
some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO:
60. In some alternatives, the scFv is encoded by an IL13Ra2 (hu08) VhV1 scFv nucleotide
sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in
SEQ ID NO: 68. In some alternatives, the scFv comprises an IL13Ra2 hu08 VhV1 scFv
amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set
forth in SEQ ID NO: 69

(MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMS

WVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDT
 AVYYCARQGTTALATRFVDVWGQGLTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS
 LSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYSASYRSTGVPSRFSGSGS
 GTDFTLTISSLQPEDFATYYCQHHYSAPWTFGGGTKVEIKESK). In some alternatives,
 the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding
 the amino acid sequence of SEQ ID NO: 69. In some alternatives, the scFv is encoded by an
 IL13Ra2 (hu07) VIVh scFv nucleotide sequence. In some alternatives, the scFv is encoded
 by a nucleic acid sequence set forth in SEQ ID NO: 70
 (atgcttctctggtgacaagccttctgctctgtgagttaccacacccagcattctctctgatcccagatattcagatgacccagagccc
 gagcagcctgagcgcgagcgtggcgatcgctgaccattacctgcaccgcgagcctgagcgtgagcagcacctatctgcattggt
 atcagcagaaaccgggcaaagcgccgaaactgctgatttatagcaccagcaacctggcgagcggcgtgccgagccgctttagcgg
 cagcggcagcggcaccgattttaccctgaccattagcagcctgcagccggaagattttgcgacctattattgccatcagtatcatcgca
 gcccgtgacctttggcgggcggcaccaaagtggaaattaaaggtggtggtggttctggcgggcggcggtccggtggtggtggtctg
 aagtgcagctggtggaaagcgggcggcgctggtgcagccggcggcagcctgcgcctgagctgcgcggcgagcggctttacct
 ttaccaaatatggcgtgcattgggtgcgccaggcgccgggcaaaggcctggaatgggtggcggtgaaatggcgggcggcgagca
 ccgattataacagcgcgctgatgagccgctttaccattagccgcgataacgcgaaaaacagcctgtatctgcagatgaacagcctgc
 gcgcggaagataccgcggtgtattattgcgcgcgcgatcatcgcatggattattggggccagggcaccctggtgaccgtg
 agcagcgaatctaagta). In some alternatives, the scFv comprises an IL13Ra2 (hu07) VhVl scFv
 amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set
 forth in SEQ ID NO: 71
 (MLLLVTSLLLCELPHPAFLIPDIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWY
 QQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYHRSP
 LTFGGGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFT
 KYGVHWVRQAPGKGLEWVAVKWAGGSTDYNALMSRFTISRDNANKNSLYLQMNS
 LRAEDTAVYYCARDHRDAMDYWGQGLTVTVSSESK). In some alternatives, the scFv
 is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
 amino acid sequence of SEQ ID NO: 71. In some alternatives, the scFv is encoded by the
 IL13Ra2 (hu07) VhVl scFv nucleotide sequence. In some alternatives, the scFv is encoded
 by a nucleic acid sequence set forth in SEQ ID NO: 72
 (atgcttctctggtgacaagccttctgctctgtgagttaccacacccagcattctctctgatcccagaagtgcagctggtggaaagcg
 gcggcggcctggtgcagccggcggcagcctgcgcctgagctgcgcggcgagcggctttacctttaccaaatatggcgtgcattgg

gtgcgccaggcgccgggcaaaggcctggaatgggtggcggtgaaatgggcgggaggcagcaccgattataacagcgcgctgat
gagccgctttaccattagccgcgataacgcgaaaaacagcctgtatctgcagatgaacagcctgcgcgcggaagataccgcggtgt
attattgcgcgcgcgatcatcgcgatgcgatggattattggggccagggcacctggtgaccgtgagcagcggtggtggtggttctg
gcggcggcggctccggtggtggtggttctgatattcagatgaccagagcccagcagcctgagcgcgagcgtgggcgatcgcg
gaccattacctgcaccgcgagcctgagcgtgagcagcacctatctgcattggtatcagcagaaaccgggcaaagcgccgaaactgc
tgatttatagcaccagcaacctggcgagcggcggtgccgagccgcttagcggcagcggcagcggcaccgattttaccctgaccatta
gcagcctgcagccggaagattttgcgacctattattgccatcagtatcatcgagcccgtgacctttggcggcggcaccgaaagtga
aattaaagaatctaagta). In some alternatives, the scFv comprises an IL13Ra2 (hu07) VhV1 scFv

amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set

forth in SEQ ID NO: 73

(MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFTKYGVH
WVRQAPGKGLEWVAVKWAGGSTDYNALMSRFTISRDNALNSLYLQMNSLRAED
TAVYYCARDHRDAMDYWGQGLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSA
SVGDRVTITCTASLSVSSTYLHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTD
FTLTISLQPEDFATYYCHQYHRSPLTFGGGTKVEIKESK). In some alternatives, the

scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the

amino acid sequence of SEQ ID NO: 73. In some alternatives, the antibody or portion thereof

or scFv is encoded by the Anti-IL13Ra2 (IL13 zetakine) nucleotide sequence. In some

alternatives, the antibody or portion thereof or scFv is encoded by a sequence set forth in

SEQ ID NO: 74

(atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccaggccctgtgcctccctctacagcc
ctcaggtacctcattgaggagctggtcaacatcaccagaaccagaaggctccgctctgcaatggcagcatggtatggagcatcaac
ctgacagctggcatgtactgtgcagccctggaatccctgatcaacgtgtcaggctgcagtgccatcgagaagaccagaggatgctg
agcggattctgcccgcacaaggtctcagctgggcagttttccagcttgcattgctccgagacaccaaatacgaggtggcccagtttgtaa
aggacctgctcttacatttaaagaaacttttcgcgagggacggttcaa). In some alternatives, the antibody or

portion thereof or scFv comprises an Anti-IL13Ra2 (IL13 zetakine) amino acid sequence. In

some alternatives, the antibody or portion thereof or scFv comprises an amino acid sequence

set forth in SEQ ID NO: 75

(MLLLVTSLLLCELPHPAFLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSI
NLTAGMYCAALESINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTEKIEVAQ
FVKDLLLHLKKLFREGRF). In some alternatives, the scFv is encoded by a nucleic acid

sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 75. In some alternatives, the scFv is encoded by an oaGD2 (8B6) VIVh scFv nucleotide sequence. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 76

(atgcttctctggtgacaagccttctgctctgtgagttaccacacccagcattctctgatcccagatgttgtgatgacccaaactcca
ctctccctgcctgtcagtccttgagatcaagcctcaatctcttcagatctagtcagagccttctaaaaataatggaaacacctttttaca
ttggtacctgcagaagtcaggccagtcctcaaagctccttatctacaaagtttccaaccgactttctggggtcccagacaggttcagtgg
cagtggatcaggacatatttcacactcaagatcagcagagtgaggctgaggatctgggagtttattctgctctcaaagtacacatat
tccgtacacattcggaggggggaccaagctcgagctgaaacgaggcggagggggctctggcggcggaggatctgggggagggg
gcagcgaggtgaaactggtggagtctggaggaggcttggtgctgcctggggattctctgagactctcctgtgcaacttctgagttcac
cttcaactgattactacatgacttgggtccgcccagcctccaagaaaggcacttgagtgggtgggtttattagaaacagagctaattggttac
acaacagagtacaatccatctgtgaagggtcggttcaccatttcagagataattcccaaagcatcctctatcttcaaataaacacccctg
agaactgaggacagtgccacttattactgtgcaagagtcttaactgggcctttgactactggggccaaggcaccactctcacagtctc
ctca). In some alternatives, the scFv comprises the oaGD2 (8B6) VIVh scFv amino acid
sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ
ID NO: 77

(MLLLVTSLLLCELPHPAFLIPDVVMTQTPLSLPVSLGDQASISCRSSQSLLKNNGNT
FLHWYLQKSGQSPKLLIYKVSNRLSGVPDRFSGSGSGTYFTLKISRVEAEDLGVYFCS
QSTHIPYTFGGGTKLELKRGGGGSGGGGSGGGGSEVKLVESGGGLVLPGLSLRLSCA
TSEFTFTDYYMTWVRQPPRKALEWLGFIENRANGYTTEYNPSVKGRFTISRDNQSIL
YLQMNTLRTEDSATYYCARVSNWAFDYWGQGTTTLTVSS). In some alternatives, the
scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
amino acid sequence of SEQ ID NO: 77. In some alternatives, the scFv is encoded by an
ROR1 (R12) scFv nucleotide sequence. In some alternatives, the scFv is encoded by a
sequence set forth in SEQ ID NO: 78

(atgctgctgctggtgacaagcctgctgctgtgcgagctgccccccccgctttctgctgatccccaggaacagctcgtcgaaagc
ggcggcagactggtgacacctggcggcagcctgacctgagctgcaaggccagcggcttcgacttcagcgcctactacatgagctg
ggtccgccaggcccctggcaagggactggaatggatgccaccatctaccccagcagcggcaagacctactacgccacctgggtg
aacggacggttcaccatctccagcgacaacgcccagaacacctggacctgcagatgaacagcctgacagccgcccagccgggcc
acctacttttgcgccagagacagctacgccgacgacggcgccctgttcaacatctggggccctggcaccctggtgacaatctctagc
ggcggaggcggatctggtggcggaggaagtggcggcggaggatctgagctggtgctgacctagagccctctgtgtctgctgccc

tggaagccctgccaagatcacctgtaccctgagcagcgcccaagaccgacaccatcgactggtatcagcagctgcagggcga
ggccccagatacctgatgcaggtgcagagcgacggcagctacaccaagaggccaggcgtgcccgaccggttcagcggatctag
ctctggcgccgaccgctacctgatcatccccagcgtgcaggccgatgacgaggccgattactactgtggcgccgactacatcggcg
gctacgtgttcggcgaggcaccagctgaccgtgaccggc). In some alternatives, the scFv comprises an
amino acid sequence set forth in SEQ ID NO: 79 (ROR1 (R12) scFv amino acid sequence;
MLLLVTSLLLCELPHPAFLIPQEQLVESGGRLVTPGGSLTLSCKASGFDFSAYYMSW
VRQAPGKGLEWIA TIYPSSGKTYyatwvNGRFTISSDNAQNTVDLQMNSLTAADRA
TYFCARDSYADDGALFNIWGPGLVTISSGGGGSGGGGSGGGGSELVLTQSPSVSAA
LGSPAKITCTLSSAHKTD TIDWYQQLQGEAPRYLMQVQSDGSYTKRPGVPDRFSGSS
SGADRYLIIPSVQADDEADYYCGADYIGGYVFGGGTQLTVTG). In some alternatives,
the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding
the amino acid sequence of SEQ ID NO: 79. In some alternatives, the scFv comprises an
amino acid sequence set forth in SEQ ID NO: 80 (CD33 (h2H12) VhVl scFv nucleotide
sequence

(atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccaggttcagctggtgcagctctgga
gctgaggtgaagaagcctggggcctcagtgaggctcctgcaaggcttctggttacacctttaccaattatgatataaattgggtgag
acagggccctggacaagggcctgagtggttgatggatttatcctggagatggtagtaccaaataatgagaaattcaaggccaag
gctaccctgacagctgacacatccaccagcacagcctacatggagctgaggagcctgagatctgatgacacagctgtgtattactgtg
cttctggatatgaagatgctatggactactgggggcaagggaccacagtcacagtctcctca). In some alternatives, the
scFv is specific for CD33. In some alternatives, the scFv comprises an amino acid sequence
set forth in SEQ ID NO: 81 (CD33 (h2H12) VhVl scFv amino acid sequence;
MLLLVTSLLLCELPHPAFLIPQVQLVQSGAEVKKPGASVKVSCKASGYTFTNYDIN
WVRQAPGQGLEWIGWIYPGDGSTKYNEKFKAKATLTADTSTSTAYMELRSLRSDDT
AVYYCASGYEDAMDYWGQGTTVTVSS). In some alternatives, the scFv is encoded by a
nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence
of SEQ ID NO: 81. In some alternatives, the scFv is encoded by a sequence set forth in SEQ
ID NO: 82 (CD33 (h2H12) VhVl scFv nucleotide sequence:
atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagacatccagatgaccagctcctcat
cctcactgtctgcatctgtaggagacagagtcacatcaattgtaaggctagtcaggacattaatagctatttgagctggttcagcaga
aaccagggaaagcccctaagaccctgatctatagagcaaataagattggtagatgggggtcccatcaagggttctctggcagtggtatctgg
gcaagattatactctcaccatcagcagcctgcagcctgaagattttgcaacttattactgcttgagtagtatgagtttctctcacatttgg

aggagggaccaaggtggagatcaaa). In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 83 (CD33 (h2H12) VIVh scFv amino acid sequence: MLLLVTSLLLCELPHPAFLIPDIQMTQSPSSLSASVGDRVTINCKASQDINSYLSWFQ QKPGKAPKTLIYRANRLVDGVPSRFSGSGSGQDYTLTISSLQPEDFATYYCLQYDEFP LTFGGGGTKVEIK). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 83. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 84. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 86. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 87. In some alternatives, the spacer is encoded by the nucleic acid sequence set forth in SEQ ID NO: 102 (IgG4 hinge nucleotide sequence: gagagcaagtacggaccgcccctgcccccttgccct). In some alternatives, the spacer comprises an amino acid sequence set forth in SEQ ID NO: 103 (IgG4 hinge amino acid sequence: ESKYGPPCPPCP). In some alternatives, the spacer is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 103. In some alternatives, the spacer is encoded by a nucleic acid sequence set forth in SEQ ID NO: 104. In some alternatives, a DNA spacer is between the inducible promoter and the transgene. In some alternatives, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120 (SEQ ID NO: 120; gctagcgtttaaacttaagcttggtaccgagctcggatccgccacc). In some alternatives, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives, HEA3 comprises two amino acid mutations, wherein the sequence is encoded by a sequence set forth in SEQ ID NO: 122 (atggtgtccaagctgtcccagctgcagacagaactgctggcagcactgctggaaagcggcctgagcaaagaggccctgattcagg cactcggcgaacctggaccttatctgctcgtggcgaaggccctctggataagggcgagagctgtggcggaggaagaggagagct ggccgagctgcctaacggcctgggcgagacaagaggcagcagaggacgagacagacgacgacggcgaggacttcaccccccc atcctgaaagagctggaaaacctgagccccgaggaagccgcccaccagaaagccgtggtggagacactgctgcaggaagatccc tggcgggtcgccaagatggtcaagagctacctgcagcagcacaacatccccagcgggaggtggtggacaccaccggcctgaac cagagccacctgagccagcacctgaacaagggcaccatgaaaaccagaagagagccgcccctgtacacttggtacgtgcgg aagcagagagaggtggcccagcagtttacacacgcccggccaggcgccctgatcgaggaacctaccggcgacgagctgccac caagaagggcagacggaaccggttaagtggggccctgcatctcagcagatcctgttcaggcctacgagcggcagaagaacccc

agcaaagaggaacgggagacactggtggaagagtgaaccgggcccagtgcatccagagaggcgtgagcccttctcaggctcag
ggcctcggcagcaatctggtcaccgaagtgcgggtgtacaattggttcgccaaccggcgaaagaggaagccttcgggcacaagct
gtctgctggcgatatgagagccgccaacctgtggcccagccccctgatgatcaagcggagcaagaagaacagcctggccctgagc
ctgaccgccgatcagatggtgtccgctctgctggacgccgagccccctatcctgtacagcgagtacgaccccaccagacccttcagc
gaggccagcatgatgggcctgctgaccaacctggccgaccgggagctggtgcacatgatcaactgggccaagcgggtgcccggc
ttcgtggacctgacctgcacgaccaggtccacctgctggaatgtgcctggctggaaatcctgatgatcggcctcgtgtggagaagc
atggaacaccccggaagctgctgttcgcccccaacctgctcctggaccggaaccaggggaaagtgcgtggagggcatggtggaga
tcttcgacatgctgctggccacctccagccggttcgggatgatgaacctgcagggcgaggaattcgtgtgcctgaagtccatcatcct
gctgaacagcggcgtgtacaccttctgtcatccacctgaagtccctggaagagaaggaccacatccaccgggtgctggacaaga
tcaccgacacctgatccacctgatggccaaggctggcctgacactccagcagcagcaccagagactggcccagctgctgctgatc
ctgagccacatccggcacatgagcaacaagcggatggaacacctgtacagcatgaagtgaagaacgtggtgcccctgtacgacct
gctgctcgagatgctggatgccacagactgcacgcccctacaagcagaggcggagccagcgtggaggaaaccgaccagtctca
cctggccaccgcccggcagcacaagcagccacagcctgcagaagtactacatcaccggcgaggccgagggaattccctgccaccgt
ggagttccagtacctgcccgaaccgacgaccggcaccggatcgaggaaaagcgggaagcggacctacgagacattccagagcat
catgaagaagtcccccttcagcggccccaccgatccagacccccctagaagaatcgccgtgcccagcagatctagcgccagc
gtgccaagcctgccccccagccctaccctttcaccagcagcctgagcaccatcaactacgacgagttccctaccatggtgttccca
gcgccagatctctcaggcctctgctctggcacctgctccacctcaggtgctgcctcaggccccctgctccagccccagccccctgccat
ggtgtctgcactggcccaggctccagctcctgtgcctgtgctggccccctggacctcctcaggctgtggccccctcctgcccctaaacct
accaggccgggggaggggaacactgtctgaggccctgctgcagctccagttcgacgacgaggatctgggagcactgctgggcaata
gcaccgacccccgccgtgtttaccgacctggcctccgtggacaacagcgagttccagcagctcctcaaccaggggcatccctgtcgcc
ccacacaccaccgagccccatgctgatggaataccccgaggccatcaccagactggtcacaggcgcccagaggccctccagatccag
caccagctccactgggagccccctggcctgcctaattgggctgctgtctggcgacgaggacttcgagagcattgccgacatggacttca
gcgccctgctgtcccagatcagcagc). In some alternatives, the HEA3 comprises an amino acid

sequence set forth in SEQ ID NO: 121
(MVSKLSQLQTELLAALLESGLSKEALIQALGEPGPYLLAGEGPLDKGESCGGGRGEL
AELPNGLGETRGSEDETDDDGEDFTPPILKELENLSPEEAAHQKAVVETLLQEDPWR
VAKMVKSYLQQHNIPQREVVDTTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVR
KQREVAQQFTHAGQGGLIEEPTGDELPTKKGRRNRFKWGPASQQILFQAYERQKNP
SKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRHK
LSAGDMRAANLWPSPLMIKRSKKNLALSLTADQMVSALLDAEPPILYSEYDPTRPF
SEASMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQVHILLECAWLEILMIGLV

WRSMEHPGKLLFAPNLLLDNRNQGKCEGMVEIFDMLLATSSRFRMMNLQGEEFVC
 LKSIILLNSGVYTFLSSTLKSLEEKDHIHRVLDKITDTLIHLMAKAGLTLQQQHQR
 LLAQLLLILSHIRHMSNKRMEHLYSMKCKNVVPLYDLLLEMLDAHRLHAPTSRGGASVE
 ETDQSHLATAGSTSSHSLQKYYITGEAEGFPATVEFQYLPDTDDRHRIEEKRKRTYET
 FQSIMKKSPFSGPTDPRPPRRRIAVPSRSSASVPKPAPQPYPTSSSLSTINYDEFPTMVFP
 SGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAP
 KPTQAGEGTLSEALLQLQFDDLEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIP
 VAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFESIADM
 DFSALLSQISS). This sequence comprises the sequence of HEA3 containing two point
 mutations (K310Q and S536E, positions in the RelA protein) in the p65 domain that enhance
 transcriptional activity. This is referred to as HEA3(p65/S536E/K310Q). K310Q in RelA
 corresponds to position 621 in HEA3; S536E corresponds to position 846 in HEA3. In some
 alternatives, a system for inducible expression of a chimeric antigen receptor in cells is
 provided. In some alternatives, the transgene encodes a polypeptide that regulates apoptosis.
 In some alternatives, genes that inhibit apoptosis include, for example, Bcl2, and/or CA-Akt.
 In some alternatives, the polypeptide is Bcl2 or CA-Akt. In some alternatives, genes that
 inhibit apoptosis include, for example, Survivin, Bcl2, CA-Akt, and/or dnCaspase3. In some
 alternatives, the polypeptide is Survivin, Bcl2, or CA-Akt and/or dnCaspase 3. In some
 alternatives, Survivin is encoded by a sequence set forth in SEQ ID NO: 124
 (Atgggtgccccgacgttgccccctgcctggcagccctttctcaaggaccaccgcatctctacattcaagaactggcccttcttgag
 ggctgcgcctgcacccccggagcggatggccgaggctggcttcatccactgccccactgagaacgagccagacttgccccagtgtt
 ctctctgcttcaaggagctggaaggctgggagccagatgacgaccccatagaggaaacataaaaagcattcgtccggttgcgctttcctt
 ctgtcaagaagcagtttgaagaattaacccttggtgaattttgaaactggacagagaaagagccaagaacaaaattgcaaaggaaac
 caacaataagaagaagaatttgaggaaactgtgaagaaagtgcgccgtgccatcgagcagctggctgcaatggat). In some
 alternatives, Survivin comprises the amino acid sequence set forth in SEQ ID NO: 123
 (MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFIHCPTENEPDLAQC
 FFCFKELEGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDREERAKNKIAK
 ETNNKKKEFEETVKKVRRRAIEQLAAMD).

[0299] In some alternatives, a system for inducible expression of a chimeric antigen receptor in cells is provided. The system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably

linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from the group consisting of a tumor specific molecule, a viral specific molecule, and any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte; a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer; a third polynucleotide, which encodes a transmembrane domain; and a fourth polynucleotide, which encodes an intracellular signaling domain; and b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by a drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives, the transcriptional activator is HEA3 or HEA4. In some alternatives, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional

activity in response to the drug ligand. In some alternatives, the variant of the wild type HEA3 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives, the variant of the wild type HEA3 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives, the variant of a wild type HEA4 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 4-7. In some alternatives, the variant of the wild type HEA4 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives, the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23. In some alternatives, the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor. In some alternatives, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives, the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain. In some alternatives, the second promoter is an inducible promoter. In some alternatives, the second promoter is a constitutive promoter. In some alternatives, the second promoter is the EF1 α p. In some alternatives, the transcriptional activator comprises at least one amino acid sequence set forth in SEQ ID NO: 1-11 or 121. In some alternatives, the transcriptional activator is encoded by at least one nucleic acid sequence set forth in SEQ ID NO: 12-22 or 122. In some alternatives, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives, both vectors are packaged in a viral vector. In some alternatives, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives, the first nucleic acid further comprises a fifth polynucleotide that encodes

a selectable marker. In some alternatives, the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker. In some alternatives, the selectable marker is EGFRt and/or HER2t. In some alternatives, the selectable marker confers drug resistance. In some alternatives, the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide. In some alternatives, the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide. In some alternatives, the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor. In some alternatives, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives, the DNA spacer enhances gene expression of the chimeric antigen receptor.

[0300] In some alternatives, a system for inducible expression of a gene that regulates a T cell function is provided. The system comprises a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity; and b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug; and wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3. In some alternatives of the system, the transcriptional activator is HEA3 or HEA4. In some

alternatives of the system, HEA3 is a variant of a wild type HEA3 transcription factor. In some alternatives of the system, HEA4 is a variant of a wild type HEA4 transcription factor. In some alternatives of the system, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system, HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives of the system, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand. In some alternatives of the system, HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug. In some alternatives of the system, the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand. In some alternatives of the system, the variant of a wild type HEA3 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 1-3, 8-11 and/or 121. In some alternatives of the system, the variant of the wild type HEA3 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122. In some alternatives of the system, the variant of a wild type HEA4 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 4-7. In some alternatives of the system, the variant of the wild type HEA4 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 15, 16, 17 or 18. In some alternatives of the system, the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug. In some alternatives of the system, the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8. In some alternatives of the system, the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or wild type HEA4 chimeric

transcription factor at a concentration of the drug that is at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values. In some alternatives of the system, the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero. In some alternatives of the system, the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor. In some alternatives of the system, the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss. In some alternatives of the system, the second nucleic acid further comprises: a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral molecule, or another molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte; a polynucleotide coding for a polypeptide spacer, wherein the spacer is optimized; a polynucleotide coding for a transmembrane domain; and a polynucleotide encoding an intracellular signaling domain. In some alternatives of the system, the first promoter is in opposite orientation to the second promoter. In some alternatives of the system, the ligand binding domain is an antibody fragment. In some alternatives of the system, the ligand binding domain is a single chain variable fragment. In some alternatives of the system, the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or any combination thereof. In some alternatives of the system, the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein. In some alternatives of the system, the ligand binding domain specifically binds with CD19, CD20, CD22, EGFRvIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA. In some alternatives of the system, the spacer comprises IgG4, IgG4-CH2(L235D, N297Q) or IgG4-CH3. In some alternatives of the system, the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that

encodes the chimeric antigen receptor. In some alternatives of the system, the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor. In some alternatives of the system, the DNA spacer comprises a sequence set forth in SEQ ID NO: 120. In some alternatives of the system, the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values. In some alternatives of the system, the DNA spacer enhances gene expression of the chimeric antigen receptor. In some alternatives of the system, the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector. In some alternatives of the system, both vectors are packaged in a viral vector. In some alternatives of the system, the viral vector is an adeno-associated virus or a lentivirus. In some alternatives, the chimeric antigen receptor comprises a scFv. In some alternatives, the scFv specifically binds to CD19. In some alternatives, the scFv comprises an huCD19 scFv (G01S) amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 41

(MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGRIGYADSVKGRFTISRDNKNSLFLQMNSLRAEDTAVYYCARDQGYHYDSAETHAFDIWGQGT VTVSSGGGGSGGGGSGGGGGSQSALTQPRSVSGFPGQSVTISCTGTTSDVSWYQQHPGKAPQLMLYDVSKRPSGVPHRFSGRSGRAASLIISGLQTEDEADYFCSSYAGRYNSVLFGGGTKLTVL). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 41. In some alternatives, the scFv is encoded by the muCD19 (FMC63) scFv nucleotide sequence. In some alternatives, the scFv specifically binds to CD19. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO: 42

(Atgctgctgctggtgaccagcctgctgctgtgcgagctgccccacccgcctttctgctgatcccgacatccagatgaccagacacactccagcctgagcgccagcctggcgaccgggtgaccatcagctgccgggcccagccaggacatcagcaagtacactgaactggtatcagcagaagcccgacggcaccgtcaagctgctgatctaccacaccagccggctgcacagcggcgtgccagccgggttagcggcagcgggtccggcaccgactacagcctgaccatctccaacctggaacaggaagatatcgccacctactttgccagcagggcaacacactgcctacacctttggcggcggaacaaagctggaaatcaccggcagcacctccggcagcggcaagcctggcagcggcgagggcagcaccaagggcgaggtgaagctgcaggaaagcggccctggcctggtggccccagccagagcctgagcgtgacctgca

ccgtgagcggcgtgagcctgcccgactacggcgtgagctggatccggcagccccccaggaaggcctggaatggctgggcgtga
tctggggcagcgcagaccactactacaacagcgccctgaagagccggctgaccatcatcaaggacaacagcaagagccaggtgtt
cctgaagatgaacagcctgcagaccgacgacaccgccatctactactgcgccaagcactactactacggcggcagctacgccatgg
actactggggccagggcaccagcgtgaccgtgagcagc). In some alternatives, the scFv comprises an
amino acid sequence set forth in SEQ ID NO: 43
(MLLLVTSLLLCELPHPAFLIPDIQMTQTTSSLSASLGDRVITISCRASQDISKYLNWY
QQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLP
YTFGGGKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVS
LPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIKD NSKSQVFLKMNSL
QTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS). In some alternatives, the scFv
is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
amino acid sequence of SEQ ID NO: 43. some alternatives, the scFv comprises a B7H3
(hBRCA84D) scFv nucleotide sequence. In some alternatives, the scFv binds specifically to
the B7-H3 receptor. In some alternatives, the scFv comprises the L1CAM (CE7) scFv
nucleotide sequence. In some alternatives, the scFv is specific for the epitope, CE7 epitope,
of L1CAM. In some alternatives, the scFv is encoded by a sequence set forth in SEQ ID NO:
50

(atgctgctgctggtgaccagcctgctgctgtgcgagctgccccaccccgctttctgctgatccccaggtgcagctgcagcagcct
ggcgccgagctggtgaagccaggcgccagcgtgaagctgtcctgcaaggccagcggctacaccttcaccggctactggatgcact
gggtgaagcagagacccggccacggcctggaatggatcggcgagatcaacccagcaacggccggaccaactacaacgagcgg
ttcaagagcaaggccaccctgaccgtggacaagagcagcaccaccgccttcagctgtccggcctgaccagcaggacagcgcg
ccgtgtacttctgcgccagggactactacggcaccagctacaacttcgactactggggccagggcaccacactgaccgtgagcagc
ggcggagggggctctggcgggcgaggatctgggggagggggcagcgcacatccagatgaccagagcagcagcagcttcagcg
tgagcctgggcgaccgggtgaccatcacctgtaaggccaacgaggacatcaacaaccggctggcctggtatcagcagacccccgg
caacagccccaggctgctgatcagcggcgccaccaacctggtgaccggcgtgccagccggttagcggcagcggctccggcaa
ggactacaccctgaccatcacaagcctgcaggccgaggacttcgccacctactactgccagcagtactggtccaccccttcaccttc
ggcagcggcaccgagctggaaatcaaa). In some alternatives, the scFv comprises the L1CAM (CE7)
scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence
set forth in SEQ ID NO: 51
(MLLLVTSLLLCELPHPAFLIPQVQLQQPGAELVKPGASVKLSCKASGYTFTGYWM
HWVKQRPGHGLEWIGEINPSNGRTNYNERFKSKATLTVDKSSTTAFMQLSGLTSEDS

AVYFCARDYYGTSYNFDYWQGTTTLTVSSGGGGSGGGGSGGGGSDIQMTQSSSSFS
 VSLGDRVITITCKANEDINNRLAWYQQTPGNSPRLISGATNLVTGVPSRFSGSGSGK
 DYTLTITSLQAEDFATYYCQQYWSTPFTFGSGTELEIK). In some alternatives, the scFv
 is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
 amino acid sequence of SEQ ID NO: 51. In some alternatives, the scFv is encoded by the
 EGFR (cetuximab) scFv nucleotide sequence. In some alternatives, the scFv is specific for
 EGFR. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ
 ID NO: 52

(atgcttctctggtgacaagccttctgctctgtgagttaccacacccagcattctctctgatccacaggtgcagctgaaacagagcg
 gcccgggctggtgcagccgagccagagcctgagcattacctgcaccgtgagcggcttagcctgaccaactatggcgtgcattgg
 gtgcgccagagcccgggcaaaggcctggaatggctggcggtgatttgagcggcggaacaccgattataacaccccgttacca
 gccgctgagcattaacaaagataacagcaaaagccaggtgtttttaaataaacagcctgcagagcaacgataccgcgattattat
 tgcgcgcgcgctgacctattatgattatgaatttgctattggggccagggcaccctggtgaccgtgagcggcgggcgcgcg
 cagcggcgggcgggcagcggcgggcgggcagcgatattctgctgaccagagcccgtgattctgagcgtgagcccggg
 aacgcgtgagcttagctgccgcgagccagagcattggcaccaacattcattggtatcagcagcgcaccaacggcagcccgcgc
 ctgctgattaaatatgcgagcgaaagcattagcggcattccgagccgcttagcggcagcggcagcggcaccgattttaccctgagc
 attaacagcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactggccgaccaccttggcgcgggcaccaaa
 ctggaactgaaacgcacc). In some alternatives, the scFv comprises the EGFR scFv amino acid
 sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ
 ID NO: 53

(MLLLVTSLLLCELPHPAFLIPQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHW
 VRQSPGKGLEWLGVIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIY
 YCARALTYDYEFAYWGQGLVTVSAGGGGSGGGGSGGGGSDILLTQSPVILSVSP
 GERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSI
 NSVESEDIADYYCQQNNNWPTTFGAGTKLELKRT). In some alternatives, the scFv is
 encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino
 acid sequence of SEQ ID NO: 53. In some alternatives, the scFv comprises the EGFRVIII
 (806) scFv nucleotide sequence. In some alternatives, the scFv is specific for EGFR. In some
 alternatives, the scFv is encoded by the EphA2 (2A4) scFv nucleotide sequence. In some
 alternatives, the scFv is encoded by the nucleic acid sequence set forth in SEQ ID NO: 56. In
 some alternatives, the scFv is encoded by the EpHA2 (4H5) scFv nucleotide sequence. In

some alternatives, the scFv is encoded by the nucleic acid sequence set forth in SEQ ID NO: 58. In some alternatives, the scFv is encoded by a FITC (E2) scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 60. In some alternatives, the scFv is encoded by an IL13Ra2 (hu08) VhVl scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 68. In some alternatives, the scFv comprises an IL13Ra2 hu08 VhVl scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 69 (MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRNGMS WVRQAPGKGLEWVATVSSGGSYIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDT AVYYCARQGTTALATRFDDVWGQGLTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRVTITCKASQDVGTAFAVWYQQKPGKAPKLLIYSASRSTGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCQHHYSAPWTFGGGTKVEIKESK). In some alternatives, the scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 69. In some alternatives, the scFv is encoded by an IL13Ra2 (hu07) VhVl scFv nucleotide sequence. In some alternatives, the scFv is encoded by a nucleic acid sequence set forth in SEQ ID NO: 70 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagatattcagatgacccagagccc gagcagcctgagcgcgagcgtggcgatcgctgaccattacctgcaccgcgagcctgagcgtgagcagcacctatctgcattggt atcagcagaaaccgggcaaagcgccgaaactgctgatttatagcaccagcaacctggcgagcggcgtgccgagccgcttagcgg cagcggcagcggcaccgattttacctgaccattagcagcctgcagccggaagattttgcgacctattattgccatcagtatcatcgca gcccgtgacctttggcggcgccaccaaagtggaaattaaaggtggtggtggttctggcggcgccggtccggtggtggtggttctg aagtgcagctggtggaaagcgggcgccgctggtgcagccggcgccgagcctgcgcctgagctgcgcggcgagcggcctttacct ttaccaaatatggcgtgattgggtgcgccaggcgccgggcaaaggcctggaatgggtggcggtgaaatgggcggcgccgagca ccgattataacagcgcgctgatgagccgctttaccattagccgcgataacgcgaaaaacagcctgtatctgcagatgaacagcctgc gcgcggaagataccgcggtgtattattgcgcgcgcgatcatcgcatgcatggattattggggccagggcaccctggtgaccgtg agcagcgaatctaagta). In some alternatives, the scFv comprises an IL13Ra2 (hu07) VhVl scFv amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 71 (MLLLVTSLLLCELPHPAFLLIPIQMTQSPSSLSASVGDRVTITCTASLSVSSTYLHWY QQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYHRSP

LTFGGGKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFT
 KYGVHWVRQAPGKGLEWVAVKWAGGSTDYNALMSRFTISRDNALNSLYLQMNS
 LRAEDTAVYYCARDHRDAMDYWGQGTLVTVSSESK). In some alternatives, the scFv
 is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
 amino acid sequence of SEQ ID NO: 71. In some alternatives, the scFv is encoded by the
 IL13Ra2 (hu07) VhVl scFv nucleotide sequence. In some alternatives, the scFv is encoded
 by a nucleic acid sequence set forth in SEQ ID NO: 72
 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccagaagtgcagctggtggaaagcg
 gggcgccctggtgcagccggcgccagcctgcgcctgagctgcgcggcgagcggctttacctttaccaaataatggcgtgcattgg
 gtgcgccaggcgccgggcaaaggcctggaatgggtggcggtgaaatggcgggcgccagcaccgattataacagcgcgctgat
 gagccgctttaccattagccgcgataacgcgaaaaacagcctgtatctgcagatgaacagcctgcgcgcggaagataccgcggtgt
 attattgcgcgcgcgatcatcgcgatgcgatggattattggggccagggcaccctggtgaccgtgagcagcgggtggtggttctg
 gggcgggcggtccggtggtggtggttctgatattcagatgaccagagcccgagcagcctgagcgcgagcgtgggcgatcgct
 gaccattacctgcaccgcgagcctgagcgtgagcagcacctatctgcattggtatcagcagaaaccgggcaaagcgccgaaactgc
 tgatttatagcaccagcaacctggcgagcggcggtgccgagccgctttagcggcagcggcagcggcaccgattttaccctgaccatta
 gcagcctgcagccggaagattttgcgacctattattgcatcagtatcatcgagcccgctgacctttggcgggcgccaccaaagtga
 aattaaagaatctaagta). In some alternatives, the scFv comprises an IL13Ra2 (hu07) VhVl scFv
 amino acid sequence. In some alternatives, the scFv comprises an amino acid sequence set
 forth in SEQ ID NO: 73
 (MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFTKYGVH
 WVRQAPGKGLEWVAVKWAGGSTDYNALMSRFTISRDNALNSLYLQMNSLRAED
 TAVYYCARDHRDAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSA
 SVGDRVITITCTASLSVSSTYLHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTD
 FTLTISSLQPEDFATYYCHQYHRSPLTFGGGKVEIKESK). In some alternatives, the
 scFv is encoded by a nucleic acid sequence comprising a nucleic acid sequence encoding the
 amino acid sequence of SEQ ID NO: 73. In some alternatives, the antibody or portion thereof
 or scFv is encoded by the Anti-IL13Ra2 (IL13 zetakine) nucleotide sequence. In some
 alternatives, the antibody or portion thereof or scFv is encoded by a sequence set forth in
 SEQ ID NO: 74
 (atgcttctcctggtgacaagccttctgctctgtgagttaccacacccagcattcctcctgatcccaggccctgtgcctccctctacagcc
 ctcaggtacctcattgaggagctggtcaacatcacccagaaccagaaggctccgctctgcaatggcagcatggtatggagcatcaac

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

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NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. A system for inducible expression of a chimeric antigen receptor in cells, the system comprising:
 - a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide that encodes a chimeric antigen receptor, which comprises a ligand binding domain that is specific for a ligand selected from the group consisting of a tumor specific molecule, a viral specific molecule, and any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte;
 - a second polynucleotide, which encodes a spacer, preferably an optimized polypeptide spacer;
 - a third polynucleotide, which encodes a transmembrane domain; and
 - a fourth polynucleotide, which encodes an intracellular signaling domain; and
 - b) a second nucleic acid comprising a second promoter that is operably linked to a nucleic acid encoding a transcriptional activator for the first promoter inducible by a drug, wherein the system is inducible by an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3.
2. The system of Claim 1, wherein the transcriptional activator is HEA3 or HEA4.
3. The system of Claim 2, wherein HEA3 is a variant of a wild type HEA3 transcription factor.
4. The system of Claim 2, wherein HEA4 is a variant of a wild type HEA4 transcription factor.
5. The system of any one of Claims 2-3, wherein HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and

wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand.

6. The system of any one of Claims 2-3, wherein HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug.
7. The system of Claim 2 or 4, wherein HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand.
8. The system of any one of Claims 2, 4 or 7, wherein HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug.
9. The system of any one of Claims 2-8, wherein the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand.
10. The system of any one of Claims 2, 3, 5, 6 or 9, wherein the variant of the wild type HEA3 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 1-3, 8-11 and/or 121.
11. The system of any one of Claims 2, 3, 5, 6, 9 or 10, wherein the variant of the wild type HEA3 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122.
12. The system of any one of Claims 2, 4 or 7-9, wherein the variant of a wild type HEA4 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 4-7.

13. The system of any one of Claims 2, 4, 7-9 or 12 wherein the variant of the wild type HEA4 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 15, 16, 17 or 18.
14. The system of any one of Claims 1-13, wherein the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8.
15. The system of any one of Claims 1-14, wherein the first promoter comprises a nucleic acid sequence set forth in SEQ ID NO: 23.
16. The system of any one of Claims 1-15, wherein the drug induces a high expression of the chimeric antigen receptor, such as at a concentration of at least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of drug or a concentration that is within a range defined by any two of the aforementioned values, as compared to a wild type HEA3 or a wild type HEA4 chimeric transcription factor.
17. The system of any one of Claims 1-16, wherein the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero.
18. The system of Claim 17, wherein the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage amount for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor.
19. The system of Claim 18, wherein the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss.
20. The system of any one of Claims 1-19, wherein the signaling domain comprises a CD28, 4-1BB and/or CD3 ζ domain.
21. The system of any one of Claims 1-20, wherein the second promoter is an inducible promoter.

22. The system of any one of Claims 1-20, wherein the second promoter is a constitutive promoter.
23. The system of Claim 22, wherein the second promoter is the EF1 α p.
24. The system of any one of Claims 1-23, wherein the transcriptional activator comprises at least one amino acid sequence set forth in SEQ ID NO: 1-11 or 121.
25. The system of any one of Claims 1-24, wherein the transcriptional activator is encoded by at least one nucleic acid sequence set forth in SEQ ID NO: 12-22 or 122.
26. The system of any one of Claims 1-25, wherein the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector.
27. The system of Claim 26, wherein both vectors are packaged in a viral vector.
28. The system of Claim 27, wherein the viral vector is an adeno-associated virus or a lentivirus.
29. The system of any one of Claims 1-28, wherein the first nucleic acid further comprises a fifth polynucleotide that encodes a selectable marker.
30. The system of any one of Claims 1-29, wherein the second nucleic acid further comprises a sixth polynucleotide that encodes a selectable marker.
31. The system of Claim 29 or 30, wherein the selectable marker is EGFR_t and/or HER2_t.
32. The system of Claim 29 or 30, wherein the selectable marker confers drug resistance.
33. The system of any one of Claims 1-32, wherein the first nucleic acid further comprises a seventh polynucleotide that encodes a self-cleaving peptide.
34. The system of Claim 33, wherein the self-cleaving peptide comprises a P2A, T2A, E2A or F2A peptide.

35. The system of any one of Claims 1-34, wherein the spacer is optimized for increased T cell proliferation and/or cytokine production in response to the ligand as compared to a reference chimeric receptor.
36. The system of any one of Claims 1-35, wherein the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor.
37. The system of Claim 36, wherein the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor.
38. The system of any one of Claims 36 or 37, wherein the DNA spacer comprises a sequence set forth in SEQ ID NO: 120.
39. The system of any one of Claims 36-37, wherein the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values.
40. The system of any one of Claims 36-39, wherein the DNA spacer enhances gene expression of the chimeric antigen receptor.
41. A system for inducible expression of a gene that regulates a T cell function comprising:
 - a) a first nucleic acid comprising a first promoter inducible by a drug, wherein the first nucleic acid is operably linked to a first polynucleotide, which encodes a cytokine, a chemokine receptor, a chimeric cytokine receptor, a polypeptide that regulates apoptosis, a polypeptide that modulates the extracellular environment, a polypeptide that modulates checkpoint signaling, a microRNA product that regulates growth, a microRNA product that regulates survival, or a microRNA product that regulates cytolytic capacity; and

b) a second nucleic acid comprising a second promoter, wherein the second nucleic acid is operably linked to a third nucleic acid that encodes a transcriptional activator for the first promoter inducible by drug; and

wherein the system is configured to respond to an amount of the drug that is less than a comparable system utilizing a wild type HEA3 chimeric transcription factor or a wild type HEA4 chimeric transcription factor, or the system has an enhanced transcriptional expression at a given concentration of the drug compared to a system utilizing a wild type HEA3.

42. The system of Claim 41, wherein the transcriptional activator is HEA3 or HEA4.
43. The system of Claim 42, wherein HEA3 is a variant of a wild type HEA3 transcription factor.
44. The system of Claim 42, wherein HEA4 is a variant of a wild type HEA4 transcription factor.
45. The system of Claim 42 or 43, wherein HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, and wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand.
46. The system of Claim 42 or 43, wherein HEA3 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug.
47. The system of Claim 42 or 44, wherein HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the estrogen receptor ligand binding domain comprises at least one mutation that allows selective binding to the drug ligand.
48. The system of Claim 42 or 44, wherein HEA4 comprises an estrogen receptor ligand binding domain (ER-LBD) and a RelA (p65) transactivation domain, wherein the

- RelA (p65) transactivation domain comprises at least one mutation that enhances transcriptional activity in response to a drug.
49. The system of any one of Claims 42-48, wherein the variant of the HEA3 or HEA4 chimeric transcription factor comprises p65 phosphomimetic and/or acetylmimetic amino acid substitutions that enhance transcriptional activity in response to the drug ligand.
50. The system of any one of Claims 42, 43, 45, 46 or 49, wherein the variant of a wild type HEA3 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 1-3, 8-11 and/or 121.
51. The system of any one of Claims 42, 43, 45, 46, 49 or 50 wherein the variant of the wild type HEA3 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 12, 13, 14, 19, 20, 21, 22 or 122.
52. The system of any one of Claims 42 or 47-49, wherein the variant of a wild type HEA4 transcription factor comprises an amino acid sequence set forth in SEQ ID Nos: 4-7.
53. The system of any one of Claims 42, 47-49 or 52, wherein the variant of the wild type HEA4 transcription factor is encoded by a nucleic acid sequence set forth in SEQ ID NO: 15, 16, 17 or 18.
54. The system of any one of Claims 2-3, 5, 6, 9-11, 14-40, 42, 43, 45, 46 or 49-51, wherein the transcriptional regulator is a variant of a HEA3 chimeric transcription factor, and wherein the variant comprises amino acid substitutions that allow selective binding to the drug.
55. The system of any one of Claims 41-54, wherein the drug is tamoxifen and/or its metabolites, 4-hydroxytamoxifen, fulvestrant or other estrogen analogs, or CMP8.
56. The system of any one of Claims 41-54, wherein the drug induces a level of expression of the chimeric antigen receptor that is greater than a wild type HEA3 or wild type HEA4 chimeric transcription factor at a concentration of the drug that is at

least 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 or 500 nM of the drug or at a concentration that is within a range defined by any two of the aforementioned values.

57. The system of any one of Claims 41-56, wherein the drug induces expression of the chimeric antigen receptor at a non-toxic dosing of drug at less than 10 nM drug but not zero.
58. The system of Claim 57, wherein the non-toxic dosing of drug decreases, eliminates or prevents drug side effects in comparison to a dosage for inducing expression with a wild type HEA3 or wild type HEA4 chimeric transcription factor.
59. The system of Claim 58, wherein the side effects are cataracts, constipation, diarrhea, edema, fatigue, fluid retention, flushing, headache, hot flashes, high blood pressure, muscle pain, nausea, shortness of breath, vomiting or weight loss.
60. The system of any one of Claims 1-59, wherein the second nucleic acid further comprises:
 - a polynucleotide encoding a chimeric antigen receptor comprising a ligand binding domain, which is a tumor specific molecule, viral specific molecule, or any other selected molecule expressed on a target cell population, wherein the ligand elicits recognition, modulation, inhibition, and/or elimination by a lymphocyte;
 - a polynucleotide coding for a polypeptide spacer, wherein the spacer is optionally optimized;
 - a polynucleotide coding for a transmembrane domain; and
 - a polynucleotide encoding an intracellular signaling domain.
61. The system of anyone of Claims 41-60, wherein the polypeptide that regulates apoptosis is Survivin.
62. The system of Claim 61, wherein Survivin is encoded by a sequence set forth in SEQ ID NO: 124.

63. The system of Claim 61 or 62, wherein Survivin comprises an amino acid sequence set forth in SEQ ID NO: 123.
64. The system of any one of Claims 1-63, wherein the first promoter is in opposite orientation to the second promoter.
65. The system of any one of Claims 1-64, wherein the ligand binding domain is an antibody fragment, such as a binding fragment.
66. The system of Claim 65, wherein the ligand binding domain is a single chain variable fragment.
67. The system of any one of Claims 1-41 or 63-66, wherein the tumor specific molecule is selected from the group consisting of CD19, CD20, CD22, CD23, CD123, CS-1, ROR1, CE7, EGFR, hB7H3, mesothelin, c-Met, PSMA, Her2, GD-2, and MAGE A3 TCR or any combination thereof.
68. The system of any one of Claims 1-41 or 63-66, wherein the viral specific molecule is an HIV envelope protein or HIV envelope protein complex, Hepatitis C virus NS3 protein, HbcAg protein, HIV-1 GP120 protein, or Epstein-Barr virus early antigen protein.
69. The system of any one of Claims 65 or 66, wherein the ligand binding domain specifically binds with CD19, CD20, CD22, EGFR_vIII, EGFR, EphA2, IL13Ra2, L1CAM, oaGD2, Her2, B7H3, CD33, Mesothelin, ROR-1, FITC and/or VAR2CSA
70. The system of any one of Claims 1-69, wherein the spacer comprises IgG4, IgG4-CH2(L235D, N297Q) or IgG4-CH3.
71. The system of any one of Claims 41-70, wherein the first nucleic acid further comprises a DNA spacer, wherein the spacer is operably linked to the first promoter and operably linked to the first polynucleotide that encodes the chimeric antigen receptor.

72. The system of Claim 71, wherein the DNA spacer is between the first promoter and the first polynucleotide that encodes the chimeric antigen receptor.
73. The system of any one of Claims 71 or 72, wherein the DNA spacer comprises a sequence set forth in SEQ ID NO: 120.
74. The system of any one of Claims 71-73, wherein the DNA spacer comprises a sequence that has 95%, 90%, 85% or 80% sequence identity to SEQ ID NO: 120 or any percent sequence identity in between a range defined by any two aforementioned values.
75. The system of any one of Claims 72-74, wherein the DNA spacer enhances gene expression of the chimeric antigen receptor.
76. The system of any one of Claims 41-75, wherein the first nucleic acid further comprises a first vector and the second nucleic acid further comprises a second vector.
77. The system of Claim 76, wherein both vectors are packaged in a viral vector.
78. The system of Claim 77, wherein the viral vector is an adeno-associated virus or a lentivirus.
79. A chimeric receptor polypeptide coded for by the first nucleic acid of the system of any one of Claims 1-35 or 54-68 or the second nucleic acid of the system of any one of Claims 41-78.
80. A host cell, such as a mammalian cell, comprising a system of any one of Claims 1-78.
81. The host cell of Claim 80, wherein the host cell is a precursor T cell.
82. The host cell of Claim 81, wherein the precursor T cell is a hematopoietic stem cell.

83. The host cell of Claim 80, wherein the host cell is a CD8⁺ T cytotoxic lymphocyte cell selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells and bulk CD8⁺ T cells.
84. The host cell according to Claim 80, wherein the host cell is a CD4⁺ T helper lymphocyte cell that is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells, and bulk CD4⁺ T cells.
85. A composition comprising a host cell of any one or more of Claims 80-84, in a pharmaceutically acceptable excipient.
86. The composition of Claim 85, comprising a host cell of Claim 81 and a host cell of Claim 82.
87. The composition of Claim 85, comprising a host cell of Claim 81 or 82 and a host cell of Claim 83 or 84.
88. An *in vitro* method for preparing a host cell of any one of Claims 80-84 comprising:
- a) providing a system of any one of Claims 1-78; and
 - b) introducing the system into an isolated T lymphocyte population and expanding each T lymphocyte population *in vitro*.
89. The method of Claim 88, wherein the T lymphocytes are expanded, and wherein the method further comprises culturing the cells in the presence of anti-CD3 and/or anti CD28, and at least one homeostatic cytokine until the cells expand sufficiently for use as a cell infusion.
90. The method of Claim 88 or 89, wherein the isolated T lymphocyte population comprises precursor T cells.
91. The method of Claim 90, wherein the precursor T cells are hematopoietic stem cells.
92. The method of any one of Claims 88-91, wherein the lymphocyte is CD8⁺ or CD4⁺.

93. A method of treating, inhibiting, or ameliorating a disease in a subject in need thereof comprising:

administering to the subject the host cell, preferably a mammalian host cell, of any one of Claims 80-84 or at least one composition or product combination of any one or more of Claims 85-87.

94. The method of Claim 93, wherein the disease is cancer or a viral disease.

95. The method of Claim 93 or 94, wherein the subject is identified or selected to receive an anti-cancer therapy.

96. The method of any one of Claims 93-95, wherein the subject is identified or selected to receive an anti-viral therapy.

97. The method of any one of Claims 93-96, further comprising measuring or evaluating an inhibition of the disease.

98. The method of any one of Claims 93-97, further comprising providing said subject an additional anti-cancer therapy or anti-viral therapy before, during, or after administration of the cells of any one or more of Claims 80-84 or at least one composition or product combination of any one or more of Claims 85-87.

99. The method of any one or more of Claims 93-98, wherein the cells of any one or more of Claims 80-84 or at least one composition or product combination of any one or more of Claims 85-87 are administered to said subject by adoptive cell transfer.

100. The method of any one or more of Claims 93-99, wherein the host cells of any one or more of Claims 80-84 or at least one composition or product combination of any one or more of Claims 85-87 are administered to said subject after said subject has received another form of anti-cancer therapy or anti-viral therapy.

101. The method of any one or more of Claims 93-100, further comprising administering a drug that induces expression of a transgene in the host cell, such as a mammalian cell, or composition for the treatment of cancer or a viral infection.

102. The method of Claim 101, wherein the drug is tamoxifen and/or its metabolites, fulvestrant and/or other estrogen analogs or CMP8.
103. The method of Claim 102, wherein tamoxifen is administered at a dose range of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mg/dose or within a range defined by any two of the aforementioned values.
104. The method of Claim 102, wherein the fulvestrant is administered at a dose range of 250, 300, 350, 400, 450 or 500 mg/dose mg/dose or within a range defined by any two of the aforementioned values.
105. The method of Claim 102, wherein the CMP8 is administered at a dose range to provide 30, 40 or 50 nM of drug levels in serum, or within a range defined by any two of the aforementioned values.
106. The method of any one of Claims 94-105, wherein the cancer is a solid tumor or hematologic malignancy.
107. The method of 106, wherein the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer.
108. A use of the host cell of any one of or more of Claims 80-84 or at least one composition or product combination of any one or more of Claims 85-88 in combination with a drug that induces expression of a transgene in the host cell or composition for the treatment of cancer or a viral infection.
109. The use of Claim 108, wherein the drug is tamoxifen and/or its metabolites, fulvestrant and/or other estrogen analogs or CMP8.
110. The use of Claim 109, wherein tamoxifen is administered at a dose range of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mg/dose or within a range defined by any two of the aforementioned values.

111. The use of Claim 109, wherein the fulvestrant is administered at a dose range of 250, 300, 350, 400, 450 or 500 mg/dose or within a range defined by any two of the aforementioned values.
112. The use of claim 109, wherein the CMP8 is administered at a dose range to provide 30, 40 or 50 nM of drug levels in serum, or within a range defined by any two of the aforementioned values.
113. The use of any one of Claims 108-112, wherein the cancer is a solid tumor or hematologic malignancy.
114. The use of Claim 113, wherein the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer.
115. A method of performing cellular immunotherapy in a subject having cancer or a viral infection comprising: administering the host cells of any one or more of Claims 80-84 or at least one composition or product combination of any one or more of Claims 85-87 to the subject and administering a drug that induces expression of a transgene in the composition or the host cells.
116. The method of Claim 115, wherein the cancer is selected from a solid tumor or hematologic malignancy.
117. The method of Claim 116, wherein the solid tumor is selected from the group consisting of a breast cancer, brain cancer, lung cancer, colon cancer, renal cancer, pancreatic cancer, prostate cancer, and ovarian cancer.
118. Use of the host cell of any one of or more of Claims 80-84 or at least one composition or product combination of any one or more of Claims 85-87 as a medicament.

HEA3 (low affinity) RelA single mutant variants

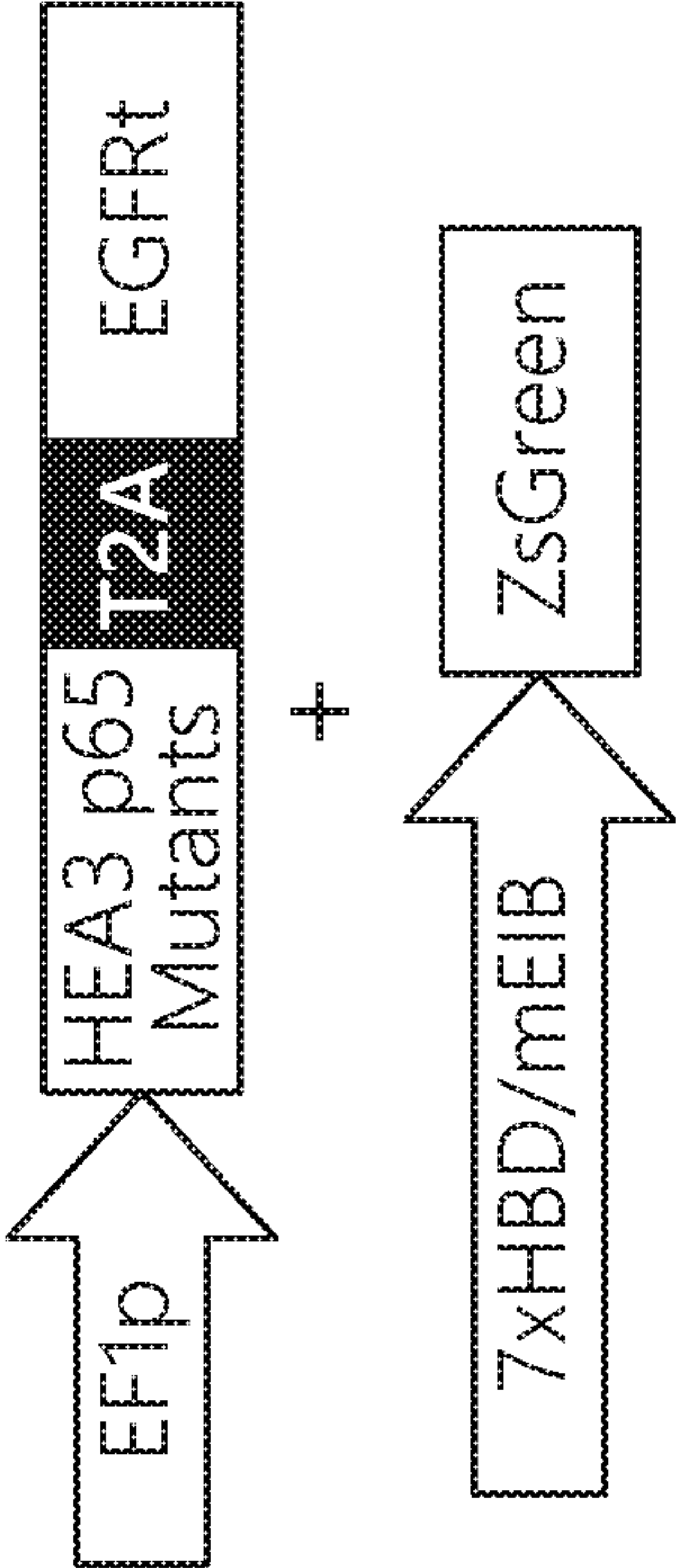


FIG. 1A

HEA4 (high affinity) RelA single mutant variants

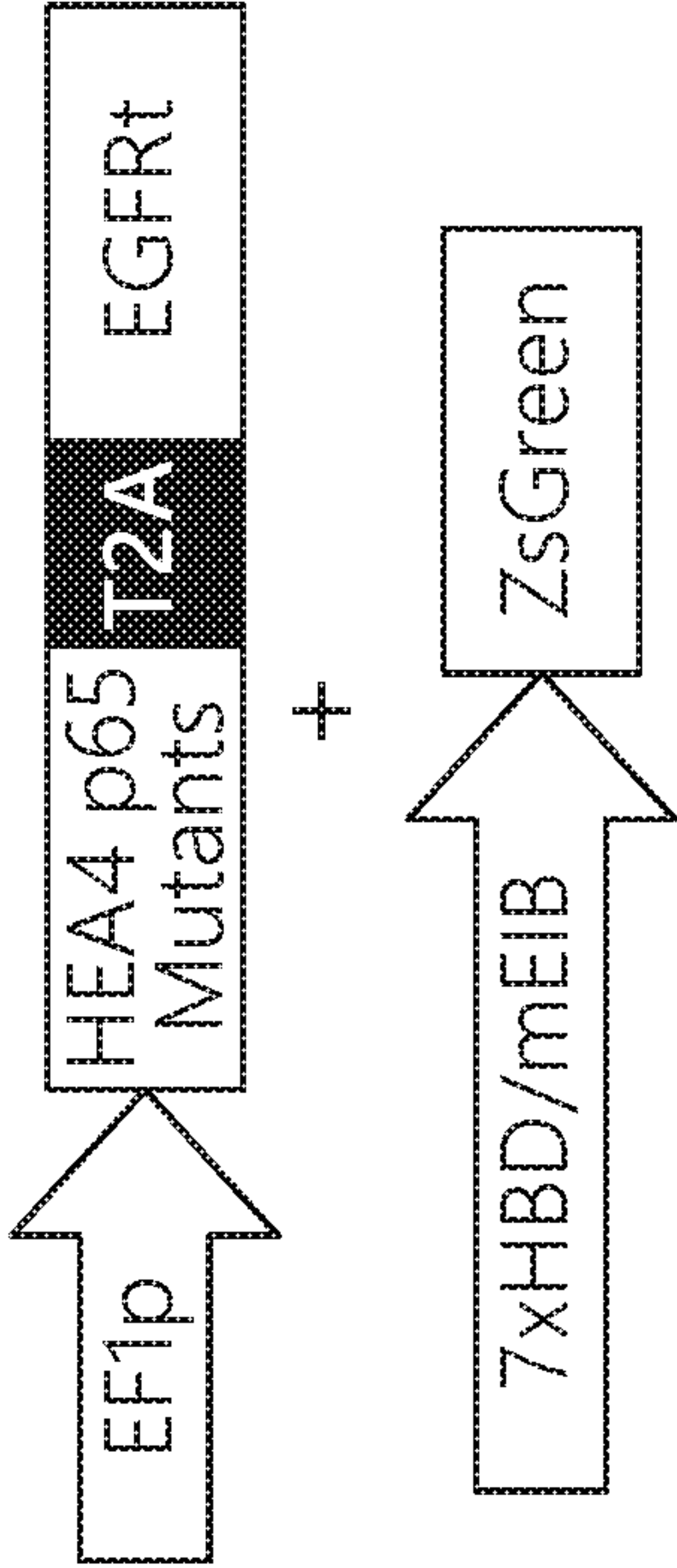


FIG. 1B

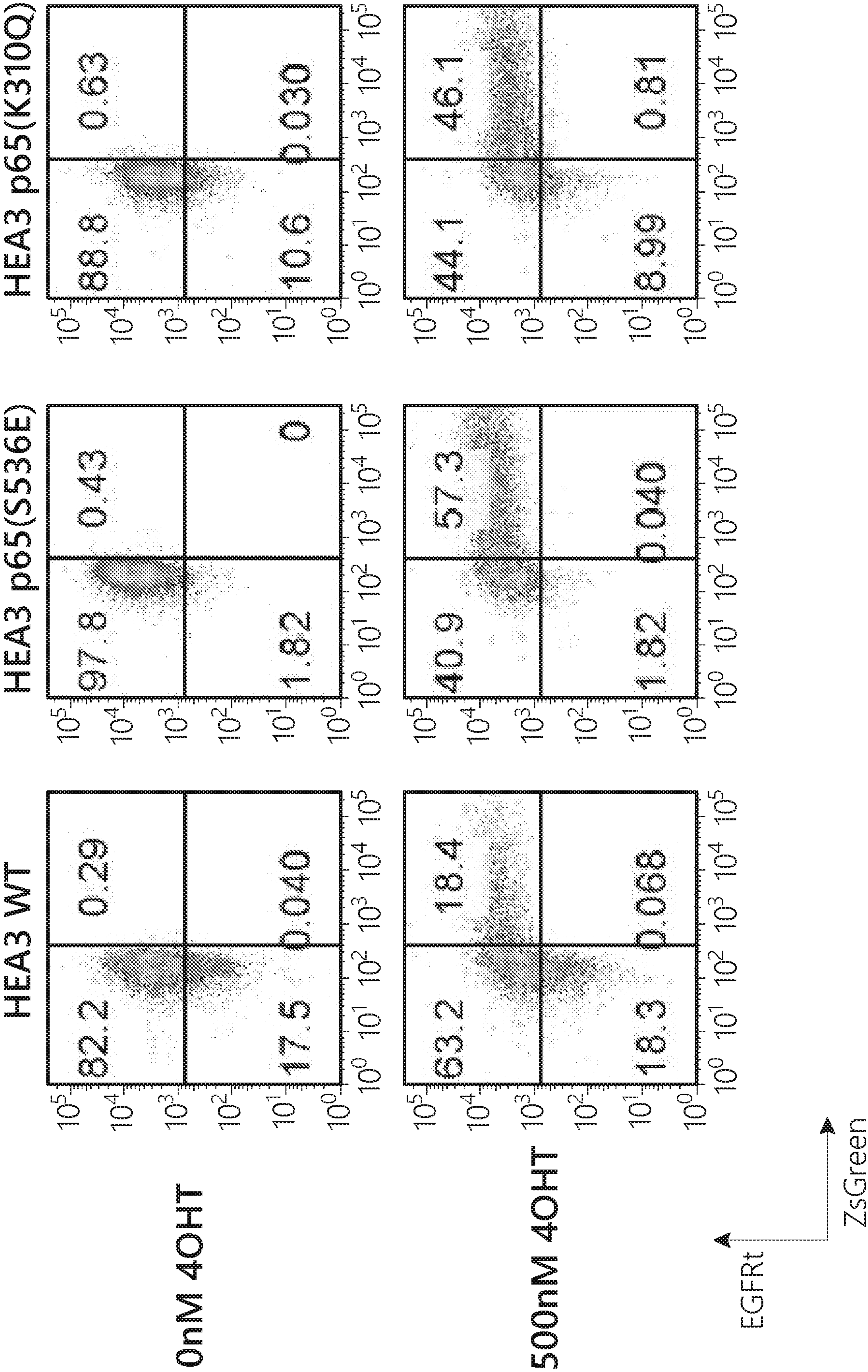


FIG. 1C

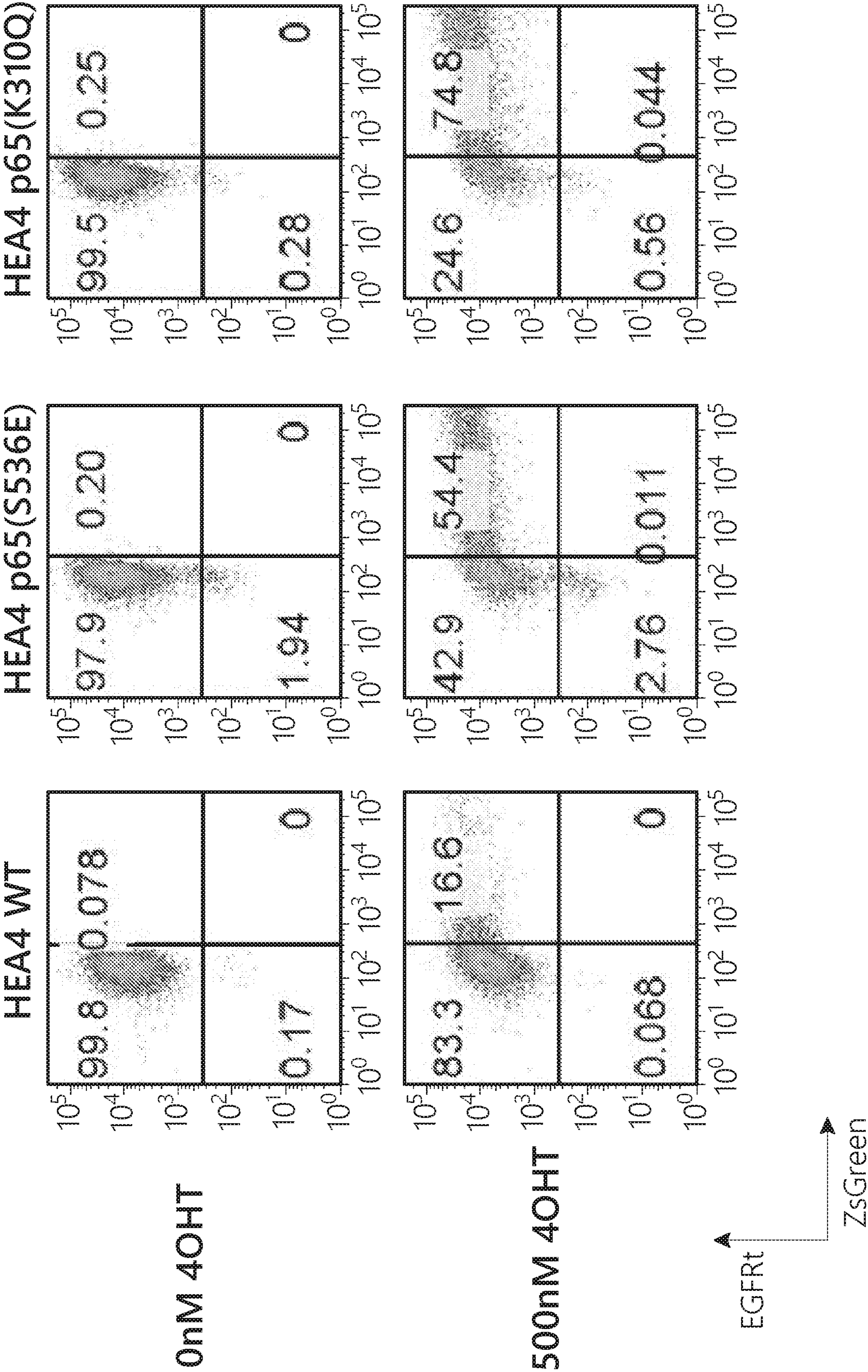
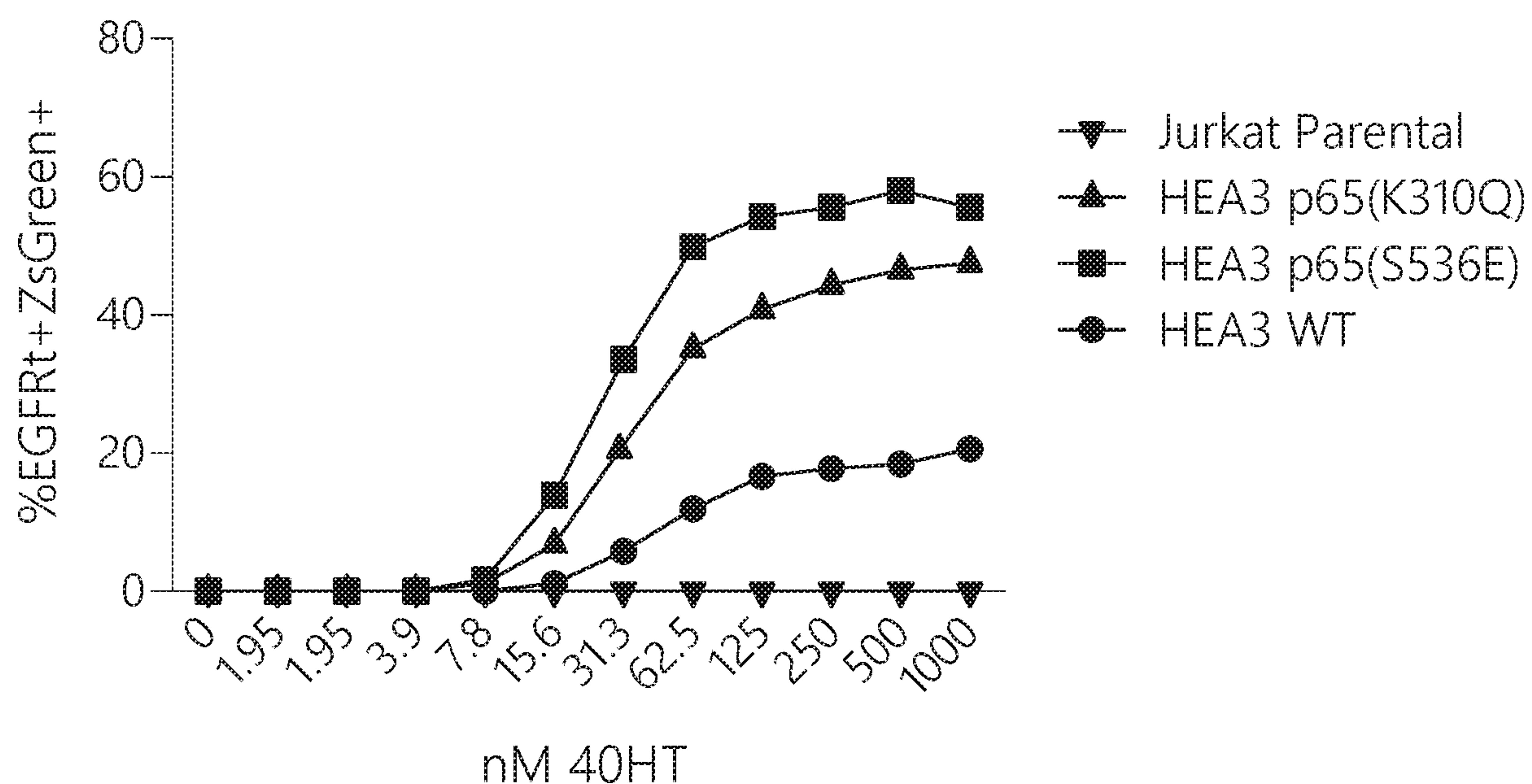


FIG. 1D

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HEA3 p65 Mutant 40HT Dose Response



HEA3 p65 Mutant 40HT Dose Response (MFI)

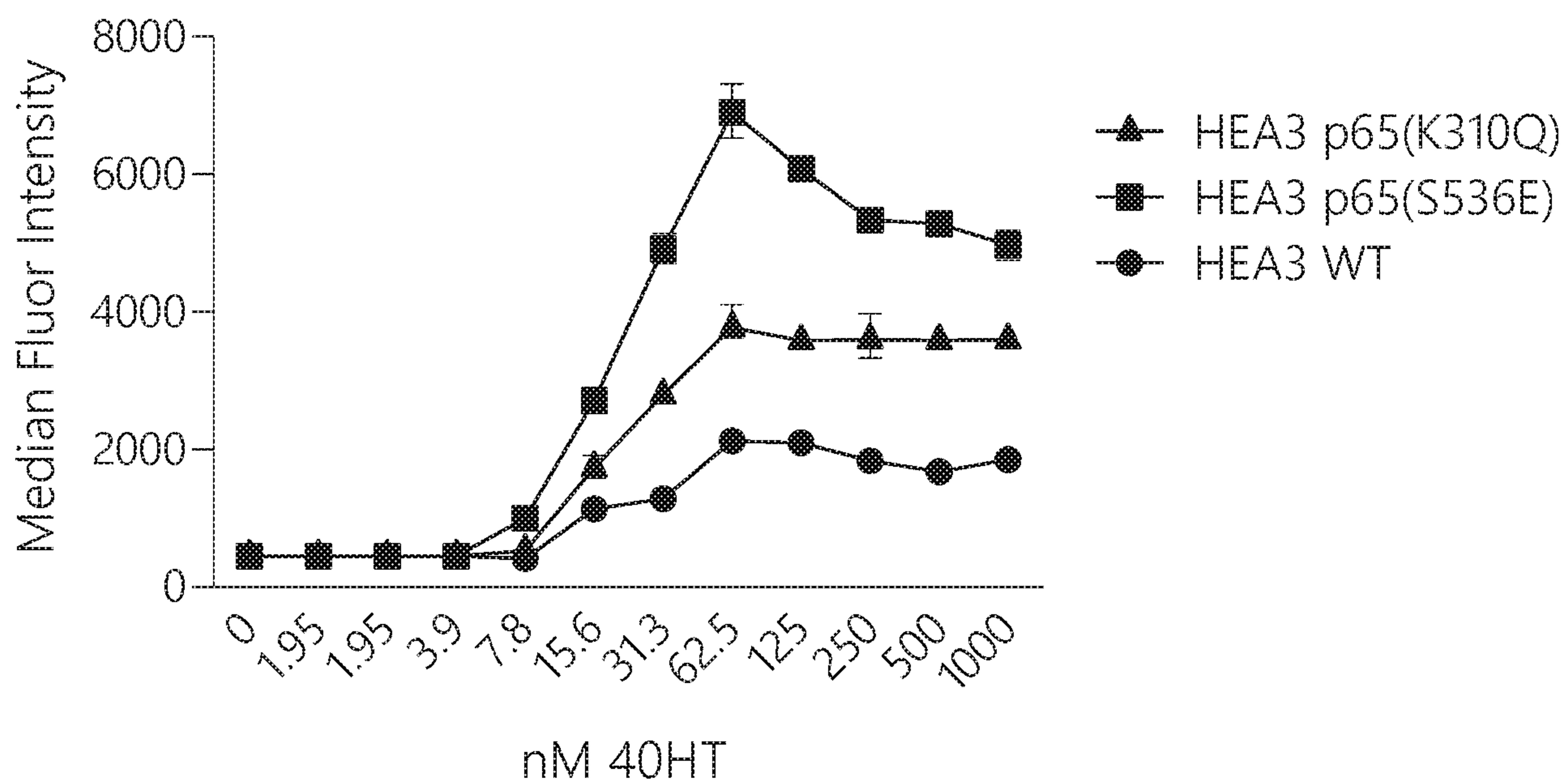
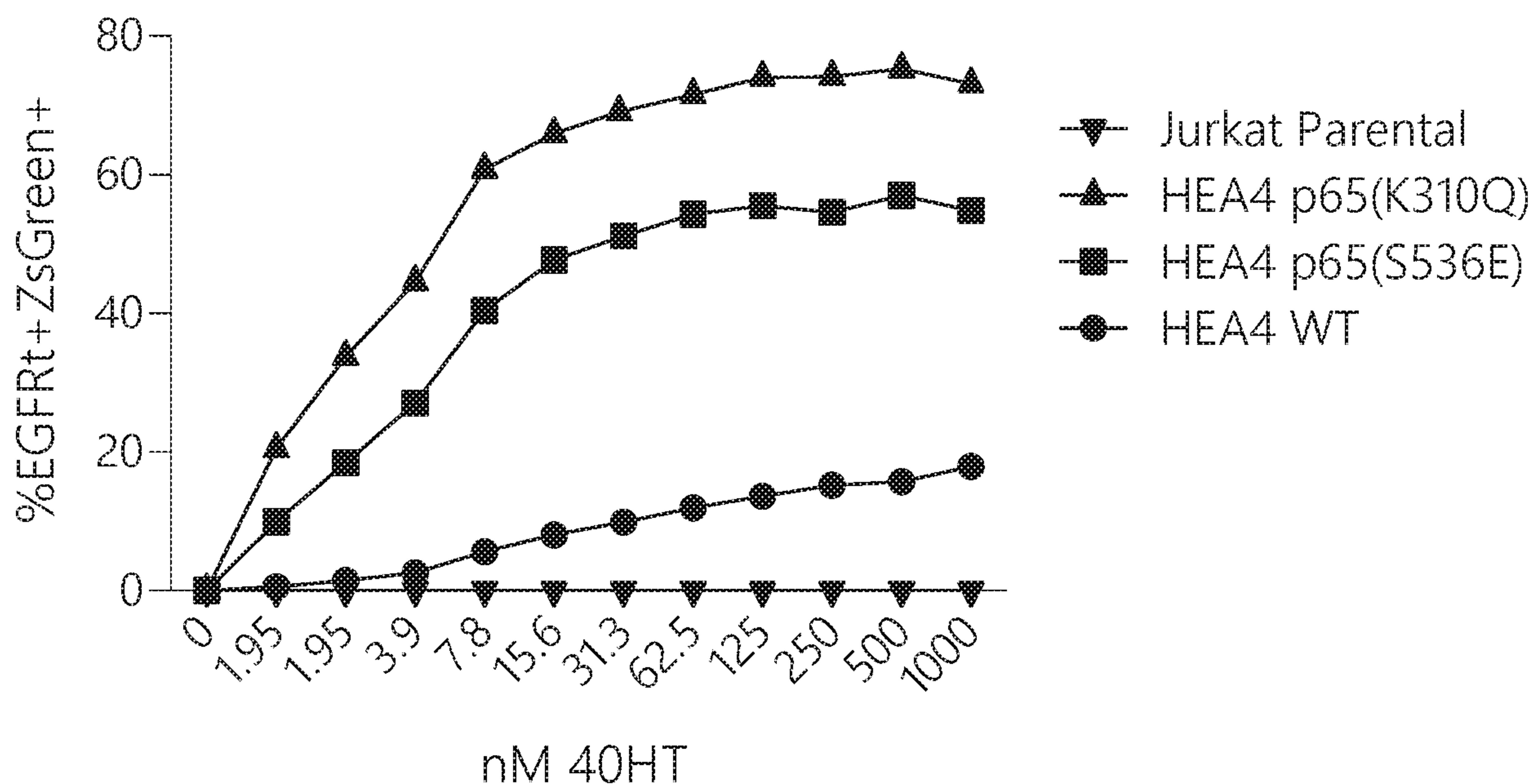


FIG. 1E

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HEA4 p65 Mutant 40HT Dose Response



HEA4 p65 Mutant 40HT Dose Response

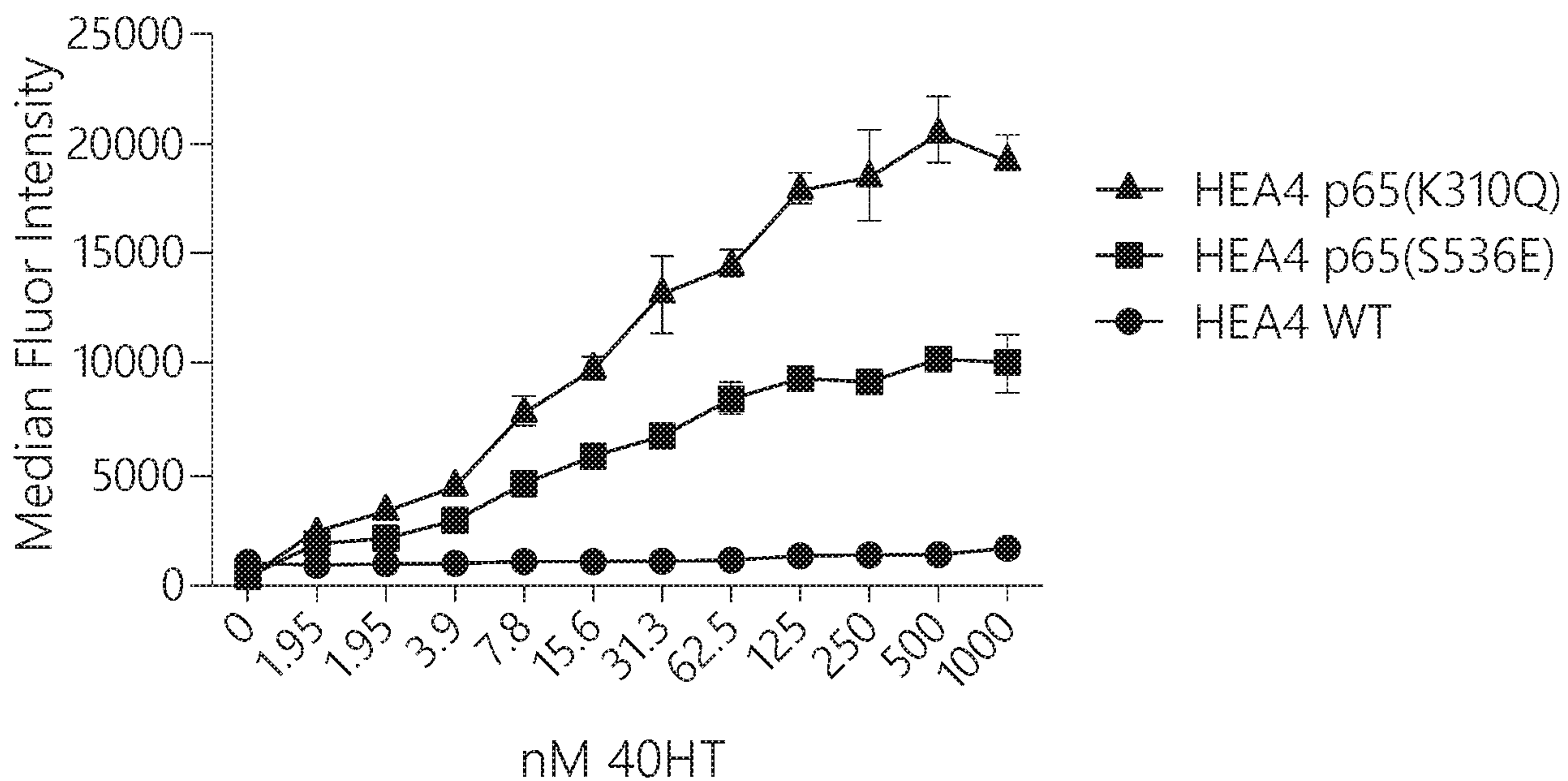


FIG. 1F

HEA4 (high affinity) RelA double mutant variants

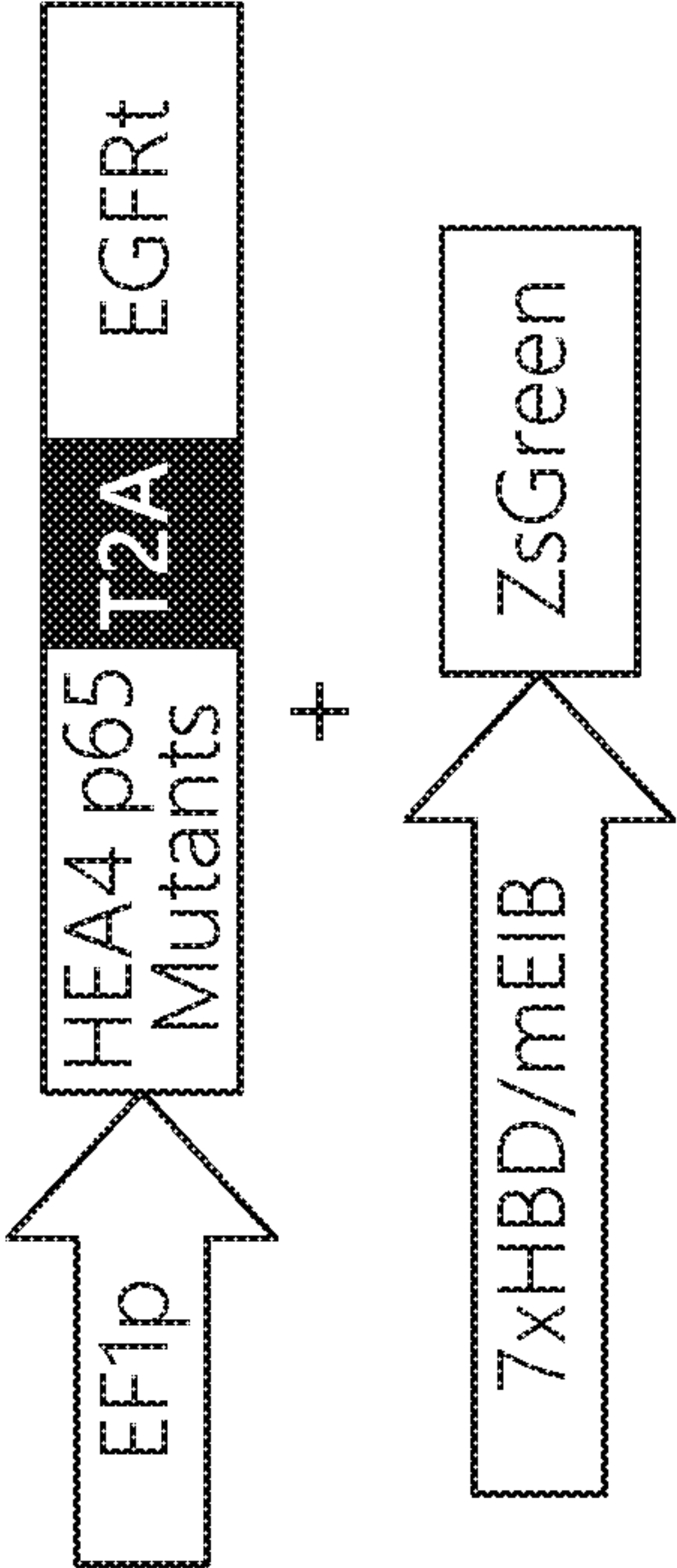


FIG. 2A

CMP8-responsive HEA3 RelA variant

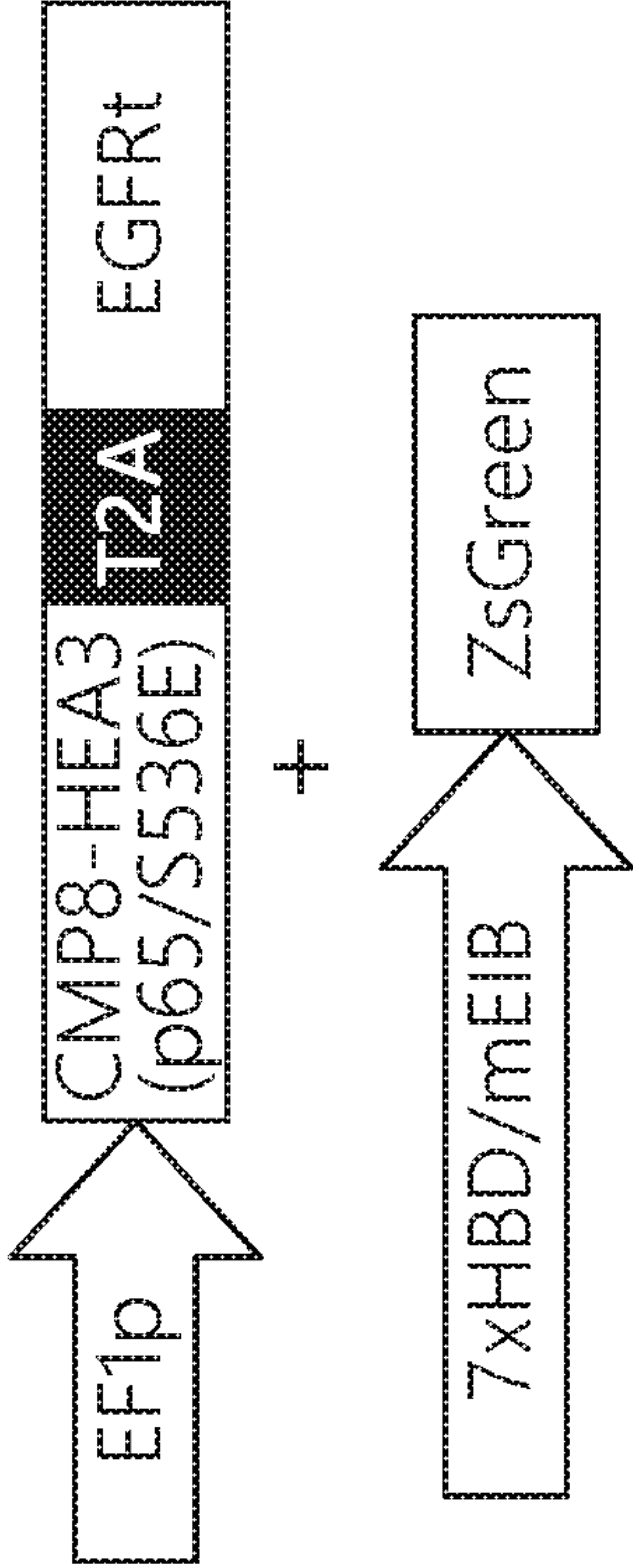


FIG. 2B

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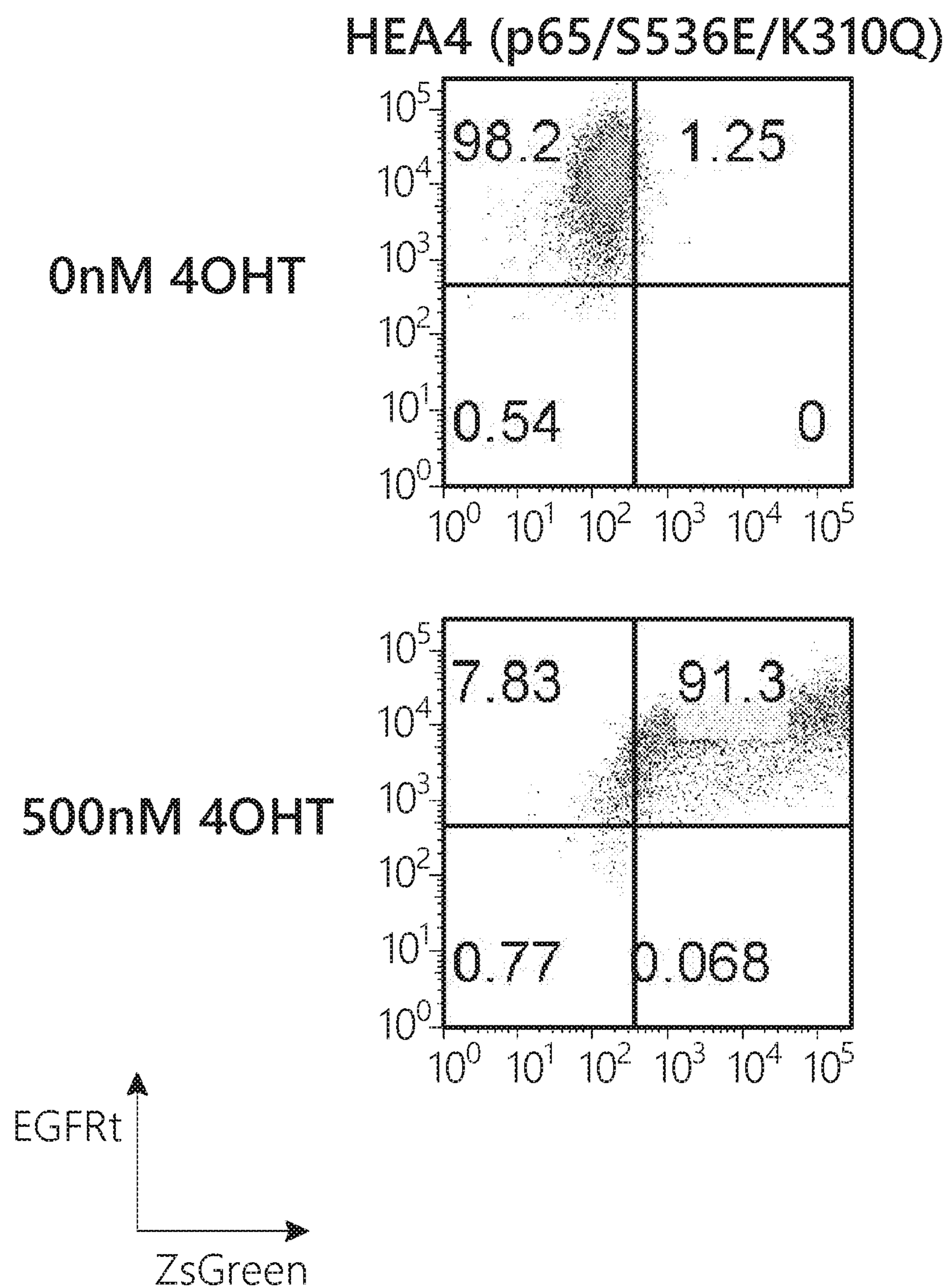


FIG. 2C

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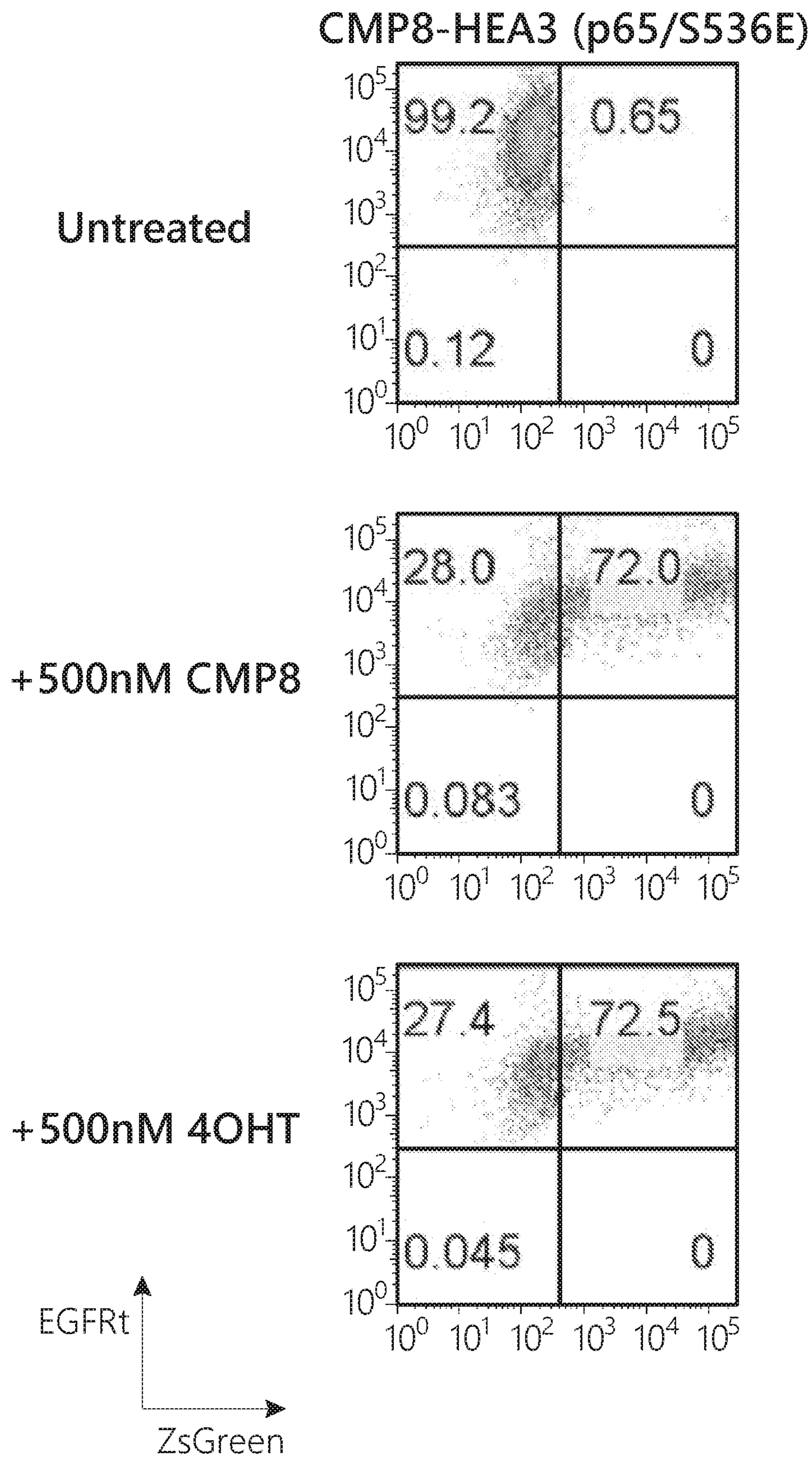
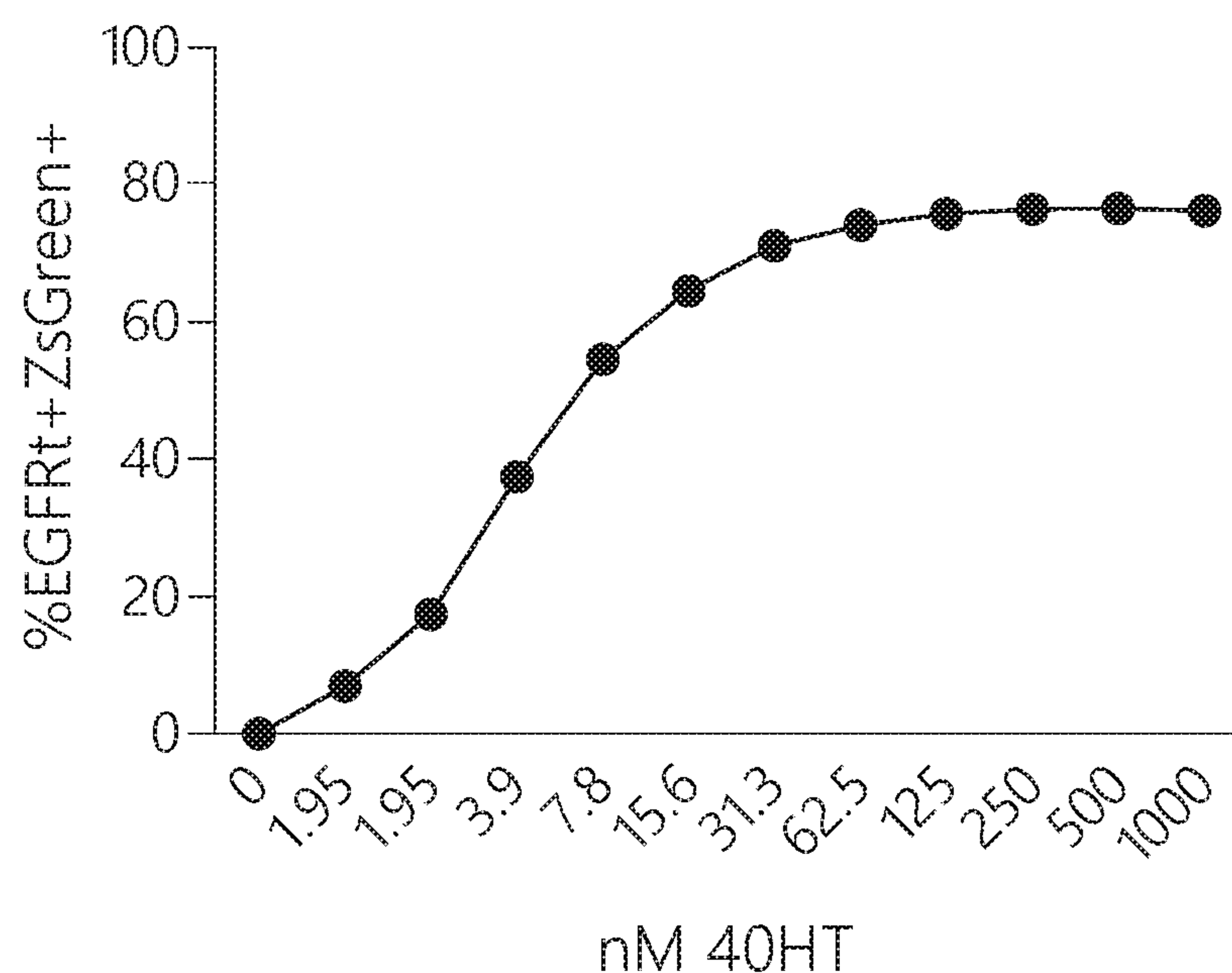


FIG. 2D

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HEA4 p65(S536E/K310Q) 40HT Dose Response



HEA4 p65(S536E/K310Q) 40HT Dose Response MFI

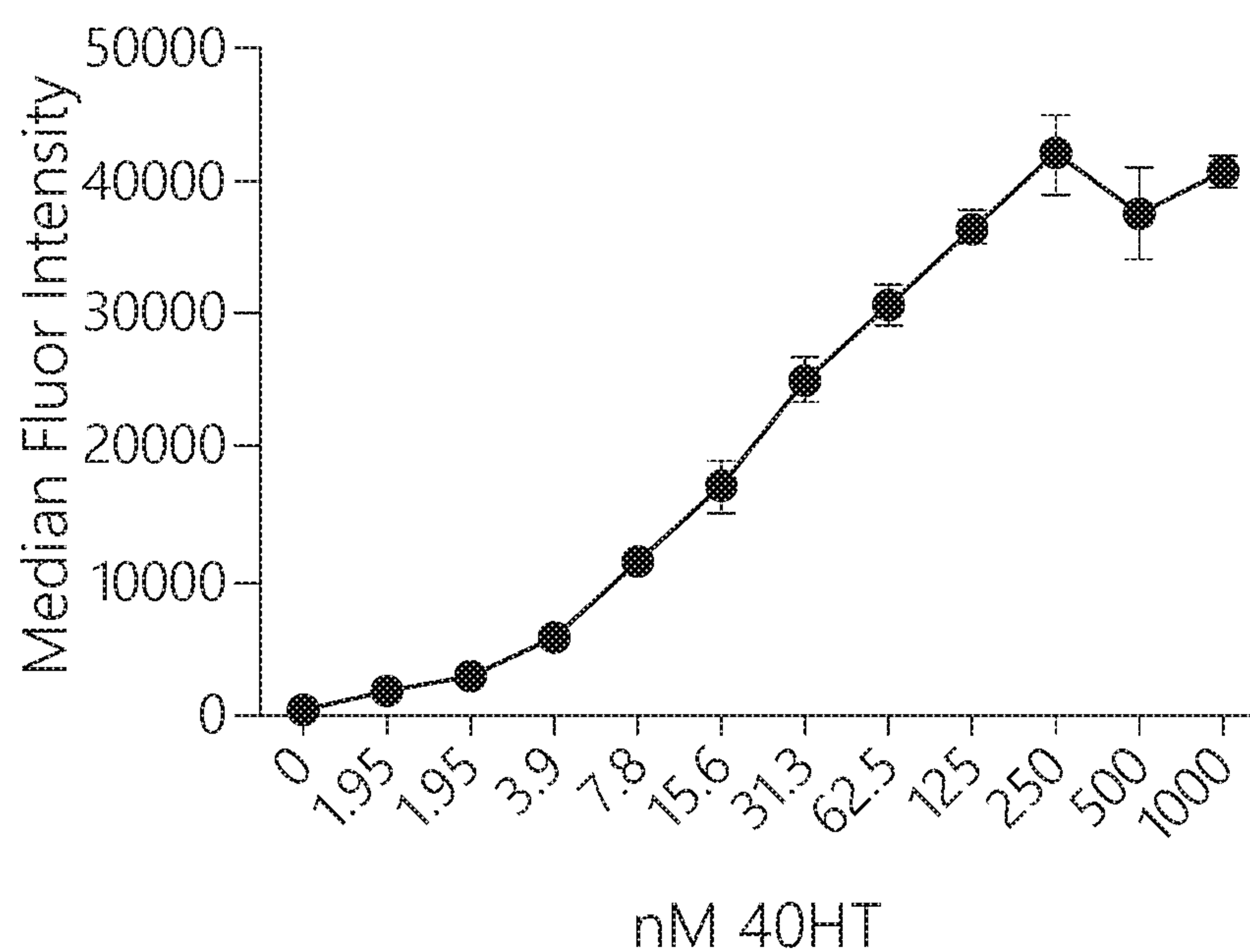


FIG. 2E

SUBSTITUTE SHEET (RULE 26)

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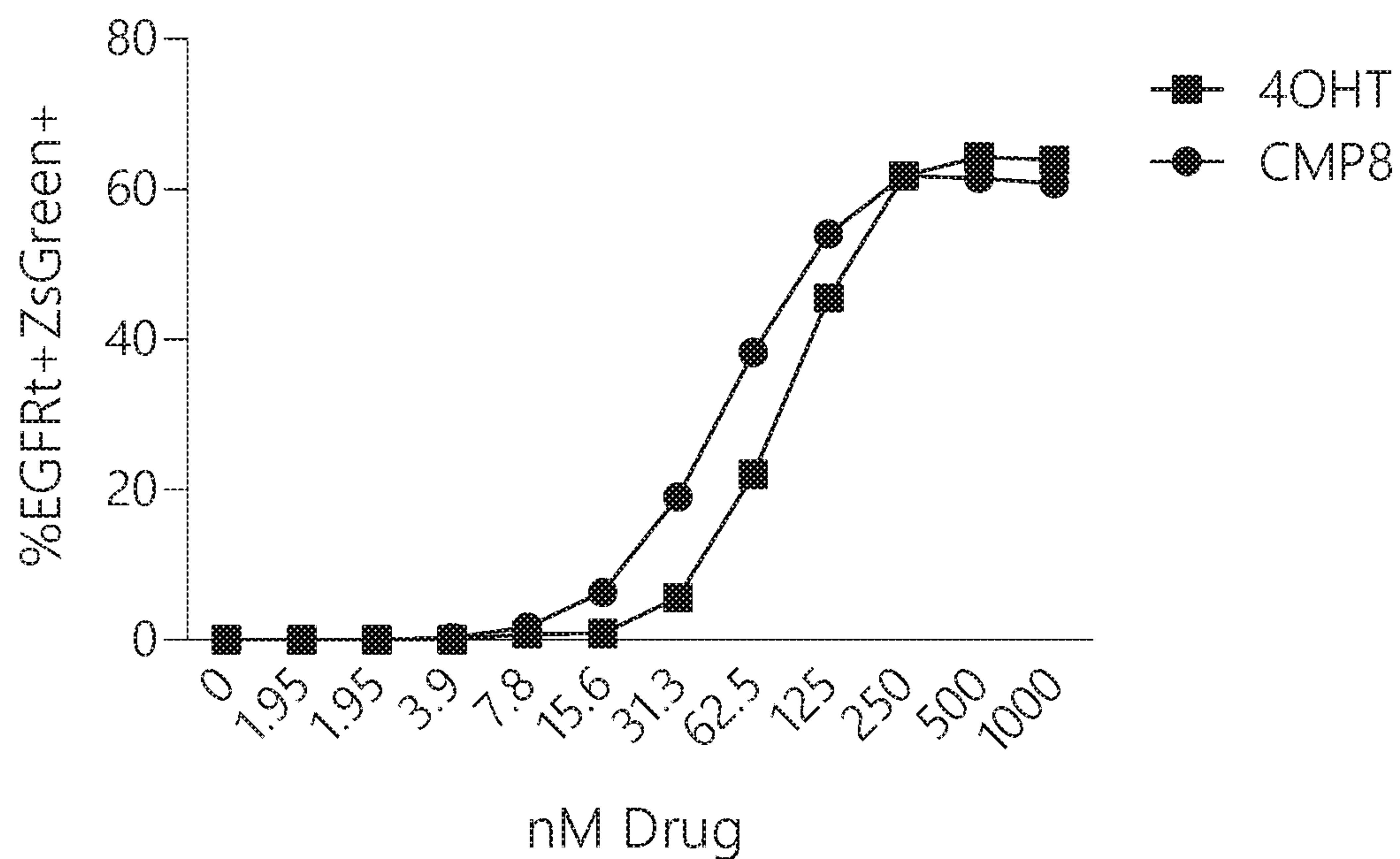
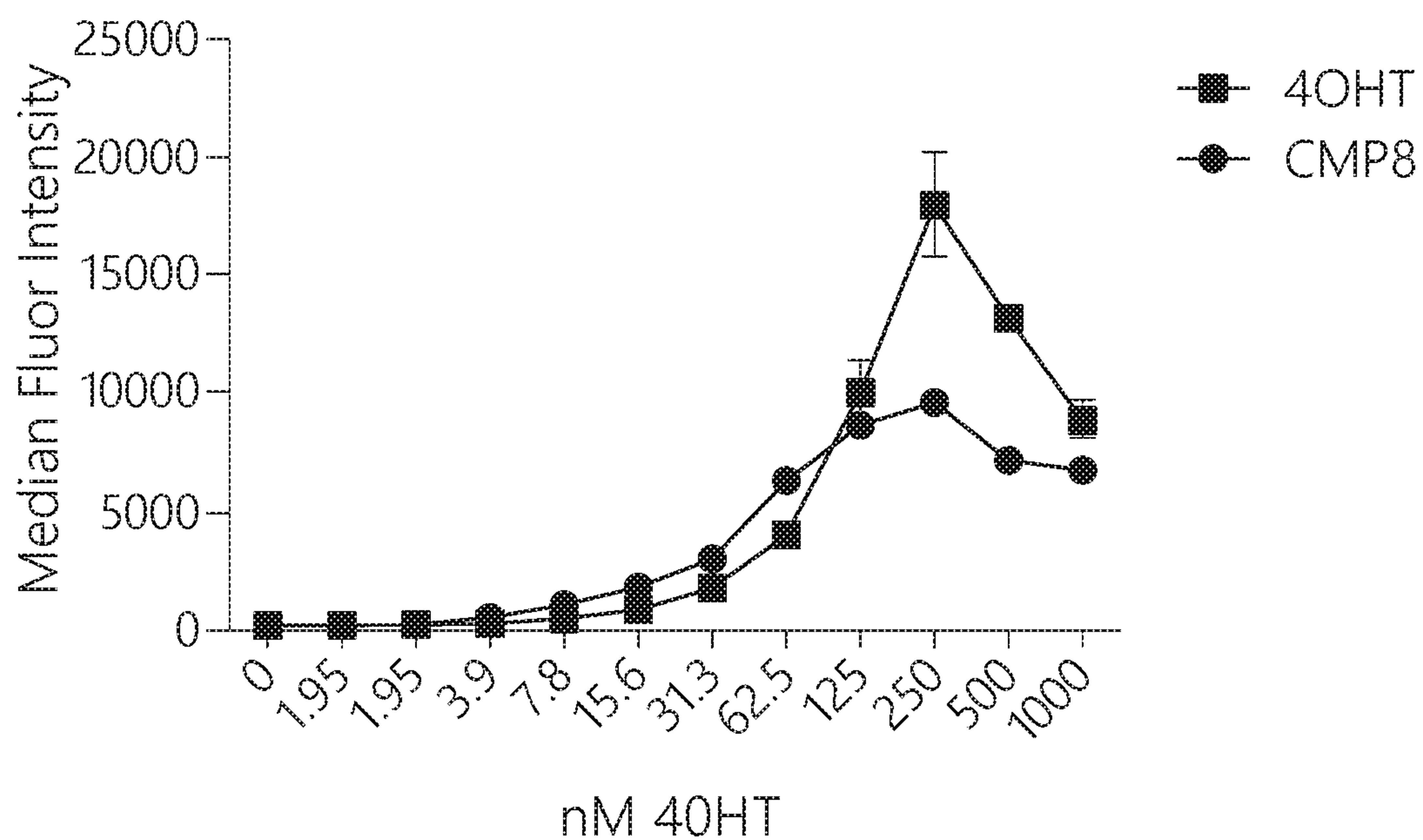
CMP8-HEA3(p65/S536E) Dose Response**CMP8-HEA3(p65/S536E) Dose Response MFI**

FIG. 2F

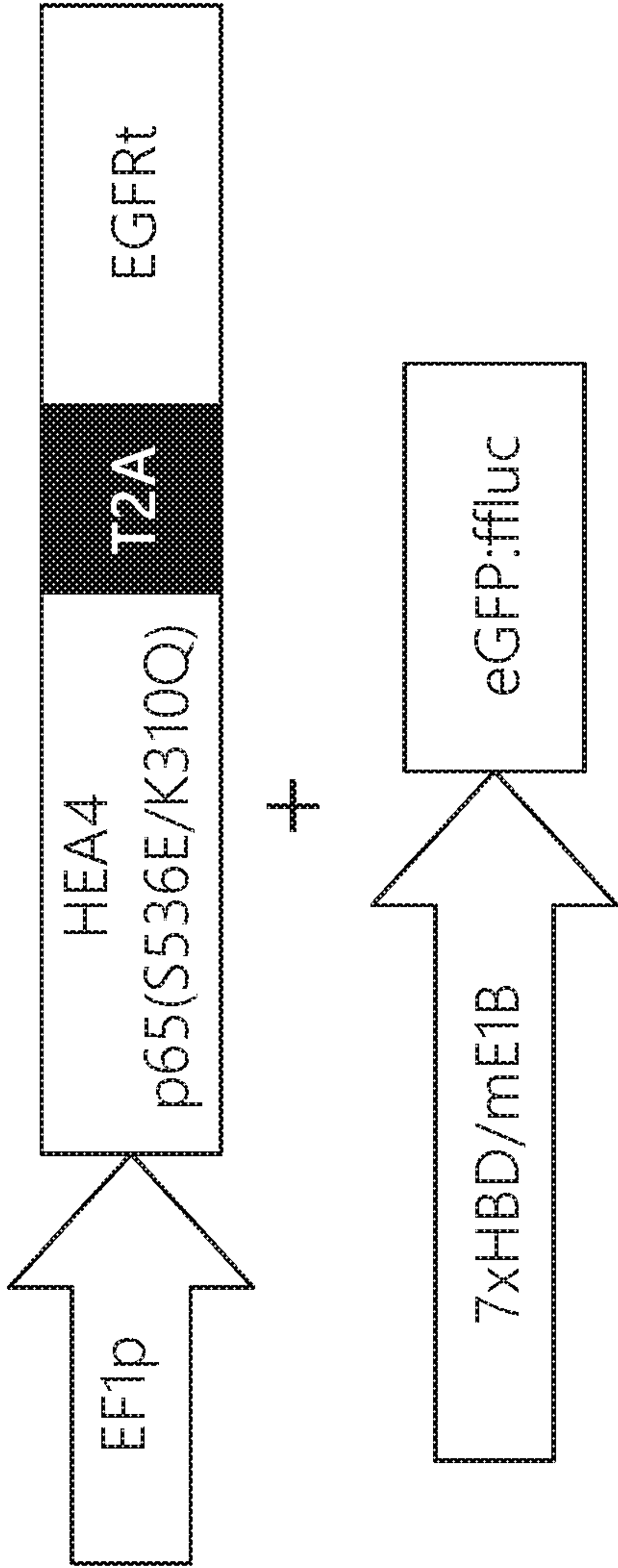


FIG. 3A

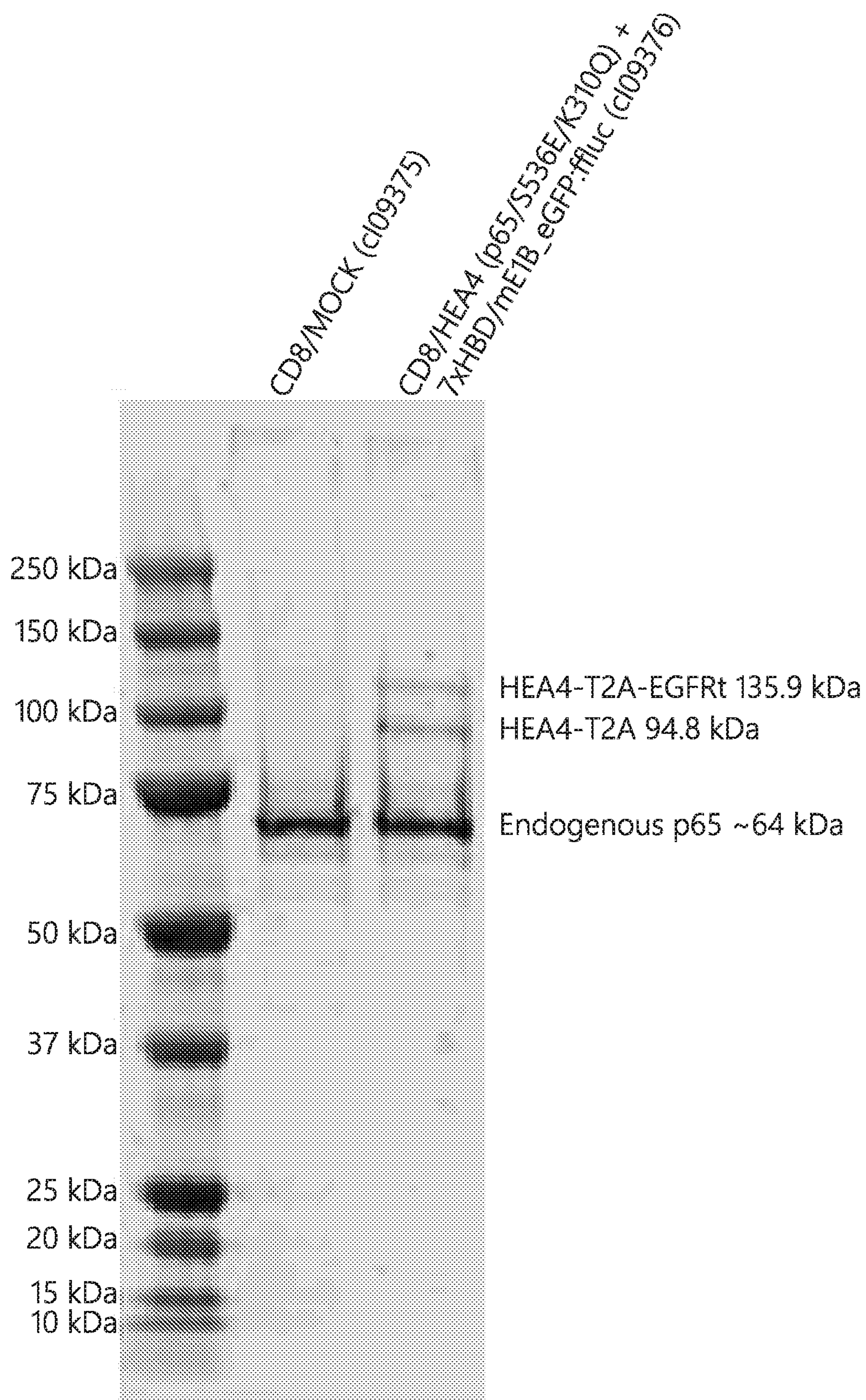
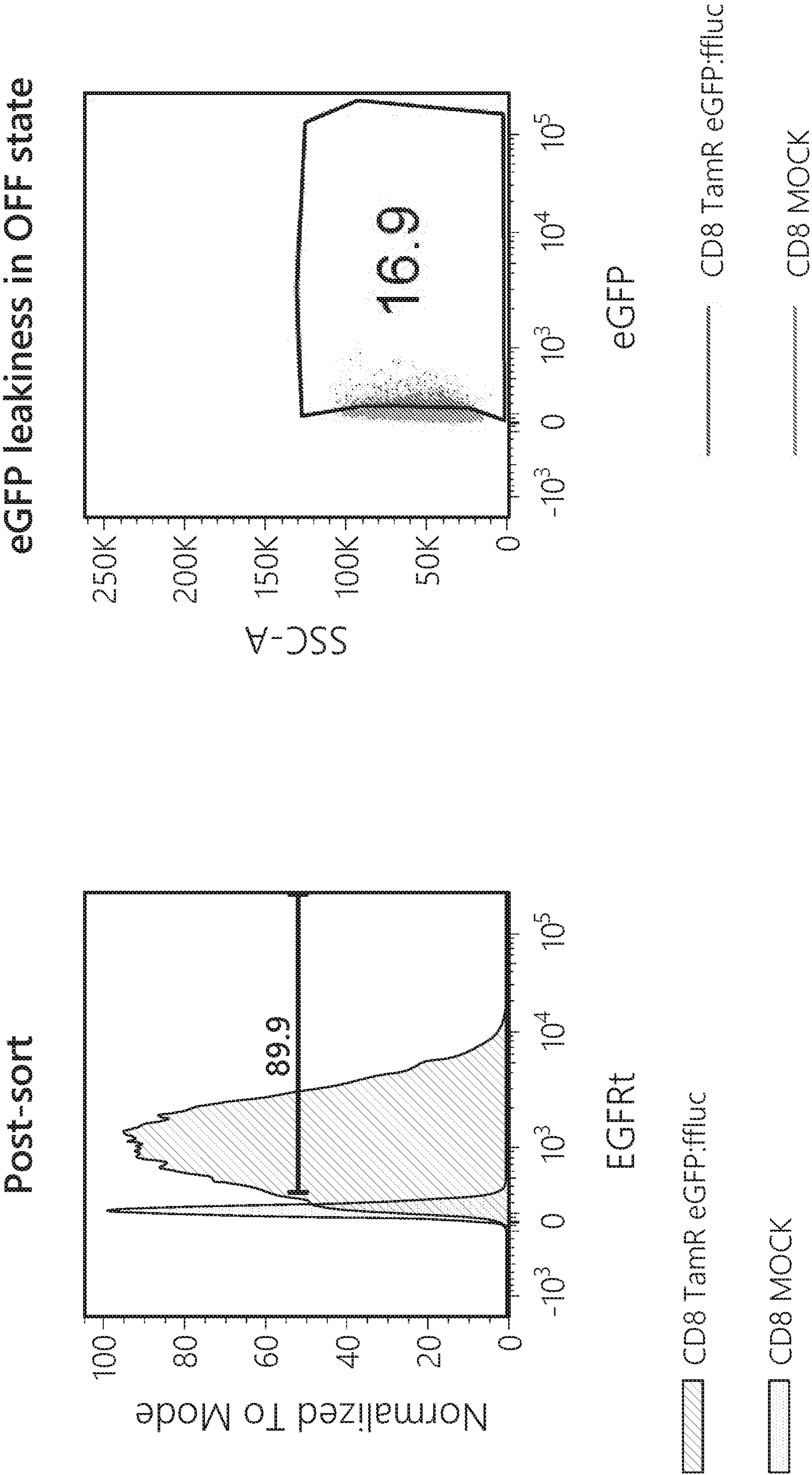
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FIG. 3B

FIG. 3C



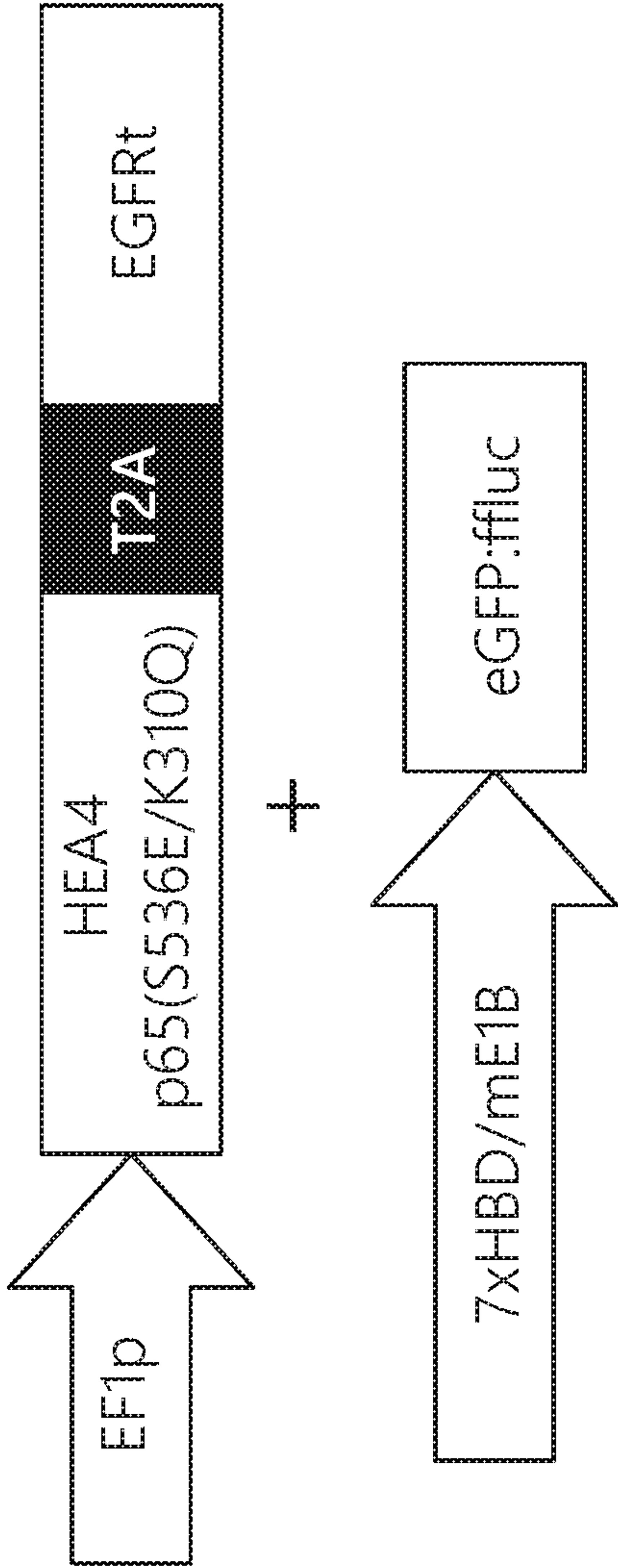
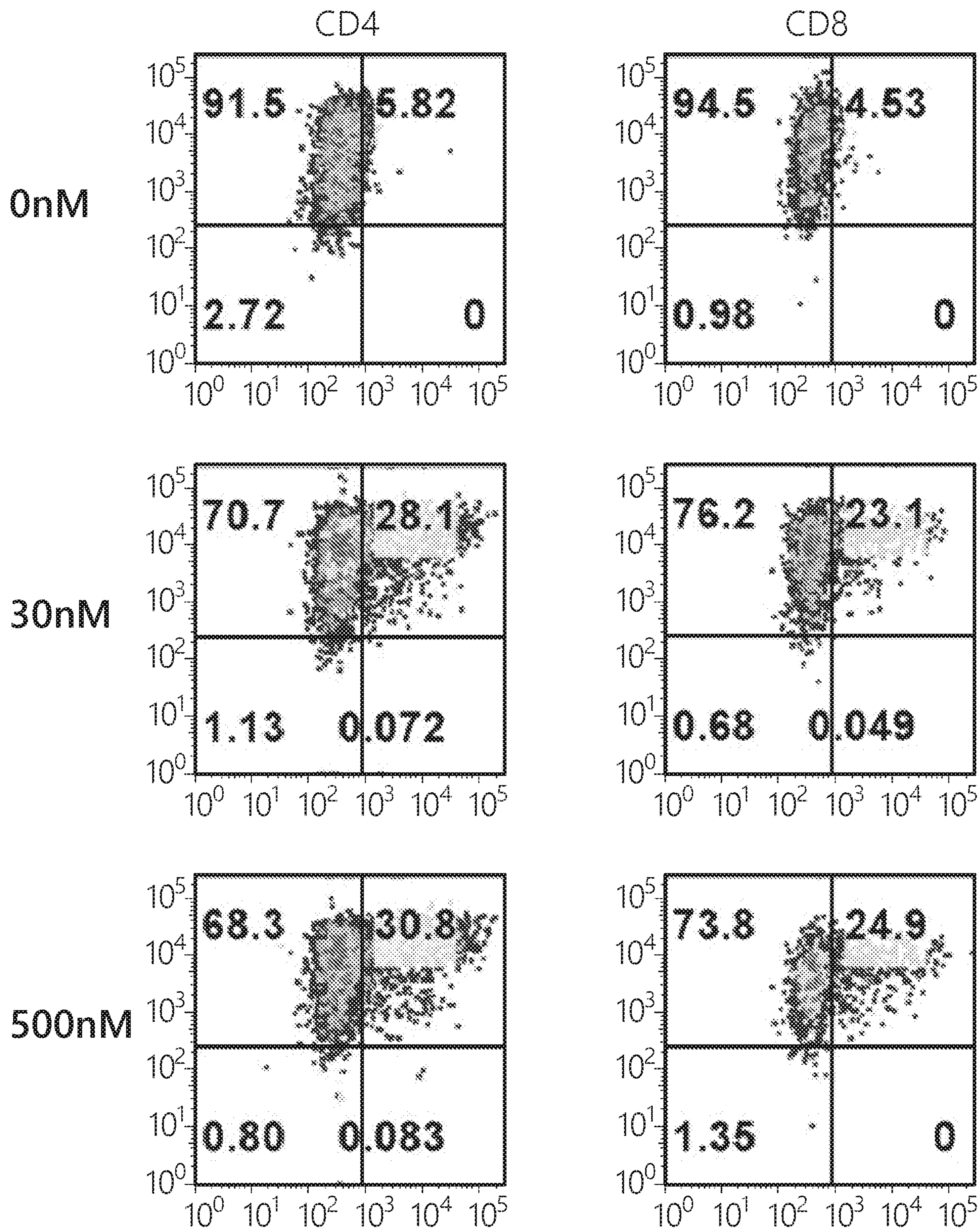


FIG. 4A

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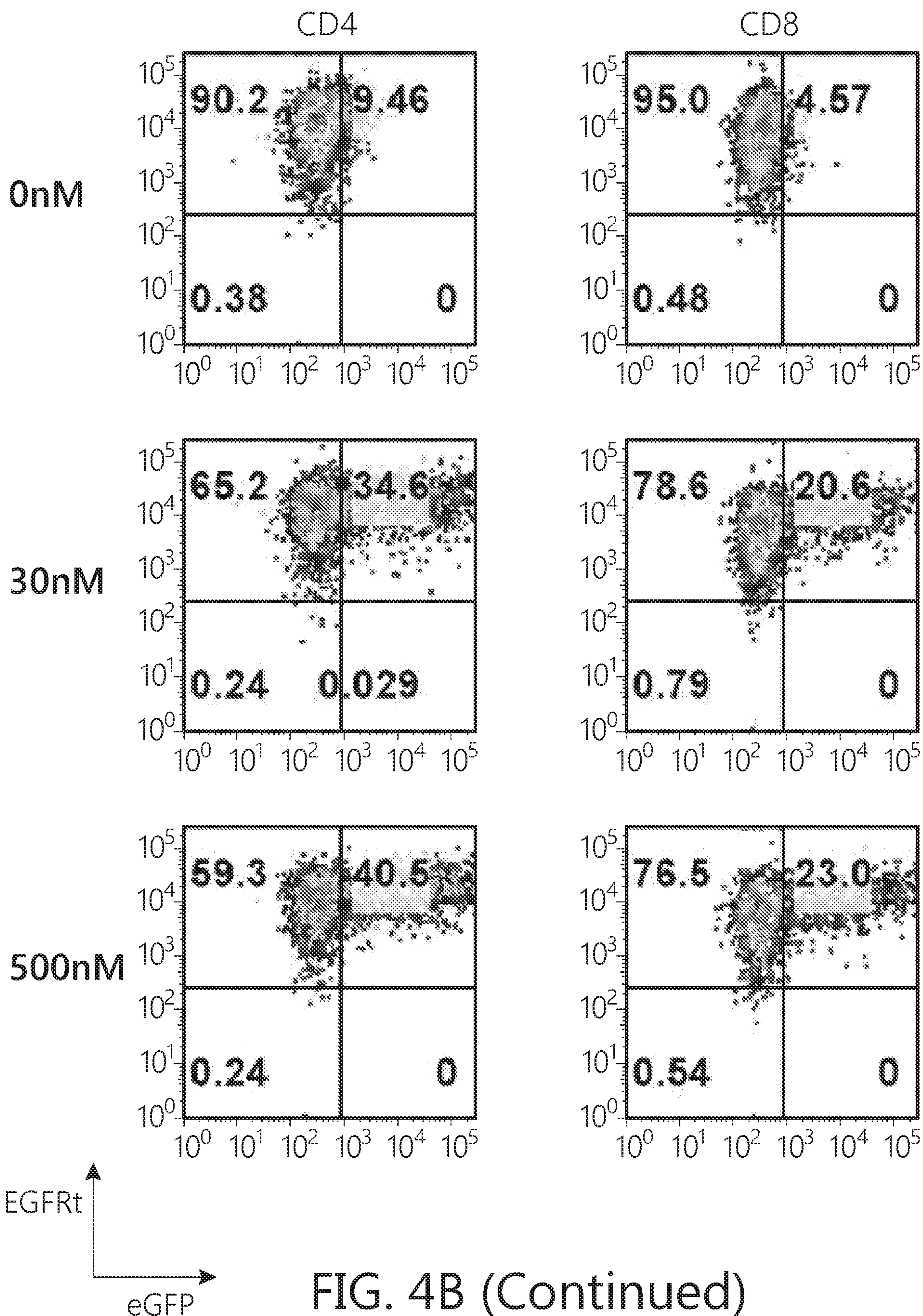
Beads (2:1 Bead:Cell)



EGFRt
eGFP

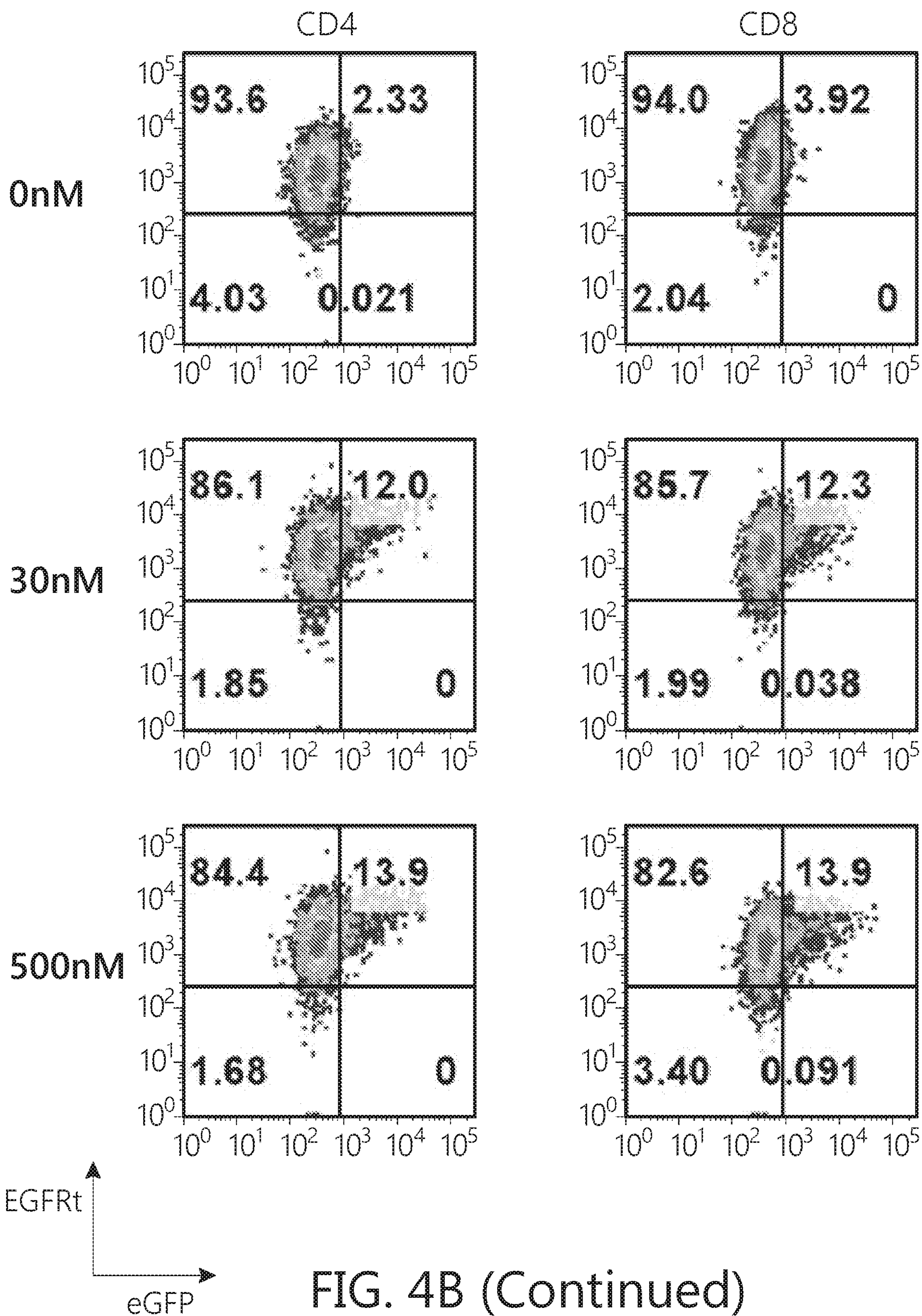
FIG. 4B

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PMA (2uM)
Ionomycin (20ng/mL)

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No TCR Stimulation



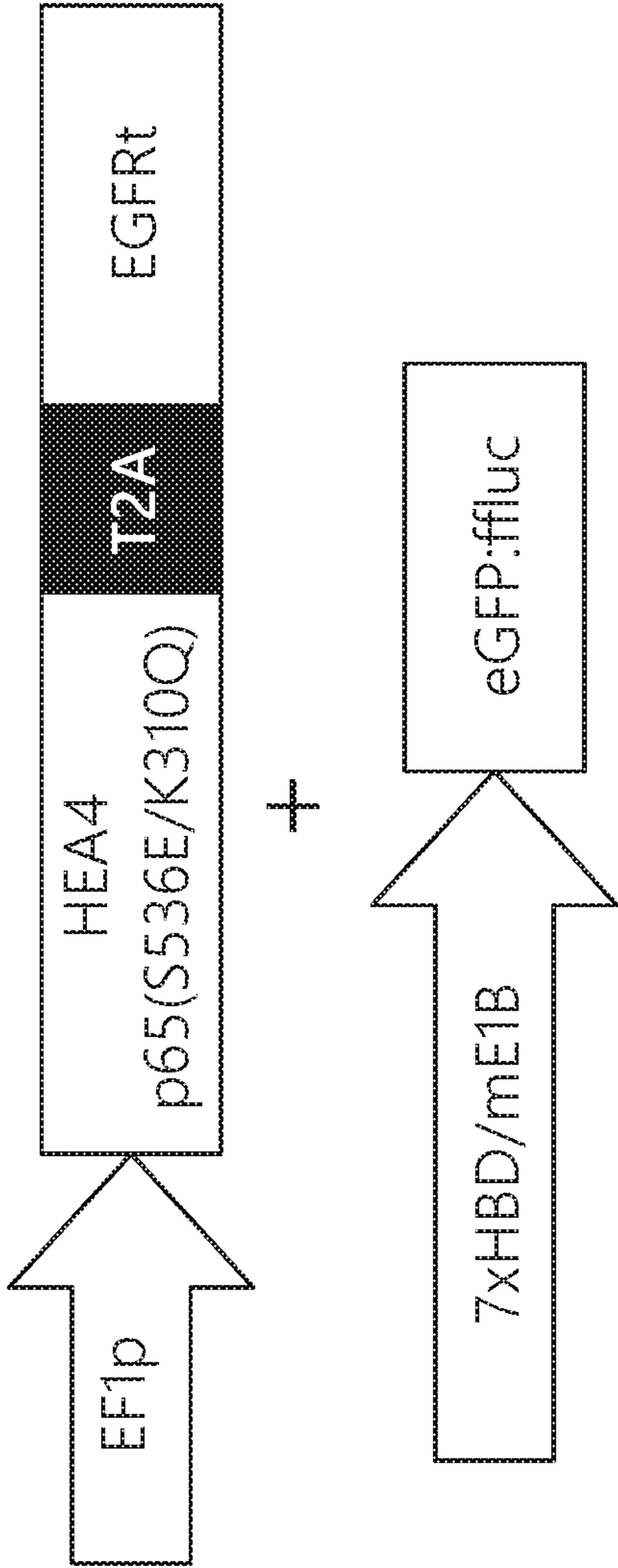


FIG. 5A

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Response of CD4 T Cells

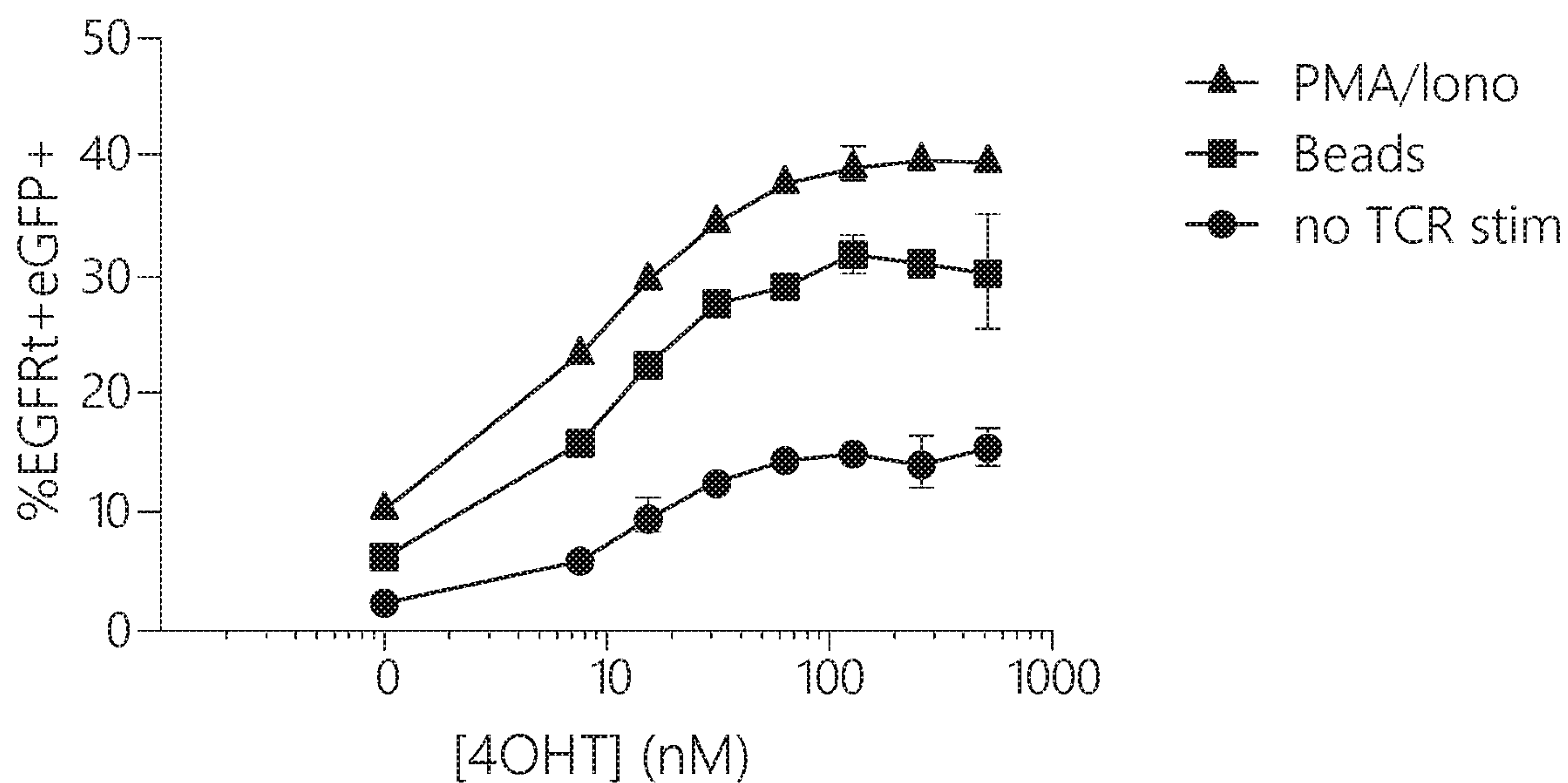


FIG. 5B

Response of CD8 T Cells

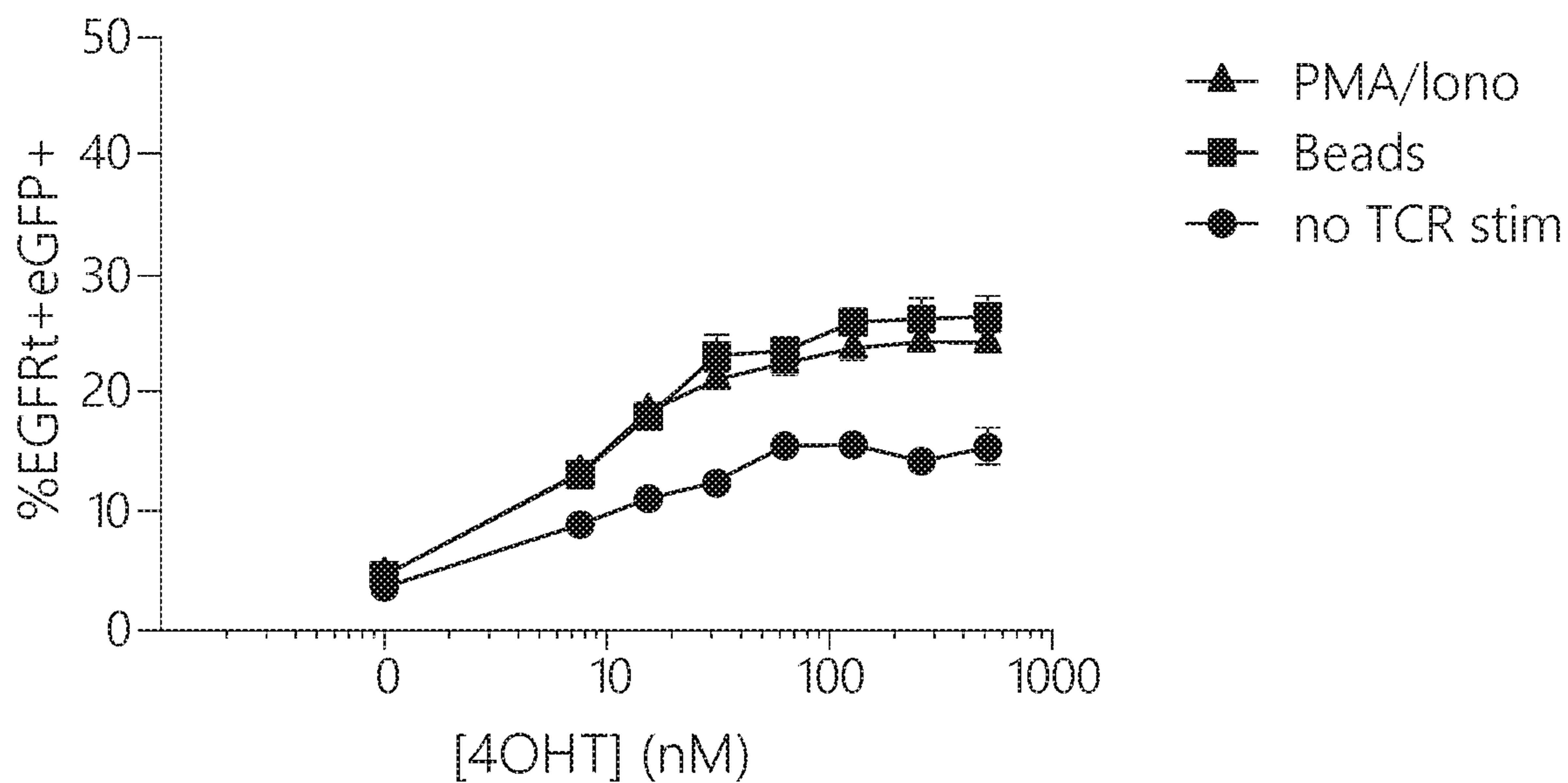


FIG. 5C

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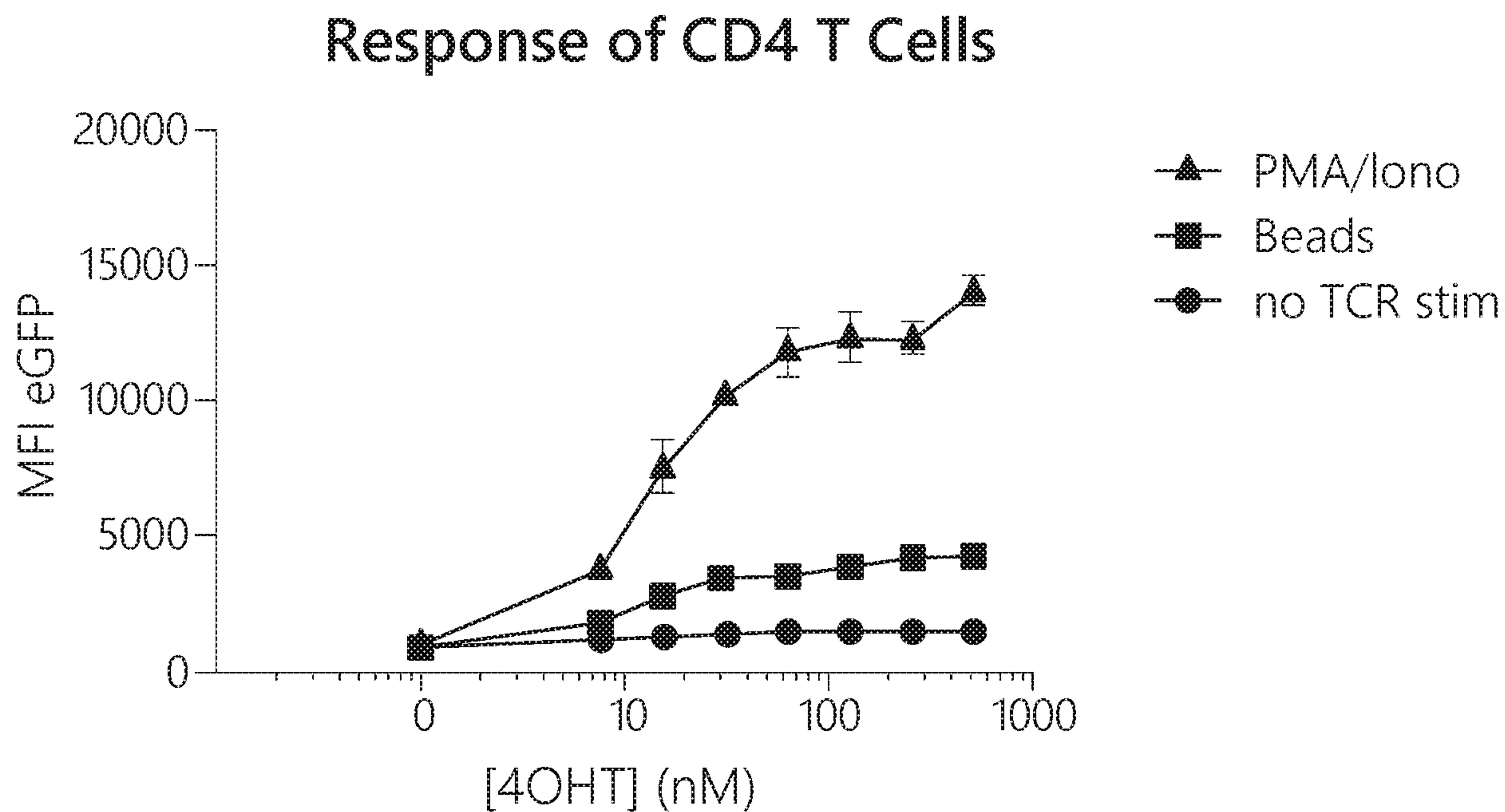


FIG. 5D

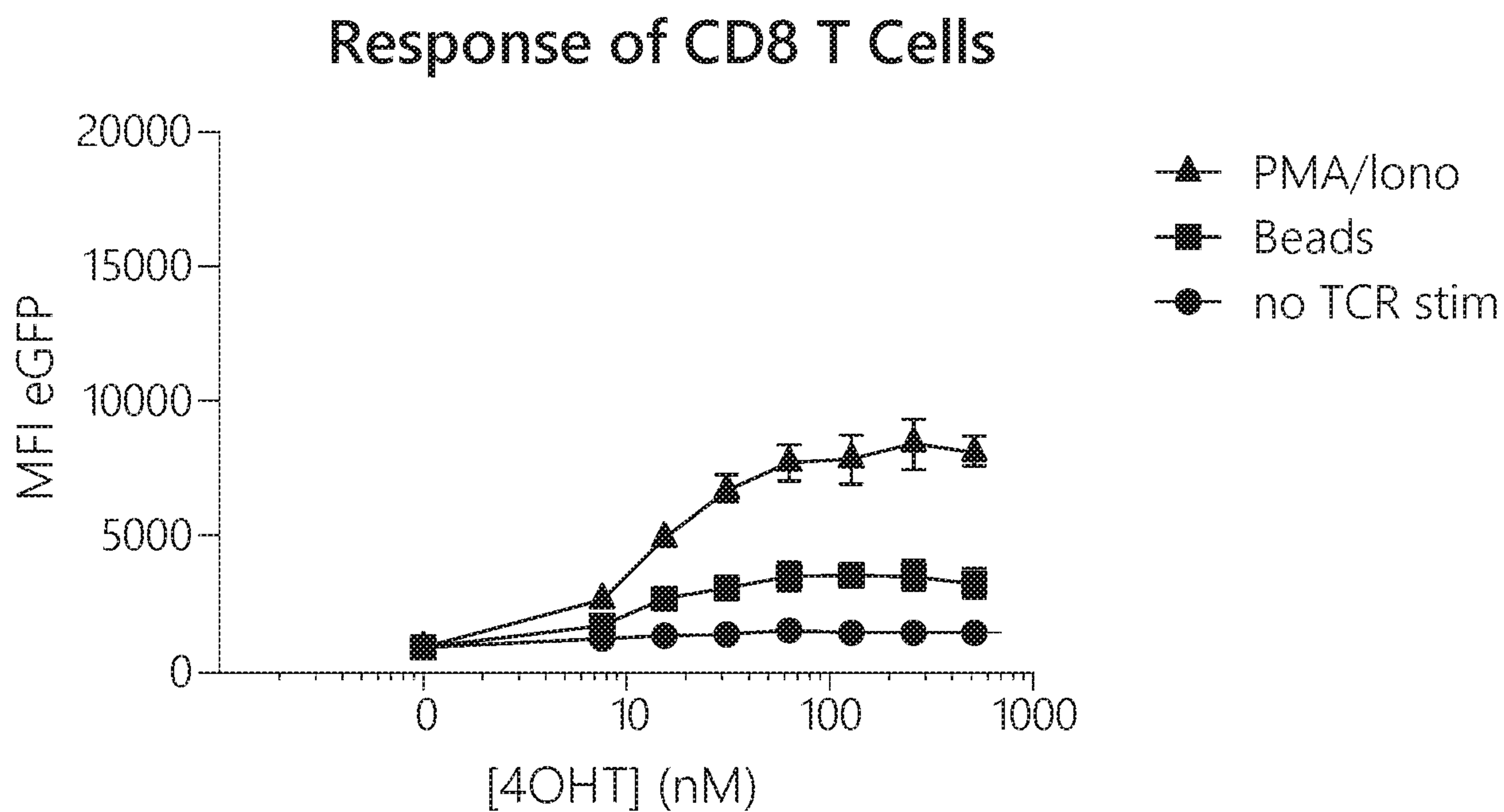


FIG. 5E

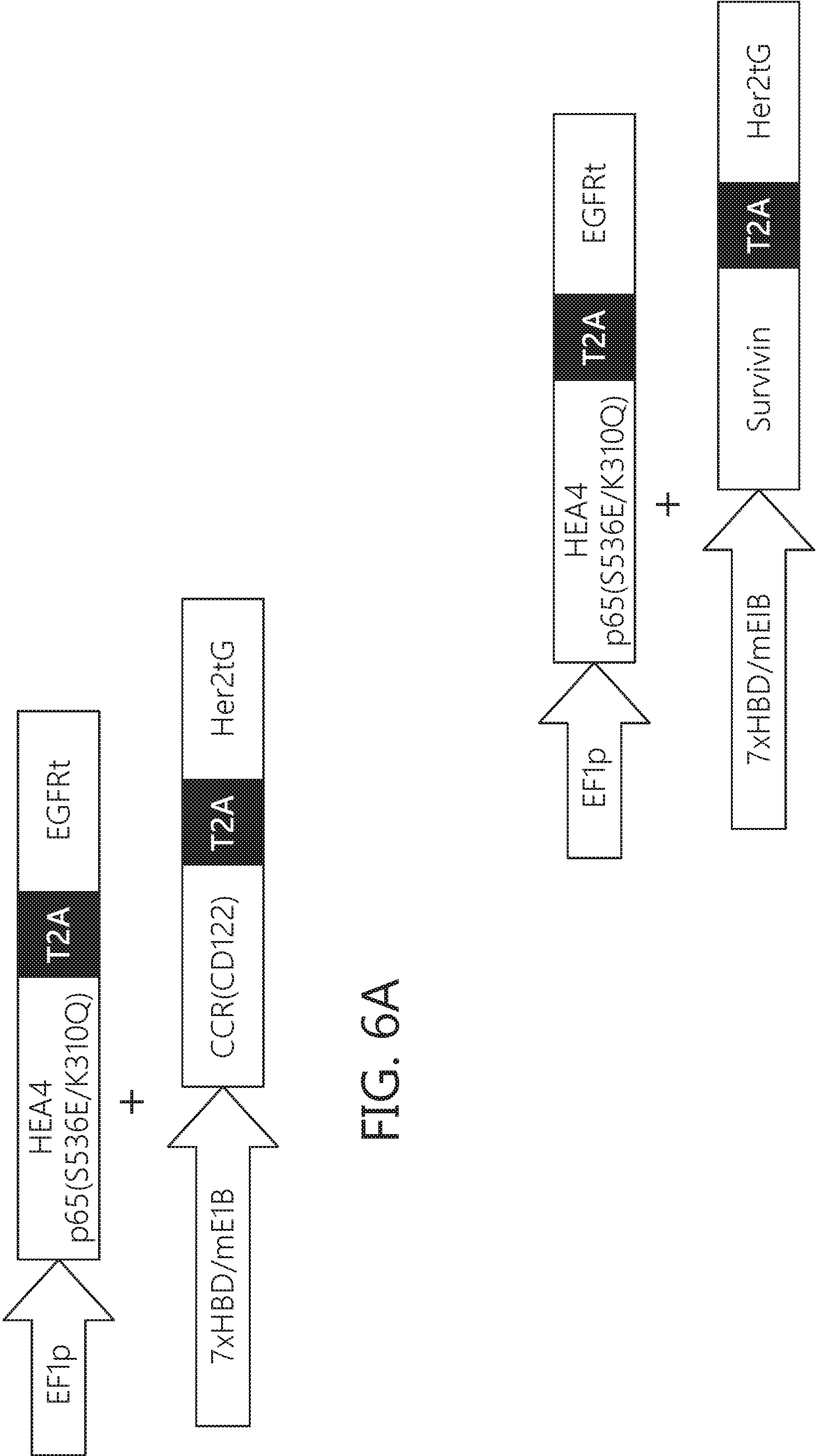


FIG. 6A

FIG. 6B

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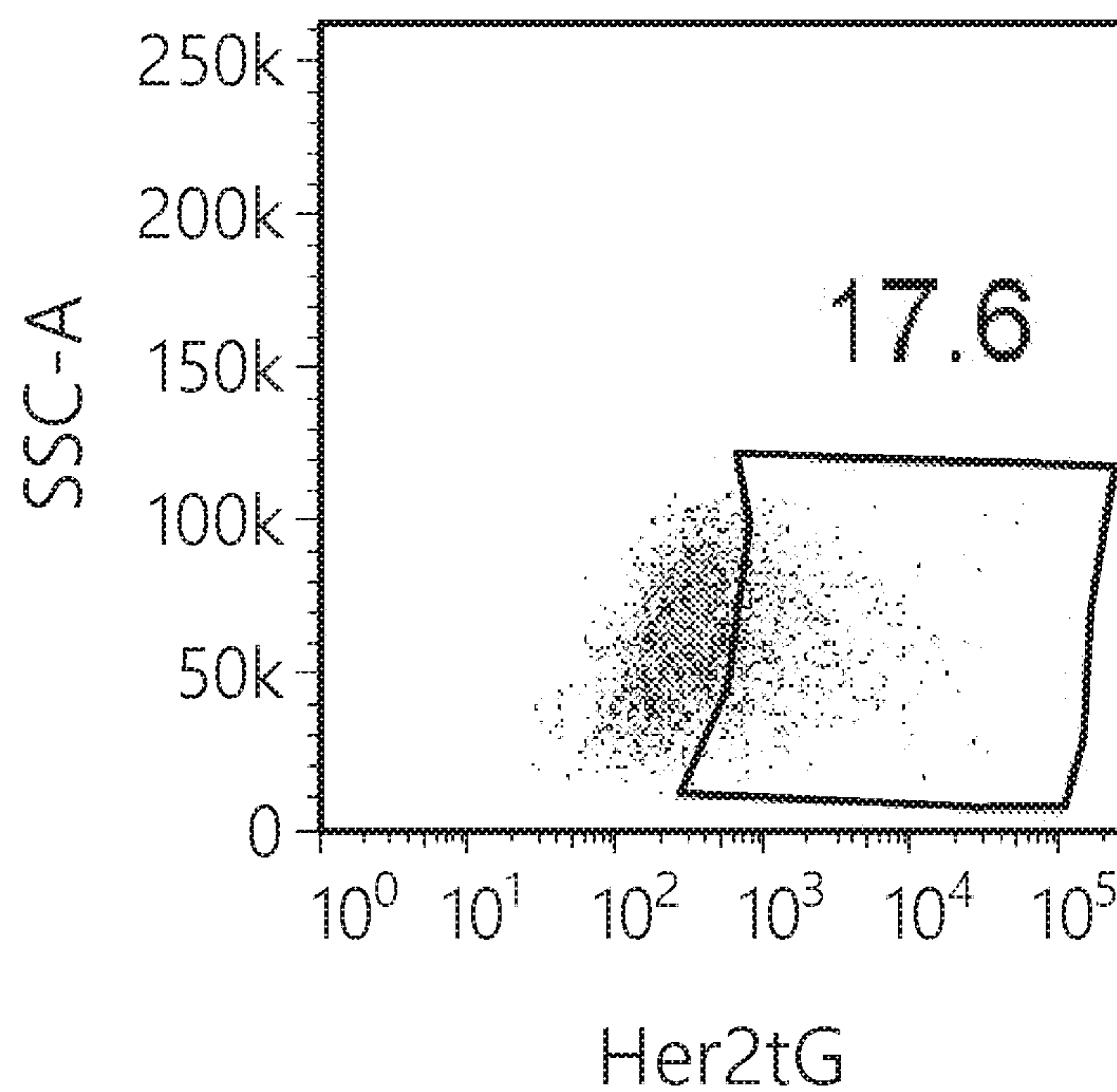


FIG. 6C

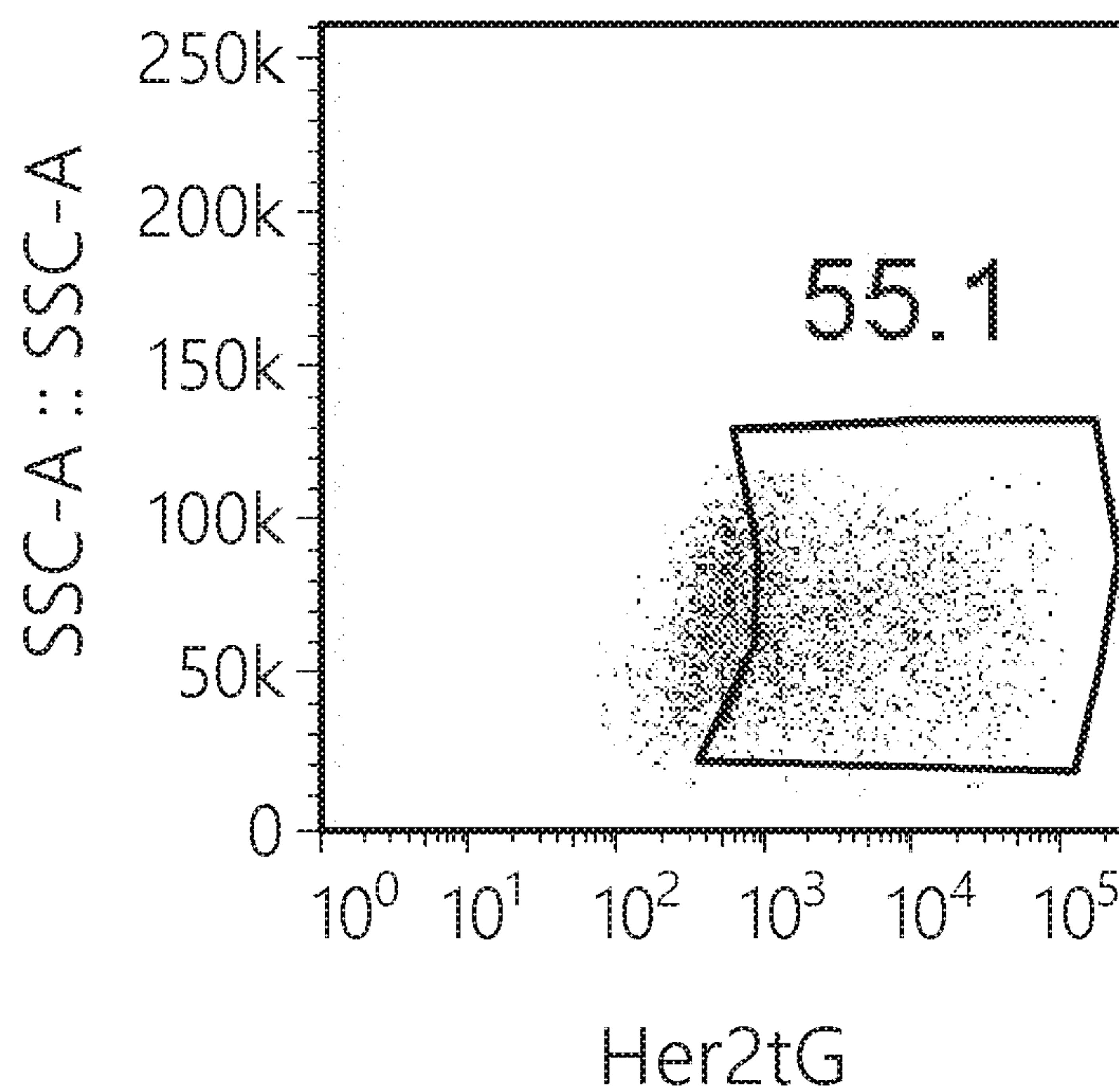


FIG. 6CD

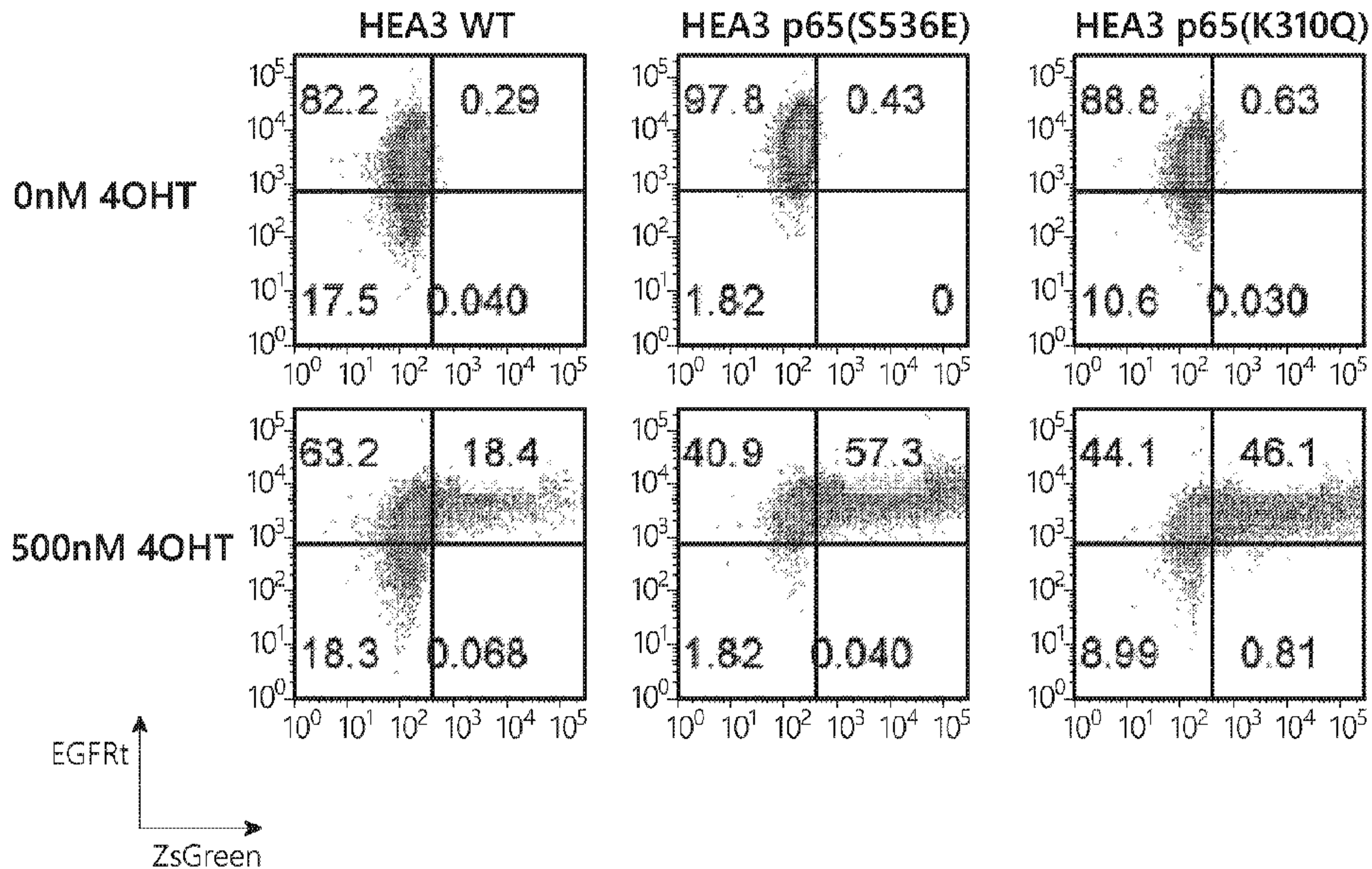


FIG. 1C