CD28 antibodies in the presence of IL-2, and finally infusing the expanded cell population back to the tumor-bearing patient (together with chemotherapy and repetitive administration of IL-2) (Dudley *et al*., *Science 298*:850, 2002). This form of adoptive T cell therapy with tumor infiltrating lymphocytes is technically cumbersome and leads to complete remission in only a minor fraction of patients with melanoma and is rarely effective in other cancers (Besser *et al*., *Clin. Cancer Res. 16*:2646, 2010).

Isolation of tumor-reactive T cell clones led to the development of another immunotherapeutic approach – the generation of recombinant T cell receptors (TCRs) specific for particular antigens, which are introduced into T cells using a vector delivery system to confer specificity for a tumor-associated peptide presented by an MHC molecule expressed on a tumor cell. A similar approach introduces a synthetic receptor, termed a chimeric antigen receptor (CAR), which contains an antigen-binding domain, which, *e.g*., in the context of anti-tumor therapy can bind to a tumor-specific or associated antigen, linked to one or more intracellular component comprising an effector domains, such as a TCR and/or costimulatory signaling domains. Unlike TILs, the basic procedure for TCR or CAR T cell immunotherapy is to genetically modify human T cells with a transgene encoding a tumor targeting moiety, *ex vivo* expansion of the recombinant T cells, and transfusing the expanded recombinant T cells back into patients. In the case of adoptive therapy with CAR T cells, the composition of the synthetic CAR structure, as well as the quality and purity of the genetically engineered T cells, will determine therapeutic efficacy against tumors *in vivo*. But, there are challenges to expanding and selecting the recombinant cell populations, as well as making sure the cells are effective and specific enough *in vivo* to avoid serious autoimmune side effects.

Currently, there remains a need in the immunotherapy field for compositions and methods for identifying, efficiently isolating/sorting, selectively expanding, *in vivo* tracking and controlling or eliminating engineered cells, such as engineered immune cells (*e.g*., T cells).

~~BRIEF~~ SUMMARY

In one aspect, the present disclosure refers to a single chain fusion protein, comprising a transmembrane domain disposed between an extracellular component and an intracellular component, wherein: the extracellular component comprises a plurality of tag cassettes and a connector region comprising a hinge, and wherein at least one of the plurality of tag cassettes comprises a Strep tag; and the intracellular component comprises an effector domain.

In another aspect, the present disclosure refers to a nucleic acid molecule encoding a single chain fusion protein as disclosed herein.

In another aspect, the present disclosure refers to a vector comprising a nucleic acid molecule as disclosed herein.

In another aspect, the present disclosure refers to a host cell, comprising a nucleic acid molecule encoding a single chain fusion protein as disclosed herein; optionally wherein the host cell is a T cell.

In another aspect, the present disclosure refers to an *in vitro* or *ex vivo* method for activating a cell, comprising contacting a cell comprising a single chain fusion protein as disclosed herein and/or the nucleic acid molecule as disclosed herein with a binding domain specific for the tag cassette.

In another aspect, the present disclosure refers to an *in vitro* or *ex vivo* method for promoting cell proliferation, comprising contacting a non-natural cell comprising a single chain fusion protein as disclosed herein and/or the nucleic acid molecule as disclosed herein with a binding domain specific for the tag cassette and a growth factor cytokine for a time sufficient to allow cell growth.

In another aspect, the present disclosure refers to an *in vitro* method for identifying a cell, comprising: contacting a sample comprising a cell comprising a single chain fusion protein as disclosed herein and/or the nucleic acid molecule as disclosed herein with a binding domain specific for the tag cassette, wherein the binding domain specific for the tag cassette comprises a detectable moiety, and detecting the presence of the cell in the sample.

In another aspect, the present disclosure refers to an *in vitro* method for sorting or selecting a cell or population of cells, the method comprising: contacting a sample comprising a cell comprising a single chain fusion protein as disclosed herein and/or the nucleic acid molecule as disclosed herein, with a binding domain specific for the tag cassette, and selecting or sorting away from other cells that are specifically bound by the binding domain, thereby selecting or sorting away from other cells the cell or population of cells.

In another aspect, the present disclosure refers to an *in vitro* or *ex vivo* method for enriching for or isolating a cell or population thereof, the method comprising contacting a sample comprising the cell comprising the nucleic acid molecule as disclosed herein and/or the single chain fusion protein as disclosed herein with a binding domain specific for the tag cassette and enriching for or isolating the cell away from other cells not expressing the fusion protein in the sample.

In another aspect, the present disclosure refers to an *in vitro* method for depleting cells, comprising contacting a cell comprising a nucleic acid molecule as disclosed herein or a single chain fusion protein as disclosed herein with a binding domain specific for the tag cassette and comprising a cell-toxic reagent, wherein binding of the binding domain to the tag cassette leads to death of the cells expressing the single chain fusion protein

In yet another aspect, the present disclosure refers to the use of a host cell as disclosed herein in the manufacture of a medicament for treating a disease in a subject, wherein the disease is selected from a cancer, an infectious disease, an autoimmune disease, an inflammatory disease, or an aging-related disease.

In certain ~~aspects~~examples, the present disclosure is directed to a single chain fusion protein, comprising an extracellular component and an intracellular component connected by a hydrophobic portion, wherein the extracellular component comprises a binding domain that specifically binds a target, a tag cassette, and a connector region comprising a hinge, and wherein the intracellular component comprises an effector domain.

In some ~~aspects~~examples, the present disclosure is directed to a chimeric antigen receptor molecule, comprising a fusion protein having one or more extracellular tag cassettes (a) located at the amino-terminus of an extracellular binding domain, (b) imbedded within an extracellular binding domain, or (c) disposed between and connecting an extracellular binding domain and an intracellular component comprising an effector domain.

In further examples~~aspects~~, the present disclosure is directed to a single chain fusion protein, comprising a hydrophobic portion disposed between and connecting an extracellular component and an intracellular component, wherein the extracellular component comprises a tag cassette and a connector region comprising a hinge, and wherein the intracellular component comprises an effector domain.

In still further examples~~aspects~~, the present disclosure is directed to a method for activating a cell, such as a T cell (*e.g.*, a non-natural T cell), comprising contacting a cell with a binding domain specific for a tag cassette, wherein the cell comprises a nucleic acid molecule encoding a fusion protein according to this disclosure and the binding domain specific for the tag cassette is attached to a solid surface.

In yet further examples~~aspects~~, the present disclosure is directed to a method for promoting cell proliferation, such as T cell proliferation, comprising contacting a cell (*e.g.*, a non-natural T cell) with a binding domain specific for a tag cassette and a growth factor cytokine for a time sufficient to allow cell growth, wherein the cell comprises a nucleic acid molecule encoding a fusion protein according to this disclosure and the binding domain specific for the tag cassette is attached to a solid surface.

In certain other examples~~aspects~~, the present disclosure is directed to a method for identifying cell, such as a T cell, comprising contacting a sample comprising a cell, such as a T cell (*e.g.*, a non-natural T cell) with a binding domain specific for a tag cassette, wherein the cell comprises a nucleic acid molecule encoding a fusion protein according to this disclosure and the binding domain specific for the tag cassette comprises a detectable moiety, and detecting the presence of the cell expressing a fusion protein in the sample.

In certain further examples~~aspects~~, the present disclosure is directed to a method for sorting a T cell, comprising contacting a sample comprising a non-natural T cell with a binding domain specific for a tag cassette, wherein the non-natural T cell comprises a nucleic acid molecule encoding a fusion protein according to this disclosure and the binding domain specific for the tag cassette comprises a detectable moiety, and sorting

the non-natural T cell expressing a fusion protein from other cells not expressing a fusion protein in the sample.

In certain examples~~aspects~~, the present disclosure is directed to a method for enriching or isolating a T cell, comprising contacting a sample comprising a non-natural T cell with a binding domain specific for a tag cassette, wherein the non-natural T cell comprises a nucleic acid molecule encoding a fusion protein according to this disclosure and the binding domain specific for the tag cassette comprises a detectable moiety, and enriching for or isolating the non-natural T cell expressing a fusion protein away from other cells not expressing a fusion protein in the sample.

In further examples~~aspects~~, the present disclosure is directed to a method for depleting certain T cells, comprising contacting a non-natural T cell with a binding domain specific for a tag cassette, wherein the non-natural T cell comprises a nucleic acid molecule encoding a fusion protein according to this disclosure and wherein binding of the binding domain specific for the tag cassette leads to cell death of the T cells expressing a fusion protein.

These and other ~~aspects~~ examples of the present ~~invention~~ disclosure will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A – 1H show illustrations of various single chain chimeric effector molecules containing one or more affinity tag cassettes (A-D, referred to herein as a Key-ChEMs), and optionally containing one or more specific binding domains (E-G, referred to herein as a T-ChARMs). The single chain ChEMs and ChARMs contain an intracellular domain. The tag cassettes may be any type of affinity tag, such as Strep tag® II (SEQ ID NO.:1), Myc tag (SEQ ID NO.:7), V5 tag (SEQ ID NO.:8), Flag® tag (SEQ ID NO.:3), His tag, or other peptides or molecules, which are recognized by a non-endogenous cognate binding partner (*e.g.*, receptor, protein, antibody). As shown, a Key-ChEM may contain (A, B) one tag cassette, (C) two tag cassettes (Key-ChEM^2), (D) three tag cassettes (Key-ChEM^3), or more. In addition, the chimeric molecules may have multiple effector domains (*e.g.*, the molecules of A and C-G have two, while the molecule shown in B has three effector domains), and the tag cassettes may be placed in various different areas of a Key-ChEM or T-ChARM molecule. In these particular examples, T-ChARMs have one tag cassette located between the specific binding domain and the effector domain (E), at the distal end (*e.g.*, amino-terminus) of the

CLAIMS

What is claimed is:

1. A single chain fusion protein, comprising a transmembrane domain disposed between an extracellular component and an intracellular component ~~connected by a hydrophobic portion~~, wherein:

the extracellular component comprises ~~a binding domain that specifically binds a target,~~ a plurality of tag cassettes~~,~~ and a connector region comprising a hinge, and wherein at least one of the plurality of tag cassettes comprises a Strep tag; and

~~wherein~~ the intracellular component comprises an effector domain.

2. The single chain fusion protein according to claim 1, wherein each of the plurality of tag cassettes comprises a Strep tag.

3. The single chain fusion protein according to claim 1, wherein two to five of the plurality of tag cassettes comprises a Strep tag.

~~2. The single chain fusion protein according to claim 1, wherein the binding domain is a scFv, scTCR, receptor ectodomain, or ligand.~~

~~3. The single chain fusion protein according to claim 1 or 2, wherein the target comprises CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR, FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, mesothelin, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor or pathogen associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, WT-1 peptide bound to HLA, LTβR, LIFRβ, LRP5, MUC1, OSMRβ, TCRα, TCRβ, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, Robo1, α-fetoprotein (AFP), Frizzled, OX40, or CD79b.~~

4. The single chain fusion protein according to any one of claims 1-3, wherein the connector region further comprises:

(a) a linker module.; and/or

~~5. The single chain fusion protein according to claim 4, wherein the~~(b) a linker module, wherein the linker module comprises one or more ~~is a (~~Gly_xSer_y~~)n~~, wherein ~~n is an integer from 1 to 10, and~~ x and y are independently an integer from 0 to 10 provided that x and y are not both 0.; and/or

(c) a linker module, wherein the linker module is a $(Gly_xSer)_n$, wherein x is an integer from 1 to 5 and n is an integer from 1 to 10; and/or

~~6. The single chain fusion protein according to claim 4, wherein~~(d) a linker module, wherein the linker module is a CH2CH3 or a CH3.

~~7~~5. The single chain fusion protein according to any one of claims 1-~~6~~4, wherein:

one or more of the plurality of tag cassettes are located within the connector region ~~comprises one or more of the tag cassettes.~~; or

~~8. The single chain fusion protein according to any one of claims 1-6, wherein the connector region comprises from one to five tag cassettes.~~

~~9. The single chain fusion protein according to claim 7 or 8, wherein the connector region comprises from one to five tag cassettes,~~ one or more of the plurality of tags are located within the connector region, wherein each tag ~~cassette~~ is connected to one or two linker modules comprising a $(Gly_xSer_y)_n$, wherein n is an integer from 1 to 10, and x and y are independently an integer from 0 to 10 provided that x and y are not both 0.; or

~~10. The single chain fusion protein according to claim 9,~~ one or more of the plurality of tags are located within the connector region, wherein each tag is connected to one or two ~~wherein the~~ linker module~~s~~ ha~~ving~~s ~~an~~the amino acid sequence of Gly-

Gly-Gly-Gly-Ser (SEQ ID NO.:10), (Gly-Gly-Gly-Gly-Ser)$_2$ (SEQ ID NO.:11), (Gly-Gly-Gly-Ser)$_2$-Gly-Gly-Ser (SEQ ID NO.:12), or any combination thereof~~.~~; or

one to five of the plurality of tags are located within the connector region; or

one to five of the plurality of tags are located within the connector region, wherein each tag is connected to one or two linker modules comprising a $(Gly_xSer_y)_n$, wherein n is an integer from 1 to 10, and x and y are independently an integer from 0 to 10 provided that x and y are not both 0; or

the connector region comprises from one to five tags, wherein each tag is connected to one or two linker modules having the amino acid sequence of Gly-Gly-Gly-Gly-Ser (SEQ ID NO:10), (Gly-Gly-Gly-Gly-Ser)$_2$ (SEQ ID NO:11), (Gly-Gly-Gly-Ser)$_2$-Gly-Gly-Ser (SEQ ID NO:12), or any combination thereof.

~~11. The single chain fusion protein according to any one of claims 1-10, wherein the binding domain comprises one or more tag cassettes.~~

~~12. The single chain fusion protein according to any one of claims 1-10, wherein the tag cassette is located amino-terminal to the binding domain, carboxy-terminal to the binding domain, or both.~~

~~13. The single chain fusion protein according to claim 11, wherein the binding domain is a scFv or scTCR comprising a variable region linker, wherein the variable region linker comprises one or more tag cassettes.~~

~~14~~6. The single chain fusion protein according to any one of claims 1-~~13~~5, wherein the single chain fusion protein further ~~tag cassette is or~~ comprises a Strep tag, His tag, Flag® tag, Xpress® tag, Avi tag, Calmodulin tag, Polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, X tag, SBP tag, Softag, V5 tag, CBP, GST, MBP, GFP, Thioredoxin tag, or any combination thereof.

~~15~~7. The single chain fusion protein according to any one of claims ~~14~~1-6, wherein one or more of the plurality of ~~the~~ tag cassettes ~~is~~are or comprise~~s~~ a Strep tag having amino acid sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2).

~~16. The single chain fusion protein according to any one of claims 1-15, wherein the connector region further comprises a linker module adjacent to one or more tag cassettes, wherein the linker module and adjacent tag cassette collectively have an amino acid sequence of (Gly-Gly-Gly-Gly-Ser)$_2$ Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:20), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Gly-Ser)$_2$ (SEQ ID NO.:21), (Gly-Gly-Gly-Gly-Ser)$_2$ Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Ser)$_2$ Gly-Gly-Ser Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:22), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Ser)$_2$ Gly-Gly-Ser Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Gly-Ser)$_2$ (SEQ ID NO.:23), (Gly-Gly-Gly-Gly-Ser)$_2$ Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Ser)$_2$ Gly-Gly-Ser Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Gly-Ser)$_2$ Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:24), or Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Gly-Ser)$_2$ Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Ser)$_2$ Gly-Gly-Ser Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-(Gly-Gly-Gly-Gly-Ser)$_2$ (SEQ ID NO.:25).~~

~~17. The single chain fusion protein according to any one of claims 1-16, wherein the hydrophobic portion is a transmembrane domain.~~

~~18~~8. The single chain fusion protein according to any one of claims ~~17~~1-7, wherein the transmembrane domain is a CD4, CD8, CD28 or CD27 transmembrane domain.

~~19~~9. The single chain fusion protein according to any one of claims 1-~~18~~8, wherein:

(a) the effector domain ~~or effector portion thereof~~ is from ~~a~~ 4-1BB (~~CD137),~~ CD3ε, CD3δ, CD3ζ, ~~CD25,~~ CD27, CD28, CD79A, CD79B, CD134, CD137, CARD11, DAP10, FcRα, FcRβ, FcRγ, Fyn, HVEM, ICOS, Lck, LAG3, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, ~~OX40 (CD134),~~ ROR2, Ryk, SLAMF1, Slp76, pTα, TCRα, TCRβ, TRIM, Zap70, PTCH2, or any combination thereof~~.~~;

~~20. The single chain fusion protein according to any one of claims 1-19, wherein~~(b) the effector domain ~~or effector portion thereof comprises~~is from (i) CD3ζ and (ii) one or more of 4-1BB (CD137), CD27, CD28, and OX40 (CD134)~~.~~; or

(c) the effector domain is from LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, ROR2, or Ryk.

~~21~~10. The single chain fusion protein according to any one of claims 1-~~20~~9, wherein the fusion protein comprises from amino-terminus to carboxy-terminus:

(a) ~~an extracellular binding domain,~~ a tag cassette, a connector region comprising a hinge, a transmembrane domain~~hydrophobic portion~~, and an intracellular component comprising an effector domain~~.~~; or

~~22. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: an extracellular binding domain,~~(b) a first connector region, a tag-~~cassette~~, a second connector region comprising a hinge, a transmembrane domain~~hydrophobic portion~~, and an intracellular component comprising an effector domain~~.~~; or

~~23. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: an extracellular binding domain,~~(c) a first tag-~~cassette~~, a first connector region, a second tag-~~cassette~~, a second connector region comprising a hinge, a transmembrane domain~~hydrophobic portion~~, and an intracellular component comprising an effector domain~~.~~; or

~~24. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: an extracellular binding domain,~~(d) a first tag-~~cassette~~, a first connector region, a second tag-~~cassette~~, a second connector region, a third tag-~~cassette~~, a third connector region comprising a hinge, a transmembrane domain~~hydrophobic portion~~, and an intracellular component comprising an effector domain~~.~~; or

~~25. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: a tag cassette, an extracellular binding domain, a connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain.~~

~~26. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: an extracellular binding domain,~~(e) two to five tag~~s cassettes~~, a connector region comprising a hinge, a ~~hydrophobic portion~~transmembrane domain, and an intracellular component comprising an effector domain~~.~~; or

~~27. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: an extracellular scFv or scTCR binding domain comprising a variable region linker disposed between the variable regions and containing a tag cassette, a connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain.~~

~~28. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: an extracellular scFv or scTCR binding domain,~~ (f) a tag ~~cassette~~, a connector region comprising an IgG hinge, a transmembrane domain, and an intracellular component comprising an effector domain from ~~, wherein the effector domain comprises~~ 4-1BB and CD3ζ, CD27 and CD3ζ, CD28 and CD3ζ, ~~OX40 and CD3ζ,~~ or CD28, 4-1BB and CD3ζ~~, OX40, 4-1BB and CD3ζ, or CD28, OX40 and CD3ζ.~~; or

(g) a tag, a connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain from LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, ROR2, or Ryk.

~~29. The single chain fusion protein according to any one of claims 1-20, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: an extracellular binding domain comprising a receptor ectodomain, a tag cassette, a connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain, wherein the effector domain comprises 4-1BB, CD27, CD28, or OX40.~~

11. The single chain fusion protein according to any one of claims 1-10, wherein the extracellular component further comprises a binding domain that specifically binds a target.

12. The single chain fusion protein according to claim 11, wherein the binding domain comprises a scFv, scTCR, receptor ectodomain, or ligand.

13. The single chain fusion protein of claim 11 or 12, wherein the fusion protein comprises from amino-terminus to carboxy-terminus:

(a) an extracellular binding domain, a tag, a connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain; or

(b) an extracellular binding domain, a first connector region, a tag, a second connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain; or

(c) an extracellular binding domain, a first tag, a first connector region, a second tag, a second connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain; or

(d) an extracellular binding domain, a first tag, a first connector region, a second tag, a second connector region, a third tag, a third connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain; or

(e) a tag, an extracellular binding domain, a connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain; or

(f) an extracellular binding domain, two to five tags, a connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain; or

(g) an extracellular scFv or scTCR binding domain comprising a variable region linker disposed between the variable regions and containing a tag, a connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain; or

(h) an extracellular scFv or scTCR binding domain, a tag, a connector region comprising an IgG hinge, a transmembrane domain, and an intracellular component

comprising an effector domain, wherein the effector domain is from (A) 4-1BB and CD3ζ, (B) CD27 and CD3ζ, (C) CD28 and CD3ζ, (D) OX40 and CD3ζ, (E) CD28, 4-1BB and CD3ζ, (F) OX40, 4-1BB and CD3ζ, or (G) CD28, OX40 and CD3ζ; or

(i) an extracellular binding domain comprising a receptor ectodomain, a tag, a connector region comprising a hinge, a transmembrane domain, and an intracellular component comprising an effector domain, wherein the effector domain is from 4-1BB, CD27, CD28, or OX40.

14. The single chain fusion protein according to any one of claims 1-10, wherein the fusion protein further comprises:

(a) a binding domain non-covalently associated with the extracellular component; and/or

(b) a binding domain non-covalently associated with the extracellular component, wherein the non-covalently associated binding domain associates with a tag; and/or

(c) a binding domain non-covalently associated with the extracellular component, wherein the non-covalently associated binding domain is a scFv, scTCR, receptor ectodomain, or ligand; and/or

(d) a bispecific binding domain non-covalently associated with the extracellular component, wherein a first binding end of the bispecific binding domain is specific for a tag and a second binding end of the bispecific binding domain is specific for a target other than the tag; and/or

(e) a bispecific binding domain non-covalently associated with the extracellular component, wherein both binding ends of the bispecific binding domain are specific for a tag; and/or

(f) a multispecific binding domain non-covalently associated with the extracellular component, wherein a first end of the multispecific binding domain binds to a tag and a second end of the multispecific binding domain is specific for one or more targets other than the tag.

15. The single chain fusion protein according to any one of claims 11-14, wherein the binding domain is specific for CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR, FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, mesothelin, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor or pathogen associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, WT-1 peptide bound to HLA, LTβR, LIFRβ, LRP5, MUC1, OSMRβ, TCRα, TCRβ, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, Robo1, α-fetoprotein (AFP), Frizzled, OX40, or CD79b.

~~30. A chimeric antigen receptor molecule, comprising a fusion protein having one or more extracellular tag cassettes disposed between and connecting an extracellular binding domain that specifically binds to an antigen and an intracellular component comprising an effector domain.~~

~~31. The chimeric antigen receptor molecule according to claim 30, wherein the one or more tag cassettes comprises one tag cassette.~~

~~32. The chimeric antigen receptor molecule according to claim 30, wherein the one or more tag cassettes comprises two to five tag cassettes.~~

~~33. The chimeric antigen receptor molecule according to any one of claims 30-32, wherein the chimeric antigen receptor molecule further comprises one or more linker modules comprising a $(Gly_xSer_y)_n$, wherein n is an integer from 1 to 10, and x and y are independently an integer from 0 to 10 provided that x and y are not both 0.~~

~~34. The chimeric antigen receptor molecule according to any one of claims 30-32, wherein the fusion protein further comprises a $(Gly_xSer)_n$ linker module disposed between the binding domain and the one or more tag cassettes, wherein x is an integer from 2 to 4 and n is an integer from 1 to 3.~~

35. The chimeric antigen receptor molecule according to any one of claims 30-32, wherein the fusion protein further comprises an extracellular $(Gly_xSer)_n$ linker module disposed between the one or more tag cassettes and the intracellular component comprising an effector domain, wherein x is an integer from 2 to 4 and n is an integer from 1 to 3.

36. The chimeric antigen receptor molecule according to any one or more of claims 30-32, wherein the fusion protein further comprises two extracellular $(Gly_xSer)_n$ linker modules, wherein x is an integer from 2 to 4 and n is an integer from 1 to 3, and wherein the first linker module is amino-terminal to at least one of the one or more tag cassettes and the second linker module is carboxy-terminal to at least one of the one or more tag cassettes.

37. The chimeric antigen receptor molecule according to claim 30, wherein the one or more tag cassettes comprises two tag cassettes and the molecule further comprises two extracellular $(Gly_xSer)_n$ linker modules, wherein x is an integer from 2 to 4 and n is an integer from 1 to 3, and wherein a first tag cassette is disposed between the binding domain and the first linker module, a second tag cassette is disposed between the first and second linker modules, and the second linker module disposed between the second tag cassette and the effector domain.

38. The chimeric antigen receptor molecule according to claim 37, wherein the fusion protein further comprises a third tag cassette and a third extracellular $(Gly_xSer)_n$ linker module, wherein the third tag cassette is disposed between the second linker module and the third linker module, and the third linker module is disposed between the third tag cassette and the effector domain.

39. The chimeric antigen receptor molecule according to any one of claims 30-38, wherein the fusion protein further comprises an extracellular hinge and extracellular CH2CH3 linker module, wherein the hinge is adjacent to the binding domain, the CH2CH3 linker module is adjacent to the intracellular component comprising an effector domain, and at least one of the one or more tag cassettes is disposed between the hinge and the CH2CH3 linker module.

40. The chimeric antigen receptor molecule according to any one of claims 30-38, wherein the fusion protein further comprises an extracellular hinge and extracellular CH3 linker module, wherein the hinge is adjacent to the binding domain, the CH3 linker module is adjacent to the intracellular component comprising an effector domain, and at least one of and/or each of the one or more tag cassette is disposed between the hinge and the CH3 linker module.

41. The chimeric antigen receptor molecule according to any one of claims 30-40, wherein at least one of the one or more tag cassettes is or comprises a Strep tag, His tag, Flag® tag, Xpress® tag, Avi tag, Calmodulin tag, Polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, X tag, SBP tag, Softag, V5 tag, CBP, GST, MBP, GFP, Thioredoxin tag, or any combination thereof.

42. The chimeric antigen receptor molecule according to claim 41, wherein at least one of the one or more tag cassette is or comprises a Strep tag having amino acid sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2).

43. The chimeric antigen receptor molecule according to any one of claims 30-40, wherein the binding domain is a scFv, scTCR, receptor ectodomain, or ligand.

44. The chimeric antigen receptor molecule according to any one of claims 30-43, wherein the antigen is or comprises CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR, FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, mesothelin, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor or pathogen associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, WT-1 peptide bound to HLA, LTβR, LIFRβ, LRP5, MUC1, OSMRβ, TCRα, TCRβ, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, Robo1, α-fetoprotein (AFP), Frizzled, OX40, or CD79b.

45. The chimeric antigen receptor molecule according to any one of claims 30-44, wherein the effector domain is a 4-1BB, CD3ε, CD3δ, CD3ζ, CD27, CD28, CD79A, CD79B, CARD11, DAP10, FcRα, FcRβ, FcRγ, Fyn, HVEM, ICOS, Lck, LAG3, LAT, NKG2D, OX40, SLAMF1, Slp76, pTα, TCRα, TCRβ, TRIM, Zap70, PTCH2, or any combination thereof.

46. The chimeric antigen receptor molecule according to any one of claims 30-45, wherein the effector domain comprises CD3ζ and one or more of 4-1BB, CD27, CD28, and OX40.

47. The chimeric antigen receptor molecule according to any one of claims 30-45, wherein the effector domain comprises 4-1BB and CD3ζ, CD27 and CD3ζ, CD28 and CD3ζ, or CD28, 4-1BB and CD3ζ.

48. The chimeric antigen receptor molecule according to any one of claims 30-45, wherein the binding domain comprises a receptor ectodomain and the effector domain comprising 4-1BB, CD27, CD28, or OX40.

49. The chimeric antigen receptor molecule according to claim 48, wherein the tag cassette is located carboxy-terminal to the receptor ectodomain.

50. A single chain fusion protein, comprising a hydrophobic portion disposed between an extracellular component and an intracellular component, wherein the extracellular component comprises a tag cassette and a connector region comprising a hinge, and wherein the intracellular component comprises an effector domain.

51. The single chain fusion protein according to claim 50, wherein the connector region further comprises a linker module.

52. The single chain fusion protein according to claim 51, wherein the linker module is a $(Gly_xSer)_n$, wherein x is an integer from 1 to 5 and n is an integer from 1 to 10.

53. The single chain fusion protein according to claim 51, wherein the linker module is a CH2CH3 or a CH3.

54. The single chain fusion protein according to any one of claims 50-53, wherein one or more tag cassettes are amino terminal to the connector region.

55. The single chain fusion protein according to any one of claims 50-54, wherein the fusion protein comprises from one to five tag cassettes.

56. The single chain fusion protein according to claim 55, wherein each tag cassette is connected to one or two linker modules comprising a $(Gly_xSer_y)_n$, wherein n is an integer from 1 to 10, and x and y are independently an integer from 0 to 10 provided that x and y are not both 0.

57. The single chain fusion protein according to claim 56, wherein the linker module has an amino acid sequence of Gly-Gly-Gly-Gly-Ser (SEQ ID NO.:10), $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO.:11), $(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Gly-Gly-Ser (SEQ ID NO.:12), or any combination thereof.

58. The single chain fusion protein according to any one of claims 50-57, wherein one or more tag cassettes are linked to the connector region by a linker module.

59. The single chain fusion protein according to claim 58, wherein the linker module is or comprises a $(Gly_xSer)_n$, wherein x is an integer from 1 to 5 and n is an integer from 1 to 10.

60. The single chain fusion protein according to claim 58, wherein the linker module is or comprises $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO.:11).

61. The single chain fusion protein according to any one of claims 50-60, wherein one or more tag cassettes are or comprise a Strep tag, His tag, Flag® tag, Xpress® tag, Avi tag, Calmodulin tag, Polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, X tag, SBP tag, Softag, V5 tag, CBP, GST, MBP, GFP, Thioredoxin tag, or any combination thereof.

62. The single chain fusion protein according to claim 61, wherein one or more tag cassettes are or comprise a Strep tag having amino acid sequence Trp-Ser-His-

Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2).

63. The single chain fusion protein according to any one of claims 50-62, wherein the fusion protein further comprises a linker module adjacent to one or more tag cassettes, wherein the linker module and the adjacent tag cassette have an amino acid sequence of $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:20), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO.:21), $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Gly-Gly-Ser-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:22), Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Gly-Gly-Ser-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO.:23), $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Gly-Gly-Ser-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:24), or Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$-Gly-Gly-Ser-Trp-Ser-His-Pro-Gln-Phe-Glu-Lys-$(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_2$ (SEQ ID NO.:25).

64. The single chain fusion protein according to any one of claims 50-63, wherein the hydrophobic portion is a transmembrane domain.

65. The single chain fusion protein according to claim 64, wherein the transmembrane domain is a CD4, CD8, CD28 or CD27 transmembrane domain.

66. The single chain fusion protein according to any one of claims 1-18, wherein the effector domain is a CD3ε, CD3δ, CD3ζ, CD27, CD28, CD79A, CD79B, CD134, CD137, CARD11, DAP10, FcRα, FcRβ, FcRγ, Fyn, HVEM, ICOS, Lck, LAG3, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, ROR2, Ryk, SLAMF1, Slp76, pTα, TCRα, TCRβ, TRIM, Zap70, PTCH2, or any combination thereof.

67. The single chain fusion protein according to any one of claims 50-66, wherein the effector domain comprises CD3ζ and one or more of 4-1BB (CD137), CD27, CD28, and OX40 (CD134).

68. The single chain fusion protein according to any one of claims 50-66, wherein the effector domain comprises LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, ROR2, or Ryk.

69. The single chain fusion protein according to any one of claims 50-68, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: a tag cassette, a connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain.

70. The single chain fusion protein according to any one of claims 50-68, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: a first connector region, a tag cassette, a second connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain.

71. The single chain fusion protein according to any one of claims 50-68, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: a first tag cassette, a first connector region, a second tag cassette, a second connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain.

72. The single chain fusion protein according to any one of claims 50-68, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: a first tag cassette, a first connector region, a second tag cassette, a second connector region, a third tag cassette, a third connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain.

73. The single chain fusion protein according to any one of claims 50-68, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: two to five tag cassettes, a connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain.

74. The single chain fusion protein according to any one of claims 50-68, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: a tag cassette, a connector region comprising an IgG hinge, a transmembrane domain, and an

intracellular component comprising an effector domain comprising 4-1BB and CD3ζ, CD27 and CD3ζ, CD28 and CD3ζ, or CD28, 4-1BB and CD3ζ.

75. The single chain fusion protein according to any one of claims 50-68, wherein the fusion protein comprises from amino-terminus to carboxy-terminus: a tag cassette, a connector region comprising a hinge, a hydrophobic portion, and an intracellular component comprising an effector domain comprising LRP, NOTCH1, NOTCH2, NOTCH3, NOTCH4, ROR2, or Ryk.

76. The single chain fusion protein according to any one of claims 50-75, wherein the fusion protein further comprises a non-covalently-associated binding domain.

77. The single chain fusion protein according to claim 76, wherein the non-covalently associated binding domain associates with a tag cassette.

78. The single chain fusion protein according to claim 76 or 77, wherein the non-covalently associated binding domain is a scFv, scTCR, receptor ectodomain, or ligand.

79. The single chain fusion protein according to any one of claims 76-78, wherein the non-covalently associated binding domain is bi-specific, wherein the first binding end is specific for the tag cassette and the second binding end is specific for a target other than the tag cassette.

80. The single chain fusion protein according to claim 79, wherein the non-covalently associated binding domain is specific for CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR, FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, mesothelin, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor or pathogen associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, WT-1 peptide bound to HLA, LTβR, LIFRβ, LRP5, MUC1, OSMRβ, TCRα, TCRβ, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD81, CD86, CD123,

~~CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, Robo1, α-fetoprotein (AFP), Frizzled, OX40, or CD79b.~~

~~81. The single chain fusion protein according to any one of claims 76-78, wherein the binding domain is bi-specific, wherein the first and second binding ends are specific for the tag cassette.~~

~~82. The single chain fusion protein according to any one of claims 76-78, wherein the binding domain is multispecific, wherein a first end binds to the tag cassette and a second end is specific for one or more targets other than the tag cassette.~~

~~83. The single chain fusion protein according to claim 82, wherein at least one binding domain is specific for CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR, FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A, mesothelin, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor or pathogen associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, WT-1 peptide bound to HLA, LTβR, LIFRβ, LRP5, MUC1, OSMRβ, TCRα, TCRβ, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, Robo1, α fetoprotein (AFP), Frizzled, OX40, or CD79b.~~

~~84. The single chain fusion protein according to claim 76, wherein the binding domain is an antibody.~~

~~85. The single chain fusion protein according to claim 76, wherein the tag cassette is a Strep tag having amino acid sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2), and the associated binding domain is a biotin binding protein.~~

~~86. The single chain fusion protein according to any one of claims 76-85, wherein the binding domain further comprises a cytotoxic, radioisotope, radiometal, or detectable agent.~~

~~87. A fusion protein according to any one of claims 1-85, wherein the fusion protein further comprises a cytotoxic, radioisotope, radiometal, or detectable agent.~~

~~88~~16. A nucleic acid molecule encoding a single chain fusion protein according to any one of claims 1-15~~85 or 50-87, or a chimeric antigen receptor according to any one of claims 30-49~~.

~~89~~17. A vector comprising a nucleic acid molecule according to claim ~~88~~16.

~~90~~18. The vector according to claim ~~89~~17, wherein the vector is a viral vector; optionally ~~.~~

~~91. The vector according to claim 90, wherein the viral vector is~~ a retroviral vector or lentiviral vector.

~~92~~19. A host cell, comprising a nucleic acid molecule encoding a single chain fusion protein ~~or chimeric antigen receptor~~ according to any one of claims 1-~~85~~15~~.~~; optionally

~~93. The host cell according to claim 92,~~ wherein the host cell is a T cell.

~~94~~20. An *in vitro* or *ex vivo* method for activating a cell, comprising contacting a cell comprising a single chain fusion protein ~~or chimeric antigen receptor~~ according to any one of claims 1-~~85~~15 and/or the nucleic acid molecule of claim ~~88~~16 with a binding domain specific for the tag cassette.

~~95. The method of claim 94, wherein the binding domain specific for the tag cassette is attached to a solid surface and/or the binding domain specific for the tag cassette is a cognate receptor, an anti-tag antibody, and/or an anti-tag scFv.~~

~~96. The method according to claim 94 or 95, wherein the tag cassette is a Strep tag having amino acid sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2).~~

~~97. The method according to one of claims 94-96, wherein the binding domain specific for the tag cassette is a biotin binding protein or an anti-Strep tag antibody.~~

~~98. The method according to any one of claims 94-97, wherein the binding domain is attached to a planar surface, agarose, resin, 3D fabric matrix, or a bead.~~

~~99. The method according to any one of claims 94-98, wherein the binding domain is attached to a microbead or a nanobead.~~

~~100. The method according to any one of claims 94-99, wherein the activation is performed *in vivo* or *ex vivo*.~~

~~101. The method according to any one of claims 94-100, wherein the cell is a T cell and/or is a human T cell.~~

~~102~~21. An *in vitro* or *ex vivo* method for promoting cell proliferation, comprising contacting a non-natural cell comprising a single chain fusion protein ~~or chimeric antigen receptor of~~according to any one of claims 1-~~85~~15 and/or the nucleic acid molecule of claim ~~88~~16 with a binding domain specific for the tag cassette and a growth factor cytokine for a time sufficient to allow cell growth.

~~103. The method of claim 102, wherein the binding domain specific for the tag cassette is attached to a solid surface and/or the binding domain specific for the tag cassette is a cognate receptor, an anti-tag antibody, or an anti-tag scFv.~~

~~104. The method according to claim 102 or 103, wherein the tag cassette is a Strep tag having amino acid sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2).~~

~~105. The method according to one of claims 102-104, wherein the binding domain specific for the tag cassette is a biotin-binding protein or an anti-Strep tag antibody.~~

~~106. The method according to any one of claims 102-105, wherein the binding domain is attached to a planar surface, agarose, resin, 3D fabric matrix, or a bead.~~

~~107. The method according to any one of claims 102-106, wherein the binding domain is attached to a microbead or a nanobead.~~

~~108. The method according to any one of claims 102-107, wherein the growth factor cytokine is IL2, IL15, or both.~~

~~109. The method according to any one of claims 102-108, wherein the method further comprises incubating the cells with an anti-CD27 binding domain, an anti-CD28 binding domain, an anti-CD137 binding domain, an anti-OX40 binding domain or any combination thereof, wherein the binding domains are attached to a solid surface.~~

~~110. The method according to claim 109, wherein the anti-CD27, anti-CD28, anti-CD137 binding domain, anti-OX40 binding domain or any combination thereof are attached to a planar surface, agarose, resin, 3D fabric matrix, or a bead.~~

~~111. The method according to any one of claims 102-110, wherein the proliferation is induced *in vivo* or *ex vivo*.~~

~~112. The method according to any one of claims 102-111, wherein the cell is a T cell and/or the cell is a human T cell.~~

~~113. The method according to claim 112, wherein the T cell is a functional T cell.~~

~~114. The method according to claim 113, wherein the functional T cell is a virus-specific T cell, a tumor antigen specific cytotoxic T cell, a memory stem T cell, a central memory T cell, or a CD4+ CD25+ regulatory T cell.~~

~~115. The method according to any one of claims 102-114, wherein the proliferation is induced *in vivo* when the binding domain of a fusion protein or chimeric antigen receptor of any one of claims 1-85 binds a target cell ligand.~~

~~116. The method according to claim 115, wherein the target cell ligand is a T cell suppressor cell ligand.~~

~~117. The method according to claim 116, wherein the T cell suppressor cell ligand is PD-L1 or PD-L2.~~

~~118~~22. An *in vitro* method for identifying a cell, comprising:

contacting a sample comprising a cell comprising~~s~~ a~~a~~ single chain fusion protein ~~or chimeric antigen receptor of~~according to any one of claims 1-~~85~~15 and/or the nucleic acid molecule of claim ~~88~~16 with a binding domain specific for the tag cassette, wherein the binding domain specific for the tag cassette comprises a detectable moiety, and

detecting the presence of the cell in the sample.

~~119~~23. An *in vitro* method for sorting or selecting a cell or population of cells, the method comprising:

contacting a sample comprising a ~~T~~ cell comprising a single chain fusion protein ~~or chimeric antigen receptor of~~ according to any one of claims 1-~~85~~15 and/or the nucleic acid molecule of claim ~~88~~16, with a binding domain specific for the tag cassette, and

selecting or sorting away from other cells ~~cell(s)~~ that are specifically bound by the binding domain, thereby selecting or sorting away from other cells the cell or population of cells.

~~120. The method according to claim 118 or 119, wherein the binding domain comprises a detectable moiety, which moiety is fluorescent marker.~~

~~121. The method according to any one of claims 118-120, wherein the binding domain comprises a detectable moiety, which is APC or FITC.~~

~~122. The method according to any one of claims 118-121, wherein the sample is blood.~~

~~123. The method according to any one of claims 118-122, wherein the cell is detected or sorted using flow cytometry.~~

~~124. The method according to any one of claims 118-123, wherein the cell is a non-natural cell, is a T cell, and/or is a human T cell.~~

~~125~~24. An *in vitro* or *ex vivo* method for enriching for or isolating a cell or population thereof, the meth~~t~~od comprising contacting a sample comprising the cell comprising the nucleic acid molecule of claim ~~88~~16 and/or the single chain fusion protein ~~or receptor of~~according to any one of claims 1-~~85~~15 with a binding domain specific for the tag cassette and enriching for or isolating the cell away from other cells not expressing the fusion protein ~~or receptor~~ in the sample.

~~126. The method according to claim 125, wherein the binding domain specific for the tag cassette is a cognate receptor, an anti-tag antibody, or an anti-tag scFv.~~

~~127. The method according to claim 125 or 126, wherein the tag cassette is a Strep tag having amino acid sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2).~~

~~128. The method according to one of claims 125-127, wherein the binding domain specific for the tag cassette is a biotin binding protein or an anti-Strep tag antibody.~~

~~129. The method according to any one of claims 125-128, wherein the binding domain is attached to a planar surface, agarose, resin, 3D fabric matrix, or a bead.~~

~~130. The method according to any one of claims 125-129, wherein the binding domain is attached to a microbead or a nanobead.~~

~~131. The method according to any one of claims 125-130, wherein the activation is performed *ex vivo*.~~

~~132. The method according to any one of claims 125-131, further comprising a step of expanding the population of cells in the sample according to any one of claims 102-117 prior to enrichment or isolation.~~

~~133. The method according to any one of claims 125-132, wherein the cell is a non-natural cell, is a T cell, and/or is a human T cell.~~

~~134. The method according to any one of claims 125-132, whereinthe cell is enriched or isolated from other components of the sample by magnetic column chromatography.~~

~~135. The method according to any one of claims 125-134, further comprising a step of identifying the enriched or isolated cell or population of cells, wherein the step of identifying comprises contacting the cells with a binding domain specific for the tag cassette and having a detectable moiety.~~

~~136. The method according to claim 135, wherein the detectable moiety is fluorescent marker.~~

~~137. The method according to claim 135 or 136, wherein the detectable moiety is APC, PE, Pacific blue, Alex fluor, or FITC.~~

~~138. The method according to any one of claims 135-137, wherein cell or populationis detected using flow cytometry.~~

~~139. The method according to any one of claims 125-138, wherein the sample is blood or a blood-derived sample.~~

~~140~~25. An *in vitro* method for depleting cells, comprising contacting a cell comprising a nucleic acid molecule of claim ~~88~~16 or ~~the~~ a single chain fusion protein ~~or receptor of~~according to any one of claims 1-~~85~~15 with a binding domain specific for the tag cassette and comprising a cell-toxic reagent, wherein binding of the binding domain

~~specific for~~ to the tag cassette leads to ~~cell~~ death of the cells expressing the single chain fusion protein ~~or chimeric receptor~~.

~~141. The method according to claim 140, wherein the binding domain specific for the tag cassette is a cognate receptor, an anti-tag antibody, an anti-tag scFv, or a cell with an anti-tag binding domain on its cell surface.~~

~~142. The method according to claim 140 or 141, wherein the tag cassette is a Strep tag having amino acid sequence Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO.:1) or Trp-Arg-His-Pro-Gln-Phe-Gly-Gly (SEQ ID NO.:2).~~

~~143. The method according to one of claims 140-142, wherein the binding domain specific for the tag cassette is a biotin binding protein, an anti-Strep tag antibody, or a cell expressing an anti-Strep tag binding domain on its cell surface.~~

~~144. The method according to one of claims 140-143, wherein the binding domain specific for the tag cassette is a bispecific binding domain, wherein a first binding domain is specific for the tag cassette and the second binding domain is specific for CD3.~~

~~145. The method according to any one of claims 140-143, wherein the binding domain specific for the tag cassette further comprises a cytotoxic, radioisotope, radiometal, or detectable agent.~~

~~146. The method according to claim 145, further comprising tracking the non-natural T cell *in vivo*, wherein the binding domain specific for the tag cassette comprises a detectable agent.~~

~~147. The method according to any one of claims 140-146, wherein the binding domain specific for a tag cassette is administered to a subject.~~

~~148. The method according to claim 147, wherein the subject is human.~~

~~149. The method according to claim 147 or 148, wherein the method further comprises monitoring cytokine levels in the subject after administering the binding domain specific for the tag cassette.~~

~~150. The method according to any one of claims 147-149, wherein the method further comprises tracking the cells in the subject.~~

~~151. The method according to claim 150, wherein the *in vivo* tracking comprises the use of the binding domain specific for the tag cassette conjugated to magnetic particles, superparamagnetic iron oxide (SPIO), fluorodeoxyglucose (18F), fluorescent compounds, or any combination thereof.~~

~~152. The method according to claim 150 or 151, wherein the *in vivo* tracking comprises use of MRI, PET, or near infrared imaging.~~

~~153. A method for generating a desired cell population, comprising contacting a sample comprising a non-natural progenitor cell with a binding domain specific for a tag cassette and a growth factor for a time sufficient to allow cell growth and differentiation, wherein the non-natural progenitor cell comprises a nucleic acid molecule encoding a fusion protein according to any one of claims 50-85 and the binding domain specific for the tag cassette is attached to a solid surface.~~

~~154. The method according to claim 153, wherein the progenitor cell is a stem cell.~~

~~155. The method according to claim 153, wherein the expanded progenitor cell population is further isolated or enriched using a method according to any one of claims 125-139.~~

~~156~~26. Use of ~~A method of treating a disease in a subject, comprising administering to a subject~~ a host cell according to claim ~~92 or 93~~19 in the manufacture of a medicament for treating a disease in a subject, wherein the disease is selected from a cancer, an infectious disease, an autoimmune disease, an inflammatory disease, or an aging-related disease.

27. The use according to claim 26, wherein the disease is a cancer selected from a solid tumor, a leukemia, and a B-cell cancer.

28. The use according to claim 26, wherein the disease is an infectious disease selected from a viral infection, bacterial infection, or protozoan infection.

~~157. The method according to claim 156, wherein the disease is a viral, bacterial, cancer, inflammatory, immune, or aging-associated disease.~~

~~158. The method according to claim 156 or 157, wherein the subject is human.~~

~~159. The method according to any one of claims 156-158, wherein the host cell is a T cell or an autologous T cell.~~

~~160. The method according to claim 159, wherein the T cell is a regulatory T cell.~~

~~161. The method according to claim 159, wherein the T cell is a $CD8^+$ T cell or a $CD4^+$ T cell.~~

~~162. The method according to any one of claims 156-158, wherein the host cell is a stem cell.~~

~~163. A *in vivo* method for local activation of an immune cell, comprising administering to a subject a matrix composition comprising a binding domain for a tag cassette and a binding domain for a co-stimulatory molecule, and administering a host cell according to claim 92, wherein association of the binding domains in the matrix composition with the host cell activates of the host cell.~~

~~164. The *in vivo* method for local activation, wherein the matrix composition comprises alginate, basement membrane matrix, or bioploymer.~~

~~165. The *in vivo* method for local activation, wherein the immune cell is a T cell.~~

~~166. A *in vivo* method for local activation of an immune cell, comprising administering to a subject a device comprising a binding domain for a tag cassette and a binding domain for a co-stimulatory molecule, and administering a host cell according to claim 92, wherein association of the binding domains in the matrix composition with the host cell activates of the host cell.~~

~~167. The *in vivo* method for local activation, wherein the device comprises a planar surface, an agarose bead, a resin, a 3D fabric matrix, or a bead.~~

~~168. The *in vivo* method for local activation, wherein the immune cell is a T cell.~~

~~169. A method of tracking cells, comprising administering to a subject a binding molecule comprising a detectable moiety, wherein said subject has been administered cells according to claim 92 or 93, or the method further comprises administration of cells according to claim 92 or 93, and said binding molecule specifically binds to the tag cassette(s) comprised within the fusion protein or chimeric receptor, and detecting the presence of said molecule in said subject *in vivo* or in a sample obtained from said subject subsequent to said administration, thereby detecting said cells in said subject or a tissue or fluid thereof.~~

~~170. The method of claim 169, wherein the method further comprises said administration of said cells, wherein said cells and said binding molecule are administered simultaneously.~~

~~171. The method of claim 170, wherein said binding molecule and said cells are administered as a complex.~~

~~172. The method according to claim 150, wherein the binding molecule is conjugated to magnetic particles, superparamagnetic iron oxide (SPIO), fluorodeoxyglucose (18F), fluorescent compounds, or any combination thereof.~~

~~173. The method according to claim 150 or 151, wherein the tracking is carried out *in vivo* and comprises use of MRI, PET, or near infrared imaging.~~

~~174. A matrix composition comprising a binding domain for a tag cassette and a binding domain for an immune cell co-stimulatory molecule.~~

~~175. The matrix composition of claim 174, further comprising alginate, basement membrane matrix, or bioploymer.~~

~~176. A device comprising a binding domain for a tag cassette and a binding domain for an immune cell co-stimulatory molecule.~~

~~177. The device of claim 176, wherein one or both of said binding domains are disposed on a surface, an agarose bead, a resin, a 3D fabric matrix, or a bead.~~