

PROSTATE CANCER CHIMERIC ANTIGEN RECEPTORS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Provisional Application No. 63/130,529, filed December 24, 2020 which is hereby incorporated herein by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] The present disclosure relates to the field of cell therapy, and more specifically, to CARs and/or TCRs that target antigens present on prostate cancer cells.

REFERENCE TO SEQUENCE LISTING

[0003] This application incorporates by reference the Sequence Listing submitted in Computer Readable Form as file K-1096-WO-PCT__SL.txt, created on December 14, 2021 and containing 301,341 bytes.

BACKGROUND

[0004] Human cancers are by their nature comprised of normal cells that have undergone a genetic or epigenetic conversion to become abnormal cancer cells. In doing so, cancer cells begin to express proteins and other antigens that are distinct from those expressed by normal cells. These aberrant tumor antigens can be used by the body's innate immune system to specifically target and kill cancer cells. However, cancer cells employ various mechanisms to prevent immune cells, such as T and B lymphocytes, from successfully targeting cancer cells.

[0005] Current therapies T cell therapies rely on enriched or modified human T cells to target and kill cancer cells in a patient. To increase the ability of T cells to target and kill a particular cancer cell, methods have been developed to engineer T cells to express constructs which direct T cells to a particular target cancer cell. Chimeric antigen receptors (CARs) and engineered T cell receptors (TCRs), which comprise binding domains capable of interacting with a particular tumor antigen, allow T cells to target and kill cancer cells that express the particular tumor antigen. A need exists for CARs and TCRs for targeting and killing cancer cells and, in particular, solid tumor cells, such as prostate cancer cells.

SUMMARY

[0006] Disclosed is an antibody, or antigen binding fragment thereof comprising an anti-PSMA binding domain, wherein the anti-PSMA binding domain comprises sequences of three heavy

chain complementarity determining regions (HCDRs) of any one of the heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NOs: 1, 25, 49, and 73, and sequences of three light chain CDRs (LCDRs) of the light chain variable region (LCVR) selected from the group consisting of SEQ ID NOs: 12, 36, 60, and 84.

[0007] In certain embodiments, the anti-PSMA binding domain comprises a first domain comprising three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and a second domain comprising three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein (i) the HCDR1 has a sequence according to any one of SEQ ID NOs: 3-5, 27-29, 51-53, and 75-77; (ii) the HCDR2 has a sequence according to any one of SEQ ID NOs: 6-8, 30-32, 54-56, and 78-80; (iii) the HCDR3 has a sequence according to any one of SEQ ID NOs: 9-11, 33-35, 57-59, and 81-83; (iv) the LCDR1 has a sequence according to any one of SEQ ID NOs: 14-16, 38-40, 62-64, and 86-88; (v) the LCDR2 has a sequence according to any one of SEQ ID NOs: 17-19, 41-43, 65-67, and 89-91; and (vi) the LCDR3 has a sequence according to any one of SEQ ID NOs: 20-22, 44-46, 68-70, and 92-94.

[0008] In certain embodiments, the HCDRs comprise: (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; (ii) an HCDR1 according to any of SEQ ID NOs: 27-29; an HCDR2 according to any of SEQ ID NOs: 30-32; an HCDR3 according to any one of SEQ ID NOs: 33-35; (iii) an HCDR1 according to any of SEQ ID NOs: 51-53; an HCDR2 according to any of SEQ ID NOs: 54-56; an HCDR3 according to any one of SEQ ID NOs: 57-59; or (iv) an HCDR1 according to any of SEQ ID NOs: 75-77; an HCDR2 according to any of SEQ ID NOs: 78-80; an HCDR3 according to any one of SEQ ID NOs: 81-83; and the LCDRs comprise: (i) an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22; (ii) an LCDR1 according to any of SEQ ID NOs: 38-40; an LCDR2 according to any of SEQ ID NOs: 41-43; an LCDR3 according to any one of SEQ ID NOs: 44-46; (iii) an LCDR1 according to any of SEQ ID NOs: 62-64; an LCDR2 according to any of SEQ ID NOs: 65-67; an LCDR3 according to any one of SEQ ID NOs: 68-70; or (iv) an LCDR1 according to any of SEQ ID NOs: 86-88; an LCDR2 according to any of SEQ ID NOs: 89-91; an LCDR3 according to any one of SEQ ID NOs: 92-94.

[0009] In certain embodiments, the antigen binding system, antibody, or antigen binding fragment thereof comprises a first domain comprising three heavy chain complementarity determining regions (HCDRs) and a second domain comprising three light chain complementarity determining regions (LCDRs), wherein: the HCDRs and LCDRs comprise: (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2

according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22; (ii) an HCDR1 according to any of SEQ ID NOs: 27-29; an HCDR2 according to any of SEQ ID NOs: 30-32; an HCDR3 according to any one of SEQ ID NOs: 33-35; an LCDR1 according to any of SEQ ID NOs: 38-40; an LCDR2 according to any of SEQ ID NOs: 41-43; an LCDR3 according to any one of SEQ ID NOs: 44-46; (iii) an HCDR1 according to any of SEQ ID NOs: 51-53; an HCDR2 according to any of SEQ ID NOs: 54-56; an HCDR3 according to any one of SEQ ID NOs: 57-59; an LCDR1 according to any of SEQ ID NOs: 62-64; an LCDR2 according to any of SEQ ID NOs: 65-67; an LCDR3 according to any one of SEQ ID NOs: 68-70; or (iv) an HCDR1 according to any of SEQ ID NOs: 75-77; an HCDR2 according to any of SEQ ID NOs: 78-80; an HCDR3 according to any one of SEQ ID NOs: 81-83; an LCDR1 according to any of SEQ ID NOs: 86-88; an LCDR2 according to any of SEQ ID NOs: 89-91; an LCDR3 according to any one of SEQ ID NOs: 92-94.

[0010] In certain embodiments, the antibody, or antigen binding fragment thereof comprises a first heavy chain variable domain comprising three HCDRs and a light chain variable domain comprising three LCDRs, wherein: (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1 SEQ ID NO: 25, SEQ ID NO: 49, and SEQ ID NO: 73; and (ii) the light chain variable domain is at least 80% identical to SEQ ID NO: 12, SEQ ID NO: 36, SEQ ID NO: 60, and SEQ ID NO: 84.

[0011] In certain embodiments, the antibody, or antigen binding fragment thereof comprises a first heavy chain variable domain comprising three HCDRs and a light chain variable domain comprising three LCDRs, wherein: (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1 and the light chain variable domain is at least 80% identical to SEQ ID NO: 12; (ii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 25 and the light chain variable domain is at least 80% identical to SEQ ID NO: 36; (iii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 49 and the light chain variable domain is at least 80% identical to SEQ ID NO: 60; or (iv) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 73 and the light chain variable domain is at least 80% identical to SEQ ID NO: 84.

[0012] In certain embodiments, the three HCDRs and the three LCDRs are comprised by a single polypeptide. In certain embodiments, the antigen binding fragment thereof comprises an scFv.

[0013] Disclosed is a nucleic acid encoding a disclosed antibody, or antigen binding fragment thereof.

[0014] Disclosed is chimeric antigen receptor, comprising the antibody, or antigen binding fragment thereof disclosed herein.

[0015] In embodiments the chimeric antigen receptor comprises an antibody, or antigen binding fragment thereof comprising comprises a heavy chain variable domain that is at least 80%

identical to SEQ ID NO: 1 and the light chain variable domain that is at least 80% identical to SEQ ID NO: 12; (ii) a heavy chain variable domain that is at least 80% identical to SEQ ID NO: 25 and a light chain variable domain that is at least 80% identical to SEQ ID NO: 36; (iii) a heavy chain variable domain that is at least 80% identical to SEQ ID NO: 49 and a light chain variable domain that is at least 80% identical to SEQ ID NO: 60; (iv) a heavy chain variable domain that is at least 80% identical to SEQ ID NO: 73 and a light chain variable domain that is at least 80% identical to SEQ ID NO: 84; or (v) or a heavy chain variable domain that is at least 80% identical to SEQ ID NO: 97 and a light chain variable domain that is at least 80% identical to SEQ ID NO: 103.

[0016] In embodiments the chimeric antigen receptor comprises a transmembrane domain of 4-1BB/CD137, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a zeta chain of a T cell receptor, or any combination thereof.

[0017] Disclosed is a nucleic acid comprising a chimeric antigen receptor disclosed herein. Disclosed is recombinant vector comprising a nucleic acid disclosed herein. In embodiments the nucleic acid encoding the antibody, antigen binding fragment thereof or chimeric antigen receptor is operatively connected to a constitutively active promoter or a conditionally activated promoter. In embodiments the conditionally activated promoter comprises at least one transcriptional activator binding site. In embodiments the transcriptional activator binding site comprises one or more GAL4 binding sites.

[0018] In embodiments a disclosed recombinant vector or nucleic acid further comprises a nucleic acid encoding a dominant negative TGF β receptor (DN TGF β R), comprising: an extracellular domain (ECD) from a TGF- β receptor and a transmembrane domain (TMD), wherein the recombinant polypeptide lacks amino acid residues responsible for signaling and phosphorylation present in a wild-type TGF- β receptor. In embodiments the ECD is selected from TGF- β RI or TGF- β RII. In embodiments the TMD is selected from TGF- β RI, TGF- β RII, PDGFR, CD4, CD8, CD28, CD127, CD132, CD3 ζ , 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, IL-5, IL-7, IL-7R α , BTLA or mutants of any of the foregoing. In embodiments the DN TGF β R further comprises a heterologous intracellular domain (ICD) which lacks amino acid residues responsible for signaling and phosphorylation present in wild-type TGF- β receptor. In embodiments the DN TGF β R binds TGF- β 1.

[0019] Disclosed is a synthetic notch (synNotch) receptor polypeptide comprising from N to C terminus: an extracellular anti-PSCA binding domain, a Notch core domain comprising one or more proteolytic cleavage sites, and an intracellular domain comprising a transcriptional activator comprising a DNA binding domain and a transactivation domain, wherein binding of the binding

extracellular anti-PSCA binding domain to PSCA induces cleavage of the Notch core domain at the one or more proteolytic cleavage sites, thereby releasing the intracellular domain and the transcriptional regulator.

[0020] In embodiments the anti-PSCA binding domain comprising a first domain comprising three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and a second domain comprising three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein (i) the HCDR1 has a sequence according to any one of SEQ ID NOs: 152-154, (ii) the HCDR2 has a sequence according to any one of SEQ ID NOs: 155-157; (iii) the HCDR3 has a sequence according to any one of SEQ ID NOs: 158-160; (iv) the LCDR1 has a sequence according to any one of SEQ ID NOs: 163-165; (v) the LCDR2 has a sequence according to any one of SEQ ID NOs: 166-168; and (vi) the LCDR3 has a sequence according to any one of SEQ ID NOs: 169-171. In embodiments the synNotch receptor polypeptide comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein: (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 150; and (ii) the light chain variable domain is at least 80% identical to SEQ ID NO: 161.

In embodiments the transcriptional regulator is a transcriptional activator. In embodiments the transcriptional activator comprises GAL4, HNF1 alpha or HNF1 beta. In embodiments the transcriptional activator comprises a transactivation domain selected from the group consisting of VP64, RelA (p65), YAP, WWTR1(TAZ), CREB3(LZIP), and MyoD.

[0021] Disclosed is a nucleic encoding a disclosed synNotch receptor polypeptide.

[0022] Disclosed is a recombinant vector comprising a disclosed synNotch receptor polypeptide.

[0023] Disclosed is a host cell transformed with a disclosed nucleic acid or disclosed recombinant vector. In embodiments the host cell comprises a T cell or an NK cell.

[0024] Disclosed is a pharmaceutical composition comprising a T cell and/or an NK cell transformed with a disclosed nucleic acid or disclosed recombinant vector.

[0025] Disclosed is a method of treating disease in a patient in need of thereof, comprising administering a T cell and/or an NK cell transformed with a disclosed nucleic acid or disclosed recombinant vector or the pharmaceutical composition comprising the same to the patient. In embodiments the disease is prostate cancer.

[0026] Disclosed is a method of inducing an immune response in a subject or immunizing a subject against a prostate cancer, the method comprising administering to the subject a T cell and/or an NK cell transformed with a disclosed nucleic acid or disclosed recombinant vector or the pharmaceutical composition comprising the same to the patient. In embodiments the T cell

and/or the NK cell is allogeneic to the patient. In embodiments the T cell and/or the NK cell is allogeneic to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figure 1 is a graph of cytotoxicity vs time for the indicated canonical CARs.

[0028] Figure 2 is a graph of cytotoxicity vs time for the indicated canonical CARs.

[0029] Figure 3 is a graph of cytotoxicity vs time for the indicated canonical CARs.

[0030] Figure 4 is a graph of cytotoxicity vs time for the indicated synNotch activated CARs.

[0031] Figure 5 is a graph of cytotoxicity vs time for the indicated synNotch activated CARs.

[0032] Figure 6 is a graph of cytotoxicity vs time for the indicated synNotch activated CARs.

DETAILED DESCRIPTION OF THE INVENTION

Terms

[0033] In order for the present disclosure to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the Specification.

[0034] As used in this Specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

[0035] Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive and covers both “or” and “and”.

[0036] The term “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0037] The term “e.g.,” as used herein, is used merely by way of example, without limitation intended, and should not be construed as referring only those items explicitly enumerated in the specification.

[0038] The terms “or more”, “at least”, “more than”, and the like, e.g., “at least one” are understood to include but not be limited to at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 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, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114,

115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000 or more than the stated value. Also included is any greater number or fraction in between.

[0039] Conversely, the term “no more than” includes each value less than the stated value. For example, “no more than 100 nucleotides” includes 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, and 0 nucleotides. Also included is any lesser number or fraction in between.

[0040] The terms “plurality”, “at least two”, “two or more”, “at least second”, and the like, are understood to include but not limited to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 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, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000 or more. Also included is any greater number or fraction in between.

[0041] Throughout the specification the word “comprising,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. It is understood that wherever aspects are described herein with the language “comprising,” otherwise analogous aspects described in terms of “consisting of” and/or “consisting essentially of” are also provided.

[0042] Unless specifically stated or evident from context the term “about” refers to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” or “comprising essentially of” can mean within one or more than one standard deviation per the practice in the art. “About” or “comprising essentially of” can mean a range of up to 10% (*i.e.*, $\pm 10\%$). Thus, “about” can be understood to be within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.01%, or 0.001% greater or less than the stated value. For

example, about 5 mg can include any amount between 4.5 mg and 5.5 mg. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the instant disclosure, unless otherwise stated, the meaning of “about” or “comprising essentially of” should be assumed to be within an acceptable error range for that particular value or composition.

[0043] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to be inclusive of the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[0044] Units, prefixes, and symbols used herein are provided using their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range.

[0045] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, Juo, “The Concise Dictionary of Biomedicine and Molecular Biology”, 2nd ed., (2001), CRC Press; “The Dictionary of Cell & Molecular Biology”, 5th ed., (2013), Academic Press; and “The Oxford Dictionary Of Biochemistry And Molecular Biology”, Cammack *et al.* eds., 2nd ed, (2006), Oxford University Press, provide those of skill in the art with a general dictionary for many of the terms used in this disclosure.

[0046] “Administering” refers to the physical introduction of an agent to a subject, such as a modified T cell disclosed herein, using any of the various methods and delivery systems known to those skilled in the art. Exemplary routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase “parenteral administration” means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, *e.g.*, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0047] The terms, “activated” and “activation” refer to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. In one embodiment, activation may also be associated with induced cytokine production, and detectable effector functions. The

term "activated T cells" refers to, among other things, T cells that are proliferating. Signals generated through the TCR alone may be insufficient for full activation of the T cell and one or more secondary or costimulatory signals may also be required. Thus, T cell activation comprises a primary stimulation signal through the TCR/CD3 complex and one or more secondary costimulatory signals. Costimulation may be evidenced by proliferation and/or cytokine production by T cells that have received a primary activation signal, such as stimulation through the TCR/CD3 complex.

[0048] The term "agent" may refer to a molecule or entity of any class comprising, or a plurality of molecules or entities, any of which may be, for example, a polypeptide, nucleic acid, saccharide, lipid, small molecule, metal, cell (such as a T cell or NK cell or progenitor of such cells), or organism (for example, a fraction or extract thereof) or component thereof. In some embodiments, an agent may be utilized in isolated or pure form. In some embodiments, an agent may be utilized in a crude or impure form. In some embodiments, an agent may be provided as a population, collection, or library, for example that may be screened to identify or characterize members present therein.

[0049] The term "allogeneic" refers to any material derived from one individual which is then introduced to another individual of the same species, *e.g.*, allogeneic T cell transplantation.

[0050] The term "antibody" (Ab) includes, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen. In general, an antibody can comprise at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding molecule thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region comprises one constant domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the Abs may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system. In general, human antibodies are approximately 150 kD tetrameric agents composed of two identical heavy (H) chain polypeptides (about 50 kD each) and two identical light (L) chain polypeptides (about 25 kD each) that associate with each other into

what is commonly referred to as a “Y-shaped” structure. The heavy and light chains are linked or connected to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, *e.g.*, on the CH₂ domain.

[0051] The term “human antibody” is intended to comprise antibodies having variable and constant domain sequences generated, assembled, or derived from human immunoglobulin sequences, or sequences indistinguishable therefrom. In some embodiments, antibodies (or antibody components) may be considered to be “human” even though their amino acid sequences comprise residues or elements not encoded by human germline immunoglobulin sequences (*e.g.*, variations introduced by *in vitro* random or site-specific mutagenesis or introduced by *in vivo* somatic mutation). The term “humanized” is intended to comprise antibodies having a variable domain with a sequence derived from a variable domain of a non-human species (*e.g.*, a mouse), modified to be more similar to a human germline encoded sequence. In some embodiments, a “humanized” antibody comprises one or more framework domains having substantially the amino acid sequence of a human framework domain, and one or more complementary determining regions having substantially the amino acid sequence as that of a non-human antibody. In some embodiments, a humanized antibody comprises at least a portion of an immunoglobulin constant region (Fc), generally that of a human immunoglobulin constant domain. In some embodiments, a humanized antibodies may comprise a C_H1, hinge, C_H2, C_H3, and, optionally, a C_H4 region of a human heavy chain constant domain.

[0052] Antibodies can include, for example, monoclonal antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, engineered antibodies, humanized antibodies, chimeric antibodies, immunoglobulins, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, antibody fusions (sometimes referred to herein as “antibody conjugates”), heteroconjugate antibodies, single domain antibodies, monovalent antibodies, single chain antibodies or single-chain Fvs (scFv), camelized antibodies, affybodies, Fab fragments, F(ab')₂ fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-anti-Id antibodies), minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), and antigen binding fragments of any of the above. In certain embodiments, antibodies described herein refer to polyclonal antibody populations. Antibodies may also comprise, for example, Fab' fragments, Fd' fragments, Fd fragments, isolated CDRs, single chain Fvs, polypeptide-Fc fusions, single domain antibodies (*e.g.*, shark single domain

antibodies such as IgNAR or fragments thereof), camelid antibodies, single chain or Tandem diabodies (TandAb®), Anticalins®, Nanobodies® minibodies, BiTE®s, ankyrin repeat proteins or DARPINs®, Avimers®, DARTs, TCR-like antibodies, Adnectins®, Affilins®, Transbodies®, Affibodies®, TrimerX®, MicroProteins, Fynomers®, Centyrins®, and KALBITOR®s.

[0053] An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG, IgE and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. “Isotype” refers to the Ab class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term “antibody” includes, by way of example, both naturally occurring and non-naturally occurring Abs; monoclonal and polyclonal Abs; chimeric and humanized Abs; human or nonhuman Abs; wholly synthetic Abs; and single chain Abs. A nonhuman Ab may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term “antibody” also includes an antigen binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain Ab.

[0054] An “antigen binding molecule,” “antigen binding portion,” “antigen binding fragment,” or “antibody fragment” or “antigen binding domain” refers to any molecule that comprises the antigen binding parts (*e.g.*, CDRs) of the antibody from which the molecule is derived. An antigen binding molecule can include the antigenic complementarity determining regions (CDRs). Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, dAb, linear antibodies, scFv antibodies, and multispecific antibodies formed from antigen binding molecules. Peptibodies (*i.e.*, Fc fusion molecules comprising peptide binding domains) are another example of suitable antigen binding molecules. In some embodiments, the antigen binding molecule binds to an antigen on a tumor cell. In some embodiments, the antigen binding molecule binds to an antigen on a cell involved in a hyperproliferative disease or to a viral or bacterial antigen. In certain embodiments an antigen binding molecule is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In certain embodiments, the antigen binding molecule or domain binds to prostate stem cell antigen (PSCA) or prostate-specific membrane antigen (PSMA). In certain embodiments, the antigen binding molecule or domain is an antibody fragment that specifically binds to the antigen, including one or more of the complementarity determining regions (CDRs) thereof. In further embodiments, the antigen binding molecule is a single chain variable fragment (scFv). In some embodiments, the antigen binding molecule or domain comprises or consists of avimers.

[0055] In some instances, a CDR is substantially identical to one found in a reference antibody (*e.g.*, an antibody of the present disclosure) and/or the sequence of a CDR provided in the present

disclosure. In some embodiments, a CDR is substantially identical to a reference CDR (*e.g.*, a CDR provided in the present disclosure) in that it is either identical in sequence or contains between 1, 2, 3, 4, or 5 (*e.g.*, 1-5) amino acid substitutions as compared with the reference CDR. In some embodiments a CDR is substantially identical to a reference CDR in that it shows at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In some embodiments a CDR is substantially identical to a reference CDR in that it shows at least 96%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments a CDR is substantially identical to a reference CDR in that one amino acid within the CDR is deleted, added, or substituted as compared with the reference CDR while the CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments a CDR is substantially identical to a reference CDR in that 2, 3, 4, or 5 (*e.g.*, 2-5) amino acids within the CDR are deleted, added, or substituted as compared with the reference CDR while the CDR has an amino acid sequence that is otherwise identical to the reference CDR. In various embodiments, an antigen binding fragment binds a same antigen as a reference antibody. In various embodiments, an antigen binding fragment cross-competes with the reference antibody, for example, binding to substantially the same or identical epitope as the reference antibody.

[0056] An antigen binding fragment may be produced by any means. For example, in some embodiments, an antigen binding fragment may be enzymatically or chemically produced by fragmentation of an intact antibody. In some embodiments, an antigen binding fragment may be recombinantly produced (such as by expression of an engineered nucleic acid sequence). In some embodiments, an antigen binding fragment may be wholly or partially synthetically produced. In some embodiments, an antigen binding fragment may have a length of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 amino acids or more; in some embodiments at least about 200 amino acids (*e.g.*, 50-100, 50-150, 50-200, or 100-200 amino acids).

[0057] The term “variable region” or “variable domain” is used interchangeably. The variable region typically refers to a portion of an antibody, generally, a portion of a light or heavy chain, typically about the amino-terminal 110 to 120 amino acids in the mature heavy chain and about 90 to 115 amino acids in the mature light chain, which differ extensively in sequence among antibodies and are used in the binding and specificity of a particular antibody for its particular antigen. The variability in sequence is concentrated in those regions called complementarity determining regions (CDRs) while the more highly conserved regions in the variable domain are called framework regions (FR). Without wishing to be bound by any particular mechanism or

theory, it is believed that the CDRs of the light and heavy chains are primarily responsible for the interaction and specificity of the antibody with antigen. In certain embodiments, the variable region is a human variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and human framework regions (FRs). In embodiments, the variable region is a primate (*e.g.*, non-human primate) variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and primate (*e.g.*, non-human primate) framework regions (FRs).

[0058] The terms “VL” and “VL domain” are used interchangeably to refer to the light chain variable region of an antibody or an antigen-binding molecule thereof.

[0059] The terms “VH” and “VH domain” are used interchangeably to refer to the heavy chain variable region of an antibody or an antigen-binding molecule thereof.

[0060] A number of definitions of the CDRs are commonly in use: Kabat numbering, Chothia numbering, AbM numbering, or contact numbering. The AbM definition is a compromise between the two used by Oxford Molecular’s AbM antibody modelling software. The contact definition is based on an analysis of the available complex crystal structures.

Table 1: CDR Numbering

Loop	Kabat	AbM	Chothia	Contact
L1	L24--L34	L24--L34	L24--L34	L30--L36
L2	L50--L56	L50--L56	L50--L56	L46--L55
L3	L89--L97	L89--L97	L89--L97	L89--L96
H1	H31--H35B (Kabat Numbering)	H26--H35B	H26--H32..34	H30--H35B
H1	H31--H35 (Chothia Numbering)	H26--H35	H26--H32	H30--H35
H2	H50--H65	H50--H58	H52--H56	H47--H58
H3	H95--H102	H95--H102	H95--H102	H93--H101

[0061] The term “Kabat numbering” and like terms are recognized in the art and refer to a system of numbering amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen-binding molecule thereof. In certain aspects, the CDRs of an antibody can be determined according to the Kabat numbering system (*see, e.g.*, Kabat EA & Wu TT (1971) *Ann NY Acad Sci* 190: 382-391 and Kabat EA *et al.*, (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Using the Kabat numbering system, CDRs within an antibody heavy chain molecule are

typically present at amino acid positions 31 to 35, which optionally can include one or two additional amino acids, following 35 (referred to in the Kabat numbering scheme as 35A and 35B) (CDR1), amino acid positions 50 to 65 (CDR2), and amino acid positions 95 to 102 (CDR3). Using the Kabat numbering system, CDRs within an antibody light chain molecule are typically present at amino acid positions 24 to 34 (CDR1), amino acid positions 50 to 56 (CDR2), and amino acid positions 89 to 97 (CDR3). In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Kabat numbering scheme.

[0062] In certain aspects, the CDRs of an antibody can be determined according to the Chothia numbering scheme, which refers to the location of immunoglobulin structural loops (*see, e.g.,* Chothia C & Lesk AM, (1987), J Mol Biol 196: 901-917; Al-Lazikani B *et al.*, (1997) J Mol Biol 273: 927-948; Chothia C *et al.*, (1992) J Mol Biol 227: 799-817; Tramontano A *et al.*, (1990) J Mol Biol 215(1): 175-82; and U.S. Patent No. 7,709,226). Typically, when using the Kabat numbering convention, the Chothia CDR-H1 loop is present at heavy chain amino acids 26 to 32, 33, or 34, the Chothia CDR-H2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDR-H3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDR-L1 loop is present at light chain amino acids 24 to 34, the Chothia CDR-L2 loop is present at light chain amino acids 50 to 56, and the Chothia CDR-L3 loop is present at light chain amino acids 89 to 97. The end of the Chothia CDR-HI loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Chothia numbering scheme.

[0063] The terms “constant region” and “constant domain” are interchangeable and have a meaning common in the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with the Fc receptor. The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an immunoglobulin variable domain.

[0064] The term “heavy chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, alpha (α), delta (δ), epsilon (ϵ), gamma (γ) and mu (μ), based on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG₁, IgG₂, IgG₃ and IgG₄.

[0065] The term “light chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains. Light

chain amino acid sequences are well known in the art. In specific embodiments, the light chain is a human light chain.

[0066] An “antigen” refers to a compound, composition, or substance that may stimulate the production of antibodies or a T cell response in a human or animal, including compositions (such as one that includes a tumor-specific protein) that are injected or absorbed into a human or animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous antigens, such as the disclosed antigens. A “target antigen” or “target antigen of interest” is an antigen that is not substantially found on the surface of other normal (desired) cells and to which a binding domain of a TCR or CAR contemplated herein, is designed to bind. A person of skill in the art would readily understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. An antigen can be endogenously expressed, *i.e.* expressed by genomic DNA, or can be recombinantly expressed. An antigen can be specific to a certain tissue, such as a cancer cell, or it can be broadly expressed. In addition, fragments of larger molecules can act as antigens. In one embodiment, antigens are tumor antigens. In one particular embodiment, the antigen is all or a fragment of prostate stem cell antigen (PSCA) or prostate-specific membrane antigen (PSMA). A “target” is any molecule bound by a binding motif, antigen binding system, CAR or antigen binding agent, *e.g.*, an antibody.

[0067] “Antigen-specific targeting region” (ASTR) refers to the region of the CAR which targets specific antigens. The targeting regions on the CAR are extracellular. In some embodiments, the antigen-specific targeting regions comprise an antibody or a functional equivalent thereof or a fragment thereof or a derivative thereof and each of the targeting regions target a different antigen. The targeting regions may comprise full length heavy chain, Fab fragments, single chain Fv (scFv) fragments, divalent single chain antibodies or diabodies, each of which are specific to the target antigen. There are, however, numerous alternatives, such as linked cytokines (which leads to recognition of cells bearing the cytokine receptor), affibodies, ligand binding domains from naturally occurring receptors, soluble protein/peptide ligand for a receptor (for example on a tumor cell), peptides, and vaccines to prompt an immune response, which may each be used in various embodiments of this disclosure. In fact, almost any molecule that binds a given antigen with high affinity can be used as an antigen-specific targeting region, as will be appreciated by those of skill in the art.

[0068] “Antigen presenting cell” or “APC” refers to cells that process and present antigens to T cells. Exemplary APCs comprise dendritic cells, macrophages, B cells, certain activated epithelial cells, and other cell types capable of TCR stimulation and appropriate T cell costimulation.

[0069] An “anti-tumor effect” refers to a biological effect that can present as a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, a decrease

in the number of metastases, an increase in overall or progression-free survival, an increase in life expectancy, or amelioration of various physiological symptoms associated with the tumor. An anti-tumor effect can also refer to the prevention of the occurrence of a tumor.

[0070] Two events or entities are “associated” with one another if the presence, level, and/or form of one is correlated with that of the other. For example, an entity (*e.g.*, polypeptide, genetic signature, metabolite, microbe, etc.) is considered to be associated with a disease, disorder, or condition, if its presence, level, and/or form correlates with incidence of and/or susceptibility to the disease, disorder, or condition (*e.g.*, across a relevant population). For example, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another (*e.g.*, bind). In additional examples, two or more entities that are physically associated with one another are covalently linked or connected to one another, or non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0071] The term “autologous” refers to any material derived from the same individual to which it is later to be re-introduced. For example, the engineered autologous cell therapy (eACT™) method described herein involves collection of lymphocytes from a patient, which are then engineered to express, *e.g.*, a CAR construct, and then administered back to the same patient.

[0072] “Binding affinity” generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured and/or expressed in a number of ways known in the art, including, but not limited to, equilibrium dissociation constant (K_D), and equilibrium association constant (K_A). The K_D is calculated from the quotient of k_{off}/k_{on} , whereas K_A is calculated from the quotient of k_{on}/k_{off} . k_{on} refers to the association rate constant of, *e.g.*, an antibody to an antigen, and k_{off} refers to the dissociation of, *e.g.*, an antibody to an antigen. The k_{on} and k_{off} can be determined by techniques known to one of ordinary skill in the art, such as BIAcore® or KinExA.

[0073] The term “ K_D ” (M) refers to the dissociation equilibrium constant of a particular antibody-antigen interaction, or the dissociation equilibrium constant of an antibody or antibody-binding fragment binding to an antigen. There is an inverse relationship between K_D and binding affinity, therefore the smaller the K_D value, the higher, i.e. stronger, the affinity. Thus, the terms “higher affinity” or “stronger affinity” relate to a higher ability to form an interaction and therefore a smaller K_D value, and conversely the terms “lower affinity” or “weaker affinity” relate to a lower

ability to form an interaction and therefore a larger K_D value. In some circumstances, a higher binding affinity (or K_D) of a particular molecule (e.g. antibody) to its interactive partner molecule (e.g. antigen X) compared to the binding affinity of the molecule (e.g. antibody) to another interactive partner molecule (e.g. antigen Y) may be expressed as a binding ratio determined by dividing the larger K_D value (lower, or weaker, affinity) by the smaller K_D (higher, or stronger, affinity), for example expressed as 5-fold or 10-fold greater binding affinity, as the case may be.

[0074] The term " k_d " (sec⁻¹ or 1/s) refers to the dissociation rate constant of a particular antibody-antigen interaction, or the dissociation rate constant of an antibody or antibody-binding fragment. Said value is also referred to as the k_{off} value.

[0075] The term " k_a " (M⁻¹ x sec⁻¹ or 1/M) refers to the association rate constant of a particular antibody-antigen interaction, or the association rate constant of an antibody or antibody-binding fragment.

[0076] The term " K_A " (M⁻¹ or 1/M) refers to the association equilibrium constant of a particular antibody-antigen interaction, or the association equilibrium constant of an antibody or antibody binding fragment. The association equilibrium constant is obtained by dividing the k_a by the k_d .

[0077] The term "binding" generally refers to a non-covalent association between or among two or more entities. Direct binding involves physical contact between entities or moieties. "Indirect" binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities may be assessed in any of a variety of contexts, e.g., where interacting entities or moieties are studied in isolation or in the context of more complex systems (e.g., while covalently or otherwise associated with a carrier entity and/or in a biological system such as a cell).

[0078] The terms "immunospecifically binds," "immunospecifically recognizes," "specifically binds," and "specifically recognizes" are analogous terms in the context of antibodies and refer to molecules that bind to an antigen (e.g., epitope or immune complex) as such binding is understood by one skilled in the art. For example, a molecule that specifically binds to an antigen may bind to other peptides or polypeptides, generally with lower affinity as determined by, e.g., immunoassays, BIACORE[®], KinExA 3000 instrument (Sapidyne Instruments, Boise, ID), or other assays known in the art. In a specific embodiment, molecules that specifically bind to an antigen bind to the antigen with a K_A that is at least 2 logs, 2.5 logs, 3 logs, 4 logs or greater than the K_A when the molecules bind to another antigen. Binding may comprise preferential association of a binding motif, antibody, or antigen binding system with a target of the binding motif, antibody, or antigen binding system as compared to association of the binding motif, antibody, or antigen binding system with an entity that is not the target (*i.e.* non-target). In some embodiments, a binding motif, antibody, or antigen binding system selectively binds a target if binding between

the binding motif, antibody, or antigen binding system and the target is greater than 2-fold, greater than 5-fold, greater than 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or greater than 100-fold as compared with binding of the binding motif, antibody, or antigen binding system and a non-target. In some embodiments, a binding motif, antibody, or antigen binding system selectively binds a target if the binding affinity is less than about 10^{-5} M, less than about 10^{-6} M, less than about 10^{-7} M, less than about 10^{-8} M, or less than about 10^{-9} M.

[0079] In another embodiment, molecules that specifically bind to an antigen bind with a dissociation constant (K_d) of about 1×10^{-7} M. In some embodiments, the antigen binding molecule specifically binds an antigen with “high affinity” when the K_d is about 1×10^{-9} M to about 5×10^{-9} M. In some embodiments, the antigen binding molecule specifically binds an antigen with “very high affinity” when the K_d is 1×10^{-10} M to about 5×10^{-10} M. In one embodiment, the antigen binding molecule has a K_d of 10^{-9} M. In one embodiment, the off-rate is less than about 1×10^{-5} . In embodiments, the antigen binding molecule binds prostate stem cell antigen (PSCA) or prostate-specific membrane antigen (PSMA) with a K_d of about 1×10^{-10} M to about 5×10^{-10} M.

[0080] In certain embodiments, provided herein is an antibody or an antigen binding molecule thereof that binds to the target human antigen, *e.g.*, In certain embodiments, the antigen binding molecule binds to prostate stem cell antigen (PSCA) or prostate-specific membrane antigen (PSMA) with a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or higher affinity than to another species of the target antigen as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay. In a specific embodiment, an antibody or an antigen binding molecule thereof described herein, which binds to a target human antigen, will bind to another species of the target antigen with less than 10%, 15%, or 20% of the binding of the antibody or an antigen binding molecule thereof to the human antigen as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay.

[0081] “Cancer” refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A “cancer” or “cancer tissue” can include a tumor. In some embodiments, the methods of the present disclosure can be used to reduce the tumor size of a tumor derived from, for example, prostate cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the

cervix, carcinoma of the vagina, carcinoma of the vulva, multiple myeloma, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers. In one particular embodiment, the cancer is prostate cancer. The particular cancer can be responsive to chemo- or radiation therapy or the cancer can be refractory. A refractory cancer refers to a cancer that is not amendable to surgical intervention and the cancer is either initially unresponsive to chemo- or radiation therapy or the cancer becomes unresponsive over time.

[0082] “Chemokines” are a type of cytokine that mediates cell chemotaxis, or directional movement. Examples of chemokines include, but are not limited to, IL-8, IL-16, eotaxin, eotaxin-3, macrophage-derived chemokine (MDC or CCL22), monocyte chemotactic protein 1 (MCP-1 or CCL2), MCP-4, macrophage inflammatory protein 1 α (MIP-1 α , MIP-1a), MIP-1 β (MIP-1b), gamma-induced protein 10 (IP-10), and thymus and activation regulated chemokine (TARC or CCL17).

[0083] “Chimeric antigen receptor” or “CAR” refers to a molecule engineered to comprise a binding motif and a means of activating immune cells (for example T cells such as naive T cells, central memory T cells, effector memory T cells, NK cells or combination thereof) upon antigen binding. CARs are also known as artificial T cell receptors, chimeric T cell receptors or chimeric immunoreceptors. In some embodiments, a CAR comprises a binding motif, an extracellular domain, a transmembrane domain, one or more co-stimulatory domains, and an intracellular signaling domain. A T cell that has been genetically engineered to express a chimeric antigen receptor may be referred to as a CAR T cell. Similarly an NK cell that has been genetically engineered to express a chimeric antigen receptor may be referred to as a CAR NK cell.

[0084] By “decrease” or “lower,” or “lessen,” or “reduce,” or “abate” refers generally to the ability of a composition contemplated herein to produce, elicit, or cause a lesser physiological response (i.e., a downstream effect) compared to the response caused by either the vehicle alone (i.e., an

active moiety) or a control molecule/composition. A "decrease" or "reduced" amount is typically a "statistically significant" amount, and may include an decrease that is 1.1, 1.2, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the response (reference response) produced by vehicle, a control composition.

[0085] "Extracellular domain" (or "ECD") refers to a portion of a polypeptide that, when the polypeptide is present in a cell membrane, is understood to reside outside of the cell membrane, in the extracellular space.

[0086] The term "extracellular ligand-binding domain," as used herein, refers to an oligo- or polypeptide that is capable of binding a ligand, e.g., a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state (e.g., cancer). Examples of cell surface markers that may act as ligands include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

[0087] The binding domain of the CAR may be followed by a "spacer," or, "hinge," which refers to the region that moves the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation (Patel et al., Gene Therapy, 1999; 6: 412-419). The hinge region in a CAR is generally between the transmembrane (TM) and the binding domain. In certain embodiments, a hinge region is an immunoglobulin hinge region and may be a wild type immunoglobulin hinge region or an altered wild type immunoglobulin hinge region. Other exemplary hinge regions used in the CARs described herein include the hinge region derived from the extracellular regions of type I membrane proteins such as CD8alpha, CD4, CD28 and CD7, which may be wild-type hinge regions from these molecules or may be altered.

[0088] The "transmembrane" region or domain is the portion of the CAR that anchors the extracellular binding portion to the plasma membrane of the immune effector cell, and facilitates binding of the binding domain to the target antigen. The transmembrane domain may be a CD3zeta transmembrane domain, however other transmembrane domains that may be employed include those obtained from CD8alpha, CD4, CD28, CD45, CD9, CD16, CD22, CD33, CD64, CD80, CD86, CD134, CD137, and CD154. In one embodiment, the transmembrane domain is the transmembrane domain of CD137. In certain embodiments, the transmembrane domain is synthetic in which case it would comprise predominantly hydrophobic residues such as leucine and valine.

[0089] The "intracellular signaling domain" or "signaling domain" refers to the part of the chimeric antigen receptor protein that participates in transducing the message of effective CAR binding to a target antigen into the interior of the immune effector cell to elicit

effector cell function, e.g., activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound target cell, or other cellular responses elicited with antigen binding to the extracellular CAR domain. The term "effector function" refers to a specialized function of the cell. Effector function of the T cell, for example, may be cytolytic activity or help or activity including the secretion of a cytokine. Thus, the terms "intracellular signaling domain" or "signaling domain," used interchangeably herein, refer to the portion of a protein which transduces the effector function signal and that directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire domain. To the extent that a truncated portion of an intracellular signaling domain is used, such truncated portion may be used in place of the entire domain as long as it transduces the effector function signal. The term intracellular signaling domain is meant to include any truncated portion of the intracellular signaling domain sufficient to transducing effector function signal. The intracellular signaling domain is also known as the, "signal transduction domain," and is typically derived from portions of the human CD3 or FcRy chains.

[0090] It is known that signals generated through the T cell receptor alone are insufficient for full activation of the T cell and that a secondary, or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequences: those that initiate antigen dependent primary activation through the T cell receptor (primary cytoplasmic signaling sequences) and those that act in an antigen independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic signaling sequences). Cytoplasmic signaling sequences that act in a costimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motif or ITAMs.

[0091] Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the disclosure include those derived from DAP10, DAP12, TCRzeta, FcRgamma, FcRbeta, CD3gamma, CD3delta, CD3epsilon, CD5, CD22, CD79a, CD79b and CD66d.

[0092] As used herein, the term, "costimulatory signaling domain," or "costimulatory domain", refers to the portion of the CAR comprising the intracellular domain of a costimulatory molecule. Costimulatory molecules are cell surface molecules other than antigen receptors or Fc receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen. Examples of such co-stimulatory molecules include CD27, CD28, 4-1 BB (CD137), OX40 (CD134), CD30, CD40, PD-1, ICOS (CD278), LFA-1, CD2, CD7, LIGHT, NKD2C, B7-H2 and a ligand that specifically binds CD83. Accordingly, while the present disclosure provides exemplary costimulatory domains derived from 4-1 BB, other costimulatory domains are contemplated for use with the CARs described herein. The inclusion of one or more

co stimulatory signaling domains may enhance the efficacy and expansion of T cells expressing CAR receptors. The intracellular signaling and costimulatory signaling domains may be linked in any order in tandem to the carboxyl terminus of the transmembrane domain.

[0093] Although scFv-based CARs engineered to contain a signaling domain from CD3 or FcRgamma have been shown to deliver a potent signal for T cell activation and effector function, they are not sufficient to elicit signals that promote T cell survival and expansion in the absence of a concomitant costimulatory signal. Other CARs containing a binding domain, a hinge, a transmembrane and the signaling domain derived from CD3zeta or FcRgamma together with one or more costimulatory signaling domains (e.g., intracellular costimulatory domains derived from 4-1BB, CD28, CD137, CD134 and CD278) may more effectively direct antitumor activity as well as increased cytokine secretion, lytic activity, survival and proliferation in CAR expressing T cells in vitro, and in animal models and cancer patients (Milone et al., *Molecular Therapy*, 2009; 17: 1453-1464; Zhong et al., *Molecular Therapy*, 2010; 18: 413-420; Carpenito et al., *PNAS*, 2009; 106:3360-3365).

[0094] A “costimulatory signal” refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to a T cell response, such as, but not limited to, proliferation and/or upregulation or down regulation of key molecules.

[0095] A “costimulatory ligand” includes a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T cell. Binding of the costimulatory ligand provides a signal that mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A costimulatory ligand induces a signal that is in addition to the primary signal provided by a stimulatory molecule, for instance, by binding of a T cell receptor (TCR)/CD3 complex with a major histocompatibility complex (MHC) molecule loaded with peptide. A co-stimulatory ligand can include, but is not limited to, 3/TR6, 4-1BB ligand, agonist or antibody that binds Toll ligand receptor, B7-1 (CD80), B7-2 (CD86), CD30 ligand, CD40, CD7, CD70, CD83, herpes virus entry mediator (HVEM), human leukocyte antigen G (HLA-G), ILT4, immunoglobulin-like transcript (ILT) 3, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), ligand that specifically binds with B7-H3, lymphotoxin beta receptor, MHC class I chain-related protein A (MICA), MHC class I chain-related protein B (MICB), OX40 ligand, PD-L2, or programmed death (PD) L1. A co-stimulatory ligand includes, without limitation, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited to, 4-1BB, B7-H3, CD2, CD27, CD28, CD30, CD40, CD7, ICOS, ligand that specifically binds with CD83, lymphocyte function-associated antigen-1 (LFA-1), natural killer cell receptor C (NKG2C), OX40, PD-1, or tumor necrosis factor superfamily member 14 (TNFSF14 or LIGHT).

[0096] A “costimulatory molecule” is a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules include, but are not limited to, A “costimulatory molecule” is a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules include, but are not limited to, 4-1BB/CD137, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD 33, CD 45, CD100 (SEMA4D), CD103, CD134, CD137, CD154, CD16, CD160 (BY55), CD18, CD19, CD19a, CD2, CD22, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 (alpha; beta; delta; epsilon; gamma; zeta), CD30, CD37, CD4, CD40, CD40a, CD49D, CD49f, CD5, CD64, CD69, CD7, CD80, CD83 ligand, CD84, CD86, CD8alpha, CD8beta, CD9, CD96 (Tactile), CD1-la, CD1-lb, CD1-lc, CD1-lf, CDS, CEACAM1, CRT AM, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-2, ICOS, Ig alpha (CD79a), IL2R beta, IL2R gamma, IL7R alpha, integrin, ITGA4, ITGA5, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGBL, KIRDS2, LAT, LFA-1, LFA-3, LIGHT, LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), MHC class I molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRK1), OX40, PAG/Cbp, PD-1, PSGL1, SELPLG (CD162), signaling lymphocytic activation molecule, SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Ly108), SLAMF7, SLP-76, TNF, TNF α , TNFR2, Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or fragments, truncations, or combinations thereof.

[0097] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). In certain embodiments, one or more amino acid residues within a CDR(s) or within a framework region(s) of an antibody or antigen-binding molecule thereof can be replaced with an amino acid residue with a similar side chain. In general, two sequences are generally considered to be “substantially similar” if they contain a conservative amino acid substitution in corresponding positions. For example, certain amino acids are generally classified as “hydrophobic” or “hydrophilic” amino acids, and/or as having “polar” or “non-polar” side chains. Substitution of one amino acid for another of the same type may be

considered a conservative substitution. Exemplary amino acid categorizations are summarized in Tables 2 and 3 below:

Table 2

Amino Acid	3-Letter	1-Letter	Property	Property	Hydropathy Index
Alanine	Ala	A	nonpolar	neutral	1.8
Arginine	Arg	R	polar	positive	-4.5
Asparagine	Asn	N	polar	neutral	-3.5
Aspartic acid	Asp	D	polar	negative	-3.5
Cysteine	Cys	C	nonpolar	neutral	2.5
Glutamic acid	Glu	E	polar	negative	-3.5
Glutamine	Gln	Q	polar	neutral	-3.5
Glycine	Gly	G	nonpolar	neutral	-0.4
Histidine	His	H	polar	positive	-3.2
Isoleucine	Ile	I	nonpolar	neutral	4.5
Leucine	Leu	L	nonpolar	neutral	3.8
Lysine	Lys	K	polar	positive	-3.9
Methionine	Met	M	nonpolar	neutral	1.9
Phenylalanine	Phe	F	nonpolar	neutral	2.8
Proline	Pro	P	nonpolar	neutral	-1.6
Serine	Ser	S	polar	neutral	-0.8
Threonine	Thr	T	polar	neutral	-0.7
Tryptophan	Trp	W	nonpolar	neutral	-0.9
Tyrosine	Tyr	Y	polar	neutral	-1.3
Valine	Val	V	nonpolar	neutral	4.2

Table 3

Ambiguous Amino Acids	3-Letter	1-Letter
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z
Leucine or Isoleucine	Xle	J
Unspecified or unknown amino acid	Xaa	X

[0098] “Combination therapy” refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (*e.g.*, two or more therapeutic moieties). In some embodiments, the two or more regimens may be administered simultaneously; in some embodiments, such regimens may be administered sequentially (*e.g.*, all “doses” of a first regimen are administered prior to administration of any doses of a second regimen); in some embodiments, such agents are administered in overlapping dosing regimens. In some embodiments, “administration” of combination therapy may involve administration of one or more agent(s) or modality(ies) to a subject receiving the other agent(s) or modality(ies) in the combination. For clarity, combination therapy does not require that individual agents be administered together in a single composition (or even necessarily at the same time), although in some embodiments, two or more agents, or active moieties thereof, may be administered together in a combination composition, or even in a combination compound (*e.g.*, as part of a single chemical complex or covalent entity).

[0099] “Corresponding to” may be used to designate the position/identity of a structural element in a molecule or composition through comparison with an appropriate reference molecule or composition. For example, in some embodiments, a monomeric residue in a polymer (*e.g.*, an amino acid residue in a polypeptide or a nucleic acid residue in a polynucleotide) may be identified as “corresponding to” a residue in an appropriate reference polymer. For example, for purposes of simplicity, residues in a polypeptide may be designated using a canonical numbering system based on a reference related polypeptide, so that an amino acid “corresponding to” a residue at position 100, for example, need not actually be the 100th amino acid in an amino acid chain provided it corresponds to the residue found at position 100 in the reference polypeptide. Various sequence alignment strategies are available, comprising software programs such as, for example, BLAST, CS-BLAST, CUDASW++, DIAMOND, FASTA, GGSEARCH/GLSEARCH, Genoogle, HMMER, HHpred/HHsearch, IDF, Infernal, KLAST, USEARCH, parasail, PSI-BLAST, PSI-Search, ScalaBLAST, Sequilab, SAM, SSEARCH, SWAPHI, SWAPHI-LS, SWIMM, or SWIPE that may be utilized, for example, to identify “corresponding” residues in polypeptides and/or nucleic acids in accordance with the present disclosure.

[0100] An antigen binding molecule, such as an antibody, an antigen binding fragment thereof, CAR or TCR, “cross-competes” with a reference binding molecule, such as an antibody or an antigen binding fragment thereof, if the interaction between an antigen and the first antigen binding molecule blocks, limits, inhibits, or otherwise reduces the ability of the reference binding molecule to interact with the antigen. Cross competition can be complete, *e.g.*, binding of the antigen binding molecule to the antigen completely blocks the ability of the reference binding molecule to bind the antigen, or it can be partial, *e.g.*, binding of the antigen binding molecule to

the antigen reduces the ability of the reference antigen binding molecule to bind the antigen. In certain embodiments, an antigen binding molecule that cross-competes with a reference antigen binding molecule binds the same or an overlapping epitope as the reference antigen binding molecule. In other embodiments, the antigen binding molecule that cross-competes with a reference antigen binding molecule binds a different epitope than the reference antigen binding molecule. Numerous types of competitive binding assays can be used to determine if one antigen binding molecule competes with another, for example: solid phase direct or indirect radioimmunoassay (RIA); solid phase direct or indirect enzyme immunoassay (EIA); sandwich competition assay (Stahli et al., 1983, *Methods in Enzymology* 9:242-253); solid phase direct biotin-avidin EIA (Kirkland et al., 1986, *J. Immunol.* 137:3614-3619); solid phase direct labeled assay, solid phase direct labeled sandwich assay (Harlow and Lane, 1988, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press); solid phase direct label RIA using 1-125 label (Morel et al., 1988, *Molec. Immunol.* 25:7-15); solid phase direct biotin-avidin EIA (Cheung, et al., 1990, *Virology* 176:546-552); and direct labeled RIA (Moldenhauer et al., 1990, *Scand. J. Immunol.* 32:77-82).

[0101] A “cytokine,” refers to a non-antibody protein that is released by one cell in response to contact with a specific antigen, wherein the cytokine interacts with a second cell to mediate a response in the second cell. A cytokine can be endogenously expressed by a cell or administered to a subject. Cytokines may be released by immune cells, including macrophages, B cells, T cells, and mast cells to propagate an immune response. Cytokines can induce various responses in the recipient cell. Cytokines can include homeostatic cytokines, chemokines, pro-inflammatory cytokines, effectors, and acute-phase proteins. For example, homeostatic cytokines, including interleukin (IL) 7 and IL-15, promote immune cell survival and proliferation, and pro-inflammatory cytokines can promote an inflammatory response. Examples of homeostatic cytokines include, but are not limited to, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12p40, IL-12p70, IL-15, and interferon (IFN) gamma. Examples of pro-inflammatory cytokines include, but are not limited to, IL-1a, IL-1b, IL-6, IL-13, IL-17a, tumor necrosis factor (TNF)-alpha, TNF-beta, fibroblast growth factor (FGF) 2, granulocyte macrophage colony-stimulating factor (GM-CSF), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, and placental growth factor (PLGF). Examples of effectors include, but are not limited to, granzyme A, granzyme B, soluble Fas ligand (sFasL), and perforin. Examples of acute phase-proteins include, but are not limited to, C-reactive protein (CRP) and serum amyloid A (SAA).

[0102] The term “domain” refers to a portion of an entity. In some embodiments, a “domain” is associated with a structural and/or functional feature of the entity, *e.g.*, so that, when the domain

is physically separated from the rest of its parent entity, it substantially or entirely retains the structural and/or functional feature. In some embodiments, a domain may comprise a portion of an entity that, when separated from that (parent) entity and linked or connected with a different (recipient) entity, substantially retains and/or imparts on the recipient entity one or more structural and/or functional features, *e.g.*, that characterized it in the parent entity. In some embodiments, a domain is a portion of a molecule (*e.g.*, a small molecule, carbohydrate, lipid, nucleic acid, or polypeptide). In some embodiments, a domain is a section of a polypeptide; in some such embodiments, a domain is characterized by a structural element (*e.g.*, an amino acid sequence or sequence motif, α -helix character, β -sheet character, coiled-coil character, random coil character, etc.), and/or by a functional feature (*e.g.*, binding activity, enzymatic activity, folding activity, signaling activity, etc.).

[0103] The term “dosage form” may be used to refer to a physically discrete unit of an active agent (*e.g.*, an antigen binding system or antibody) for administration to a subject. Generally, each such unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population. The total amount of a therapeutic composition or agent administered to a subject is determined by one or more medical practitioners and may involve administration of more than one dosage forms.

[0104] The term “dosing regimen” may be used to refer to a set of one or more unit doses that are administered individually to a subject. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, a dosing regimen comprises a plurality of doses and consecutive doses are separated from one another by time periods of equal length; in some embodiments, a dosing regimen comprises a plurality of doses and consecutive doses are separated from one another by time periods of at least two different lengths. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen is periodically adjusted to achieve a desired or beneficial outcome.

[0105] “Effector cell” refers to a cell of the immune system that expresses one or more Fc receptors and mediates one or more effector functions. In some embodiments, effector cells may comprise, without limitation, one or more of monocytes, macrophages, neutrophils, dendritic

cells, eosinophils, mast cells, platelets, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, T-lymphocytes, and B-lymphocytes. Effector cells may be of any organism comprising, without limitation, humans, mice, rats, rabbits, and monkeys.

[0106] “Effector function” refers to a biological result of interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions comprise, without limitation, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), and complement-mediated cytotoxicity (CMC). An effector function may be antigen binding dependent, antigen binding independent, or both. ADCC refers to lysis of antibody-bound target cells by immune effector cells. Without wishing to be bound by any theory, ADCC is generally understood to involve Fc receptor (FcR)-bearing effector cells recognizing and subsequently killing antibody-coated target cells (*e.g.*, cells that express on their surface antigens to which an antibody is bound). Effector cells that mediate ADCC may comprise immune cells, comprising yet not limited to, one or more of natural killer (NK) cells, macrophages, neutrophils, eosinophils.

[0107] The term “engineered Autologous Cell Therapy,” which can be abbreviated as “eACT™,” also known as adoptive cell transfer, is a process by which a patient's own T cells are collected and subsequently genetically altered to recognize and target one or more antigens expressed on the cell surface of one or more specific tumor cells or malignancies. T cells can be engineered to express, for example, chimeric antigen receptors (CAR) or T cell receptor (TCR). CAR positive (+) T cells are engineered to express an extracellular single chain variable fragment (scFv) with specificity for a particular tumor antigen linked to an intracellular signaling part comprising at least one costimulatory domain and at least one activating domain. The costimulatory domain can be derived from a naturally-occurring costimulatory domain, or a variant thereof, *e.g.*, a variant having a truncated hinge domain (“THD”), and the activating domain can be derived from, *e.g.*, CD3-zeta. In certain embodiments, the CAR is designed to have two, three, four, or more costimulatory domains. The CAR scFv can be designed to target, for example, PSMA, which is a transmembrane protein expressed prostate tissue, including carcinoma.

[0108] In some embodiments, the CAR is engineered such that the costimulatory domain is expressed as a separate polypeptide chain. Example CAR T cell therapies and constructs are described in U.S. Patent Publication Nos. 2013/0287748, 2014/0227237, 2014/0099309, and 2014/0050708, which are incorporated by reference in their entirety. “Adoptive cell therapy” or “ACT” involves transfer of immune cells with anti-tumor activity into a subject, *e.g.*, a cancer patient. In some embodiments, ACT is a treatment approach that involves the use of lymphocytes (*e.g.*, engineered lymphocytes) with anti-tumor activity.

[0109] An “epitope” refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear

or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody binds can be determined by, *e.g.*, NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays, and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.*, (1994) *Acta Crystallogr D Biol Crystallogr* 50(Pt 4): 339-350; McPherson A (1990) *Eur J Biochem* 189: 1-23; Chayen NE (1997) *Structure* 5: 1269-1274; McPherson A (1976) *J Biol Chem* 251: 6300-6303). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; see *e.g.* *Meth Enzymol* (1985) volumes 114 & 115, eds Wyckoff HW *et al.*; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) *Acta Crystallogr D Biol Crystallogr* 49(Pt 1): 37-60; Bricogne G (1997) *Meth Enzymol* 276A: 361-423, ed Carter CW; Roversi P *et al.*, (2000) *Acta Crystallogr D Biol Crystallogr* 56(Pt 10): 1316-1323). Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See, e.g.*, Champe M *et al.*, (1995) *J Biol Chem* 270: 1388-1394 and Cunningham BC & Wells JA (1989) *Science* 244: 1081-1085 for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques.

[0110] “Endogenous” with reference to a gene, protein, and/or nucleic acid refers to the natural presence of that gene, protein, and/or nucleic acid in a cell, such as an immune cell.

[0111] “Exogenous” refers to an introduced agent, such as a nucleic acid, gene, or protein, into a cell, for example from an outside source. A nucleic acid introduced into a cell is exogenous even if it encodes a protein which is naturally found in the cell. Such exogenous introduction of a nucleic acid encoding a protein can be used to increase the expression of the protein over the level that would naturally be found in the cell under similar conditions, *e.g.* without introduction of the exogenous nucleic acid.

[0112] The term “excipient” refers to an agent that may be comprised in a composition, for example to provide or contribute to a desired consistency or stabilizing effect. In some embodiments, a suitable excipient may comprise, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, or the like.

[0113] A “fragment” or “portion” of a material or entity as described herein has a structure that comprises a discrete portion of the whole, *e.g.*, of a physical entity or abstract entity. In some

embodiments, a fragment lacks one or more moieties found in the whole. In some embodiments, a fragment consists of or comprises a characteristic structural element, domain or moiety found in the whole. In some embodiments, a polymer fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (*e.g.*, residues) as found in the whole polymer. In some embodiments, a polymer fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of the monomeric units (*e.g.*, residues) found in the whole polymer (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). The whole material or entity may in some embodiments be referred to as the “parent” of the fragment.

[0114] The term “fusion polypeptide” or “fusion protein” generally refers to a polypeptide comprising at least two segments. Generally, a polypeptide containing at least two such segments is considered to be a fusion polypeptide if the two segments are moieties that (1) are not comprised in nature in the same peptide, and/or (2) have not previously been linked or connected to one another in a single polypeptide, and/or (3) have been linked or connected to one another through action of the hand of man. In embodiments, a CAR is a fusion protein. In embodiments, a synthetic Notch receptor (synNotch receptor) is a fusion protein.

[0115] The term “gene product” or “expression product” generally refers to an RNA transcribed from the gene (pre-and/or post-processing) or a polypeptide (pre- and/or post-modification) encoded by an RNA transcribed from the gene.

[0116] The term “genetically engineered” or “engineered” refers to a method of modifying the genome of a cell, including, but not limited to, deleting a coding or non-coding region or a portion thereof or inserting a coding region or a portion thereof. In some embodiments, the cell that is modified is a lymphocyte, *e.g.*, a T cell or NK cell, which can either be obtained from a patient or a donor. The cell can be modified to express an exogenous construct, such as, *e.g.*, a chimeric antigen receptor (CAR) or a T cell receptor (TCR), which is incorporated into the cell's genome. Engineering generally comprises manipulation by the hand of man. For example, a polynucleotide is considered to be “engineered” when two or more sequences, that are not linked or connected together in that order in nature, are manipulated by the hand of man to be directly linked or connected to one another in the engineered polynucleotide. In the context of manipulation of cells by techniques of molecular biology, a cell or organism is considered to be “engineered” if it has been manipulated so that its genetic information is altered (*e.g.*, new genetic material not previously present has been introduced, for example by transformation, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or

removed, for example by substitution or deletion mutation, or by other protocols). In some embodiments, a binding agent is a modified lymphocyte, *e.g.*, a T cell or NK cell, may be obtained from a patient or a donor. An engineered cell may be modified to express an exogenous construct, such as, *e.g.*, a chimeric antigen receptor (CAR) or a T cell receptor (TCR), which is incorporated into the cell's genome. Progeny of an engineered polynucleotide or binding agent are generally referred to as “engineered” even though the actual manipulation was performed on a prior entity. In some embodiments, “engineered” refers to an entity that has been designed and produced. The term “designed” refers to an agent (i) whose structure is or was selected by the hand of man; (ii) that is produced by a process requiring the hand of man; and/or (iii) that is distinct from natural substances and other known agents.

[0117] A “T cell receptor” or “TCR” refers to antigen-recognition molecules present on the surface of T cells. During normal T cell development, each of the four TCR genes, α , β , γ , and δ , may rearrange leading to highly diverse TCR proteins.

[0118] The term “heterologous” means from any source other than naturally occurring sequences. For example, a heterologous sequence included as a part of a costimulatory protein is amino acids that do not naturally occur as, *i.e.*, do not align with, the wild type human costimulatory protein. For example, a heterologous nucleotide sequence refers to a nucleotide sequence other than that of the wild type human costimulatory protein-encoding sequence.

[0119] Term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Methods for the calculation of a percent identity as between two provided polypeptide sequences are known. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, may be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps may be introduced in one or both of a first and a second sequences for optimal alignment and non-identical sequences may be disregarded for comparison purposes). The nucleotides or amino acids at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (*e.g.*, nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, optionally taking into account the number of gaps, and the length of each gap, which may need to be introduced for optimal alignment of the two sequences. Comparison or alignment of sequences and determination of percent identity between two sequences may be accomplished using a mathematical algorithm, such as BLAST (basic local alignment search tool). In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%,

85%, 90%, 95%, or 99% identical (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%).

[0120] To calculate percent identity, the sequences being compared are typically aligned in a way that gives the largest match between the sequences. One example of a computer program that can be used to determine percent identity is the GCG program package, which includes GAP (Devereux et al., 1984, Nucl. Acid Res. 12:387; Genetics Computer Group, University of Wisconsin, Madison, Wis.). The computer algorithm GAP is used to align the two polypeptides or polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the “matched span,” as determined by the algorithm). In certain embodiments, a standard comparison matrix (*see*, Dayhoff et al., 1978, Atlas of Protein Sequence and Structure 5:345-352 for the PAM 250 comparison matrix; Henikoff et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919 for the BLOSUM 62 comparison matrix) is also used by the algorithm. Other algorithms are also available for comparison of amino acid or nucleic acid sequences, comprising those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410, 1990; Altschul, et al., Methods in Enzymology; Altschul, et al., “Gapped BLAST and PSI-BLAST: a new generation of protein database search programs,” Nucleic Acids Res. 25:3389-3402, 1997; Baxevanis, et al., Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener, et al., (eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying similar sequences, the programs mentioned above generally provide an indication of the degree of similarity. In some embodiments, two sequences are considered to be substantially similar if at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more of their corresponding residues are similar and/or identical over a relevant stretch of residues (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400, at least 425, at least 450, at least 475, at least 500 or more residues. Sequences with substantial sequence similarity may be homologs of one another.

[0121] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0122] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

[0123] The terms "improve," "increase," "inhibit," and "reduce" indicate values that are relative to a baseline or other reference measurement. In some embodiments, an appropriate reference measurement may comprise a measurement in certain system (*e.g.*, in a single individual) under otherwise comparable conditions absent presence of (*e.g.*, prior to and/or after) an agent or treatment, or in presence of an appropriate comparable reference agent. In some embodiments, an appropriate reference measurement may comprise a measurement in comparable system known or expected to respond in a comparable way, in presence of the relevant agent or treatment.

[0124] An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0125] The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. Examples of immunotherapy include, but are not limited to, NK cells and T cell therapies. T cell therapy can include adoptive T cell therapy, tumor-infiltrating lymphocyte (TIL) immunotherapy, autologous cell therapy, engineered autologous cell therapy (eACT™), and allogeneic T cell transplantation. However, one of skill in the art would recognize that the conditioning methods disclosed herein would enhance the

effectiveness of any transplanted T cell therapy. Examples of T cell therapies are described in U.S. Patent Publication Nos. 2014/0154228 and 2002/0006409, U.S. Patent No. 5,728,388, and International Publication No. WO 2008/081035.

[0126] The T cells or NK cells of the immunotherapy can come from any source known in the art. For example, T cells and NK cells can be differentiated *in vitro* from a hematopoietic stem cell population or can be obtained from a subject. T cells and NK cells can be obtained from, *e.g.*, peripheral blood mononuclear cells (PBMCs), bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In addition, the T cells can be derived from one or more T cell lines available in the art. T cells can also be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation and/or apheresis. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Publication No. 2013/0287748, which is herein incorporated by references in its entirety.

[0127] The term “*in vitro*” refers to events occurring in an artificial environment, *e.g.*, in a test tube, reaction vessel, cell culture, etc., rather than within a multi-cellular organism. The term “*in vitro* cell” refers to any cell which is cultured *ex vivo*. In particular, an *in vitro* cell can include a T cell or an NK cell. The term “*in vivo*” refers to events that occur within a multi-cellular organism, such as a human or a non-human animal.

[0128] The term “isolated” refers to a substance that (1) has been separated from at least some components with which it was associated at an earlier time or with which the substance would otherwise be associated, and/or (2) is present in a composition that comprises a limited or defined amount or concentration of one or more known or unknown contaminants. An isolated substance, in some embodiments, may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) of other non-substance components with which the substance was associated at an earlier time, *e.g.*, other components or contaminants with which the substance was previously or otherwise would be associated. In certain instances, a substance is isolated if it is present in a composition that comprises a limited or reduced amount or concentration of molecules of a same or similar type. For instance, in certain instances, a nucleic acid, DNA, or RNA substance is isolated if it is present in a composition that comprises a limited or reduced amount or concentration of non-substance nucleic acid, DNA, or RNA molecules. For instance, in certain instances, a polypeptide substance is isolated if it is present in a composition that comprises a limited or reduced amount or concentration of non-substance polypeptide molecules. In certain embodiments, an amount may be, *e.g.*, an amount

measured relative to the amount of a desired substance present in a composition. In certain embodiments, a limited amount may be an amount that is no more than 100% of the amount of substance in a composition, *e.g.*, no more than 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the amount of substance in a composition (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain instances, a composition is pure or substantially pure with respect to a selected substance. In some embodiments, an isolated substance is about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). A substance is "pure" if it is substantially free of other components or of contaminants. In some embodiments, a substance may still be considered "isolated" or even "pure," after having been combined with certain other components such as, for example, one or more carriers or excipients (*e.g.*, buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without comprising such carriers or excipients.

[0129] "Linker" (L) or "linker domain" or "linker region" refers to an oligo- or polypeptide region from about 1 to 100 amino acids in length, for example linking together any of the domains/regions of a CAR, a synNotch receptor, a DN TFGbeta receptor and/or scFv, or ever one of more of those polypeptides together. Linkers may be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers may be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Linkers may be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSELsequences of porcine teschovirus (P2A), virus (T2A) or combinations, variants and functional equivalents thereof. In other embodiments, the linker sequences may comprise Asp-Val/Ile-Glu-X-Asn-Pro-Gly^(2A)-Pro^(2B) motif (SEQ ID NO: 255), which results in cleavage between the 2A glycine and the 2B proline. Other linkers will be apparent to those of skill in the art and may be used in connection with this disclosure. A linker may be a portion of a multi-element agent that connects different elements to one another. For example, a polypeptide comprises two or more functional or structural domains may comprise a stretch of amino acids between such domains that links them to one another. In some embodiments, a polypeptide comprising a linker element has an overall structure of the general form S1-L-S2, wherein S1 and S2 may be the same or different and represent two domains associated with one another by the linker. A linker may connect or link together any of the domains/regions of a CAR or a synNotch receptor. In some embodiments, a polypeptide linker is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length (*e.g.*, 1 to 10, 1 to 20, 1 to 30, 1 to 40, 1 to 50, 1 to 60, 1 to 70, 1 to 80, 1 to 90, 1 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 10 to 60, 10 to 70, 10 to 80, 10 to 90, or 10 to 100 amino acids in length). In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, and instead provides flexibility to the polypeptide. In another example it may be used to connect to or more polypeptides to be expressed, such as a CAR, a synNotch receptor and/or a TGF β -DNR as disclosed herein. In some examples, the CAR, the synNotch receptor and/or the DN TGF β -R are connected by a cleavable linker.

[0130] Other linkers include non-cleavable linkers. A number of linkers are employed to realize the subject invention including “flexible linkers.” The latter are rich in glycine. Klein et al., *Protein Engineering, Design & Selection* Vol. 27, No. 10, pp. 325–330, 2014; Priyanka et al., *Protein Sci.*, 2013 Feb; 22(2): 153–167.

[0131] In some embodiments, the linker is a synthetic linker. A synthetic linker can have a length of from about 10 amino acids to about 200 amino acids, *e.g.*, from 10 to 25 amino acids, from 25 to 50 amino acids, from 50 to 75 amino acids, from 75 to 100 amino acids, from 100 to 125 amino acids, from 125 to 150 amino acids, from 150 to 175 amino acids, or from 175 to 200 amino acids. A synthetic linker can have a length of from 10 to 30 amino acids, *e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids. A synthetic linker can have a length of from 30 to 50 amino acids, *e.g.*, from 30 to 35 amino acids, from 35 to 40 amino acids, from 40 to 45 amino acids, or from 45 to 50 amino acids.

[0132] In some embodiments, the linker is a flexible linker. In some embodiments, the linker is rich in glycine (Gly or G) residues. In some embodiments, the linker is rich in serine (Ser or S) residues. In some embodiments, the linker is rich in glycine and serine residues. In some embodiments, the linker has one or more glycine-serine residue pairs (GS), *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GS pairs.

[0133] The term “lymphocyte” includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represent a component of the inherent immune system. NK cells reject tumors and cells infected by viruses. It works through the process of apoptosis or programmed cell death. They were termed “natural killers” because they do not require activation in order to kill cells. T cells play a role in cell-mediated-immunity (no antibody involvement). Its T cell receptors (TCR) differentiate themselves from other lymphocyte types. The thymus, a specialized organ of the immune system, is primarily responsible for the T cell’s maturation. There are six types of T cells, namely: Helper T cells (*e.g.*, CD4⁺ cells), Cytotoxic T cells (also known as TC, cytotoxic T lymphocyte, CTL, T-killer cell, cytolytic T cell, CD8⁺ T

cells or killer T cell), Memory T cells ((i) stem memory T_{SCM} cells, like naive cells, are CD45RO⁺, CCR7⁺, CD45RA⁺, CD62L⁺ (L-selectin), CD27⁺, CD28⁺ and IL-7Rα⁺, but they also express large amounts of CD95, IL-2Rβ, CXCR3, and LFA-1, and show numerous functional attributes distinctive of memory cells); (ii) central memory T_{CM} cells express L-selectin and the CCR7, they secrete IL-2, but not IFNγ or IL-4, and (iii) effector memory T_{EM} cells, however, do not express L-selectin or CCR7 but produce effector cytokines like IFNγ and IL-4), Regulatory T cells (Tregs, suppressor T cells, or CD4⁺CD25⁺ regulatory T cells), Natural Killer T cells (NKT) and Gamma Delta T cells. B-cells, on the other hand, play a role in humoral immunity (with antibody involvement). It makes antibodies and antigens and performs the role of antigen-presenting cells (APCs) and turns into memory B-cells after activation by antigen interaction. In mammals, immature B-cells are formed in the bone marrow, where its name is derived from.

[0134] The term “neutralizing” refers to an antigen binding molecule, scFv, antibody, or a fragment thereof, that binds to a ligand and prevents or reduces the biological effect of that ligand. In some embodiments, the antigen binding molecule, scFv, antibody, or a fragment thereof, directly blocking a binding site on the ligand or otherwise alters the ligand's ability to bind through indirect means (such as structural or energetic alterations in the ligand). In some embodiments, the antigen binding molecule, scFv, antibody, or a fragment thereof prevents the protein to which it is bound from performing a biological function.

[0135] “Nucleic acid” refers to any polymeric chain of nucleotides. A nucleic acid may be DNA, RNA, or a combination thereof. In some embodiments, a nucleic acid comprises one or more natural nucleic acid residues. In some embodiments, a nucleic acid comprises of one or more nucleic acid analogs. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long (e.g., 20 to 100, 20 to 500, 20 to 1000, 20 to 2000, or 20 to 5000 or more residues). In some embodiments, a nucleic acid is partly or wholly single stranded; in some embodiments, a nucleic acid is partly or wholly double stranded. In some embodiments a nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide.

[0136] “Operably linked” refers to a juxtaposition where the components described are in a relationship permitting them to function in their intended manner. For example, a control element “operably linked” to a functional element is associated in such a way that expression and/or

activity of the functional element is achieved under conditions compatible with the control element. In embodiments, a promotor is operably linked to nucleic a

[0137] A “patient” includes any human who is afflicted with a cancer (*e.g.*, prostate cancer). The terms “subject” and “patient” are used interchangeably herein.

[0138] The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide contains at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[0139] The term “pharmaceutically acceptable” refers to a molecule or composition that, when administered to a recipient, is not deleterious to the recipient thereof, or that any deleterious effect is outweighed by a benefit to the recipient thereof. With respect to a carrier, diluent, or excipient used to formulate a composition as disclosed herein, a pharmaceutically acceptable carrier, diluent, or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof, or any deleterious effect must be outweighed by a benefit to the recipient. The term “pharmaceutically acceptable carrier” means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting an agent from one portion of the body to another (*e.g.*, from one organ to another). Each carrier present in a pharmaceutical composition must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not deleterious to the patient, or any deleterious effect must be outweighed by a benefit to the recipient. Some examples of materials which may serve as pharmaceutically acceptable carriers comprise: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar;

buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0140] The term “pharmaceutical composition” refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in a unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant subject or population. In some embodiments, a pharmaceutical composition may be formulated for administration in solid or liquid form, comprising, without limitation, a form adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0141] The term “proliferation” refers to an increase in cell division, either symmetric or asymmetric division of cells. In some embodiments, “proliferation” refers to the symmetric or asymmetric division of T cells. “Increased proliferation” occurs when there is an increase in the number of cells in a treated sample compared to cells in a non-treated sample.

[0142] The term “reference” describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence, or value of interest is compared with a reference or control that is an agent, animal, individual, population, sample, sequence, or value. In some embodiments, a reference or control is tested, measured, and/or determined substantially simultaneously with the testing, measuring, or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Generally, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. When sufficient similarities are present to justify reliance on and/or comparison to a selected reference or control.

[0143] “Regulatory T cells” (“Treg”, “Treg cells”, or “Tregs”) refer to a lineage of CD4⁺ T lymphocytes that participate in controlling certain immune activities, *e.g.*, autoimmunity, allergy,

and response to infection. Regulatory T cells may regulate the activities of T cell populations, and may also influence certain innate immune system cell types. Tregs may be identified by the expression of the biomarkers CD4, CD25 and Foxp3, and low expression of CD127. Naturally occurring Treg cells normally constitute about 5-10% of the peripheral CD4+ T lymphocytes. However, Treg cells within a tumor microenvironment (i.e. tumor-infiltrating Treg cells), Treg cells may make up as much as 20-30% of the total CD4+ T lymphocyte population.

[0144] The term “sample” generally refers to an aliquot of material obtained or derived from a source of interest. In some embodiments, a source of interest is a biological or environmental source. In some embodiments, a source of interest may comprise a cell or an organism, such as a cell population, tissue, or animal (*e.g.*, a human). In some embodiments, a source of interest comprises biological tissue or fluid. In some embodiments, a biological tissue or fluid may comprise amniotic fluid, aqueous humor, ascites, bile, bone marrow, blood, breast milk, cerebrospinal fluid, cerumen, chyle, chime, ejaculate, endolymph, exudate, feces, gastric acid, gastric juice, lymph, mucus, pericardial fluid, perilymph, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, semen, serum, smegma, sputum, synovial fluid, sweat, tears, urine, vaginal secretions, vitreous humour, vomit, and/or combinations or component(s) thereof. In some embodiments, a biological fluid may comprise an intracellular fluid, an extracellular fluid, an intravascular fluid (blood plasma), an interstitial fluid, a lymphatic fluid, and/or a transcellular fluid. In some embodiments, a biological fluid may comprise a plant exudate. In some embodiments, a biological tissue or sample may be obtained, for example, by aspirate, biopsy (*e.g.*, fine needle or tissue biopsy), swab (*e.g.*, oral, nasal, skin, or vaginal swab), scraping, surgery, washing or lavage (*e.g.*, bronchoalveolar, ductal, nasal, ocular, oral, uterine, vaginal, or other washing or lavage). In some embodiments, a biological sample comprises cells obtained from an individual. In some embodiments, a sample is a “primary sample” obtained directly from a source of interest by any appropriate means. In some embodiments, as will be clear from context, the term “sample” refers to a preparation that is obtained by processing (*e.g.*, by removing one or more components of and/or by adding one or more agents to) a primary sample. Such a “processed sample” may comprise, for example nucleic acids or proteins extracted from a sample or obtained by subjecting a primary sample to one or more techniques such as amplification or reverse transcription of nucleic acid, isolation and/or purification of certain components, *etc.*

[0145] “Single chain variable fragment”, “single-chain antibody variable fragments” or “scFv” antibodies refer to forms of antibodies comprising the variable regions of only the heavy and light chains, connected by a linker peptide.

[0146] The term “stage of cancer” refers to a qualitative or quantitative assessment of the level of advancement of a cancer. In some embodiments, criteria used to determine the stage of a cancer

may comprise, without limitation, one or more of where the cancer is located in a body, tumor size, whether the cancer has spread to lymph nodes, whether the cancer has spread to one or more different parts of the body, etc. In some embodiments, cancer may be staged using the so-called TNM System, according to which T refers to the size and extent of the main tumor, usually called the primary tumor; N refers to the number of nearby lymph nodes that have cancer; and M refers to whether the cancer has metastasized. In some embodiments, a cancer may be referred to as Stage 0 (abnormal cells are present without having spread to nearby tissue, also called carcinoma *in situ*, or CIS; CIS is not cancer, though could become cancer), Stage I-III (cancer is present; the higher the number, the larger the tumor and the more it has spread into nearby tissues), or Stage IV (the cancer has spread to distant parts of the body). In some embodiments, a cancer may be assigned to a stage selected from the group consisting of: *in situ*; localized (cancer is limited to the place where it started, with no sign that it has spread); regional (cancer has spread to nearby lymph nodes, tissues, or organs); distant (cancer has spread to distant parts of the body); and unknown (there is not enough information to determine the stage).

[0147] “Stimulation,” refers to a primary response induced by binding of a stimulatory molecule with its cognate ligand, wherein the binding mediates a signal transduction event. A “stimulatory molecule” is a molecule on a T cell, *e.g.*, the T cell receptor (TCR)/CD3 complex, that specifically binds with a cognate stimulatory ligand present on an antigen present cell. A “stimulatory ligand” is a ligand that when present on an antigen presenting cell (*e.g.*, an APC, a dendritic cell, a B-cell, and the like) can specifically bind with a stimulatory molecule on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands include, but are not limited to, an anti-CD3 antibody (such as OKT3), an MHC Class I molecule loaded with a peptide, a superagonist anti-CD2 antibody, and a superagonist anti-CD28 antibody.

[0148] The phrase “therapeutic agent” may refer to any agent that elicits a desired pharmacological effect when administered to an organism. In some embodiments, an agent is considered to be a therapeutic agent if it demonstrates a statistically significant effect across an appropriate population. In some embodiments, the appropriate population may be a population of model organisms or human subjects. In some embodiments, an appropriate population may be defined by various criteria, such as a certain age group, gender, genetic background, preexisting clinical conditions, in accordance with presence or absence of a biomarker, etc. In some embodiments, a therapeutic agent is a substance that may be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. In some embodiments, a therapeutic agent is an agent that has been or is required to be approved by a government agency before it

may be marketed for administration to humans. In some embodiments, a therapeutic agent is an agent for which a medical prescription is required for administration to humans.

[0149] A “therapeutically effective amount,” “effective dose,” “effective amount,” or “therapeutically effective dosage” of a therapeutic agent, *e.g.*, engineered CAR T cells or NK cell, is any amount that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0150] The terms “transduction” and “transduced” refer to the process whereby foreign DNA is introduced into a cell via viral vector (*see* Jones et al., “Genetics: principles and analysis,” Boston: Jones & Bartlett Publ. (1998)). In some embodiments, the vector is a retroviral vector, a DNA vector, a RNA vector, an adenoviral vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.

[0151] “Transformation” refers to any process by which exogenous DNA is introduced into a host cell. Transformation may occur under natural or artificial conditions using various methods. Transformation may be achieved using any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. In some embodiments, some transformation methodology is selected based on the host cell being transformed and/or the nucleic acid to be inserted. Methods of transformation may comprise, yet are not limited to, viral infection, electroporation, and lipofection. In some embodiments, a “transformed” cell is stably transformed in that the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. In some embodiments, a transformed cell may express introduced nucleic acid.

[0152] “Treatment” or “treating” of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease. In one embodiment, “treatment” or “treating” includes a partial remission. In another embodiment, “treatment” or “treating” includes a complete remission. In some embodiments, treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease,

disorder, and/or condition. In some embodiments, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

[0153] The term “vector” refers to a recipient nucleic acid molecule modified to comprise or incorporate a provided nucleic acid sequence. One type of vector is a “plasmid,” which refers to a circular double stranded DNA molecule into which additional DNA may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors comprise sequences that direct expression of inserted genes to which they are operatively linked. Such vectors may be referred to herein as “expression vectors.” Standard techniques may be used for engineering of vectors, e.g., as found in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference.

[0154] A “binding protein” is a protein that is able to bind non-covalently to another molecule. A binding protein can bind to, for example, a DNA molecule (a DNA-binding protein), an RNA molecule (an RNA-binding protein) and/or a protein molecule (a protein-binding protein). In the case of a protein-binding protein, it can bind to itself (to form homodimers, homotrimers, etc.) and/or it can bind to one or more molecules of a different protein or proteins. A binding protein can have more than one type of binding activity. For example, zinc finger proteins have DNA-binding, RNA-binding and protein-binding activity.

[0155] The term “sequence” refers to a nucleotide sequence of any length, which can be DNA or RNA; can be linear, circular or branched and can be either single-stranded or double stranded. The term “donor sequence” refers to a nucleotide sequence that is inserted into a genome. A donor sequence can be of any length, for example between 2 and 10,000 nucleotides in length (or any integer value therebetween or thereabove), preferably between about 100 and 1,000 nucleotides in length (or any integer therebetween), more preferably between about 200 and 500 nucleotides in length.

[0156] A “gene,” for the purposes of the present disclosure, includes a DNA region encoding a gene product (see *infra*), as well as all DNA regions which regulate the production of the gene

product, whether or not such regulatory sequences are adjacent to coding and/or transcribed sequences. Accordingly, a gene includes, but is not necessarily limited to, promoter sequences, terminators, translational regulatory sequences such as ribosome binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites and locus control regions.

[0157] “Chimeric Notch receptor” also referred to as “Chimeric Notch receptor,” or “chimeric Notch receptor” or “synthetic Notch receptor” (synNotch receptor) as described in international patent publications WO16138034 and WO18236825, comprises, from N-terminal to C-terminal and in covalent linkage: a) an extracellular ligand binding domain, for example an antigen binding domain, that specifically binds an antigen present on the surface of a cells, such prostate specific membrane antigen (PSMA); b) wherein the synNotch receptor polypeptide has a length of from 50 amino acids to 1000 amino acids, and comprises one or more ligand-binding inducible proteolytic cleavage sites; and c) an intracellular domain, wherein the ligand binding domain is heterologous to the synNotch receptor polypeptide, and wherein binding of ligand to ligand binding domain induces cleavage of the synNotch receptor polypeptide at the one or more ligand binding-inducible proteolytic cleavage sites, thereby releasing the intracellular domain. In some cases, the synNotch receptor polypeptide has a length of from 300 amino acids to 400 amino acids.

[0158] In embodiments a synNotch receptor polypeptide comprises a linker interposed between the extracellular ligand binding domain and the Notch receptor polypeptide. In embodiments the intracellular domain is a transcriptional activator, such as a transcription factor. In embodiments, the extracellular ligand binding domain comprises an antibody or antigen binding fragment thereof, that specifically binds to prostate stem cell antigen (PSCA). In some cases, where the first member of the specific binding pair is an antibody, the antibody is a single-chain Fv (scFv), such as an scFv that specifically binds to a prostate stem cell antigen (PSCA). In embodiments, the extracellular ligand binding domain is a nanobody, a single-domain antibody, a diabody, a triabody, or a minibody.

[0159] A “transmembrane domain” is a domain of a polypeptide that includes at least one contiguous amino acid sequence that traverses a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell. For example, a transmembrane domain can include one, two, three, four, five, six, seven, eight, nine, or ten contiguous amino acid sequences that each traverse a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell. A transmembrane domain can, e.g., include at least one (e.g., two, three, four, five, six, seven, eight, nine, or ten) contiguous amino acid sequence (that traverses a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell) that has α -helical secondary structure in the lipid bilayer. In some

embodiments, a transmembrane domain can include two or more contiguous amino acid sequences (that each traverse a lipid bilayer when present in the corresponding endogenous polypeptide when expressed in a mammalian cell) that form a β -barrel secondary structure in the lipid bilayer. Non-limiting examples of transmembrane domains are described herein. Additional examples of transmembrane domains are known in the art.

[0160] The phrase “extracellular side of the plasma membrane” when used to describe the location of a polypeptide means that the polypeptide includes at least one transmembrane domain that traverses the plasma membrane and at least one domain (e.g., at least one antigen-binding domain) that is located in the extracellular space.

[0161] The disclosure may employ, unless indicated specifically to the contrary, methods of chemistry, biochemistry, organic chemistry, molecular biology, microbiology, recombinant DNA techniques, genetics, immunology, and cell biology that are within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, e.g., Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual* (1982); Ausubel *et al.*, *Current Protocols in Molecular Biology* (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience; Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford, 1985); Anand, *Techniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); *Transcription and Translation* (B. Hames & S. Higgins, Eds., 1984); Perbal, *A Practical Guide to Molecular Cloning* (1984); Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998) *Current Protocols in Immunology* Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); *Annual Review of Immunology*; as well as monographs in journals such as *Advances in Immunology*.

[0162] The present disclosure provides antigen binding agents, such as antibodies, chimeric antigen receptors (CARs) and T cell receptors (TCRs) comprising at least an anti-PSMA binding domain. Among other things, the present disclosure provides methods and compositions useful for treatment of cancer and/or for initiating or modulating immune responses. In various embodiments, one or more anti-PSMA binding domain is an scFv. Exemplary anti-PSMA binding domain amino acid sequences, and nucleic acid sequences encoding the same, are provided herein, for example in Tables 4-8. In some embodiments, an antigen binding agent of the present disclosure is a chimeric antigen receptor (CAR). In some embodiments, an antigen binding agent of the present disclosure is an engineered T cell receptor (TCR). In some embodiments, the CARs and/or TCRs are expressed with a dominant negative TFG β Receptor (DN TFG β R).

[0163] The present disclosure further provides for synthetic Notch receptors (synNotch receptors) comprising at least an anti-PSCA binding domain. In various embodiments, one or more anti-PSCA binding domain is an scFv. An exemplary anti-PSCA binding domain amino acid sequences, and nucleic acid sequence encoding the same, is provided herein, for example in Table 9. In some embodiments, the synNotch receptors CARs and/or TCRs are expressed with a dominant negative TFG β Receptor.

[0164] Various embodiments of the present disclosure provide a vector encoding an anti-PSMA binding domain or antigen anti-PSMA binding agent provided herein, *e.g.*, a vector encoding an anti-PSMA CAR. Various embodiments of the present disclosure provide a vector encoding a DN TFG β R, *e.g.*, a vector encoding an anti-PSMA CAR and a DN TFG β R. In some embodiments the DN TFG β R is encoded in a separate vector from the vector encoding the anti-PSMA CAR. In some embodiments the DN TFG β R is encoded in the same vector encoding the anti-PSMA CAR. Various embodiments of the present disclosure provide a vector encoding an anti-PSCA synNotch receptor. In some embodiments the DN TFG β R is encoded in a separate vector from the vector encoding the anti-PSCA synNotch receptor. In some embodiments the DN TFG β R is encoded in the same vector encoding the anti-PSCA synNotch receptor.

[0165] Various embodiments of the present disclosure provide an anti-PSMA antigen binding agent that is a cell encoding or expressing an anti-PSMA antigen binding agent, *e.g.*, a T cell or NK cell engineered to encode or express an anti-PSMA chimeric antigen receptor or TCR. The present disclosure provides immune cells genetically modified with an integrated gene, *e.g.*, a nucleotide sequence of interest (*e.g.*, a constitutive expression construct and/or an inducible expression construct that comprises such nucleotide sequence, for example a synthetic notch (synNotch) receptor inducible expression construct, such as an anti-PSCA synNotch receptor described herein. In embodiments the immune cells are further engineered to express an anti-PSCA synNotch receptor. In embodiments, the immune cells are further engineered to express a DN TFG β R. In some embodiments, the present disclosure provides methods of treating a subject having a tumor, such as a prostate tumor, comprising administering to the subject an anti-PSMA binding agent therapy described herein and/or a protein therapeutic described herein. In some embodiments, methods further comprise administration of one or more additional therapies (*e.g.*, a second binding agent (*e.g.*, CAR-T cell, CAR-NK cell, TCR-T cell, TIL cell, allogeneic NK cell, and autologous NK cell), an antibody-drug conjugate, an antibody, a bispecific antibody, a T cell-engaging bispecific antibody, an engineered antibody, and/or a polypeptide described herein).

[0166] Other features, objects, and advantages of the present disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description,

while indicating embodiments of the present disclosure, is given by way of illustration only, not limitation.

[0167] An anti-PSMA binding domain of the present disclosure may comprise antigen-binding sequences as found in an antibody described herein. In some instances, an anti-PSMA binding domain of the present disclosure comprises an anti-PSMA binding domain described herein, such as an scFv. Unless otherwise indicated, it is to be appreciated the references to PSMA in the present disclosure relate to human PSMA. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one heavy chain CDR (HCDR) provided herein, *e.g.*, at least one HCDR disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one light chain CDR (LCDR) provided herein, *e.g.*, at least one LCDR disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three LCDRs provided herein, *e.g.*, three LCDRs disclosed in any one of Tables 4-8.

[0168] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one HCDR provided herein, *e.g.*, at least one HCDR disclosed in any one of Tables 4-8, and at least one LCDR provided herein, *e.g.*, at least one LCDR disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises one HCDR provided herein, *e.g.*, at least one HCDR disclosed in any one of Tables 4-8, and one LCDR provided herein, *e.g.*, derived from the same Table of Tables 4-8 as the HCDR(s). In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in any one of Tables 4-8, and two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in any one of Tables 4-8, and two LCDRs provided herein, *e.g.*, derived from the same Table of Tables 4-8 as the HCDR(s). In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in any one of Tables 4-8, and three LCDRs provided herein, *e.g.*, three LCDRs disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three HCDRs provided herein,

e.g., three HCDRs disclosed in any one of Tables 4-8, and three LCDRs derived from the same Table of Tables 4-8 as the HCDR(s).

[0169] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one heavy chain framework region (heavy chain FR) of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-8.

[0170] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in any one of Tables 4-8.

[0171] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one heavy chain FR of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in any one of Tables 4-8, and at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises one heavy chain FR of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in any one of Tables 4-8, and one light chain FR of a light chain variable domain disclosed herein, *e.g.*, derived from the same Table of Tables 4-8 as the heavy chain FR(s). In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-8, and two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two heavy chain FRs of a heavy

chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-8, and two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, derived from the same Table of Tables 4-8 as the heavy chain FR(s). In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-8, and three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in any one of Tables 4-8, and three light chain FRs derived from the same Table of Tables 4-8 as the heavy chain FR(s).

[0172] Exemplary antibody sequences provided in Tables 4-8 are suitable for use in any antibody format, comprising, *e.g.*, a tetrameric antibody, a monospecific antibody, a bispecific antibody, an antigen binding fragment, or a binding motif. Heavy chain variable domains and light chain variable domains and portions thereof provided in Tables 4-8 may be comprised in anti-PSMA binding domain.

[0173] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100%, *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) to corresponding FR(s) of a heavy chain variable domain of a heavy chain variable domain disclosed in in any one of Tables 4-8. In various embodiments, an anti-PSMA binding domain of the present disclosure comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100%) to corresponding FR(s) of a light chain variable domain of a light chain variable domain disclosed in any one of Tables 4-8.

[0174] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in any one of Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two heavy chain variable domains each having at least 75% sequence identity to a heavy chain variable domain disclosed in Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), which heavy chain variable domains may be same or different.

[0175] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two light chain variable domains each having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), which light chain variable domains may be same or different.

[0176] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in any one of Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-PSMA binding domain of the present disclosure comprises one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in any one of Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), where the heavy chain variable domain and light chain variable domain are optionally derived from the same Table of Tables 4-8.

[0177] In various embodiments, an anti-PSMA binding domain of the present disclosure comprises two heavy chain variable domains each having at least 75% sequence identity to a heavy chain variable domain disclosed in Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and two light chain variable domains each having at least 75% sequence identity to a light chain variable domain disclosed in Tables 4-8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), where, in various embodiments, (i) each of the heavy chain variable domains may be same or different; (ii) each of the light chain variable domains may be same or different; (iii) at least one heavy chain variable domain and at least one light chain variable domain may be derived from the same Table

of Tables 4-8; or (iv) the two heavy chain variable domains and the two light chain variable domains are all derived from the same Table of Tables 4-8. Each of Tables 4-8 represents the heavy chain variable domain and light chain variable domain sequences of an exemplary antibody, comprising (i) the heavy chain variable domain of the exemplary antibody; (ii) a DNA sequence encoding the heavy chain variable domain (iii) three heavy chain variable domain CDRs of the heavy chain variable domain, according to IMGT, Kabat, and Chothia numbering; (iv) the light chain variable domain of the exemplary antibody; (v) a DNA sequence encoding the light chain variable domain; and (vi) three light chain variable domain CDRs of the light chain variable domain, according to IMGT, Kabat, and Chothia numbering. Information provided in each table provides framework amino acid sequences, as well as nucleotide sequences encoding each CDR amino acid sequence and nucleotide sequences encoding corresponding FR amino acid sequence.

[0178] In various embodiments an anti-PSMA binding domain may comprise a heavy chain variable domain of the present disclosure (*e.g.*, having at least 75% sequence identity to a heavy chain variable domain of any one of Tables 4-8, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), a light chain variable domain of the present disclosure (*e.g.*, having at least 75% sequence identity to a light chain variable domain of any one of Tables 4-8, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), and a linker (*e.g.*, a linker according to SEQ ID NO: 126. In various embodiments an anti-PSMA binding domain may comprise a leader sequence, a heavy chain variable domain of the present disclosure (*e.g.*, having at least 75% sequence identity to a heavy chain variable domain of any one of Tables 4-8, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), a light chain variable domain of the present disclosure (*e.g.*, having at least 75% sequence identity to a light chain variable domain of any one of Tables 4-8, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), and a linker. If provided with an amino acid or nucleotide sequence of an anti-PSMA binding domain comprising a heavy chain variable domain of the present disclosure and a light chain variable domain of the present disclosure, the linker joining the two variable domains will be apparent from the sequence in view of the present disclosure. If provided with an amino acid or nucleotide sequence of an anti-PSMA binding domain comprising a heavy chain variable domain of the present disclosure and a light chain variable domain of the present disclosure, the leader sequence will be apparent in view of the present disclosure. For the avoidance of doubt, a heavy chain variable domain and a light chain variable domain of the present disclosure may be present in any orientation, *e.g.*, an orientation in which the heavy chain variable domain is C terminal of the light chain variable domain or in which the heavy chain variable domain is N terminal of the

light chain variable domain. In various embodiments an anti-PSMA binding domain may comprise a linker according to SEQ ID NO: 126.

[0179] In certain embodiments, an anti-PSMA binding domain of the present disclosure comprises an anti-PSMA binding domain that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a linker having at least 75% sequence identity to SEQ ID NO: 8 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-PSMA binding domain of the present disclosure comprises an anti-PSMA binding domain that comprises a linker according to SEQ ID NO: 126. In certain embodiments, an anti-PSMA binding domain of the present disclosure comprises an anti-PSMA binding domain that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a leader sequence having at least 75% sequence identity to SEQ ID NO: 48 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-PSMA binding domain of the present disclosure comprises an anti-PSMA binding domain that comprises a CSF2RA leader sequence according to SEQ ID NO: 137 (MLLLVTSLLLCELPHPAFLIP; SEQ ID NO: 137). In embodiments a leader sequence may be encoded by nucleic acid sequence at least 75% sequence identity to atgcttctctggtgacaagccttctgctctgtgaattgccacaccagcattctctgattcct (SEQ ID NO: 256) (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-PSMA binding domain of the present disclosure comprises an anti-PSMA binding domain that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, a linker of the present disclosure, and a leader sequence of the present disclosure.

[0180] A binding agent of the present disclosure that is based on an exemplary antibody provided herein, such as for example Abs 1-5, may be provided in any fragment or format, comprising a heavy chain variable domain according to the indicated exemplary antibody and a light chain variable domain according to the indicated exemplary antibody.

Table 4: Exemplary Antibody Sequences 1 (Ab1)

SEQ ID NO:	Description	Sequence
1	Heavy Chain Variable Domain	EVQLVESGGGLVQPGGSMRLSCAASGFTFSDYYMAWVRQAPGKGLEWIANININYDGSNTYYADSLKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARNWDGYYGYFDVWGQGTTVTVSS
2	VH (DNA)	Gaggtgcaactgtggagagcggaggaggttagtgcaacccggaggcagcatgagactgagctgcgccgccagcggcttcacattctccgactactacatggcttgggtccgacaagctcccgaa aaggactggagtggatcgccaacatcaactacgacggctccaacacactactacgccgactctta aagggtcgttcacaatctctcgtgacaacagcaagaacactttatattacaaatgaactcttaagg gccgaggataccgccgtgtactactgcgctcgtactgggacggctactacggctacttcgacgt gtggggccaaggaaccaccgtgaccgtgagcagc
3	CDRH1 IMGT (Prot)	GFTFSDYY
4	CDRH1 Kabat (Prot)	DYYMA
5	CDRH1 Chothia (Prot)	GFTFSD
6	CDRH2 IMGT (Prot)	INYDGSNT
7	CDRH2 Kabat (Prot)	NINYDGSNTYYADSLKG
8	CDRH2 Chothia (Prot)	NINYDGSNTYYADSLKG
9	CDRH3 IMGT (Prot)	ARNWDGYYGYFDV
10	CDRH3 Kabat (Prot)	NWDGYYGYFDV

11	CDRH3 Chothia (Prot)	NWDGYYGYFDV
12	Light Chain Variable Domain	DIQLTQSPSSLSASVGDRVTITCRASSSVSHIYWYQQKPGKAP KPWIYRTSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYC QQYHTYPPTFGQGKLEIK
13	VL (DNA)	Gatatccagctgacccagtccttctctctgtctgctgtgtggcgatcgtgtcaccatcactgt tcgtgccagcagcagcgtgagccacattattggtaccaacaaaagccggcaaagcccctaag ccttgatctacagaacctccaatctggccagcggcgtgccagcagattcagcgggaagcggat ccggcaccgactacacttaacctcagctctttacagccgaggacttcgccacatactactgcc agcagtaccacacctatccccccacattcggccaaggaacaaagctggagattaag
14	CDRL1 IMGT (Prot)	SSVSH
15	CDRL1 Kabat (Prot)	RASSSVSHIY
16	CDRL1 Chothia (Prot)	RASSSVSHIY
17	CDRL2 IMGT (Prot)	RTS
18	CDRL2 Kabat (Prot)	RTSNLAS
19	CDRL2 Chothia (Prot)	RTSNLAS
20	CDRL3 IMGT (Prot)	QQYHTYPPT
21	CDRL3 Kabat (Prot)	QQYHTYPPT
22	CDRL3 Chothia (Prot)	QQYHTYPPT
23	ScFv	DIQLTQSPSSLSASVGDRVTITCRASSSVSHIYWYQQKPGKAP KPWIYRTSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYC

		QQYHTYPPTFGQGTKLEIKGSTSGSGKPGSGEGSTKGEVQLVE SGGGLVQPGGSMRLSCAASGFTFSDYYMAWVRQAPGKGLE WIANINYDGSNTYYADSLKGRFTISRDN SKNTLYLQMNSLRA EDTAVYYCARNWDGYYGYFDVWGQGTTVTVSS
24	ScFv	Gatatccagctgaccagctccccttctctctgtctgctgtgttgccgatcgtgtcaccatcacttg tcgtgccagcagcagcgtgagccacattattggtaccaacaaaagcccgcaaagcccctaag ccttgatctacagaacctccaatctggccagcggcgtgccagcagattcagcgggaagcggat ccggcaccgactacacttaaccatcagctctttacagcccaggacttcgccacatactactgcc agcagtaccacacctatccccccacattcggccaaggaacaaagctggagattaagggtccac ctccggaagcggcaaaccggtagcggcgagggtccacaaagggcgaggtgcaactgtgg agagcggaggagggttagtgcaaccggaggcagcatgagactgagctgcgccgccagcggc ttcacattctcgactactacatggcttgggtccgacaagctcccgaaaaggactggagtggatc gccaacatcaactacgacggctccaacacctactacgccgactctttaagggtcgtttcacaatct ctcgtgacaacagcaagaacactttatattacaaatgaactctttaagggccgaggataccgccgt gtactactgcgctcgtaactgggacggctactacggctacttcgacgtgtggggccaaggaacca ccgtgaccgtgagcagc

Table 5: Exemplary Antibody Sequences 2 (Ab2)

SEQ ID NO:	Description	Sequence
25	Heavy Chain Variable Domain	QVQLVQSGAEVKKPGASVKLSCKASGYTFTTYWMHWVRQA PGQGLEWIGMIHPNSGSTNYAQKFQGRATLTVDTSTSTAYME LSSLRSED TAVYYCARDPYDYGEDFDVWGQGTTVTVSS
26	VH (DNA)	Caagtgcagctggtgcagtcggcgccgaggtgaagaagcccggtgcttcctgaagctgtctt gcaaagccagcggctacaccttcaccacctattggatgcactgggtccgacaagctcccggtcaa ggtctggagtggattggcatgatccacccaactccggctccaccaactacgcccagaagttcca aggctcgtgccactttaacagtggataccagcaccagcaccgcctacatggagctgagtagttga ggagcggaggacaccgccgtgtactattgcgctcgtgaccctacgactacggcgaggacttcga cgtgtggggccaaggaacaacagtgaccgtgagcagc
27	CDRH1 IMGT (Prot)	GYTFTTYW
28	CDRH1 Kabat (Prot)	TYWMH

29	CDRH1 Chothia (Prot)	GYTFTT
30	CDRH2 IMGT (Prot)	IHPNSGST
31	CDRH2 Kabat (Prot)	MIHPNSGSTNYAQKFQG
32	CDRH2 Chothia (Prot)	MIHPNSGSTNYAQKFQG
33	CDRH3 IMGT (Prot)	ARDPYDYGEDFDV
34	CDRH3 Kabat (Prot)	DPYDYGEDFDV
35	CDRH3 Chothia (Prot)	DPYDYGEDFDV
36	Light Chain Variable Domain	DIQMTQSPSSLSASVGDRVTVTCRASQNVNTNVAWYQQKPG KAPKVLISASYSRNSGVPSRFSGSGSGTDFTLTISVQPEDFAT YYCQQYNSYPFTFGQGTKLEIK
37	VL (DNA)	Gacatccagatgaccagagccccagctctttaagtgccagcgtggcgacagagtgcagtg acttgctgctgccagccagaacgtgaataccaacgtggcttggtaccagcagaagcccggaag cccctaaggtgctgatctattccgcttctatcgtaactccggcgtgccttcgcgttttctgggtctg gtagcggcaccgacttcacttaacaatcagcagcgttcagcccgaagacttcgccacctactact gccagcagtacaacagctatcccttacttccgtcaagggaagctcgagatcaaa
38	CDRL1 IMGT (Prot)	QNVNTN
39	CDRL1 Kabat (Prot)	RASQNVNTNVA
40	CDRL1 Chothia (Prot)	RASQNVNTNVA
41	CDRL2 IMGT (Prot)	MIHPNSGSTNYAQKFQG

42	CDRL2 Kabat (Prot)	SASYRNS
43	CDRL2 Chothia (Prot)	SASYRNS
44	CDRL3 IMGT (Prot)	DPYDYGEDFDV
45	CDRL3 Kabat (Prot)	QQYNSYPFT
46	CDRL3 Chothia (Prot)	QQYNSYPFT
47	ScFv	DIQMTQSPSSLSASVGDRTVTTCRASQNVNTNVAWYQQKPG KAPKVLISASYRNSGVPSRFSGSGSGTDFTLTISSVQPEDFAT YYCQQYNSYPFTFGQGTKLEIKGSTSGSGKPGSGEGSTKGQV QLVQSGAEVKKPGASVKLSCKASGYTFTTYWMHWVRQAPG QGLEWIGMIHPNSGSTNYAQKFQGRATLTVDSTSTAYMELS SLRSEDNAVYYCARDPYDYGEDFDVWGQGTITVTVSS
48	ScFv	Gacatccagatgacccagagccccagctctttaagtccagcgtggcgacagagtacagtg actgtcgtgccagccagaacgtgaataccaacgtggcttggtaccagcagaagcccggaag cccctaaggtgctgatctattccgcgtcttatcgtaactccggcgtgccttcgcgttttctgggtctg gtagcggcaccgacttcacttaacaatcagcagcgttcagcccgaagacttcgccactactact gccagcagtacaacagctatcccttactttcgggtcaagggaaccaagctcgagatcaaaggctcca ccagcggtagcggcaaacccgggtccggcgagggctctaccaagggaagtgagctggtgc agtccggcgccgaggtgaagaagcccggtgcttcgtgaagctgtcttgcaaagccagcggcta caccttcaccacatttggtgctgctgggtccgacaagctcccggtcaaggttgagtggtgattgg catgatccaccccaactccgggtccaccaactacgccagaagttccaaggtcgtgccacttaac agtggataccagcaccagcaccgcctacatggagctgagtagttgaggagcaggacaccgc cgtgtactattgcgtcgtgaccctacgactacggcgaggacttcgacgtgtggggccaaggaa caacagtgaccgtgagcagc

Table 6: Exemplary Antibody Sequences 3 (Ab3)

SEQ ID NO:	Description	Sequence
49	Heavy Chain Variable Domain	EVQLVESGGGLVQPGGSMRLSCAASGFTFSDYYMAWVRQAPGKGLEWVANINYDGTSTYYADSLKGRFTISRDSKNTLYLQMNSLRAEDTAVYYCARALDGYGYLDVWGQGTTVTVSS
50	VH (DNA)	Gaggtgcagctggtagtccggaggaggttagtccaacccggtggcagcatgaggctgtctt gtgctgcctccggcttcactttttctgattactacatggcttgggtccgacaagctcccggaaaaggt ttagagtgggtggctaacaatcaactacgacggcaccagcacctactatgccgacagcctcaagg gcagattcaccatctctcgtgattcgtctaaaaacactttatattacaaatgaactcttaagagccga agataccgccgtgtactattgcgctcgtgccctcgacggctactacggatatttagacgtgtggggt caaggaacaaccgtgaccgtgtccagc
51	CDRH1 IMGT (Prot)	GFTFSDYY
52	CDRH1 Kabat (Prot)	DYYMA
53	CDRH1 Chothia (Prot)	GFTFSD
54	CDRH2 IMGT (Prot)	INYDGTST
55	CDRH2 Kabat (Prot)	NINYDGTSTYYADSLKG
56	CDRH2 Chothia (Prot)	NINYDGTSTYYADSLKG
57	CDRH3 IMGT (Prot)	ARALDGYGYLDV
58	CDRH3 Kabat (Prot)	ALDGYGYLDV
59	CDRH3 Chothia (Prot)	ALDGYGYLDV

60	Light Chain Variable Domain	DIQLTQSPSSLSASVGDRVLTLCRASQSSISNNLHWYQQKPGKA PKLLIKYVSQSIGIPSRFSGSLGTDFLTISVQPEDFATYYC QQSNSWPYTFGQGTKLEIK
61	VL (DNA)	Gacatccagctgaccagagccctagctcttaagcgctagcgtgggcgatagggtgactctga ctgtcgtgcgtcccaaagcattagcaacaatttacactgggtaccagcagaagcccgaaaagcc cccaagctgctgatcaaatatgtgagccagagcatctccggcatcccctctcgttttctggtagcg gactgggcaccgactttactttaacctcagcagcgtccagcccaggacttcgccacatactact gccagcagagcaacagctggccctatacttctcgccaaggaacaaagctggagatcaag
62	CDRL1 IMGT (Prot)	QSISNN
63	CDRL1 Kabat (Prot)	RASQSSISNNLH
64	CDRL1 Chothia (Prot)	RASQSSISNNLH
65	CDRL2 IMGT (Prot)	YVS
66	CDRL2 Kabat (Prot)	YVSQSSIS
67	CDRL2 Chothia (Prot)	YVSQSSIS
68	CDRL3 IMGT (Prot)	QQSNSWPYT
69	CDRL3 Kabat (Prot)	QQSNSWPYT
70	CDRL3 Chothia (Prot)	QQSNSWPYT
71	ScFv	EVQLVESGGGLVQPGGSMRLSCAASGFTFSDYYMAWVRQAP GKGLEWVANINYDGTSTYYADSLKGRFTISRDSKNTLYLQM NSLRAEDTAVYYCARALDGYYG YLDVWGQGTTVTVSSGSTS GSGKPGSGEGSTKGDIQLTQSPSSLSASVGDRVLTLCRASQSSIS

		NNLHWYQQKPGKAPKLLIKYVSQSIGIPSRFSGSGLGTDFTL TISSVQPEDFATYYCQQSNSWPYTFGQGTKLEIK
72	ScFv	gaggtgcagctgggtggagtcggaggaggttagtccaacccggtggcagcatgaggctgtctt gtgctgcctccggcttcacttttctgattactacatggcttgggtccgacaagctcccgaaaaggt ttagagtgggtggctaactcaactacgacggcaccagcacctactatgccgacagcctcaagg gcagattcaccatctctcgtgattcgtctaaaaacactttatattacaaatgaactctttaagaccga agataccgccgtgtactattgcgctcgtgccctcgacggctactacggatatttagacgtgtgggt caaggaacaaccgtgaccgtgtccagcggatccacctccggaagcggcaaacccggtagcgg cgaaggcagcaccaaaggagacatccagctgaccagagccctagctctttaagcgttagcgtg ggcgatagggtgactctgacttgcgtgcgtcccaaagcattagcaacaattacactggtaccag cagaagcccgaaaagccccaagctgctgatcaaatatgtgagccagagcatctccggcatcc cctctcgttttctggtacggactgggcaccgactttactttaaccatcagcagcgtccagcccga ggacttcgccacatactactgccagcagagcaacagctggccctatactttcgccaaggaacaa agctggagatcaag

Table 7: Exemplary Antibody Sequences 4 (Ab4)

SEQ ID NO:	Description	Sequence
73	Heavy Chain Variable Domain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYYMAWVRQAP GKGLEWVANINYDGSSTFYADSLKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCGRQVGYYDPM DYWGQGTTVTVSS
74	VH (DNA)	Gaggtgcagttgggtggagagcggaggaggactggtgcagcccggtggctctttaagactcagc tgtgccgccagcggatttacattctccgactactacatggcttgggtccgacaagccccggaaaa ggttttagagtgggtggccaacatcaactacgacggctcctccacattctacgccactctttaag ggctcgtttcaccatctctcgtgacaacagcaaaaatactttatattacaaatgaactctttaagggcc gaggacaccgccgtgtactactcggtcgtcaagttggctattacgcccattggactactgggg ccaaggaactaccgtgaccgtgagcagc
75	CDRH1 IMGT (Prot)	GFTFSDYY
76	CDRH1 Kabat (Prot)	DYYMA

77	CDRH1 Chothia (Prot)	GFTFSD
78	CDRH2 IMGT (Prot)	INYDGSST
79	CDRH2 Kabat (Prot)	NINYDGSSTFYADSLKG
80	CDRH2 Chothia (Prot)	NINYDGSSTFYADSLKG
81	CDRH3 IMGT (Prot)	GRQVGYYDPMDY
82	CDRH3 Kabat (Prot)	QVGYYDPMDY
83	CDRH3 Chothia (Prot)	QVGYYDPMDY
84	Light Chain Variable Domain	DIQLTQSPSSLSASVGDRVITTCRASSSVSHMYWYQQKPGKAP KPWIYRTSNLASGVPSRFSGSGSGTDYTLTISSMQPEDFATYY CQQYHSYPLTFGQGGTKLEIK
85	VL (DNA)	Gacatccagctgaccagtcctccagctcttatccgctagcgtggcgatagggtgaccatcac ttgtcgtgcgtcttcgtctgtgtctcatatgtactgggtaccagcagaagcccggaaggccccaag ccttgatctatcgtacatccaatcttgcaagcggcgctcccttctcgttttctggttccgggtctgga ccgactacacttaaccatcagcagcatgcagcccgaggacttcgccactactactgccagcag tatcactctatcctttaacttttgccaaggaacaaagtggagatcaag
86	CDRL1 IMGT (Prot)	SSVSH
87	CDRL1 Kabat (Prot)	RASSSVSHMY
88	CDRL1 Chothia (Prot)	RASSSVSHMY
89	CDRL2 IMGT (Prot)	RTS

90	CDRL2 Kabat (Prot)	RTSNLAS
91	CDRL2 Chothia (Prot)	RTSNLAS
92	CDRL3 IMGT (Prot)	QQYHSYPLT
93	CDRL3 Kabat (Prot)	QQYHSYPLT
94	CDRL3 Chothia (Prot)	QQYHSYPLT
95	ScFv	DIQLTQSPSSLSASVGDRVITITCRASSSVSHMYWYQQKPGKAP KPWIYRTSNLASGVPSRFSGSGSGTDYTLTISSMQPEDFATYY CQQYHSYPLTFGQGTKLEIKGSTSGSGKPGSGEGSTKGEVQL VESGGGLVQPGGSLRLSCAASGFTFSDYYMAWVRQAPGKGL EWVANINYDGSSTFYADSLKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCGRQVGYYDPMDYWGQGTTVTVSS
96	ScFv	Gacatccagctgacccagtccttccgctagcgtggcgatagggtgaccatcac ttgtcgtgctctctgtctgtctcatatgtactggtagcagaagcccggaaggccccaag ccttgatctatcgtacatccaatcttgcaagcggcgctccctctcgttttctgggtccgggtctgga ccgactacactttaaccatcagcagcatgcagcccaggacttcgccactactactgccagcag tatcactcctatcctttaacttttggccaaggaacaaagttggagatcaagggcagcacctccgga gcggaaagcccgtagcggcgagggcagcaccaaggagaggtgcagttgggtggagagcgg aggaggactggtgcagcccgtggctctttaagactcagctgtgccgccagcggattacattctc cgactactacatggcttgggtccgacaagcccccggaaaaggttagagtgggtggccaacatca actacgacggctcctccacattctacgccgactctttaaagggtcgttcacatctctcgtgacaac agcaaaaatactttatattacaatgaactctttaagggccgaggacaccgccgtgtactactgcg gtcgtcaagttggctattacgacccatggactactggggccaaggaaactaccgtgaccgtgagc agc

Table 8: Exemplary Antibody Sequences 5 (Ab5)

SEQ ID NO:	Description	Sequence
97	Heavy Chain Variable Domain	EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASG KGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELS SLRSEDTAVYYCAAGWNFDYWGQGTTVTVSS
98	VH (DNA)	Gaagtcaacttgctgcaaagcggggcagaagtgaaaaacccggggcgagcgttaaaatatctt gtaaaacaagtggtacaccttcacggagtacaccatccactgggttaacaagcttctggaaag ggacttgaatggatcggaacataaaccccaacaatgggggcactactataatcaaaagttgag gatcgggctacccctcacagtggataagtccacctccacagcttatatggaattgagtagccttagg agcgaggatacagccgtttattattgtgcggcgggctggaacttgactattgggggcaaggagac gacggtgacggtgtcctcc
99	CDRH1 IMGT (Prot)	GYTFTEYT
100	CDRH1 Kabat (Prot)	EYTIH
101	CDRH1 Chothia (Prot)	GYTFTE
102	CDRH2 IMGT (Prot)	INPNNGGT
103	CDRH2 Kabat (Prot)	NINPNNGGTTYNQKFED
104	CDRH2 Chothia (Prot)	NINPNNGGTTYNQKFED
105	CDRH3 IMGT (Prot)	AAGWNFDY
106	CDRH3 Kabat (Prot)	GWNFDY
107	CDRH3 Chothia (Prot)	GWNFDY

108	Light Chain Variable Domain	DIVMTQSPSSLSASVGDRVITITCKASQDVGTAVDWYQQKPGK APKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISLQPEDFAD YFCQQYNSYPLTFGGGTKLEIK
109	VL (DNA)	Gacattgtgatgactcagtctccttcttcttccgcttccgttggggaccgctcactataacttgta aagcgtcccaagatgtcggcaccgccgttgactggtaccagcaaaaaccgggaaagcgccga aactgctcatctactgggcttcaaccgccacacgggtgtcccggaccggttacggggagcggt agtggaaaccgatttcactctgaccatttctccttcaaccggaagattcgctgactactttgtcaa caataataattcatatcccctcacttccggagggggcacgaagtggaaataaag
110	CDRL1 IMGT (Prot)	QDVGTA
111	CDRL1 Kabat (Prot)	KASQDVGTAVD
112	CDRL1 Chothia (Prot)	KASQDVGTAVD
113	CDRL2 IMGT (Prot)	WAS
114	CDRL2 Kabat (Prot)	WASTRHT
115	CDRL2 Chothia (Prot)	WASTRHT
116	CDRL3 IMGT (Prot)	QQYNSYPLTF
117	CDRL3 Kabat (Prot)	QQYNSYPLT
118	CDRL3 Chothia (Prot)	QQYNSYPLT
119	ScFv	DIVMTQSPSSLSASVGDRVITITCKASQDVGTAVDWYQQKPGK APKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISLQPEDFAD YFCQQYNSYPLTFGGGTKLEIKGSTSGSGKPGSGEGSTKGEVQ LVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGL

		EWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLR SEDTAVYYCAAGWNFDYWGQGTITVTVSS
120	ScFv	Gacattgtgatgactcagtctccttctcttccgctccgtggggaccgcgcactataacttgta aagcgctcccaagatgtcggcaccgccgttgactggtaccagcaaaaacccgggaaagcgccga aactgctcatctactgggcttcaaccgccacacgggtgtcccgaccggttacggggagcgggt agtggaaaccgatttactctgaccatttcccttcaaccggaagatttcgctgactacttttgtaa caatataattcatatcccctcactttcggagggggcacgaagtggaaataaagggtagcacctct ggtagcggcaagcctggctctggcgagggtagtagcaaaaggagaagtcaacttgtaaagcg gggcagaagtgaataaacccggggcgagcggttaaaatatctgtaaaacaagtggctacaccttc acggagtacaccatccactgggttaacaagcttctgaaagggaactggaatggatcgggaaacat aaacccaacaatgggggcactactataatcaaaagtttgaggatcgggctacccctcacagtgg ataagtccacctccacagcttatggaattgagtagccttaggagcgaggatacagccgtttattat tgtgcggcgggctggaactttgactattgggggcaaggacgacggtgacggtgtcctcc

[0181] Chimeric antigen receptors (CARs) are engineered receptors that may direct or redirect T cells or NK cells (*e.g.*, patient or donor T or NK cells) to target a selected antigen. A CAR may be engineered to recognize an antigen and, when bound to that antigen, activate the immune cell to attack and destroy the cell bearing that antigen. When these antigens exist on tumor cells, an immune cell that expresses the CAR may target and kill the tumor cell. CARs generally comprise an extracellular binding motif that mediates antigen binding (*e.g.*, an anti-PSMA binding domain), a transmembrane domain that spans, or is understood to span, the cell membrane when the CAR is present at a cell surface or cell membrane, and an intracellular (or cytoplasmic) signaling domain.

[0182] According to at least one non-limiting view, there have been at least three “generations” of CAR compositions. In a first generation of CARs, a binding motif (*e.g.*, a single chain fragment variable, binding motif) is linked or connected to a signaling domain (*e.g.*, CD3 ζ) via a transmembrane domain, optionally comprising a hinge domain and one or more spacers. In a second generation of CARs, a costimulatory domain (CM1, such as CD28, 4-1BB, or OX-40) is introduced with the signaling domain (*e.g.*, CD3 ζ). In a third generation of CARs, a second costimulatory domain (CM2) is comprised.

[0183] TCRs are heterodimers composed of an α -chain and a β -chain. TCR signaling requires recruitment of signaling proteins that generate an immune synapse. In addition, TCR localization at the plasma membrane depends on CD3 complex, which is expressed in T cells. Engineered single chain TCRs may be generated, *e.g.*, using transmembrane and signaling domains of CAR constructs, methods and constructs for which are known (*e.g.*, sTCR and TCR-CAR molecules,

e.g., fusion of a TCR β chain with CD28 TM and CD28 and CD3 ζ signaling modules). An anti-PSMA binding system of the present disclosure may comprise one or more antigen binding motifs that bind PSMA. In some embodiments, an antigen binding system further comprises a costimulatory domain, and/or an extracellular domain (*e.g.*, a "hinge" or "spacer" region), and/or a transmembrane domain, and/or an intracellular (signaling) domain, and/or a CD3-zeta or CD3-epsilon activation domain. In some embodiments, an anti-PSMA binding system of the present disclosure comprises at least a binding motif that binds human PSMA, a costimulatory domain, an extracellular domain, a transmembrane domain, and a CD3-zeta or CD3-epsilon activating domain.

[0184] In some embodiments, an anti-PSMA CAR of the present disclosure may comprise an antigen binding system that comprises one or more, or all, of a leader peptide (P), an anti-PSMA binding domain (B), a costimulatory protein's extracellular domain (E), a transmembrane domain (T), a costimulatory domain (C), a second costimulatory domain (C'), and an activation domain (A). In some instances, an anti-PSMA CAR is configured according to the following: B E T A. In some instances, an anti-PSMA CAR is configured according to the following: P B E T A. In some instances, an anti-PSMA CAR is configured according to the following: B E T C A. In some instances, an anti-PSMA CAR is configured according to the following: P B E T C A. In some instances, an anti-PSMA CAR is configured according to the following: B E T C C' A. In some instances, an anti-PSMA CAR is configured according to the following: P B E T C C' A. In some embodiments, the an anti-PSMA CAR comprises a VH and a VL, optionally wherein the CAR is configured according to the following: P-VH-VL-E-T-C-A or P-VL-VH-E-T-C-A. In some embodiments, the VH and the VL are connected by a linker (L), optionally wherein the CAR is configured according to the following, from N-terminus to C-terminus: P-VH-L-VL-E-T-C-A or P-VH-L-VL-E-T-C-A.

[0185] One or more antigen binding motifs determine the target(s) of an antigen binding system. A binding motif of an antigen binding system may comprise any an anti-PSMA binding domain, *e.g.*, an antibody provided by the present disclosure, *e.g.*, a binding motif of the present disclosure. Binding domain are used in chimeric antigen receptors at least in part because they may be engineered to be expressed as part of a single chain along with the other CAR components. See, for example, U.S. Pat. Nos. 7,741,465, and 6,319,494 as well as Eshhar et al., Cancer Immunol Immunotherapy (1997) 45: 131-136, Krause et al., J. Exp. Med., Volume 188, No. 4, 1998 (619-626); Finney et al., Journal of Immunology, 1998, 161: 2791-2797, each of which is incorporated herein by reference with respect to binding domains in CARs. A binding domain or scFv, is a single chain antigen binding fragment comprising a heavy chain variable domain and a light chain variable domain, which heavy chain variable domain and light chain variable domain are linked

or connected together. See, for example, U.S. Pat. Nos. 7,741,465, and 6,319,494 as well as Eshhar et al., *Cancer Immunol Immunotherapy* (1997) 45: 131-136, each of which is incorporated herein by reference with respect to binding motif domains. When derived from a parent antibody, a binding motif may retain some of, retain all of, or essentially retain the parent antibody's binding of a target antigen. In some embodiments, a CAR contemplated herein comprises antigen-specific binding domain that may be a scFv (a murine, human or humanized scFv) that binds an antigen expressed on a cancer cell. In a certain embodiment, the scFv binds PSMA.

[0186] In certain embodiments, the CARs contemplated herein may comprise linker residues between the various domains, e.g., between VH and VL domains, added for appropriate spacing conformation of the molecule. CARs contemplated herein, may comprise one, two, three, four, or five or more linkers. In some embodiments, the length of a linker is about 1 to about 25 amino acids, about 5 to about 20 amino acids, or about 10 to about 20 amino acids, or any intervening length of amino acids. In some embodiments, the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more amino acids long.

[0187] Illustrative examples of linkers include glycine polymers (G)_n; glycine-serine polymers (G₁₋₅S₁₋₅)_n (SEQ ID NO: 257), where n is an integer of at least one, two, three, four, or five; glycine-alanine polymers; alanine-serine polymers; and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between domains of fusion proteins such as the CARs described herein. Glycine accesses more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Other linkers contemplated herein include Whitlow linkers (see Whitlow, *Protein Eng.* 6(8): 989-95 (1993)). The ordinarily skilled artisan will recognize that design of a CAR in some embodiments may include linkers that are all or partially flexible, such that the linker may include a flexible linker as well as one or more portions that confer less flexible structure to provide for a desired CAR structure. In one embodiment, any of the constructs described herein may comprise a "GS" linker. In another embodiment, any of the constructs described herein comprise a "GSG" linker. In an example a glycine-serine linker comprises or consists of the amino acid sequence GS (SEQ ID NO: 121), which may be encoded by the nucleic acid sequence according to ggatcc (SEQ ID NO: 122) or ggggtcc (SEQ ID NO: 123). In an example a glycine-serine linker comprises or consists of the amino acid sequence GGGSGGGS (SEQ ID NO: 124), which may be encoded by the nucleic acid sequence according to ggcggtggaagcggaggaggttcc (SEQ ID NO: 125). In another embodiment, the CARs described herein comprise the amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) of SEQ ID NO: 126

(GSTSGSGKPGSGEGSTKG (SEQ ID NO: 126). In an embodiment, a linker is encoded by a nucleic acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid sequence according to ggctccacctccggaagcggcaaacccggtagcggcgagggtccacaaagggc (SEQ ID NO: 127)

[0188] In embodiments, a CAR comprises a scFv that further comprises a variable region linking sequence. A "variable region linking sequence," is an amino acid sequence that connects a heavy chain variable region to a light chain variable region and provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. In one embodiment, the variable region linking sequence is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more amino acids long.

[0189] In embodiments, the binding domain of the CAR is followed by one or more "spacer domains," which refers to the region that moves the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation (Patel *et al.*, *Gene Therapy*, 1999; 6: 412–419). The spacer domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. In certain embodiments, a spacer domain is a portion of an immunoglobulin, including, but not limited to, one or more heavy chain constant regions, *e.g.*, CH2 and CH3. The spacer domain may include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region.

[0190] The binding domain of the CAR may generally be followed by one or more "hinge domains," which plays a role in positioning the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation. A CAR generally comprises one or more hinge domains between the binding domain and the transmembrane domain. The hinge domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The hinge domain may include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region.

[0191] In some embodiments, an Antigen binding system of the present disclosure may comprise a hinge that is, is from, or is derived from (*e.g.*, comprises all or a fragment of) an immunoglobulin-like hinge domain. In some embodiments, a hinge domain is from or derived from an immunoglobulin. In some embodiments, a hinge domain is selected from the hinge of IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, or IgM, or a fragment thereof.

[0192] A hinge may be derived from a natural source or from a synthetic source. Hinge domains suitable for use in the CARs described herein include the hinge region derived from the

extracellular regions of type 1 membrane proteins such as CD8 α , CD4, CD28 and CD7, which may be wild-type hinge regions from these molecules or may be altered, for example a truncated CD28 hinge domain. A hinge may be derived from a natural source or from a synthetic source. In some embodiments, an Antigen binding system of the present disclosure may comprise a hinge that is, is from, or is derived from (*e.g.*, comprises all or a fragment of) CD2, CD3 delta, CD3 epsilon, CD3 gamma, CD4, CD7, CD8 α , CD8 β , CD11a (ITGAL), CD11b (ITGAM), CD11c (ITGAX), CD11d (ITGAD), CD18 (ITGB2), CD19 (B4), CD27 (TNFRSF7), CD28, CD28T, CD29 (ITGB1), CD30 (TNFRSF8), CD40 (TNFRSF5), CD48 (SLAMF2), CD49a (ITGA1), CD49d (ITGA4), CD49f (ITGA6), CD66a (CEACAM1), CD66b (CEACAM8), CD66c (CEACAM6), CD66d (CEACAM3), CD66e (CEACAM5), CD69 (CLEC2), CD79A (B-cell antigen receptor complex-associated alpha chain), CD79B (B-cell antigen receptor complex-associated beta chain), CD84 (SLAMF5), CD96 (Tactile), CD100 (SEMA4D), CD103 (ITGAE), CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD158A (KIR2DL1), CD158B1 (KIR2DL2), CD158B2 (KIR2DL3), CD158C (KIR3DP1), CD158D (KIRDL4), CD158F1 (KIR2DL5A), CD158F2 (KIR2DL5B), CD158K (KIR3DL2), CD160 (BY55), CD162 (SELPLG), CD226 (DNAM1), CD229 (SLAMF3), CD244 (SLAMF4), CD247 (CD3-zeta), CD258 (LIGHT), CD268 (BAFFR), CD270 (TNFSF14), CD272 (BTLA), CD276 (B7-H3), CD279 (PD-1), CD314 (NKG2D), CD319 (SLAMF7), CD335 (NK-p46), CD336 (NK-p44), CD337 (NK-p30), CD352 (SLAMF6), CD353 (SLAMF8), CD355 (CRTAM), CD357 (TNFRSF18), inducible T cell co-stimulator (ICOS), LFA-1 (CD11a/CD18), NKG2C, DAP-10, ICAM-1, NKp80 (KLRF1), IL-2R beta, IL-2R gamma, IL-7R alpha, LFA1-1, SLAMF9, LAT, GADS (GrpL), SLP-76 (LCP2), PAG1/CBP, a CD83 ligand, Fc gamma receptor, MHC class 1 molecule, MHC class 2 molecule, a TNF receptor protein, an immunoglobulin protein, a cytokine receptor, an integrin, activating NK cell receptors, or Toll ligand receptor, or which is a fragment or combination thereof. In certain embodiments, a CAR does not comprise a CD28 hinge. In embodiments, the hinge domain comprises a CD8 α hinge region. In embodiments the CARs described herein comprise a hinge domain from CD8 α having the amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) of SEQ ID NO: 129 (TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 129)). In embodiments, hinge domain from CD8 α is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) to the nucleic acid having the sequence according to

accacgacgccagcgccgcgaccaccaacaccggcgccaccatcgcgctcgcaacccctgtccctgcgccccgaggcgtgccggcc
agcggcgggggcgcgagtgacacgagggggctggacttcgcctgtgat (SEQ ID NO: 130).

[0193] Polynucleotide and polypeptide sequences of these hinge domains are known. In some embodiments, the polynucleotide encoding a hinge domain comprises a nucleotide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a nucleotide sequence known. In some embodiments, the polypeptide sequence of a hinge domain comprises a polypeptide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a known polypeptide sequence.

[0194] In general, a “transmembrane domain” (e.g., of an antigen binding system) refers to a domain having an attribute of being present in the membrane when present in a molecule at a cell surface or cell membrane (e.g., spanning a portion or all of a cellular membrane). A costimulatory domain for an antigen binding system of the present disclosure may further comprise a transmembrane domain and/or an intracellular signaling domain. It is not required that every amino acid in a transmembrane domain be present in the membrane. For example, in some embodiments, a transmembrane domain is characterized in that a designated stretch or portion of a protein is substantially located in the membrane. Amino acid or nucleic acid sequences may be analyzed using a variety of algorithms to predict protein subcellular localization (e.g., transmembrane localization). The programs psort (PSORT.org) and Prosite (prosite.expasy.org) are exemplary of such programs.

[0195] The type of transmembrane domain comprised in an antigen binding system described herein is not limited to any type. In some embodiments, a transmembrane domain is selected that is naturally associated with a binding motif and/or intracellular domain. In some instances, a transmembrane domain comprises a modification of one or more amino acids (e.g., deletion, insertion, and/or substitution), e.g., to avoid binding of such domains to a transmembrane domain of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0196] A transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, a domain may be derived from any membrane-bound or transmembrane protein. Exemplary transmembrane domains may be derived from (e.g., may comprise at least a transmembrane domain of) an alpha, beta or zeta chain of a T-cell receptor,

CD28, CD3 epsilon, CD3 delta, CD3 gamma, CD45, CD4, CD5, CD7, CD8, CD8 alpha, CD8beta, CD9, CD11a, CD11b, CD11c, CD11d, CD16, CD22, CD27, CD33, CD37, CD64, CD80, CD86, CD134, CD137, TNFSFR25, CD154, 4-1BB/CD137, activating NK cell receptors, an Immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD276 (B7-H3), CD29, CD30, CD40, CD49a, CD49D, CD49f, CD69, CD84, CD96 (Tactile), CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LFA-1, a ligand that binds with CD83, LIGHT, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1; CD1-1a/CD18), MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), Signaling Lymphocytic Activation Molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Ly108), SLAMF7, SLP-76, TNF receptor proteins, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a fragment, truncation, or a combination thereof. In some embodiments, a transmembrane domain may be synthetic (and can, *e.g.*, comprise predominantly hydrophobic residues such as leucine and valine). In some embodiments, a triplet of phenylalanine, tryptophan and valine are comprised at each end of a synthetic transmembrane domain. In some embodiments, a transmembrane domain is directly linked or connected to a cytoplasmic domain. In some embodiments, a short oligo- or polypeptide linker (*e.g.*, between 2 and 10 amino acids in length) may form a linkage between a transmembrane domain and an intracellular domain. In some embodiments, a linker is a glycine-serine doublet. In embodiments the CARs described herein comprise a TM domain from CD8 α having the amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) of SEQ ID NO: 131 (IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 131)). In embodiments, TM domain from CD8 α is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid having the sequence according to atctacatctgggcgcccttgccgggactgtggggctctctctgtcactggttatccctttattgc (SEQ ID NO: 132).

[0197] Polynucleotide and polypeptide sequences of transmembrane domains provided herein are known. In some embodiments, the polynucleotide encoding a transmembrane domain comprises a nucleotide sequence at least about 60%, at least about 65%, at least about 70%, at least about

75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a nucleotide sequence known. In some embodiments, the polypeptide sequence of a transmembrane domain comprises a polypeptide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a polypeptide sequence known. Optionally, short spacers may form linkages between any or some of the extracellular, transmembrane, and intracellular domains of the CAR.

[0198] Intracellular signaling domains that may transduce a signal upon binding of an antigen to an immune cell are known, any of which may be comprised in an antigen binding system of the present disclosure. For example, cytoplasmic sequences of a T cell receptor (TCR) are known to initiate signal transduction following TCR binding to an antigen (see, *e.g.*, Brownlie et al., *Nature Rev. Immunol.* 13:257-269 (2013)).

[0199] In some embodiments, CARs contemplated herein comprise an intracellular signaling domain. An "intracellular signaling domain," refers to the part of a CAR that participates in transducing the message of effective CAR binding to a target antigen into the interior of the immune effector cell to elicit effector cell function, *e.g.*, activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound target cell, or other cellular responses elicited with antigen binding to the extracellular CAR domain. In some embodiments, a signaling domain and/or activation domain comprises an immunoreceptor tyrosine-based activation motif (ITAM). Examples of ITAM containing cytoplasmic signaling sequences comprise those derived from TCR zeta, FcR gamma, FcR beta, CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d (see, *e.g.*, Love et al., *Cold Spring Harb. Perspect. Biol.* 2:a002485 (2010); Smith-Garvin et al., *Annu. Rev. Immunol.* 27:591-619 (2009)). In certain embodiments, suitable signaling domains comprise, without limitation, 4-1BB/CD137, activating NK cell receptors, an Immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE,

ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LFA-1, ligand that binds with CD83, LIGHT, LIGHT, LTBR, Ly9 (CD229), Ly108), lymphocyte function-associated antigen-1 (LFA-1; CD1-1a/CD18), MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), Signaling Lymphocytic Activation Molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A, SLAMF7, SLP-76, TNF receptor proteins, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a fragment, truncation, or a combination thereof.

[0200] The term "effector function" refers to a specialized function of the cell. Effector function of the T cell, for example, may be cytolytic activity or help or activity including the secretion of a cytokine. Thus, the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and that directs the cell to perform a specialized function. While usually the entire intracellular signaling domain may be employed, in many cases it is not necessary to use the entire domain. To the extent that a truncated portion of an intracellular signaling domain is used, such truncated portion may be used in place of the entire domain as long as it transduces the effector function signal. The term intracellular signaling domain is meant to include any truncated portion of the intracellular signaling domain sufficient to transducing effector function signal.

[0201] It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or costimulatory signal may also be required. Thus, T cell activation may be said to be mediated by two distinct classes of intracellular signaling domains: primary signaling domains that initiate antigen-dependent primary activation through the TCR (e.g., a TCR/CD3 complex) and costimulatory signaling domains that act in an antigen independent manner to provide a secondary or costimulatory signal. In some embodiments, a CAR contemplated herein comprises an intracellular signaling domain that comprises one or more "costimulatory signaling domain" and a "primary signaling domain."

[0202] Illustrative examples of ITAM containing primary signaling domains that are useful in the present disclosure include those derived from TCR ζ , FcR γ , FcR β , DAP12, CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD22, CD79a, CD79b, and CD66d. In some embodiments, a CAR comprises a CD3 ζ primary signaling domain and one or more costimulatory signaling domains. The intracellular primary signaling and costimulatory signaling domains may be linked in any order in tandem to the carboxyl terminus of the transmembrane domain. In one embodiment, the CARs have a CD3 ζ domain having the amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) of SEQ ID NO: 133.

LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO: 133). In embodiments, a CD3 ζ domain is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid having the sequence according to
ttgagagtgaagttcagcaggagcgcagacgccccgcctatcagcaaggccagaaccagctctataacgagctcaatttagggcgaag
agaggagtacgatgttttgacaagaggcgtggccgggacccgaaatgggggaaagccgagaaggaagaacacctcaggaaggctt
gtacaatgaattgcagaaggataagatggcggaggcatacagtgagattgggatgaaaggcgagcgccggaggggcaaggggcacg
atggcctttatcaggtctcagtagcagccaccaaggacacctacgacgcccttcacatgcaagccctgccccctcgc (SEQ ID NO: 134).

[0203] CARs contemplated herein comprise one or more costimulatory signaling domains to enhance the efficacy and expansion of T cells expressing CAR receptors. As used herein, the term, "costimulatory signaling domain," or "costimulatory domain", refers to an intracellular signaling domain of a costimulatory molecule. In some embodiments, costimulatory molecules may include CD27, CD28, CD137(4-IBB), OX40 (CD134), CD30, CD40, PD-I, ICOS (CD278), CTLA4, LFA-1, CD2, CD7, LIGHT, TRIM, LCK3, SLAM, DAPIO, LAG3, HVEM, and NKD2C, and CD83. In embodiments, the CARs described herein comprise a 4-IBB costimulatory domain having the amino acid sequence of having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID NO: 135. KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE (SEQ ID NO: 135). In embodiments, a 4-IBB costimulatory domain is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid having the sequence according to , which may be encoded by the nucleic acid sequence according to

aaacggggcagaaagaaactcctgtatatattcaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtagctgcc
gatttcagaagaagaaggaggatgtgaa (SEQ ID NO: 136).

[0204] The engineered CARs described herein may also comprise an N-terminal signal peptide or tag at the N-terminus of the scFv or antigen binding domain. In one embodiment, a heterologous signal peptide may be used. The antigen binding domain or scFV may be fused to a leader or a signal peptide that directs the nascent protein into the endoplasmic reticulum and subsequent translocation to the cell surface. It is understood that, once a polypeptide containing a signal peptide is expressed at the cell surface, the signal peptide is generally proteolytically removed

during processing of the polypeptide in the endoplasmic reticulum and translocation to the cell surface. Thus, a polypeptide such as the CAR constructs described herein, are generally expressed at the cell surface as a mature protein lacking the signal peptide, whereas the precursor form of the polypeptide includes the signal peptide. Any suitable signal sequence known in the art may be used. Similarly any known tag sequence known in the art may also be used. In one embodiment a signal sequence is a CSF2RA signal sequence. In embodiments, the CARs described herein comprise a CSF2RA signal sequence having the amino acid sequence of having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to SEQ ID NO: 137; MLLLVTSLLLCELPHPAFLIP (SEQ ID NO: 137) SEQ ID MEWTWVFLFLSVTAGVHS (SEQ ID NO: 138), MALPVTALLLPLALLLHAARP (SEQ ID NO: 139).

[0205] Components of a CAR may be exchanged or “swapped” using routine techniques of biotechnology for equivalent components. To provide just a few non-limiting and partial examples, a CAR of the present disclosure may comprise a binding motif as provided herein in combination with a hinge provided herein and a costimulatory domain provided herein. In certain examples, a CAR of the present disclosure may comprise a leader sequence as provided herein together with a binding motif as provided herein in combination with a hinge provided herein and a costimulatory domain provided herein.

[0206] The present disclosure comprises conjugates in which an antibody of the present disclosure is associated with a therapeutic agent or a detectable moiety. In various embodiments, the therapeutic agent is an anti-cancer agent as provided herein. In certain embodiments, provided conjugate comprises one or more detectable moieties, *i.e.*, is “labeled” with one or more such moieties. In some such embodiments, a conjugate of the present disclosure is useful in diagnostic or imaging applications, *e.g.*, diagnosing or imaging cancer. Any of a wide variety of detectable moieties may be used in labeled antibody conjugates described herein. Suitable detectable moieties comprise, without limitation: various ligands, radionuclides; fluorescent dyes; chemiluminescent agents (such as, for example, acridinium esters, stabilized dioxetanes, and the like); bioluminescent agents; spectrally resolvable inorganic fluorescent semiconductors nanocrystals (*i.e.*, quantum dots); microparticles; metal nanoparticles (*e.g.*, gold, silver, copper, platinum, etc.); nanoclusters; paramagnetic metal ions; enzymes; colorimetric labels (such as, for example, dyes, colloidal gold, and the like); biotin; dioxigenin; haptens; and proteins for which antisera or monoclonal antibodies are available.

[0207] The present disclosure comprises nucleic acids encoding anti-PSMA binding domains provided herein. The present disclosure comprises nucleic acids encoding antibodies of the provided herein, comprising, without limitation, nucleic acids encoding anti-PSMA binding

domains. The present disclosure comprises nucleic acids encoding antigen binding systems provided herein, comprising without limitation nucleic acids encoding anti-PSMA chimeric antigen receptors. The nucleic acid sequence of SEQ ID NO: 2 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 1 and 3-11. The nucleic acid sequence of SEQ ID NO: 13 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 12 and 14-22. The nucleic acid sequence of SEQ ID NO: 24 comprises and provides exemplary nucleic acid sequence corresponding to and encoding SEQ ID NOs: 23.

[0208] The nucleic acid sequence of SEQ ID NO: 26 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 25 and 27-35. The nucleic acid sequence of SEQ ID NO: 37 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 36 and 38-46. The nucleic acid sequence of SEQ ID NO: 48 comprises and provides an exemplary nucleic acid sequence corresponding to and encoding SEQ ID NOs: 47.

[0209] The nucleic acid sequence of SEQ ID NO: 50 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 49 and 51-59. The nucleic acid sequence of SEQ ID NO: 61 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 60 and 62-70. The nucleic acid sequence of SEQ ID NO: 72 comprises and provides an exemplary nucleic acid sequence corresponding to and encoding SEQ ID NOs: 71.

[0210] The nucleic acid sequence of SEQ ID NO: 74 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 73 and 75-83. The nucleic acid sequence of SEQ ID NO: 85 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 84 and 86-94. The nucleic acid sequence of SEQ ID NO: 76 comprises and provides an exemplary nucleic acid sequence corresponding to and encoding SEQ ID NOs: 95.

[0211] The nucleic acid sequence of SEQ ID NO: 98 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 97 and 99-101. The nucleic acid sequence of SEQ ID NO: 109 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 108 and 110-118. The nucleic acid sequence of SEQ ID NO: 108 comprises and provides an exemplary nucleic acid sequence corresponding to and encoding SEQ ID NOs: 107.

[0212] In one embodiment described herein, an anti-PSMA CAR construct has an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or

95–100%) SEQ ID NO: 140.

DIQLTQSPSSLSASVGDRVTITCRASSSVSHIYWYQQKPGKAPKPKWIYRTSNLASGVPSR
FSGSGSGTDYTLTISSLQPEDFATYYCQQYHTYPPTFGQGKLEIKGSTSGSGKPGSGEG
STKGEVQLVESGGGLVQPGGSMRLSCAASGFTFSDYYMAWVRQAPGKGLEWIANINY
DGSNTYYADSLKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARNWDGYYGYFDV
WGQGTTVTVSSGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY
IWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL

PPR (SEQ ID NO: 140). In embodiments an anti-PSMA CAR is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid having the sequence according to
gatatccagctgaccagtcaccttctctctgtctgctgtgtggcgatcgtgcaccatcactgtcgtgccagcagcagcgtgagccac
atttattggtaccaacaaaagcccgcaagccctaagccttgatctacagaacctccaatctggccagcggcgtgccagcagattca
gcggaagcggatccggcaccgactacactttaaccatcagctctttacagcccgaggacttcgccacatactactgccagcagcagcagc
ctatccccccacattcgccaaggaacaaagctggagattaagggtccacctccggaagcggcaaacccgtagcggcgagggtcc
acaaaggcgaggtgcaacttggagagcggaggaggttttagtgcaaccggaggcagcatgagactgagctgcgccgccagcggc
ttcacattctccgactactacatggcttgggtccgacaagctcccgaaaaggactggagtggatcgccaacatcaactacgacggtcca
acacactactacgacgactctttaagggtcgttcacatctctcgtgacaacagcaagaacactttatattacaatgaactctttaagggtcc
gaggataccgcccgtgtactactgcgctcgttaactgggacggctactacggctacttcgacgtgtggggccaaggaaccaccgtgaccgtg
agcagcgggtccaccacgacgcccagcggcgaccaccaacaccggcgcccaccatcgctcgcaacccctgtccctgcgccccga
ggcgtgccggccagcggcgggggcgagtgacacgagggggctggacttcgctgtgatctctacatctggcgcccttgccggg
gacttgggggtccttctcctgtcactggttatcaccctttattgcaaacggggcagaaagaaactcctgtatatattcaaacaccattatga
gaccagtacaaactactcaagaggaagatggctgtagctgccgatttccagaagaagaaggaggatgtgaattgagagtgaagtca
gcaggagcgcagacgccccgcctatcagcaaggccagaaccagctctataacgagctcaatttagggcgaagagaggagtacgatgtt
ttggacaagaggcgtggccgggacccgaaatggggggaaagccgagaaggaagaaccctcaggaaggctgtacaatgaattgcag
aaggataagatggcgaggcatacagtgagattgggatgaaaggcgagcggcgaggggcgaaggggcacgatggcctttatcagggt
ctcagtacagccaccaaggacacctacgacgcccttcacatgcaagccctgccccctcgc (SEQ ID NO: 141).

[0213] In one embodiment described herein, an anti-PSMA CAR construct has an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%)

95–100%) SEQ ID NO: 142.

DIQMTQSPSSLSASVGDRVTITCRASQNVNTNVAWYQQKPGKAPKVLIIYSASYRNSGV
PSRFSGSGSGTDFTLTISVQPEDFATYYCQQYNSYPPTFGQGKLEIKGSTSGSGKPGSG

EGSTKGQVQLVQSGAEVKKPGASVKLSCKASGYTFTTYWMHWVRQAPGQGLEWIGM
IHPNSGSTNYAQKFQGRATLTVDSTSTAYMELSSLRSEDVAVYYCARDPYDYGEDFD
VWGQGTITVTVSSGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI
YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE
GGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPR (SEQ ID NO: 142). In embodiments an anti-PSMA CAR is encoded by a nucleic acid
having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least
95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to
the nucleic acid having the sequence according to
gacatccagatgacccagagccccagctctttaagtccagcgtggcgacagagtacagtgactgtcgtgccagccagaacgtgaat
accaacgtggcttggtaccagcagaagcccgcaaagcccctaaggtgctgatctattccgcttatcgtaactccggcgtgccttcgc
gttttctgggtctggtagcggcaccgacttcactttaacaatcagcagcgttcagcccgaagacttcgccactactactgccagcagtaca
acagctatcccttacttctcggtcaaggaccagctcgagatcaaaggctccaccagcgttagcggcaaaccgggtccggcgagggtct
ctaccaaggcccaagtgcagctggtgcagtcggcgccgaggtgaagaagcccggtgcttcgtgaagctgtcttgc aaagccagcgg
ctacacctcaccacctattggatgcactgggtccgacaagctcccggtcaaggtctggagtggattggcatgatccacccaactccggct
ccaccaactacgcccagaagtccaaggtcgtgccactttaacagtggataccagcaccagcaccgcctacatggagctgagtagttgag
gagcgaggacacccgctgtactattgcgtcgtgacctacgactacggcgaggacttcgacgtgtggggccaaggaacaacagtga
ccgtgagcagcgggtccaccagacgccagcggcgaccaccaacaccggcgcccaccatcgctgcgaacccctgtccctgcgcc
ccgaggcgtgccggccagcggcgggggcgagtcacacgagggggctggacttcgctgtgatattcatctgtggcgcccttggc
cgggactgtgggtccttctctgtcactggttatcacctttattgcaaacggggcagaaagaaactcctgtatatattcaacaaccattta
tgagaccagtacaaactactcaagaggaagatggctgtagctccgattccagaagaagaaggaggatgtgaattgagagtgaagt
tcagcaggagcgcagacgccccgcctatcagcaaggccagaaccagctctataacgagctcaatttagggcgaagaggaggtacga
tgtttggacaagaggcgtggccgggacccgaaatgggggaaagccgagaaggaagaaccctcaggaaggctgtacaatgaattg
cagaaggataagatggcggaggcatagtgagattgggatgaaaggcgagcgggaggggcaaggggcacgatggcctttatcag
ggtctcagtagacccaccaaggacacctacgacgcccttcacatgcaagccctgccccctcgc (SEQ ID NO: 143).

[0214] In one embodiment described herein, an anti-PSMA CAR construct has an amino acid
sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least
90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or
95–100%)

SEQ

ID

NO:

144.

EVQLVESGGGLVQP GGSMLSCAASGFTFSDYYMAWVRQAPGKGLEWVANINYDGTS
TYYADSLKGRFTISRDSKNTLYLQMNSLRAEDTAVYYCARALDGYGYGLDVWGQGT
TVTVSSGSTSGSGKPGSGEGSTKGDIQLTQSPSSLSASVGDRVTLTCRASQSSNNLHWY
QQKPGKAPKLLIKYVSQSIGPSRFSGSLGTDFTLTISVQPEDFATYYCQQSNSWPYT
FGQGTKLEIKGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYW

APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC
ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
R (SEQ ID NO: 144). In embodiments an anti-PSMA CAR is encoded by a nucleic acid having
at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%,
or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the
nucleic acid having the sequence according to
gaggtgcagctggtggagtcgggaggttagtccaacccggtggcagcatgaggctgtcttgctgctcctccggtcacttttctgat
tactacatggcttgggtccgacaagctcccgaaaaggttagagtggtggctaacaactacgacggcaccagcacctactatgccg
acagcctcaaggcagattcaccatctctcgtgattcgtctaaaaacatttatattacaatgaactctttaagagccgaagataccgctg
gtactattgcgctcgtgccctcgacggctactacggatatttagacgtgtgggtcaaggaacaacgtgaccgtgtccagcggatccacct
ccggaagcggcaaacccgtagcggcgaaggcagcaccaaggagacatccagctgaccagagccctagctctttaagcgtacg
tgggcgatagggtgactctgactgtcgtgcgtccaaagcatttagcaacaatttactgtgtaccagcagaagcccgaaaagcccca
agctgctgatcaaatatgtgagccagagcatctccgcatccctctcgttttctgttagcggactgggcaccgactttactttaaccatcag
cagcgtccagcccaggacttcgccacatactactgccagcagagcaacagctggccctatactttcgccaaggaacaaagctggaga
tcaaggggtccaccagcagccagcggcgaccaccaacaccggcgccaccatcgctcgcaaccctgtccctgcgccccgagg
cgtgccggccagcggcgggggcgagtcacacgagggggctggacttcgcctgtgatattctacatctgggcgcccttggccgggac
ttgtgggtccttctcgtcactgtgtatcacctttattgcaaacggggcagaagaaactcctgtatatattcaacaaccatttatgagacc
agtacaaactactcaagaggaagatggctgtagctgccgatttcagaagaagaaggaggatgtgaattgagagtgaattcagcag
gagcgcagacgccccgcctatcagcaaggccagaaccagctctataacgagctcaatttagggcgaagagaggagtacgatgttttg
acaagaggcgtggccgggaccccgaaatgggggaaagccgagaaggaagaaccctcaggaaggctgtacaatgaattgcagaag
gataagatggcggaggcatacagtgagattgggatgaaaggcgagcggcgaggggcaaggggcacgatggccttatcaggtctca
gtacagccaccaaggacacctacgacgcccttcacatgcaagccctgccccctgc (SEQ ID NO: 145).

[0215] In one embodiment described herein, an anti-PSMA CAR construct has an amino acid
sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least
90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or
95–100%) SEQ ID NO: 146.

DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGV
PDRFTGSGSGTDFTLTISLQPEDFADYFCQQYNSYPLTFGGGTKLEIKGSTSGSGKPGSG
EGSTKGEVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEWIGNINP
NNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWQGTT
VTVSSGSTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG
TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVK
FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ

ID NO: 146). In embodiments an anti-PSMA CAR is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid having the sequence according to

gacattgtgatgactcagtcctctcttcttccgcttcggtggggaccgcgtcactataacttgtaaagcgctccaagatgctggcaccgc
cggtgactggtaccagcaaaacccgggaaagcgccgaaactgctcatctactgggcttaacccgccacacgggtgtccggaccgggtt
tacggggagcggtagtggaaccgatttcactctgaccatttcctccctcaaccggaagatttcgctgactactttgtcaacaataataattcata
tccccctactttcggagggggcacgaagtggaataaagggtagcacctctggtagcggcaagcctggctctggcgagggtagtacca
aaggagaagttaactgtgcaaagcggggcagaagtgaaaaaacccggggcgagcgttaaaatatcttgtaaaacaagtggctacacct
tcacggagtagacacatccactgggttaacaagctctggaaggactgaatggatcggaacataaacccaacaatgggggcacta
cttataatcaaaagttgaggatcgggctaccctcacagtggataagtcacctccacagcttatatggaattgagtagccttaggagcgagg
atacacccgtttattattgtgcggcggtggaacttgactattgggggaaggacgacggtgacggtgtcctccgggtccaccacgac
gccagcgccgcgaccaccaacacggcgcccaccatcgctgcgaacccctgtccctgcgccccgaggcgtgccggccagcggcggg
ggggcgagtgacacagagggggctgacttcgctgtgatatctacatctggggcgcccttgccgggacttggtgggtccttctctgtc
actggttatcacctttattgcaaacggggcagaagaactcctgtatatattcaacaaccatttatgagaccagtacaaactactcaagag
gaagatggctgtagctgccgatttcagaagaagaaggaggtgtgaattgagagtgaagttcagcaggagcgcagacgcccccg
cctatcagcaaggccagaaccagctctataacgagctcaatttagggcgaagagaggagtacgatgtttggacaagaggcgtggccgg
gaccccgaaatgggggaaagccgagaaggaagaacctcaggaaggctgtacaatgaattgcagaaggataagatggcgagggca
tacagtgagattgggatgaaaggcgagcgccggaggggcaaggggcacgatggcctttatcagggtctcagtacagccaccaaggaca
cctacgacgcccttcacatgcaagccctgccccctcgc (SEQ ID NO: 147).

[0216] In one embodiment described herein, an anti-PSMA CAR construct has an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%)

SEQ	ID	NO:	148.
DIQLTQSPSSLSASVGDRVTITCRASSSVSHMYWYQKPGKAPKPWIYRTSNLASGVPS			
RFSGSGSGTDYTLTISSMQPEDFATYYCQYHSHYPLTFGQGTKLEIKGSTSGSGKPGSGE			
GSTKGEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYYMAWVRQAPGKGLEWVANIN			
YDGSSTFYADSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCGRQVGYYPDMDYW			
GQGTTVTVSSGSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI			
WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG			
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKN			
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL			

PPR (SEQ ID NO: 148). In embodiments an anti-PSMA CAR is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the

nucleic acid having the sequence according to

gacatccagctgaccagctccccagctctttatccgctagcgtggcgatagggtagaccatcactgtcgtgcgtcttcgtctgtctcata
 tgtactggtagcagcagaagcccggaaggccccaagccttgatctatcgatccaatcttgcaagcggcgtcccttctcgttttctg
 gttccgggtctggtaccgactacactttaaccatcagcagcatgcagcccaggacttcgccactactactgccagcagtatcactcctatc
 cttaacttttggccaaggaacaaagtggagatcaaggcgagcacctccgtagcggaaagcccgtagcggcgaggcgagcagcaciaa
 gggagaggtgcagttggtggagagcggaggaggactggtgcagcccggtggctctttaagactcagctgtccgcccagcggatttacat
 tctccgactactacatggcttgggtccgacaagcccccgaaaagggttagagtggtggccaacatcaactacgacggctcctccacatt
 ctacgccgactctttaagggtcgtttcaccatctctcgtgacaacagcaaaaatactttatattacaatgaactcttaaggcgccaggaca
 ccgccgtgtactactgcggtcgtcaagttggctattacgacccatggactactggggccaaggaaactaccgtgaccgtgagcagcgggt
 ccaccacgacgccagcgcgcgaccaccaacaccggcgccaccatcgctcgcaacccctgtccctgcgccccgaggcgtgccgg
 ccagcggcgggggcgagtgacacgagggggctggacttcgctgtgatctacatctgggcgccttggccgggactgtgggggt
 ccttctctgtcactggttatcaccctttattgcaaacggggcagaaagaaactcctgtatatattcaacaaccatttatgagaccagtacaaa
 ctactcaagaggaagatggctgtagctgccgattccagaagaagaaggaggatgtgaattgagagtgaagttcagcaggagcgcga
 gacgccccgcctatcagcaaggccagaaccagctctataacgagctcaatttagggcgaagagaggagtacgatgtttggacaagag
 gcgtggccgggacccccgaaatgggggaaagccgagaaggagaacccctcagggaaggctgtacaatgaattgcagaaggataagat
 ggcggaggcatacagtgagattgggatgaaaggcgagcggcgaggggcaaggggcacgatggcctttatcagggtctcagtacagc
 caccaaggacacctacgacgcccttcacatgcaagccctgccccctcgc (SEQ ID NO: 149).

[0217] Notch receptors are single pass transmembrane proteins that mediate cell-cell contact signaling and play a central role in development and other aspects of cell-to-cell communication between two contacting cells, in which one contacting cell has the Notch receptor, and the other contacting cell is a cell that exhibits a ligand on its surface which binds to the corresponding Notch receptor. The engagement of native Notch and Delta, it's native ligand, leads to two-step proteolysis of the Notch receptor that ultimately causes the release of the intracellular portion of the receptor from the membrane into the cytoplasm, where it moves to the nucleus. There the released domain alters cell behavior by functioning as a transcriptional regulator. Notch receptors are involved in and are required for a variety of cellular functions during development and are critical for the function of numerous cell-types across species.

[0218] Synthetic Notch receptors (synNotch receptors) disclosed herein can display one or more different binding moieties on the cell surface, for example, scFVs, nanobodies, single chain T-cell receptors, to name a few, that recognize a prostate specific cancer antigen (PSCA) causing the release of the intracellular, transcriptional regulatory portion of the receptor from the membrane into the cytoplasm resulting in transcriptional regulation. Engineered cells bearing synNotch receptors that encounter their specific target antigen (PSCA) will then be cleaved such that their cytosolic or intercellular fragment is free to translocate into the cell nucleus to regulate the transcription of any open reading frame (ORF) under the control of a synthetic promoter, for

example a GAL4 promoter. As disclosed herein the ORF expressed is an anti-PSMA chimeric antigen T-cell receptor (CAR-T) that targets a separate, distinct target antigen (PSMA) for target cell killing, only after the priming target antigen (prostate specific cancer antigen (PSCA) in the synNotch receptors disclosed herein) detected by the synNotch receptor has been detected. This enables highly-specific combinatorial antigen pattern recognition to allow greater discrimination between diseased or cancerous cells and healthy cells. This could greatly enable the application of engineered CAR-T cells to safely target tumors with less side-effects on healthy tissue.

[0219] SynNotch receptor polypeptides of the present disclosure comprise: a) an extracellular anti-PSCA binding domain; b) a Notch receptor core polypeptide, where the Notch receptor polypeptide has a length of from 50 amino acids to 1000 amino acids, and comprises one or more ligand-inducible (PSCA binding) proteolytic cleavage sites; and c) an intracellular domain. Binding of the anti-PSCA binding domain to PSCA, for example on the surface of a prostate cancer cell, induces cleavage of the Notch receptor core polypeptide at the one or more ligand-inducible proteolytic cleavage sites, thereby releasing the intracellular domain. Release of the intracellular domain induces the expression of an anti-PSMA CAR in a cell that produces the anti-PSCA synNotch receptor polypeptide. The extracellular domain comprises an amino acid sequence that is heterologous to the Notch core receptor polypeptide. The intracellular domain comprises an amino acid sequence that is heterologous to the Notch core receptor polypeptide.

[0220] A synNotch receptor polypeptide of the present disclosure comprises an extracellular domain that includes an antigen binding domain that specifically binds prostate specific cancer antigen (PSCA), i.e. an anti-PSCA binding domain. In embodiments the anti-PSCA binding may comprise antigen-binding sequences as found in an antibody described herein. In some instances, an anti-PSCA binding domain comprises an antigen binding fragment described herein, such as an scFv. Unless otherwise indicated, it is to be appreciated the references to PSCA in the present disclosure relate to human PSCA. In various embodiments, an anti-PSCA binding motif comprises at least one heavy chain CDR (HCDR) provided herein, *e.g.*, at least one HCDR disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises at least one light chain CDR (LCDR) provided herein, *e.g.*, at least one LCDR disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises three LCDRs provided herein, *e.g.*, three LCDRs disclosed in Table 9.

[0221] In various embodiments, an anti-PSCA binding domain comprises at least one HCDR provided herein, *e.g.*, at least one HCDR disclosed in Table 9, and at least one LCDR provided herein, *e.g.*, at least one LCDR disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises one HCDR provided herein, *e.g.*, at least one HCDR disclosed in Table 9, and one LCDR provided herein, *e.g.*, derived from the same Table of Table 9 as the HCDR(s). In various embodiments, an anti-PSCA binding domain comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in Table 9, and two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in Table 9, and two LCDRs provided herein, *e.g.*, derived from the same Table of Table 9 as the HCDR(s). In various embodiments, an anti-PSCA binding domain comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in Table 9, and three LCDRs provided herein, *e.g.*, three LCDRs disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in Table 9, and three LCDRs derived from the same Table of Table 9 as the HCDR(s).

[0222] In various embodiments, an anti-PSCA binding domain comprises at least one heavy chain framework region (heavy chain FR) of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in Table 9.

[0223] In various embodiments, an anti-PSCA binding domain comprises at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in Table 9.

[0224] In various embodiments, an anti-PSCA binding domain comprises at least one heavy chain FR of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in Table 9, and at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed

in Table 9. In various embodiments, an anti-PSCA binding domain comprises one heavy chain FR of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in Table 9, and one light chain FR of a light chain variable domain disclosed herein, *e.g.*, derived from the same Table of Table 9 as the heavy chain FR(s). In various embodiments, an anti-PSCA binding domain comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in Table 9, and two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in Table 9, and two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, derived from the same Table of Table 9 as the heavy chain FR(s). In various embodiments, an anti-PSCA binding domain comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in Table 9, and three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in Table 9. In various embodiments, an anti-PSCA binding domain comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in Table 9, and three light chain FRs derived from the same Table of Table 9 as the heavy chain FR(s).

[0225] Exemplary antibody sequences provided in Table 9 are suitable for use in any antibody format, comprising, *e.g.*, a tetrameric antibody, a monospecific antibody, a bispecific antibody, an antigen binding fragment, or a binding motif. Heavy chain variable domains and light chain variable domains and portions thereof provided in Table 9 may be comprised in a binding motif.

[0226] In various embodiments, an anti-PSCA binding domain comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100%, *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) to corresponding FR(s) of a heavy chain variable domain of a heavy chain variable domain disclosed in in Table 9. In various embodiments, an anti-PSCA binding domain comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100%) to corresponding FR(s) of a light chain variable domain of a light chain variable domain disclosed in Table 9.

[0227] In various embodiments, an anti-PSCA binding domain comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed

in Table 9 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%).

[0228] In various embodiments, an anti-PSCA binding domain comprises at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in Table 9 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%).

[0229] In various embodiments, an anti-PSCA binding domain comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in Table 9 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in Table 9 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%).

[0230] Table 9 represents the heavy chain variable domain and light chain variable domain sequences of an exemplary antibody, comprising (i) the heavy chain variable domain of the exemplary antibody; (ii) a DNA sequence encoding the heavy chain variable domain (iii) three heavy chain variable domain CDRs of the heavy chain variable domain, according to IMGT, Kabat, and Chothia numbering; (iv) the light chain variable domain of the exemplary antibody; (v) a DNA sequence encoding the light chain variable domain; and (vi) three light chain variable domain CDRs of the light chain variable domain, according to IMGT, Kabat, and Chothia numbering. Information provided in each table provides framework amino acid sequences, as well as nucleotide sequences encoding each CDR amino acid sequence and nucleotide sequences encoding corresponding FR amino acid sequence.

[0231] In various embodiments an anti-PSCA binding domain may comprise a heavy chain variable domain (*e.g.*, having at least 75% sequence identity to a heavy chain variable domain in Table 9, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), a light chain variable domain (*e.g.*, having at least 75% sequence identity to a light chain variable domain in Table 9, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), and a linker (*e.g.*, a linker according to SEQ ID NO: 126). In various embodiments a binding motif may comprise a leader sequence, a heavy chain variable domain (*e.g.*, having at least 75% sequence identity to a heavy chain variable domain of any one of Table 9, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), a light chain variable domain (*e.g.*, having at least 75% sequence identity to a light chain variable domain of any one of Table 9, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%,

90-95%, 90-100%, or 95-100%), and a linker. The linker joining the two variable domains will be apparent from the sequence in view of the present disclosure. For the avoidance of doubt, a heavy chain variable domain and a light chain variable domain may be present in any orientation, *e.g.*, an orientation in which the heavy chain variable domain is C terminal of the light chain variable domain or in which the heavy chain variable domain is N terminal of the light chain variable domain. In various embodiments an anti-PSCA binding domain may comprise a linker according to SEQ ID NO: 126.

[0232] In certain embodiments, an anti-PSCA binding domain comprises a binding domain that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a linker having at least 75% sequence identity to SEQ ID NO: 126 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-PSCA binding domain comprises a binding motif that comprises a linker according to SEQ ID NO: 126. In certain embodiments, an anti-PSCA binding domain comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a leader sequence having at least 75% sequence identity to SEQ ID NO: 137, SEQ ID NO: 138, or SEQ ID NO: 139 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-PSCA binding domain comprises a binding motif that comprises a CSF2RA leader sequence according to SEQ ID NO: 137.

[0233] In certain embodiments, an anti-PSCA binding domain comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, a linker of the present disclosure, and a leader sequence of the present disclosure.

[0234] A binding agent that is based on an exemplary antibody provided herein, such as for example Ab6, may be provided in any fragment or format, comprising a heavy chain variable domain according to the indicated exemplary antibody and a light chain variable domain according to the indicated exemplary antibody.

Table 9: Exemplary Antibody Sequences 6 (Ab6)

SEQ ID NO:	Description	Sequence
150	Heavy Chain Variable Domain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGNYWSWIRQPP GKGLEWIGEINHSGSTNYNPSLKS RVVTISVDTSKNQFSLKLSS VTAADTAVYYCARGGSYNYFDYWGQGTLVTVSS
151	VH (DNA)	Caagttcagctgcagcagctggggagctggttactgaagcctagcgagacactgtctttaacatg cgccgtgtacggcggaagcttcagcggcaactattggagctggatcagacagcctcccgttaa gggttagagtgatcggcgagatcaaccactccggctccaccaactataacccctctttaaagtc tcgtgtgaccatctccgtggacaccagcaagaaccagttctctttaaagctgagctccgtgacag ccgccgacaccgctgtgtattactgtctcgtggcggcagctacaactacttcgactactggggc caaggtaccctcgtgaccgtgtccagc
152	CDRH1 IMGT (Prot)	GGSFSGNY
153	CDRH1 Kabat (Prot)	GNYWS
154	CDRH1 Chothia (Prot)	GGSFSG
155	CDRH2 IMGT (Prot)	INHSGST
156	CDRH2 Kabat (Prot)	EINHSGSTNYNPSLKS
157	CDRH2 Chothia (Prot)	EINHSGSTNYNPSLKS
158	CDRH3 IMGT (Prot)	ARGGSYNYFDY
159	CDRH3 Kabat (Prot)	GGSYNYFDY

160	CDRH3 Chothia (Prot)	GGSYNYFDY
161	Light Chain Variable Domain	DIVMTQSPLSLPVTPGEPASISCRSSQSLHNSNGYNLVWYLQ KPGQSPQLLIYLGSIASGVPRFSFGSGSGTDFTLKISRVEAED VGVYYCMQPLQTPITFGQGTRLEIK
162	VL (DNA)	Gacatcgtgatgacacagagccctctgtctttaccggtacccccgggaacccgctagcatcag ctgcagaagctcccagctcttactccacagcaacggctacaactatttagtggtatttacagaaa ccccgccagagccccagctgctgattatctgggctcattcgtgctagcggcgtgcccgatag atttccggcagcgggaagcggcaccgacttcactttaagatctctcgtgtggaggccgaggac gtggcgctctactactgtatgcagcctctgcagacccccattaccttcggccaagggtactcgtctg gaaatcaag
163	CDRL1 IMGT (Prot)	QSLHNSNGYNY
164	CDRL1 Kabat (Prot)	RSSQSLHNSNGYNLV
165	CDRL1 Chothia (Prot)	RSSQSLHNSNGYNLV
166	CDRL2 IMGT (Prot)	LGS
167	CDRL2 Kabat (Prot)	LGSIRAS
168	CDRL2 Chothia (Prot)	LGSIRAS
169	CDRL3 IMGT (Prot)	MQPLQTPITF
170	CDRL3 Kabat (Prot)	MQPLQTPIT
171	CDRL3 Chothia (Prot)	MQPLQTPIT

172	ScFv	DIVMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLVWYLQ KPGQSPQLLIYLGSIASGVDPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQPLQTPITFGQGTRLEIKGSTSGSGKPGSGEGSTK GQVQLQQWGAGLLKPSETLSLTCAVYGGSFSGNYWSWIRQP PGKGLEWIGEINHSGSTNYPNPSLKSRVTISVDTSKNQFSLKLS SVTAADTAVYYCARGGSYNYFDYWGGQGLTVTVSS
173	ScFv	gacatcgtgatgacacagagccctctgtctttacccgttaccgccgtgaaccgctagcatcag ctgcagaagctcccagctcttactccacagcaacggctacaactatttagtggtatttacagaaa cccgccagagccccagctgctgattatctgggctcattcgtgctagcggcgtgcccgatag atttccggcagcgggaagcggcaccgacttcactttaagatctctcgtgtggaggccgaggac gtggcgcttactactgtatgcagcctctgcagacccccattacctcggccaaggctactcgtctg gaaatcaagggcagcaccagcggcagcggaaaaccggaagcggcgagggaagcaccaa aggccaagttcagctgcagcagtgaggagctggtttactgaagcctagcgagacactgtcttaa catgcgccgtgtacggcgggaagcttcagcggcaactattggagctggatcagacagcctcccg gtaaggggttagagtgatcggcgagatcaaccactccggctccaccaactataacccctctttaa agtctcgtgtgacctctccgtggacaccagcaagaaccagttctctttaaagctgagctccgtga cagccgccgacaccgctgtgtattactgtgctcgtggcggcagctacaactacttcgactactgg ggccaaggtaccctcgtgacctgtccagc

[0235] As noted above, a synNotch receptor polypeptide comprises a Notch core receptor polypeptide having a length of from 50 amino acids to 1000 amino acids and comprising one or more ligand-inducible proteolytic cleavage sites. In embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 50 amino acids to 1000 amino acids, e.g., from 50 amino acids to 75 amino acids, from 75 amino acids to 100 amino acids, from 100 amino acids to 150 amino acids, from 150 amino acids to 200 amino acids, from 200 amino acids to 250 amino acids, from 250 a to 300 amino acids, from 300 amino acids to 350 amino acids, from 350 amino acids to 400 amino acids, from 400 amino acids to 450 amino acids, from 450 amino acids to 500 amino acids, from 500 amino acids to 550 amino acids, from 550 amino acids to 600 amino acids, from 600 amino acids to 650 amino acids, from 650 amino acids to 700 amino acids, from 700 amino acids to 750 amino acids, from 750 amino acids to 800 amino acids, from 800 amino acids to 850 amino acids, from 850 amino acids to 900 amino acids, from 900 amino acids to 950 amino acids, or from 950 amino acids to 1000 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 300 amino acids to 400 amino acids. In some embodiments, the Notch core receptor polypeptide present in a

synNotch receptor polypeptide of the present disclosure has a length of from 300 amino acids to 350 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 300 amino acids to 325 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 350 amino acids to 400 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 750 amino acids to 850 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 50 amino acids to 75 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 310 amino acids to 320 amino acids, e.g., 310 amino acids, 311 amino acids, 312 amino acids, 313 amino acids, 314 amino acids, 315 amino acids, 316 amino acids, 317 amino acids, 318 amino acids, 319 amino acids, or 320 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of 315 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of from 360 amino acids to 370 amino acids, e.g., 360 amino acids, 361 amino acids, 362 amino acids, 363 amino acids, 364 amino acids, 365 amino acids, 366 amino acids, 367 amino acids, 368 amino acids, 369 amino acids, or 370 amino acids. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure has a length of 367 amino acids.

[0236] In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: IPYKIEAVKSEPVEPPLPSQLHLMYVAAAFVLLFFVGCGVLLSRKRRRQHGQL (SEQ ID NO: 174); where the Notch core receptor polypeptide comprises an S2 proteolytic cleavage site and an S3 proteolytic cleavage site; where the Notch receptor polypeptide has a length of from 50 amino acids (aa) to 65 aa, e.g., 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, or 65 aa.

[0237] In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: IPYKIEAVKSEPVEPPLPSQLHLMYVAAAFVLLFFVGCGVLLSRKRRRQLCIQKL (SEQ ID NO: 175); where the TM domain is underlined; where the Notch core receptor polypeptide

comprises an S2 proteolytic cleavage site and an S3 proteolytic cleavage site; where the Notch receptor polypeptide has a length of from 50 amino acids (aa) to 65 aa, e.g., 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, or 65 aa.

[0238] In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) a LNR-A segment; ii) a LNR-B segment; iii) a LNR-C segment; iv) an HD-N segment, v) an HD-C segment; and vi) a TM domain. A LNR-A segment, LNR-B segment, and LNR-C segment can collectively be referred to as an "LNR segment."

[0239] An LNR segment can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: PPQIEEACELPECQVDAGNKVCNLQCNNHACGWDGGDCSLNFNDPWKNCTQSLQCW KYFSDGHCDSCNSAGCLFDGFDCQLTEGQCNPLYDQYCKDHFSDGHCDQGCNSAEC EWDGLDC (SEQ ID NO:176); and can have a length of from 118 to 122 amino acids (e.g., 118, 119, 120, 121, or 122 amino acids).

[0240] An HD segment (HD-N plus HD-C) can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: AAGTLVLVLLPPDQLRNNSFHFLRELSHVLHTNVVFKRDAQGQQMIFPYYGHEEELR KHPIKRSTVGWATSSLLPGTSGGRQRRELDPMDIRGSIVYLEIDNRQCVQSSSQCFQSAT DVAAFLGALASLGSLNIPYKIEAVKSEPVEPLP (SEQ ID NO:177); and can have a length of 150, 151, 152, 153, or 154 amino acids.

[0241] A transmembrane segment can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: HLMYVAAAFAVLLFFVGCGVLLS (SEQ ID NO:178); and can have a length of 21, 22, 23, 24, or 25 amino acids.

[0242] In some embodiments, a Notch receptor polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: PPQIEEACELPECQVDAGNKVCNLQCNNHACGWDGGDCSLNFNDPWKNCTQSLQCW KYFSDGHCDSCNSAGCLFDGFDCQLTEGQCNPLYDQYCKDHFSDGHCDQGCNSAEC EWDGLDCAEHVPERLAAGTLVLVLLPPDQLRNNSFHFLRELSHVLHTNVVFKRDAQG QQMIFPYYGHEEELRKHPIKRSTVGWATSSLLPGTSGGRQRRELDPMDIRGSIVYLEIDN RQCVQSSSQCFQSATDVAAFLGALASLGSLNIPYKIEAVKSEPVEPLPSQLHLMYVAA

AAFVLLFFVGCGVLLS (SEQ ID NO: 179); and has a length of from 300 amino acids to 310 amino acids (e.g., 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, or 310 amino acids).

[0243] In some embodiments, a Notch core receptor polypeptide comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

[0244] PCVGSNPCYNQGTCEPTSENPFYRCLCPAKFNGLLCHILDYSFTGGAGRDI PPPQI
EEACELPECQVDAGNKVCNLQCNNHACGWDGGDCSLNFNDPWKNCTQSLQCWKYFS
DGHCDSCNSAGCLFDGFDCQLTEGQCNPLYDQYCKDHFSDGHCDQGCNSAECEWDG
LDCAEHVPERLAAGTLVLVLLPPDQLRNNSFHFLRELHSHVLHTNVVFKRDAQGQQMI
FPYYGHEEELRKHPKRSTVGWATSSLLPGTSGGRQRRELDPMDIRGSIVYLEIDNRQCV
QSSSQCFQSATDVA AFLGALASLGSLNIPYKIEAVKSEPVEPPLPSQLHLMYVAAA FVL
LFFVGCGVLLS (SEQ ID NO:180); and has a length of from 350 amino acids to 370 amino acids (e.g., 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, or 370 amino acids).

[0245] In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) a single EGF repeat; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain.

[0246] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following sequence: PCVGSNPCYNQGTCEPTSENPFYRCLCPAKFNGLLCH (SEQ ID NO: 181); and can have a length of 35 amino acids to 40 amino acids (e.g., 35, 36, 37, 38, 39, or 40 amino acids).

[0247] An LNR segment can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: PPQIEEACELPECQVDAGNKVCNLQCNNHACGWDGGDCSLNFNDPWKNCTQSLQCWKYFSDGHCDSCNSAGCLFDGFDCQLTEGQCNPLYDQYCKDHFSDGHCDQGCNSAECEWDGLDC (SEQ ID NO:182); and can have a length of from 118 to 122 amino acids (e.g., 118, 119, 120, 121, or 122 amino acids).

[0248] An HD segment (HD-N plus HD-C) can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: AAGTLVLVLLPPDQLRNNSFHFLRELHSHVLHTNVVFKRDAQGQQMIFPYYGHEEELRKHPKRSTVGWATSSLLPGTSGGRQRRELDPMDIRGSIVYLEIDNRQCVQSSSQCFQSAT

DVAAFLGALASLGSLNIPYKIEAVKSEPVEPPLP (SEQ ID NO: 183); and can have a length of 150, 151, 152, 153, or 154 amino acids.

[0249] A transmembrane segment can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: HLMYVAAAFAVLLFFVGCGVLLS (SEQ ID NO:184); and can have a length of 21, 22, 23, 24, or 25 amino acids.

[0250] In some embodiments, a Notch core receptor polypeptide comprises a synthetic linker. For example, In some embodiments, a Notch core receptor polypeptide comprises, in order from N-terminus to C-terminus: i) a synthetic linker; ii) an EGF repeat; iii) an LNR segment; iv) an HD-N segment; v) an HD-C segment; and vi) a TM domain.

[0251] A synthetic linker can have a length of from about 10 amino acids (aa) to about 200 aa, e.g., from 10 aa to 25 aa, from 25 aa to 50 aa, from 50 aa to 75 aa, from 75 aa to 100 aa, from 100 aa to 125 aa, from 125 aa to 150 aa, from 150 aa to 175 aa, or from 175 aa to 200 aa. A synthetic linker can have a length of from 10 aa to 30 aa, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 aa. A synthetic linker can have a length of from 30 aa to 50 aa, e.g., from 30 aa to 35 aa, from 35 aa to 40 aa, from 40 aa to 45 aa, or from 45 aa to 50 aa.

[0252] In some instances, a synthetic linker, as described herein, may include an extracellular protein structural domain or a portion thereof. Extracellular protein structural domains suitable for use as a synthetic linker include but are not limited to e.g., Ig-like extracellular structural domains, Fc extracellular structural domains, fibronectin extracellular structural domains and the like. In some instances, a synthetic linker may include a plurality of extracellular protein structural domains where the plurality may include a plurality of the same domain or a plurality of different domains.

[0253] In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) from two to eleven EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain.

[0254] In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) two EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) three EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch

receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) four EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) five EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) six EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) seven EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) eight EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) nine EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) ten EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain. In some embodiments, the Notch core receptor polypeptide present in a synNotch receptor polypeptide of the present disclosure comprises, in order from N-terminus to C-terminus: i) eleven EGF repeats; ii) an LNR segment; iii) an HD-N segment, iv) an HD-C segment; and v) a TM domain.

[0255] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to DINECVLSPCRHGASCQNTHTGGYRCHCQAGYSGRNCE; SEQ ID NO:185 and can have a length of from 35 amino acids to about 40 amino acids (aa) (e.g., 35, 36, 37, 38, 39, or 40 aa).

[0256] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to DIDDCRPNPCHNGGSCTDGINTAFCDCLPGFRGTFC; SEQ ID NO: 186 and can have a length of from 35 amino acids to about 40 amino acids (aa) (e.g., 35, 36, 37, 38, 39, or 40 aa).

[0257] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to DINECASDPCRNGANCTDCVDSYTCTCPAGFSGIHCE; (SEQ ID NO: 187 and can have a length of from 35 amino acids to about 40 amino acids (aa) (e.g., 35, 36, 37, 38, 39, or 40 aa).

[0258] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to TESSCFNNGGTCVDGINSFTCLCPPGFTGSYCQ; SEQ ID NO: 188 and can have a length of from 30 amino acids (aa) to 35 aa (e.g., 30, 31, 32, 33, 34, or 35 aa).

[0259] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to DVNECDSQPCLHGGTCQDGCGRCTCPQGYTGPNQC; SEQ ID NO: 189 and can have a length of from 35 amino acids to about 40 amino acids (aa) (e.g., 35, 36, 37, 38, 39, or 40 aa).

[0260] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to DSSPCKNGGKCWQHTHTQYRCECPGWT; SEQ ID NO: 190 and can have a length of from 25 amino acids (aa) to 30 aa, e.g., 25, 26, 27, 28, 29, or 30 aa.

[0261] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to LVDECSPSPCQNGATCTDYLGGYSCKCVAGYHGVNC; SEQ ID NO: 191 and can have a length of from 35 amino acids to about 40 amino acids (aa) (e.g., 35, 36, 37, 38, 39, or 40 aa).

[0262] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to IDECLSHPCQNGGTCLDLPNTYKCSGPRGTQGVHCE; SEQ ID NO: 192 and can have a length of from 35 amino acids to about 40 amino acids (aa) (e.g., 35, 36, 37, 38, 39, or 40 aa).

[0263] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to CFNNGTCVDQVGGYSCTCPPGFVGERCE; SEQ ID NO: 193 and can have a length of from 25 amino acids (aa) to 30 aa, e.g., 25, 26, 27, 28, 29, or 30 aa.

[0264] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to DVNECLSNPCDARGTQNCVQRVNDHFHCECRAGHTGRRCE; (SEQ ID NO: 194)

and can have a length of from 35 amino acids to about 40 amino acids (aa) (e.g., 35, 36, 37, 38, 39, or 40 aa).

[0265] An EGF repeat can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to PCVGSNPCYNQGTCEPTSENPFYRCLCPAKFNGLLCH (SEQ ID NO: 195); and can have a length of 35 amino acids to 40 amino acids (e.g., 35, 36, 37, 38, 39, or 40 amino acids).

[0266] An LNR segment can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: PPQIEEACELPECQVDAGNKVCNLQCNNHACGWDGGDCSLNFNDPWKNCTQSLQCW KYFSDGHCDSCQNSAGCLFDGFDCQLTEGQCNP LYDQYCKDHFSDGHCDQGCNSAEC EWDGLDC (SEQ ID NO: 196); and can have a length of from 118 to 122 amino acids (e.g., 118, 119, 120, 121, or 122 amino acids).

[0267] An HD segment (HD-N plus HD-C) can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: AAGTLVLVLLPPDQLRNNSFHFLREL SHVLHTNVVFKRDAQGQQMIFPYYGHEEELR KHPIKRSTVGWATSSLLPGTSGGRQRRELDPM DIRGSIVYLEIDNRQCVQSSSQCFQSAT DVAAFLGALASLGSLNIPYKIEAVKSEPVEPLP (SEQ ID NO: 197); and can have a length of 150, 151, 152, 153, or 154 amino acids.

[0268] A transmembrane segment can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: HLMYVAAAFAVLLFFVGCGLLS (SEQ ID NO: 198); and can have a length of 21, 22, 23, 24, or 25 amino acids.

[0269] In some embodiments, a Notch core receptor polypeptide comprises a synthetic linker. For example, In some embodiments, a Notch core receptor polypeptide comprises, in order from N-terminus to C-terminus: i) two to eleven EGF repeats; ii) a synthetic linker; iii) an LNR segment; iv) an HD-N segment, v) an HD-C segment; and vi) a TM domain.

[0270] A synthetic linker can have a length of from about 10 amino acids (aa) to about 200 aa, e.g., from 10 aa to 25 aa, from 25 aa to 50 aa, from 50 aa to 75 aa, from 75 aa to 100 aa, from 100 aa to 125 aa, from 125 aa to 150 aa, from 150 aa to 175 aa, or from 175 aa to 200 aa. A synthetic linker can have a length of from 10 aa to 30 aa, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 aa. A synthetic linker can have a length of from 30 aa to 50 aa, e.g., from 30 aa to 35 aa, from 35 aa to 40 aa, from 40 aa to 45 aa, or from 45 aa to 50 aa.

[0271] Notch receptor polypeptide comprising an HD-C segment and a TM domain

[0272] In some embodiments, a synNotch receptor polypeptide comprises, in order from N-terminus to C-terminus: i) an HD-C segment; and ii) a TM domain, where the synNotch receptor polypeptide does not include an LNR segment. In some embodiments, the LNR segment is replaced with a heterologous polypeptide.

[0273] A transmembrane segment can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

[0274] HLMYVAAAFAVLLFFVGCGVLLS (SEQ ID NO: 199); and can have a length of 21, 22, 23, 24, or 25 amino acids.

[0275] As discussed above, a synNotch receptor polypeptide of the present disclosure comprises: a) an extracellular anti-PSCA binding domain; b) a Notch core receptor polypeptide having a length of from 50 amino acids to 1000 amino acids, and comprising one or more ligand-inducible proteolytic cleavage sites; and c) an intracellular domain, where binding of the of extracellular anti-PSCA binding domain to PSCA induces cleavage of the Notch receptor polypeptide at the one or more ligand-inducible proteolytic cleavage sites, thereby releasing the intracellular domain.

[0276] In some embodiments, the synNotch receptor polypeptide includes only one ligand-inducible proteolytic cleavage site. In some embodiments, the synNotch receptor polypeptide includes two ligand-inducible proteolytic cleavage sites. In some embodiments, the synNotch receptor polypeptide includes three ligand-inducible proteolytic cleavage sites. For simplicity, ligand-inducible cleavage sites will be referred to herein as "SI," "S2," and "S3" ligand-inducible proteolytic cleavage sites.

[0277] In some embodiments, the synNotch receptor polypeptide includes an SI ligand-inducible proteolytic cleavage site. An SI ligand-inducible proteolytic cleavage site can be located between the HD-N segment and the HD-C segment. In some embodiments, the SI ligand-inducible proteolytic cleavage site is a furin-like protease cleavage site. A furin-like protease cleavage site can have the canonical sequence Arg-X-(Arg/Lys)-Arg, where X is any amino acid; the protease cleaves immediately C-terminal to the canonical sequence. In some embodiments, an amino acid sequence comprising an SI ligand-inducible proteolytic cleavage site can have the amino acid sequence GRRRRELDPM (SEQ ID NO: 200), where cleavage occurs between the "RE" sequence. As another example, an amino acid sequence comprising an SI ligand-inducible proteolytic cleavage site can have the amino acid sequence RQRRELDPM (SEQ ID NO: 201), where cleavage occurs between the "RE" sequence.

[0278] In some embodiments, the synNotch receptor polypeptide includes an S2 ligand-inducible proteolytic cleavage site. An S2 ligand-inducible proteolytic cleavage site can be located within

the HD-C segment. In some embodiments, the S2 ligand-inducible proteolytic cleavage site is an ADAM-17-type protease cleavage site. An ADAM-17-type protease cleavage site can comprise an Ala-Val dipeptide sequence, where the enzyme cleaves between the Ala and the Val. In some embodiments, amino acid sequence comprising an S2 ligand-inducible proteolytic cleavage site can have the amino acid sequence KIEAVKSE (SEQ ID NO: 128), where cleavage occurs between the "AV" sequence. As another example, an amino acid sequence comprising an S2 ligand-inducible proteolytic cleavage site can have the amino acid sequence KIEAVQSE (SEQ ID NO: 202), where cleavage occurs between the "AV" sequence.

[0279] In some embodiments, the synNotch receptor polypeptide includes an S3 ligand-inducible proteolytic cleavage site. An S3 ligand-inducible proteolytic cleavage site can be located within the TM domain. In some embodiments, the S3 ligand-inducible proteolytic cleavage site is a gamma- secretase (γ -secretase) cleavage site. A γ -secretase cleavage site can comprise a Gly-Val dipeptide sequence, where the enzyme cleaves between the Gly and the Val. In some embodiments, an S3 ligand-inducible proteolytic cleavage site has the amino acid sequence VGCGVLLS (SEQ ID NO: 203), where cleavage occurs between the "GV" sequence. In some embodiments, an S3 ligand-inducible proteolytic cleavage site comprises the amino acid sequence GCGVLLS (SEQ ID NO: 204).

[0280] In some embodiments, the synNotch receptor polypeptide lacks an S1 ligand-inducible proteolytic cleavage site. In some embodiments, the synNotch receptor polypeptide lacks an S2 ligand-inducible proteolytic cleavage site. In some embodiments, the synNotch receptor polypeptide lacks an S3 ligand-inducible proteolytic cleavage site. In some embodiments, the synNotch receptor polypeptide lacks both an S1 ligand-inducible proteolytic cleavage site and an S2 ligand-inducible proteolytic cleavage site. In some embodiments, the synNotch receptor polypeptide includes an S3 ligand-inducible proteolytic cleavage site; and lacks both an S1 ligand-inducible proteolytic cleavage site and an S2 ligand-inducible proteolytic cleavage site.

[0281] In some embodiments, the synNotch receptor polypeptide comprises a Notch core that comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

```
ILDYSFTGGAGRDIPPPQIEEACELPECQVDAGNKVCNLQCNNHACGWDGGDCSLNFN
DPWKNCTQSLQCWKYFSDGHCDSCNSAGCLFDGFDQCQLTEGQCNPYDQYCKDHFS
DGHCDQGCNSAECEWDGLDCAEHVPERLAAGTLVLVLLPPDQLRNNSFHFLRELSHV
LHTNVVFKRDAQGQQMIFPYYGHEEELRKHPIKRSTVGWATSSLLPGTSGGRQRRELDP
MDIRGSIVYLEIDNRQCVQSSQCFQSATDVA AFLGALASLGSLNIPYKIEAVKSEPVEPP
LPSQLHLMYVAAA FVLLFFVGCGVLLSRKRRRQHGQLWFPEGFKVSEASKKKRREPL
```

GEDSVGLKPLKNASDGALMDDNQNEWGDEDELETKKFRFEPPV (SEQ ID NO: 205). In embodiments a notch core is encoded by a nucleic acid having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid having the sequence according to:

atcctggactacagcttcacaggtggcgctggcgcgacattccccaccgcagattgaggaggcctgtgagctgcctgagtgccaggt
ggatgcaggcaataaggtctgcaacctgcagtgtataatcacgcatgtggctgggatgggtggcgactgctccctcaactcaatgacccct
ggaagaactgcacgcagctctctacagtgtggaagtatttttagcgacggccactgtgacagccagtgaactcggccggctgcctctttgat
ggcttcgactgccagctcaccgagggacagtgaacccctgtatgaccagtactgaaggaccacttcagtgtgcccactgcgaccag
ggctgtaacagtgccgaatgtgagtgaggatggcctagactgtgctgagcatgtacccgagcggctggcagccggcacccctggtgctggt
ggtgctgctccacccgaccagctacggaacaactcctccactttctgctgggagctcagccacgtgctgcacaccaacgtggtcttcaag
cgtgatgcgaaggccagcagatgatctccgtactatggccacgaggaagagctgcgcaagcaccatcaagcgctctacagtggg
ttggccacctcttactgcttctgttacaagtgtggggcgccagcgcagggagctggacccatggacatccgtggctccattgtctac
ctggagatgcacaacggcaatgtgtgcagtcactctgcagtgcttcagagtgccaccgatgtggctgccttctaggtgcttctgcgtc
actggcagcctcaatattccttacaagattgaggccgtgaagagtgcgagcgggtggagcctccgctgccctgcagctgcacctcatgtac
gtggcagcagccgcttctgctcctgttcttggggctgtgggggtgctgctgtcccgcaagcgccggcgg (SEQ ID NO: 206).

[0282] In some embodiments, the chimeric Notch receptor polypeptide contains all or a portion of human Notch1, Notch2, Notch3, or Notch4. In some embodiments, the chimeric Notch receptor polypeptide contains all or a portion of SEQ ID NO: 244 SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, or SEQ ID NO: 248. In some embodiments, a “portion” of Notch comprises the three NLR domains, the transmembrane domain, and a short cytosolic fragment including the native Nuclear Localization Sequence (NLS) of Notch.

[0283] Human neurogenic locus notch homolog protein 1 preprotein NP_060087.3: MPPLLAPLLCLALLPALAARGPRCSQPGETCLNGGKCEAANGTEACVCGGAFVGPGRQC
DPNPCLSTPCKNAGTCHVVDRRGVADYACSCALGFSGPLCLTPLDNACLTPCRNNGGT
CDLLTLTEYKCRCPGWSGKSCQQADPCASNPCANGGQCLPFEASYTCHCPPSFHGPTC
RQDVNECGQKPGLCRHGGTCHNEVGSYRCVCRAHTHTGPNCEPYPVPCSPSPCQNGGTC
RPTGDVTHEACLPFGFTGQNCENIDDCPGNNCKNGGACVDGVNTYNCRCPPWTGQ
YCTEDVDECQLMPNACQNGGTCHNTHGGYNCVCVNGWTGEDCSENIDDCASAACFH
GATCHDRVASFYCECPHGRTGLLCHLNDACISNPCNEGSNCDTNPVNGKATCTCPSGY
TGPACSQDVDEC SLGANPCEHAGKCINTLGSFECQLQGYTGPRCEIDVNECVSNPCQN
DATCLDQTGEFQCTCM PGYEGVHCEVNTDECASSPCLHNGRCLDKINEFQCECPTGFT
GHLQCYDVDECASTPCKNGAKCLDGPNTYTCVCTEGYTGTHCEVDIDECDDPDPCHYGS
CKDGVATFTCLCRPGYTGHHCETNINECSSQPCRHGGTCQDRDNAYLCFCLKGTTPGN
CEINLDDCASSPCDSGTCLDKIDGYECACEPGYTGSMCNINIDECAGNPCHNGGTCEDEGI

NGFTCRCPEGYHDPTCLSEVNECNSNPCVHGACRDSLNGYKCDGDPGWSGTNCDINNN
 ECESNPCVNGGTCKDMTSGYVCTCREGFSGPNCQTNINECASNPCLNQGTCTCDDVAGY
 KCNCLLPYTGATCEVVLAPCAPSPCRNGGECRQSEDIYESFSCVCPTGWQGGTCEVDINE
 CVLSPCRHGASCQNTHTGGYRCHCQAGYSGRNCETDIDDCRPNPCHNGGSCTDGINATF
 CDCLPGFRGTFCEDINECASDPCRNGANCTDCVDSYTCTCPAGFSGIHCENNTPDCTES
 SCFNGGTCTVDGINSFTCLCPPGFTGSYCQHDVNECDSQPCLHGGTCTQDGCGRSYR'CTCPQ
 GYTGPNCQNLVHWCDSSPCKNGGKWCWQTHTQYRCECPSGWTGLYCDVPSVSCEVAA
 QRQGVDFVARLCQHGGCLVDAGNTHHCRCQAGYTGSYCEDLVDECSPSPCQNGATCTD
 YLGGYSCKCVAGYHGVNCSEEIDECLSHPCQNGGTCLDLPNTYKCS'PRGTQGVHCEI
 NVDDCNPPVDPVSRSPKCFNNGTCTVDQVGGYSCTCPPGFVGERCEGDVNECLSNPCDA
 RGTQNCVQRVNDFHCECRAGHTGRRCESVINGCKGKPKCKNGGTCAVASNTARGFTCK
 CPAGFEGATCENDARTCGSLRCLNGGTCTISGPRSPCTCLCLGPFTGPECQFPASSPCLGGN
 PCYNQGTCEPTSES'PFYRCLCPAKFNGLLCHILDYSFGGGAGRDIPPLIEEACELPECQE
 DAGNKVCSLQCNNHACGWDGGDCSLNFNDPWKNCTQSLQCWKYFSDGHCDSDQCNSA
 GCLFDGFDCQRAEGQCNPLYDQYCKDHFSDGHCDQGCNSAECEWDGLDCAEHVPERL
 AAGTLVVVVLMPPEQLRNSSFHFLRELSRVLHTNVVFKRDAHGQQMIFPYYGREEELR
 KHPIKRAAEGWAAPDALLGQVKASLLPGGSEGRRRRRELDPMDVGRSIVYLEIDNRQC
 VQASSQCFQSATDVAAFLGALASLGS'LNIPYKIEAVQSETVEPPPPAQLHFMYVAAAAF
 VLLFFVGC'GVLLSRKRRRQHGQLWFPEGFKVSEASKKKRREPLGEDSVGLKPLKNASD
 GALMDDNQNEWGDEDLETKKFRFE'PVLPDLDDQTDHRQWTQQHLDAADLRMSA
 MAPTPPQGEVDADCMDVNVVRGPDGFTPLMIASCSGGGLETGNSEEEEDAPAVISDFIYQ
 GASLHNQTDRTGETALHLAARYSRSDAAKRLLEASADANIQDNMGRTP'PLHAAVSADA
 QGVFQILIRN'RTDLDARMHDGTTPLILAA'RLAVEGMLEDLINSHADVNAVDDLKSA
 LHWAAA'VNNVDA'AVVLLKNGANKDMQNNREETPLFLAAREGSYETAKVLLDHFANR
 DITDHMDRLPRDIAQERMHHDIVRL'LD'EYNLVRSPQLHGAPLGGTPTLSPPLCSPNGYL
 GSLKPGVQGGKVRKPSKGLACGSKEAKDLKARRKKSQDGKGCLLDSSGMLSPVDSLE
 SPHGYLSDVASP'LLPSPFQQSPSVPLNHLPGMPDTHLGTGHLNVA'AKPEMAALGGGG
 RLAFETGPPRLSHLPVASGTSTVLGSSSGGALNFTVGGSTSLNGQCEWLSRLQSGMVPN
 QYNPLRGSVAPG'PLSTQAPSLQHGMVGPLHSSLAASALSQMMSYQGLPSTRLATQPHL
 VQTQQVQPNLQMQQQNLQ'PANIQQQSLQPPPPPPQPHLGVSSAASGHLGRSFLSGEP
 SQADVQPLGPSSLA'VHTILPQESPALPTSLPSSLVPPVTAAQFLTPPSQHSYSSPVDNTPSH
 QLQVPEHPFLTPSPESPDQWSSSSPHSNVSDWSEGVSSPPTSMQSQIAR'PEAFK (SEQ ID
 NO: 244).

[0284] Human neurogenic locus notch homolog protein 2 isoform 1 preprotein NP_077719.2:
 SMPALRPALLWALLALWLCCAAPAHALQCRDGYEPCVNEGMCV'TYHNGTGYCKCPE

GFLGEYCQHRDPCEKNRCQNGGTCVAQAMLGKATCRCASGFTGEDCQYSTSHPCFVS
RPCLNGGTCHMLSRDTYECTCQVGFTGKECQWTDACLSHPCANGSTCTTVANQFSCK
CLTGFTGQKCETDVNECDIPGHCQHGGTCLNLPGSYQCQCPQGFTGQYCDSLYVPCAP
SPCVNGGTCTRQTGDTFECNCLPGFEGSTCERNIDDCPNHRCQNGGVCVDGVNTYNCR
CPPQWTGQFCTEDVDECLLQPNACQNGGTCANRNGGYGCVCVNGWSGDDCSENIDDC
AFASCTPGSTCIDRVASFSCMCPEGKAGLLCHLDDACISNPCHKGALCDTNPLNGQYTC
TCPQGYKGADCTEDVDECAMANSNPCEHAGKCVNTDGAHFHCECLKGYAGPRCEMDIN
ECHSDPCQNDATCLDKTGFTCLCMPGFGKVHCELEINECQSNPCVNNGQCVDKVNRF
QCLCPPGFTGPVCQIDIDDCSSTPCLNGAKCIDHPNGYECQCATGFTGVLCEENIDNCDP
DPCHHGGCQDGDIDSYTCICNPGYMGATCSDQIDECYSSPCLNDGRCIDLVNGYQCNCQP
GTSGVNCEINFDDCASNPCHGTCMDGINRYSCVCSPGFTGQRCNIDIDECASNPCRKGA
TCINGVNGFRCTCPEGPHHPSCYSQVNECLSNPCIHGNCTGGLSGYKCLCDAGWVGINC
EVDKNECLSNPCQNGGTCDNLVNGYRCTCKKGFKGYNCQVNIDECASNPCLNQGTCTF
DDISGYTCHCVLPYTGKNCQTVLAPCSPNPCENAAVCKESP NFESYTCLCAPGWQQR
CTIDIDECISKPCMNHGLCHNTQGSYMCECPPGFSGMDCEEDIDDCLANPCQNGGSCMD
GVNTFSCCLCPGFTGDKCQTDMECLSEPCKNGGTCSDYVNSYTCKCQAGFDGVHCE
NNINECTESSCFNGGTCTVDGINSFSCCLCPVGFTGSFCLHEINECSSHPCLNEGTCVDGLGT
YRCSCLPYTGKNCQTLVNLCSRSPCKNKGTCVQKKAESQCLCPSGWAGAYCDVPNV
SCDIAASRRGVLVEHLCQHSGVCINAGNTHYQCPLGYTGSYCEEQLDEECASNPCQHG
ATCSDFTGGYRCECVPGYQGVNCEYEVEDECQNQPCQNGGTCLDLVNHFKCSCPPGTRG
LLCEENIDDCARGPHCLNGGQCMDRTGGYSCRCLPGFAGERCEGDINECLSNPCSSEGS
LDCIQLTNDYLCVCRSAFTGRHCETFDVCPQMPCLNGGTCAVASNMPDGFTCRCPG
FSGARCQSSCGQVKCRKGEOCVHTASGPRCFPCSPRDCESGCASSPCQHGGSCHPQRQP
PYYSQCAPPFSGSRCELYTAPPSTPPATCLSQYCADKARDGVCDEACNSHACQWDGG
DCSLIMENPWANCSSPLPCWDYINNQCDELNTVECLFDNFECQGNSTCKYDKYCAD
HFKDNHCDQGCNSEECGWDGLDCAADQENLAEGTLVIVVLMPPPEQLLQDARSFLRA
LGTLLHTNLRKRSQGELMVYPYYGEKSAAMKKQRMTRRSLPGEQEQEVAGSKVFLE
IDNRQCVQDSDHCFKNTDAAAALLASHAIQGTLSYPLVSVVSESLTPERTQLLYLLAVA
VVIIIFHILLGVIMAKRKRKHGSLWLPEGFTLRRDASNHRREPVGQDAVGLKNLSVQV
SEANLTGTGTSEHWVDDEGPQPKKVKAEDEALLSEEDDPIDRRPWTQQHLEAADIRRT
PSLALTPPQAEQEVLDVLDVNVVRGPDGCTPLMLASLRGGSSDLSDEDEDAEDSSANIITDL
VYQGASLQAQTDRTGEMALHLAARYSRADAARKLLDAGADANAQDNMGRCPHLHAA
VAADAQGVFQILIRNRVTDLDARMNDGTTPLILAAARLAVEGMVAELINCQADVNAVD
DHGKSALHWAAAVNNVEATLLLLKNGANRDMQDNKEETPLFLAAREGSYEAAKILLD
HFANRDITDHMDRLPRDVARDRMHHDIVRLLEYNVTPSPPGTVLTSALSPVTCGPNRS

FLSLKHTPMGKKSRRPSAKSTMPTSLPNLAKEAKDAKGSRRKKSLSEK VQLSESSVTL
 PVDSLESPTYVSDTTSSPMITSPGILQASPNPMLATAAPPAPVHAQHLSFSNLHEMQP
 LAHGASTVLPVSQLLSHHHIVSPGSGSAGSLRLHPVPVPADWMNRMEVNETQYNEM
 FGMVLAPAEGTHPGIAPQSRPPEGKHITTPREPLPPIVTFQLIPKGSIAQAPAGAPQPQSTCP
 PAVAGPLPTMYQIPEMARLPSVAFPTAMMPQQDGQVAQTILPAYHPFPASVGKYPTPPS
 QHSYASSNAAERTPSHSGHLQGEHPYLTPSPESPDQWSSSSPHSASDWSDVTTSPTPGGA
 GGGQRGPGTHMSEPPHNNMQVYA (SEQ ID NO: 245).

[0285] Human neurogenic locus notch homolog protein 2 isoform 2 precursor
 NP_001186930.1:

MPALRPALLWALLALWLCCAAPAHALQCRDGYEPCVNEGMCVTYHNGTGYCKCPEG
 FLGEYCQHRDPCEKNRCQNGGTCVAQAMLGKATCRCASGFTGEDCQYSTSHPCFVSRP
 CLNGGTCHMLSRDITYECTCQVGFTGKECQWIDACLSHPCANGSTCTTVANQFSCKCLT
 GFTGQKCETDVNECDIPGHCQHGGTCLNLPGSYQCQCPQGFTGQYCDSLYVPCAPSPC
 VNGGTQRQTGDFTECNCLPGFEGSTCERNIDDCPNHRCQNGGVCVDGVNTYNCRCPP
 QWTGQFCTEDVDECLLQPNACQNGGTCANRNGGYGCVCVNGWSGDDCSENIDDCAF
 ASCTPGSTCIDRVASFSCMCPEGKAGLLCHLDDACISNPCHKGALCDTNPLNGQYTCTC
 PQGYKGADCTEDVDECAMANSNPCEHAGKCVNTDGAHFCECLKGYAGPRCEMDINEC
 HSDPCQNDATCLDKTGGFTCLCMPGFGKVHCELEINECQSNPCVNNGQCVDKVNRFQC
 LCPPGFTGPVCQIDIDDCSSTPCLNGAKCIDHPNGYECQCATGFTGVLCEENIDNCDPDP
 CHHGQCQDGDISTYTCICNPGYMGATCSDQIDECYSSPCLNDGRCIDLVNGYQCNCQPGT
 SGVNCEINFDDCASNPCHGTCMDGINRYSCVCSPGFTGQRCNIDIDECASNPCRKGATC
 INGVNGFRCTCPEGPHHPSCYSQVNECLSNPCIHGNCCTGGLSGYKCLCDAGWVGINCEV
 DKNECLSNPCQNGGTCDNLVNGYRCTCKKGFKGYNCQVNIDECASNPCLNQGTCFDDI
 SGYTCHCVLPYTGKNCQTVLAPCSPNPCENAAVCKESPNEFESYTCLCAPGWQGQRCTI
 DIDECISKPCMNHGLCHNTQGSYMCECPPGFSGMDCEEDIDDCLANPCQNGGSCMDGV
 NTFSCLCPLPGFTGDKCQTDMECLSEPCKNGGTCSYVNSYTCCKQAGFDGVHCENNI
 NECTESSCFNGGTCDVGINSFSCLCPLVGFTGSFCLHEINECSSHPCLNEGTCVDGLGTyr
 CSCPLGYTGKNCQTLVNLCSRSPCKNKGTCVQKKAESQCLCPSGWAGAYCDVPNVSC
 DIAASRRGVLVEHLCQHSGVCINAGNTHYCQCPLGYTGSYCEEQLDECASNPCQHGT
 CSDFTGGYRCECVPGYQGVNCEYEVEDECQNQPCQNGGTCDLVNHFKCSCPPGTRGM
 KSSLSIFHPGHCLKL (SEQ ID NO: 246).

[0286] Human neurogenic locus notch homolog protein 3 precursor NP_000426.2;
 MGPGARGRRRRRRPMSPPPPPPPVRALPLLLLLAGPGAAAPPCLDGSPCANGGRCTQLP
 SREAACLCPGWVGERCQLEDPCSHGSPCAGRGVCQSSVAGTARFSCRCPRGFRGPDC
 SLPDPCLSSPCAHGARCSVGPDGRFLCSCPPGYQGRSCRSDVDECRVGEPCRHHGGTCLN

TPGSFRCQCPAGYTGPLCENPAVPCAPSPCRNGGTCRQSGDLTYDCACLPGFEGQNCEV
NVDDCPGHRCLNGGTCVDGVNTYNCQCQPEWTGQFCTEDVDECQLQPNACHNGGTCTF
NTLGGHSCVCVNGWTGESCSQNIDDCATAVCFHGATCHDRVASFYCACPMGKTGLLC
HLDDACVSNPCHEDATCDTNPVNGRATCTCPPGFTGGACDQDVDECSTGANPCEHLGR
CVNTQGSFLCQCGRGYTGPRCETDVNECLSGPCRNQATCLDRTGQFTCTCMAGFTGT
CEVDIDECQSSPCVNGGVCKDRVNGFSCTCPSGFSGSTCQLDVDECASTPCRNGAKCVD
QPDGYECRCAEGFEGTLCDRNVDDCSPDPCHHGRCVDGIAFSCACAPGYTGTRCESQ
VDECRSQPCRHHGKCLDLVDKYLRCRPSGTTGVNCEVNIDDCASNPTFGVCRDGINR
YDCVCQPGFTGPLCNVEINECASSPCGEGGSCVDGENGFRCCLCPPGSLPPLCLPPSHPCA
HEPCSHGTCYDAPGGFRCVCEPGWSGPRCSQSLARDACESQPCRAGGTCSDDGMGFHC
TCPPGVQGRQCELLSPCIPNPCEHGGRCESAPGQLPVCSCPQGWQGPRCQQDVDECAGP
APCGPHGTCTNLASFSCTCHGGYTGPSCDQDINDCDPNPCLNGGSCQDGVGSFSCSCL
PGFAGPRCARDVDECLSNPCGPGTCTDHVASFTCTCPPGYGGFHCEQDLPDCSPSSCFN
GGTCVDGVNSFSCLCRPGYTGAHCQHEADPCLSRPCLHGGVCSAAHPGFRCTCLESFT
GPQCQTLVDWCSRQPCQNGGRCVQTGAYCLCPPGWSGRLCDIRSLPCREAAAQTGVR
LEQLCQAGGQCVDEDEDSSHVCVPEGRTGSHCEQEVDPCLAQPCQHGGTCRGYMGGY
MCECLPGYNGDNCEDDVDECASQPCQHGGSCIDL VARYLCSCPPGTLGVLCEINEDDC
GPGPPLDSGPRCLHNGTCVDLVGGFRCTCPPGYTGLRCEADINECRSGACHAAHTRDCL
QDPGGGFRCLCHAGFSGPRCQTVLSPCESQPCQHGGQCRPSPGPGGGLTFTCHCAQPFW
GPRCERVARSCRELQCPVGVPCQQTTPRGPRCACPPGLSGPSCRSPGSPPGASNASCAAA
PCLHGGSCRPAFLAPFFRCACAQGWTPRCEAPAAPEVSEEPRCPRAACQAKRGDQR
CDRECNSPGCGWDGGDCSLSVGDPWRQCEALQCWRLFNNRCDPACSSPACLYDNFD
CHAGGRERTCNPVYEKYNADHFADGRCDQGCNTEECGWDGLDCASEVPALLARGVL
VLTVLLPPEELLRSSADFLQRLSAILRTSLRFLDAHGQAMVFPYHRPSPGSEPRARREL
APEVTGSSVVMLEIDNRLCLQSPENDHCFPDAQSAADYLGALSAVERLDFPYPLRDVRGE
PLEPPEPSVPLLPLLVAGAVLLLVLVLGVMVARRKREHSTLWFPEGFSLHKDVASGHK
GRREPVGQDALGMKNMAKGESLMGEVATDWMDETCPEAKRLKVEEPPMGAEAAVD
CRQWTQHHLVAADIRVAPAMALTPPQGDADADGMDVNVVRGPDGFTPLMLASF CGGA
LEPMPTEEDEADDTASIIISDLTCQGAQLGARTDRTGETALHLAARYARADA AKRLLD
AGADTNAQDHSGRTPLHTAVTADAQGVFQILIRNRSTDLDARMADGSTALILAA RLAV
EGMVEELIASHADVNAVDELGKSALHWAAAVNNVEATLALLKNGANKDMQDSKEET
PLFLAAREGSYEA AKLLLDHFANREITDHLDRLPDVAQERLHQDIVRLLDQPSGPRSP
GPHGLGPLLCPPGAFLPGLKAAQSGSKKSRRPPGKAGLGPQGPRGRGKLTACPGPLA
DSSVTLSPVDSLSPRPFGGPPASPGGFLEGPYAAATATAVSLAQLGGPGRAGLGRQPP
GGCVLSLGLLNPVAVPLDWARLPPPAPGPSFLLPLAPGPQLLNPGTPVSPQERPPPYLA

VPGHGEEYPAAGAHSSPPKARFLRVPSEHPYLTPSPESPEHWASPSPPSLSDWSESTPSPA
TATGAMATTTGALPAQPLPLSVPSSLAQAQTQLGPQPEVTPKRQVLA (SEQ ID NO: 247).
[0287] Human neurogenic locus notch homolog protein 4 preprotein NP_004548.3:
MQPPSLLLLLLLLLLLLLCVSVVRPRGLLCGSFPEPCANGGTCLSLSLGQGTCQCAPGFLGE
TCQFPDPCQNAQLCQNGGSCQALLPAPLGLPSSPSPLTPSFLCTCLPGFTGERCQAKLED
PCPPSFCSKRGRCHIQASGRPQCSCMPGWTGEQCQLRDFCSANPCVNGGVCLATYPQIQ
CHCPPGFEGHACERDVNECFQDPGPCPKGTSCHTLGSFQCLCPVGQEGPRCEL RAGPC
PPRGCSNGGTCQLMPEKDSTFHLCLCPPGFTGPDCEVNP DNCVSHQCQNGGTCQDGLD
TYTCLCPETWTGWDCSEDVDECETQGPPHCRNGGTCQNSAGSFHCVCVSGWGGTSCE
ENLDDCIAATCAPGSTCIDRVGSFSLCPPGRTGLLCHLED MCLSQPCHGDAQCSTNPLT
GSTLCLCQPGYSGPTCHQDLDECLMAQQGPSPCEHGGSC LNTPGSFNCLCPPGYTG SRC
EADHNECLSQPCHPGSTCLDLLATFHCLCPPGLEGLCEVETNEC ASAPCLNHADCHDL
LNGFQCTCLPGFSGTRCEEDIDECRSSPCANGGQCQDQPGAFHCKCLPGFEGPRCQTEV
DECLSDPCPVGASCLDLPGAFFCLCPSGFTGQLCEVPLCAPNLCQPKQTCKDQKDKANC
LCPDGSPGCAPPEDNCTCHHGHCCRSSCVCDVGTGPECEAELGGCISAPCAHGGTCY
PQPSGYNCTCPTGYTGPTCSEEMTACHSGPCLNGGSCNPSPGGYYCTCPPSHTGPQCQT
STDYCVSAPCFNGGTCVNRPGTFSCLCAMGFQGPRCEGKLRPSCADSPCRNRATCQDSP
QGPRCLCPTGYTGGSQTLMDLCAQKPCPRNSHCLQTGPSFHCLCLQGTGPLCNLPL
SSCQKAALSQGIDVSSLCHNGGLCVDSGPSYFCHCPPGFQGS LQDHVNPCESRPCQNG
ATCMAQPSGYLCQCAPGYDGQNC SKELDACQSQPCHNHGTCTPKPGGFHCACPPGFV
GLRCEGDVDECLDQPCHTGTAAACHSLANAFYCQCLPGHTGQWCEVEIDPCHSQPCFH
GGTCEATAGSPLGFTCHCPKGFEGPTCSHRAPSCGFHHCHHGGLCLPSPKPGFPPRCACL
SGYGGPDCLTPPAPKGC GPPSPCLYNGSCSETTGLGGPGFRCSCPHSSPGPRCQKPGAKG
CEGRSGDGACDAGCSGPGGNWDGGDCSLGVPDPWKGCPSHSRCWLLFRDGGQCHPQC
DSEEC LFDGYDCETPPACTPAYDQYCHDHFHNGHCEKGCNTAECGWDGGDCRPEDGD
PEWGPSLALLVVLSPPALDQQLFALARVLSLTLRVGLWVRKDRDGRDMVYPYPGARA
EEKLGGIRDPTYQERAAPQTQPLGKETDSL SAGFVVVMGVDL SRCGPDHPASRCPWDP
GLLLRFLAAMAAVGALEPLLPGPLLA VHPHAGTAPPANQLPWPVLCSPVAGVILLALG
ALLVLQLIRRRRREHGALWLPPGFTRRPRTQSAPHRRRPPLGEDSTGLKALKPKAEVDE
DGVVMCSGPEEGEEVGQAEETGPPSTCQLWSLSGGCGALPQAAMLIPPQESEMEAPDL
DTRGPDGVTP LMSAVCCGEVQSGTFQGAWLGCPEPWEPLLDGGACPAHTVGTGETP
LHLAARFSRPTAARRLLEAGANPNQPD RAGRTPLHAAVAADAREVCQLLLRSRQTAVD
ARTEDGTTPLMLAARLAVEDLVEELIAAQADV GARDKWGKTALHWAAAVNNARAAR
SLLQAGADKDAQDNREQTPFLAAREGAVEVAQLLLGLGAARELRDQAGLAPADV AH
QRNHWDLLTLLEGAGPPEARHKATPGREAGPFPRARTVS VSVPPHGGGALPRCRTL SA

GAGPRGGGACLQARTWSVDLAARGGGAYSHCRSLSGVGAGGGPTPRGRRFSAGMRGP
RPNPAIMRGRYGVAAGRGGRVSTDDWPCDWVALGACGSASNIPIPPCLTPSPERGSPQ
LDCGPPALQEMPINQGGEGKK (SEQ ID NO: 248).

[0288] In some embodiments, the Notch core of the chimeric Notch receptor polypeptide contains a portion of SEQ ID NO: 244 SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, or SEQ ID NO: 248. In some embodiments, the chimeric Notch receptor polypeptide contains 50 to 1000 amino acids of SEQ ID NO: 244 SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, or SEQ ID NO: 248. In some embodiments, the chimeric Notch receptor polypeptide contains 50 to 900 amino acids, 100 to 800 amino acids, 200 to 700 amino acids, 300 to 600 amino acids, 400 to 500 amino acids of SEQ ID NO: 244 SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, or SEQ ID NO: 248. In some embodiments, the amino acid sequence of Notch, as described herein, is at least 80% identical to a corresponding amino acid sequence in SEQ ID NO: 244 SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, or SEQ ID NO: 248. In some embodiments, the amino acid sequence of Notch is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a corresponding amino acid sequence in SEQ ID NO: 244 SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, or SEQ ID NO: 248. In some embodiments, the amino acid sequence of Notch, as described herein, can vary from the amino acid sequence of SEQ ID NO: 244 SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, or SEQ ID NO: 248 by 1 to 50 amino acids, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids. Human Notch1 core regulatory domain (human Notch1 core regulatory region, hN1 c): ile1427 after the last EGF repeat on NECD of human Notch1(NP_060087) was used as the N-terminus of hN1 c. The last Arg1762 of the 5 consecutive basic amino acids (RKRRR (SEQ ID NO: 249)) at the C-terminus of the transmembrane domain serves as the C-terminus of hN1 C. The sequences of hN1c and mN1c were highly similar.

ILDYSFGGGAGRDIPPLIEEACELPECQEDAGNKVCSLQCNNHACGWDGGDCSLNFND
PWKNCTQSLQCWKYFSDGHCDSDQCN SAGCLFDGFD CQRAEGQCNPLYDQYCKDHFSD
GHCDQGCNSAECEWDGLDCAEHVPERLAAGTLVVVVLMPPEQLRNSSFHFLRELSRVL
HTNVVFKRDAHGQQMIFPYYGREEELRKHPIKRAAEGWAAPDALLGQVKASLLPGGSE
GGRRRRELDPM DV RGSIVYLEIDNRQCVQASSQCFQSATDVAAFLGALASLGS LNIPYK
IEAVQSETVEPPPPAQLHFMVAAAAFVLLFFVGC GVLLSRKRRRNRR (SEQ ID NO:
250) with hN1c at positions 1-294, 295-336 is TMD. Human Notch2 core regulatory domain
(human Notch2 core regulatory region, hN2 c): leu1413 after the last EGF repeat on NECD of
human Notch2(NP_077719) was used as the N-terminus of hN2 c. The last Arg1704 of the 4

consecutive basic amino acids (KRKR (SEQ ID NO: 258)) at the C-terminus of the transmembrane domain serves as the C-terminus of hN 2C.

LYTAPPSTPPATCLSQYCADKARDGVCDEACNSHACQWDGGDCSLTMENPWANCSSP
LPCWDYINNQCDELNTVECLFDNFECQGNSKTCKYDKYCADHFKDNDHCDQGCNSEE
CGWDGLDCAADQPENLAEGTLVIVVLMPPPEQLLQDARSFLRALGTLLHTNLRJKRDSQ
GELMVYPYGEKSAAMKKQRMTRRSLPGEQEVEVAGSKVFLEIDNRQCVQDSDFHCFK
NTDAAAALLASHAIQGTLSYPLVSVVSESLTPERTQLLYLLAVAVVILFIILLGVIMAKR
KR (SEQ ID NO: 251). Human Notch3 core regulatory domain (human Notch3 core regulatory region, hN3 c): pro1375 after the last EGF repeat on NECD of human Notch3 (NP-000426) was used as the N-terminus of hN3 c. The last Arg1669 of the 4 consecutive basic amino acids (RRKR (SEQ ID NO: 259)) at the C-terminus of the transmembrane domain served as the C-terminus of hN 3C.

PAAAPEVSEEPKCPRAACQAKRGDQRCDCRENSPGCGWDGGDCSLSVGDPWRQCEAL
QCWRLFNNRCDPACSSPACLYDNFDCCHAGGRERTCNPVYEKYCADHFADGRCDQGC
NTEECGWDGLDCASEVPALLARGVLVLTVLLPPEELLRSSADFLQRLSAILRTSLRFRDL
AHGQAMVFPYHRPSPGSEPRARRELAPEVIGSVVMLEIDNRLCLQSPENDHCFPDQAQA
ADYLGALSAVERLDFFPYPLRDVRGEPLPEPSVPLLPLLVAGAVLLLVLVGLVMVAR
RRR (SEQ ID NO: 252). Human Notch4 core regulatory domain (human Notch4 core regulatory region, hN4 c): pro1162 after the last EGF repeat on NECD of human Notch4 (NP-004548) was used as the N-terminus of hN4 c. The last Arg1476 of the 5 consecutive basic amino acids (RRRRRRR (SEQ ID NO: 253)) at the C-terminus of the transmembrane domain serves as the C-terminus of hN 4C.

PHSSPGPRCQKPGAKGCEGRSGDGACDAGCSGPGGNWDGGDCSLGVDPWPWKGCPSHS
RCWLLFRDGQCHPQCDSEECCLFDGYDCETPPACTPAYDQYCHDFHNGHCEKGCNTA
ECGWDGGDCRPEDGDPEWGPSLALLVVLSPPALDQQLFALARVLSLTLRVGLWVRKD
RDGRDMVYPYPGARAEELGGTRDPTYQERAAPQTQPLGKETDLSAGFVVVMGVDL
SRCGPDHPASRCPWDPGLLLRLAAMAAGALEPLLPGPLLAVHHPHAGTAPPANQLPW
PVLCSVPVAGVILLALGALLVLQLIRRRRR (SEQ ID NO: 254).

[0289] As noted above, a synNotch receptor polypeptide of the present disclosure comprises an intracellular domain that is released following binding of the anti-PSCA binding domain to PSCA, where binding of the synNotch receptor polypeptide to PSCA induces cleavage of an above-mentioned proteolytic cleavage site. The intracellular domain comprises an amino acid sequence that is heterologous to the synNotch receptor polypeptide. In other words, the intracellular domain comprises an amino acid sequence that is not naturally present in a synNotch receptor polypeptide.

[0291] In some cases, a transcription factor may be an artificial transcription factor (ATF) including but not limited to e.g., Zinc-finger-based artificial transcription factors (including e.g., those described in Sera T. *Adv Drug Deliv Rev.* 2009 61(7-8):513-26; Collins et al. *Curr Opin Biotechnol.* 2003 14(4):371-8; Onori et al. *BMC Mol Biol.* 2013 14:3 the disclosures of which are incorporated herein by reference in their entirety). In some embodiments, the intracellular domain is a transcriptional activator.

[0293] In some cases, the intracellular domain comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following GAL4-VP64 sequence:
MKLLSSIEQACDICRLKKLKCSKEPKCAKCLKNNWECRYSPTKRSPLTRAHLTEVES
RLERLEQLFLLIFPREDLDMILKMDSLQDIKALLTGLFVQDNVNKDAVTDRLASVETDM
PLTLRQHRISATSSSESSNKGQRQLTVS (SEQ ID NO: 207)

[0295] In some cases, the intracellular domain comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following GAL4-VP64 sequence: MKLLSSIEQACDICRLKCLKCSKEPKCAKCLKNNWECRYSPTKRSPLTRAHLTEVES

RRLERLEQLFLLIFPREDLDMILKMDSLQDIKALLTGLFVQDNVNKDAVTDRLASVETDM
PLTLRQHRISATSSSEESSNKGQRQLTVSGGGSGGGSDALDDFDLDMLGSDALDDFDLDM
MLGSDALDDFDLDMLGSDALDDFDLDM (SEQ ID NO: 209); and has a length of from 208
to 214 amino acids (e.g., 203, 203, 204, 205, 206, 207, or 208 amino acids).

[0296] The present disclosure comprises nucleic acids encoding antigen binding systems provided herein, comprising without limitation nucleic acids encoding anti-PSCA synNotch antigen receptors. The nucleic acid sequence of SEQ ID NO: 151 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 150 and 152-160. The nucleic acid sequence of SEQ ID NO: 162 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 161 and 163-191. The nucleic acid sequence of SEQ ID NO: 173 comprises and provides exemplary nucleic acid sequence corresponding to and encoding SEQ ID NOs: 172.

[0297] In an embodiment an anti-PSCA synNotch receptor peptide comprises or consist of an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLVWYLQKPGQSPQLLIYLGSIAS
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQPLQTPITFGQGTRLEIKGSTSGSGKP
GSGEGSTKGQVQLQWAGALLKPSSETLSLTCAVYGGSFSGNYWSWIRQPPGKGLEWIG
EINHSGSTNYPNLSKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGSYNYFDYWG
QGTLVTVSSILDYSFTGGAGRDIPPPQIEEACELPECQVDAGNKVCNLCNNHACGWDG
GDCSLNFNDPWKNCTQSLQCWKYFSDGHCDSCNSAGCLFDGFDGCLTEGQCNPLYD
QYCKDHFSDGHCDQGCNSAECEWDGLDCAEHVPERLAAGTLVLVLLPPDQLRNNSF
HFLRELSHVLHTNVVFKRDAQGGQMIFPYYGHEEELRKHPIKRSTVGWATSSLLPGTSG
GRQRRELDPMDIRGSIVYLEIDNRQCVQSSSQCFQSATDVAAFLGALASLGLNIPYKIE
AVKSEPVEPPLPSQLHLMYVAAAFAVLLFFVGCGLVLLSRKRRRQHGQLWFPEGFKVSE
ASKKKRREPLGEDSVGLKPLKNASDGAALMDDNQNEWGDEDLETKKFRFEPPVVGSMK
LLSSIEQACDICRLKCLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLTRAHLTEVESRLE
RLEQLFLLIFPREDLDMILKMDSLQDIKALLTGLFVQDNVNKDAVTDRLASVETDMPLT
LRQHRISATSSSEESSNKGQRQLTVSGGGSGGGSDALDDFDLDMLGSDALDDFDLDM
GSDALDDFDLDMLGSDALDDFDLDM (SEQ ID NO: 210). In embodiments an anti-PSCA
synNotch receptor is encoded by a nucleic acid having at least 75% sequence identity to (such as,
at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%,
85–100%, 90–95%, 90–100%, or 95–100%) to the nucleic acid having the sequence according to
gacatcgatgacacagagccctctgtctttacccgttaccgccggtgaacccgtagcatcagctgcagaagctcccagcttttactccac
agcaacggctacaactatttagtggtatttacagaaacccggccagagcccccagctgctgattatctgggctccattcgtgtagcggc

gtgcccgatagattttccggcagcgggaagcggcaccgacttcactttaaatctctcgtgtggaggccgaggacgtggcgcttactact
gtatgcagcctctgcagacccccattaccttcggccaaggctactcgtctggaaatcaagggcagcaccagcggcagcggaacccggga
agcggcgagggaagcaccaaaggccaagttcagctgcagcagtggggagctgggttactgaagcctagcgagacactgtctttaacatg
cgccgtgtacggcggaagcttcagcggcaactattggagctggatcagacagcctcccggtaagggttagagtggtatcgcgagatca
accactccggctccaccaactataacccctctttaagtctcgtgtgacctctccgtggacaccagcaagaaccagtctctttaagtga
gtccgtgacagccgccacaccgctgtgtattactgtgctcgtggcgagctacaactacttcgactactggggccaaggtacctctgt
gaccgtgtccagcatcctggactacagcttcacaggtggcgctggcgcgacattccccaccgcagattgaggaggcctgtgagctgc
ctgagtgccaggtggatgcaggcaataaggtctgcaacctgcagtgtaataatcacgcatgtggctgggatgggtggcgactgctccctcaa
cttaatgacccttgaagaactgcacgcagctctacagtgtggaagtattttagcgacggccactgtgacagccagtgaactcggcc
ggctgctctttgatggcttcgactgccagctcaccgaggagcagtgcaacccctgtatgaccagtactgcaaggaccacttcagtgtg
gccactgcgaccagggtgtaacagtgcgaatgtgagtggtggatggcctagactgtgctgagcatgtaccgagcggctggcagccgg
caccttggtgctggtggtgctgctccacccgaccagctacggaacaactcctccactttctcgggagctcagccacgtgtgcacacc
aacgtggtcttaagcgtgatgcgaaggccagcagatgatcttccgtactatggccacgaggaagagctgcgaagcaccaatcaa
gcgctctacagtggttggccacctcttactgcttctggtacaagtgtggggcgccagcgaggagctggacccatggacatccg
tggctcattgtctacctggagatcgacaaccggcaatgtgtgcagtcacctcgcagtgcttccagagtccaccgatgtggtgcttctc
aggtgctcttgcgtcacttggcagcctcaatattccttacaagattgaggccgtgaagagtgcgagcgggtggagcctccgctgcccctgcag
ctgcacctcatgtacgtggcagcagccgcttctgctcctgttcttggggctgtgggtgctgctgtcccgaagcgccggcggcagc
acggtcaactttggttccagaaggttcaaggtctccgaagcctccaagaaaagcgaagggaaccactcggggaagacagtgtaggg
ttgaaacctttgaagaacgccagcagtgagccttgatggatgataacaaaaatgaatggggtgatgaagacctggaaacaaaaagtctc
gcttgtaggaacctgtggttagatccatgaaactccttagcagcatcgaaagcgttgcgacatctgcaggtgaaaaaactcaagtgtca
aaagaaaagcctaagtgcgcaaagtgccttaaaaacaattgggaatgtcgtatagcccaagacaaaaggcggacctctcacgagagc
acacctgactgaggtagaatctcgttgagaggtggaacagcttttctgcttatttccacgcgaggatctcgatatgatcctcaaatg
gactccctccaggacatcaaagctctgctgactggactgtttgtacaggataatgtgaacaaggacgctgtgacagacagattggcaagcg
tgagagccgatatgccctgaccttagacagcaccggatcagtgccaccttcttagcgaggaaagttcaataaaggacagcgccagc
tgacgggtgagtgcggtggaagcggaggaggtccgacgctcttgatgatttcgatctcgacatgctgggatcagacgctctcgacgactt
cgatttgacatgcttgatccgacgctctcgatgatttcgacctcgacatgctcgatccgatgctctgggatgactttgatcttgatgctg
(SEQ ID NO: 221)

[0298] As disclosed herein the intracellular domain is a polypeptide that, when released upon binding of the anti-PSCA binding domain to PSCA, induces production, in a cell that expresses the synNotch polypeptide, of an anti-PSMA CAR.

As the intracellular domain of a synNotch receptor polypeptide of the present disclosure, when released upon binding of the first member of the specific binding pair to a second member of the specific binding pair, may induce the expression of various polypeptides as described herein, in some instances, induced expression of two or more polypeptides may generate a logic gated circuit. Such logic gated circuits may include but are not limited to e.g., "AND gates", "OR gates",

"NOT gates" and combinations thereof including e.g., higher order gates including e.g., higher order AND gates, higher order OR gates, higher order NOT gates, higher order combined gates (i.e., gates using some combination of AND, OR and/or NOT gates).

In some embodiments an anti-PSCA synNotch receptor comprises a Myc tag, such as having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the amino acid sequence set forth as SEQ ID NO. 75 (EQKLISEEDL: SEQ ID NO: 222). In an embodiment, a Myc tag is encoded by the nucleic acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to gagcagaagctgattagcgaggaggattta (SEQ ID NO: 223).

[0299] Both engineered T cell receptors (TCR) and chimeric antigen receptor (CAR) therapies harness the specificity and immunotherapeutic effect of T cells for the treatment of a wide variety of malignancies. Some studies suggest that these therapies may be susceptible to the suppressive factors in the TME that result from T cell suppression by TGF- β (Bendle et al., J Immunol, 191:3232–3239 (2013) and Vong et al., Blood, 130:1791 (2017)). The present disclosure contemplates the use of the DN TGF- β Receptors described herein in combination with either TCR or CAR therapies as a way to maintain, or in some cases, restore TCR and/or CAR expansion in the presence of TGF- β suppression.

[0300] Chimeric antigen receptor (CAR) T cell therapy provides another therapeutic approach against tumor progression. Clinically, investigators have demonstrated that CAR expansion and persistence is correlated with therapeutic efficacy. Without being bound by any theory, it is believed that TGF- β repressed T cell populations found in the TME may be limiting CAR T cell expansion and persistence in patients who do not respond to CAR therapy. The resulting inhibitory cytokines in the TME are believed to limit CAR cell function and expansion. Thus, TGF- β could limit the efficacy of therapeutic engineered T cells.

[0301] Combining any CAR constructs or TCRs as described herein with a DN TGF- β receptors may restore, maintain or enhance the therapeutic effect of CAR T therapy challenged by TGF- β suppression. Thus, in one embodiment described herein, the DN TGF- β receptors, for example DN TGF- β RI or RII, are co-expressed in a T cell or an NK cell with an anti-PSMA CAR, either a constitutively or conditionally expressed as described herein. In some embodiment, the DN TGF- β receptors, for example DN TGF- β RI or RII, are co-expressed in a T cell or NK cell with an anti-PSMA CAR, such as described herein. In some embodiments the DN TGF- β receptors, for example DN TGF- β RI or RII, are co-expressed in a T cell or NK cell with an anti-PSMA CAR and an anti-PSCA synNotch receptor, such as described herein. DN TGF- β receptors are

described in International Patent Publication No. PCT/US2020/070157, which is hereby incorporated herein by reference in its entirety.

[0302] Dominant negative TGF- β Receptors are designed to inhibit the immunosuppressive effects of TGF- β in the TME. These constructs may also stimulate cytokine signaling to enhance T cell or NK function in the TME. The constructs described herein may be used alone or in combination with each other, and/or in combination with other immunotherapies, in order to inhibit TGF- β induced immunosuppression.

[0303] The engineered TGF- β receptors may comprise an N-terminal signal peptide at the N-terminus, for example at the N-terminus of the extracellular ligand binding domain of DN TGF- β RI. In one embodiment, a heterologous signal peptide may be used. The extracellular domain of a DN TGF- β RI may be fused to a leader or a signal peptide that directs the nascent protein into the endoplasmic reticulum and subsequent translocation to the cell surface. It is understood that, once a polypeptide containing a signal peptide is expressed at the cell surface, the signal peptide is generally proteolytically removed during processing of the polypeptide in the endoplasmic reticulum and translocation to the cell surface. Thus, a polypeptide such as a DN TGF- β RI is generally expressed at the cell surface as a mature protein lacking the signal peptide, whereas the precursor form of the polypeptide includes the signal peptide. Any suitable signal sequence may be used. In one embodiment described herein, the DN TGF- β RI comprises the amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) of SEQ ID NO: 224 or a portion thereof. MEAAVAAPRPRLLLLVLAAAAAAAALLPGATA (SEQ ID NO: 224).

[0304] A signal peptide or leader may facilitate the glycosylation of DN TGF- β RI. The signal sequence or leader is a peptide sequence generally present at either the N-terminus or C-terminus of newly synthesized proteins that directs their entry into the secretory pathway. In the present disclosure, the signal peptide is joined to the N-terminus of the extracellular antigen-binding domain of the DN TGF- β RI as a fusion protein. In one embodiment, the DN TGF- β RI comprises an extracellular ligand binding domain having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the wild-type TGF- β RI and a signal peptide at the N-terminus of the extracellular domain TGF- β RI, having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 225.

[0305] MEAAVAAPRPRLLLLVLAAAAAAAALLPGATALQCFCHLCTKDNFTCVTDGL CFVSVTETTDKVIHNSMCIAEIDLIPDRPFVVCAPSSKTGSVTTTTYCCNQDHCNKIELPTT VKSSPGLGPVEL (SEQ ID NO: 225).

[0306] The engineered DN TGF- β RII constructs may also comprise an N-terminal signal peptide at the N-terminus of the extracellular ligand binding domain of TGF- β RII. In one embodiment, a heterologous signal peptide may be used. The extracellular domain of a DN TGF- β RII may be fused to a leader or a signal peptide that directs the nascent protein into the endoplasmic reticulum and subsequent translocation to the cell surface. It is understood that, once a polypeptide containing a signal peptide is expressed at the cell surface, the signal peptide is generally proteolytically removed during processing of the polypeptide in the endoplasmic reticulum and translocation to the cell surface. Thus, a polypeptide such as a DN TGF- β RII is generally found at the cell surface as a mature protein lacking the signal peptide, whereas the precursor form of the polypeptide includes the signal peptide. Any suitable signal sequence may be used. In one embodiment described herein, the DN TGF- β RII constructs described herein comprise a signal sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 226 or a portion thereof. MGRGLLRGLWPLHIVLWTRIAS (SEQ ID NO: 226). In another embodiment, the signal sequence is derived from Colony Stimulating Factor 2 Receptor Alpha subunit (CSF2R α) comprising the amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) of SEQ ID NO: 137 or a portion thereof. MLLLVTSLLLCELPHPAFLIP (SEQ ID NO: 137). The signal sequences described herein may also be optionally used with any suitable protein tag, including but not limited to: V5-tag, myc-tag, HA-tag, Spot-tag, NE-tag. In one embodiment described herein, the signal sequence and tag comprise the amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID NO: 227. MLLLVTSLLLCELPHPAFLIPEQKLISEEDL (SEQ ID NO: 227).

[0307] It is understood that use of this signal peptide is exemplary. Any suitable signal peptide, as are well known in the art, may be applied to the DN TGF- β RI or RII to provide cell surface expression in an immune cell. Useful signal peptides may be derived from cell surface proteins naturally expressed in the T cell NK cell or precursor cell thereof, including any of the signal peptides of the polypeptides disclosed herein. Thus, any suitable signal peptide may be utilized to direct the DN TGF- β RI RII to be expressed at the cell surface of a T cell or NK cell.

[0308] In embodiments, a DN TGF- β RI comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 228.

[0309] MEAAVAAPRPRLLLLVLAAAAAAAALLPGATALQCFCHLCTKDNFTCVTDGL
CFVSVTETTDKVIHNSMCIAEIDLIPDRPFVFCAPSSKTGSVTTTCCNQDHCNKIELPTT
VKSSPGLGPVELAAVIAGPVCFVCISLMLMVYIRVNRQ (SEQ ID NO: 228).

[0310] In one embodiment a DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 229:

[0311] MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFC
DVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILE
DAASPKCIMKEKKKPGETFFMCSSDECNDNIIFSEEYNTSNPDLLL VIFQVTGISLLPPL
GVAISVIIIIFYCYRVNRQ (SEQ ID NO: 229).

[0312] In an embodiment a DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 230.

[0313] TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFC D V R F S T C D N Q K S C M S N C S I T S I C
E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C
S C S S D E C N D N I I F S E E Y N T S N P D (SEQ ID NO: 230).

[0314] In one embodiment described herein, the DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the the wild-type TGF- β RII as shown in the amino acid sequence of SEQ ID NO: 231.

[0315] TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFC D V R F S T C D N Q K S C M S N C S I T S I C
E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C
S C S S D E C N D N I I F S E E Y N T S N P D L L L V I F Q V T G I S L L P P L G V A I S V I I F Y C Y (SEQ ID NO: 231).

[0316] In one embodiment described herein, the DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 232.

[0317] TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMC
SCSSDECNDNIIFSEYNTSNPDSGPILLTISILSFFSVALLVIL (SEQ ID NO: 232).

[0318] In one embodiment described herein, the DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) as shown in the amino acid sequence of SEQ ID NO: 233.

[0319] TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMC
SCSSDECNDNIIFSEYNTSNPDSGPILLTCTISILSFFSVALLVIL (SEQ ID NO: 233).

[0320] T In one embodiment described herein, the DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID NO: 234.

[0321] ACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDI
QARDEVEGFLQDTPPQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGN
VSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPSLQSGILTLNPVAQG
QPILTSLGSNQEEAYVTMSSFYQ (SEQ ID NO: 234).

[0322] In one embodiment described herein, the DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 235.

[0323] TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMC
SCSSDECNDNIIFSEYNTSNPDSGPILLTISILSFFSVALLVILACVLWKKRIKPIVWPSLP
DHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTPPQLEESE
KQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKN
GPHVYQDLLLLSLGTTNSTLPPPSLQSGILTLNPVAQGPILTSLGSNQEEAYVTMSSFYQ
NQ (SEQ ID NO: 235).

[0324] In one embodiment described herein, the DN TGF- β RII comprises an amino acid sequence at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) to the amino acid sequence of SEQ ID NO: 236.

[0325] TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMC

SCSSDECNDNIIFSEEYNTSNPDSPGILLTCPTISILSFFSVALLVILACVLWKKRIKPIVWP
SLPDHKKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQRDEVEGFLQDTFPQQL
SEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGK
NGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLNPVAQGGQPILTSLGSNQEEAYVTMSSFY
QNQ (SEQ ID NO: 236).

[0326] In an embodiment an engineered DN TGF- β RII comprises an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) the amino acid sequence of SEQ ID NO: 237.

[0327] MLLLVTSLLLCELPHPAFLLIPTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFC
D VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 237).

[0328] In an embodiment an engineered DN TGF- β RII comprises an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID NO: 238.

[0329] MLLLVTSLLLCELPHPAFLLIPEQKLISEEDLTIPPHVQKSVNNDMIVTDNNGAVK
FPQLCKFC DVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPK
LPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID
NO: 238).

[0330] In an embodiment an engineered DN TGF- β RII comprises an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID: 239.

[0331] MLLLVTSLLLCELPHPAFLLIPTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFC
D VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDSPGILLTISILSFFSVALL
VILACVLWKKRIKPIVWPSLPDHKKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQA
RDEVEGFLQDTFPQQLSEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSA
CDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLNPVAQGGQPIL
TSLGSNQEEAYVTMSSFYQNQ (SEQ ID NO: 239).

[0332] In an embodiment an engineered DN TGF- β RII comprises an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID: 240.

[0333] MLLLVTSLLLCELPHPAFLLIPEQKLISEEDLTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGPILLTISILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTPPQQLLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPSLQSGILT LNPVAQGQPILTSLSNQEEAYVTMSSFYQNNQ (SEQ ID NO: 240).

[0334] In an embodiment an engineered DN TGF- β RII comprises an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID: 241.

[0335] MLLLVTSLLLCELPHPAFLLIPTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGPILLTISILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTPPQQLLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPSLQSGILT LNPVAQGQPILTSLSNQEEAYVTMSSFYQNNQ (SEQ ID NO: 241).

[0336] In an embodiment an engineered DN TGF- β RII comprises an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID: 242.

[0337] MLLLVTSLLLCELPHPAFLLIPEQKLISEEDLTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGPILLTPTISILSFFSVALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFLQDTPPQQLLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPSLQSGILT LNPVAQGQPILTSLSNQEEAYVTMSSFYQNNQ (SEQ ID NO: 242).

[0338] In an embodiment an engineered DN TGF- β RII comprises an amino acid sequence having at least 75% sequence identity to (such as, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85–90%, 85–95%, 85–100%, 90–95%, 90–100%, or 95–100%) SEQ ID: 243.

[0339] MLLLVTSLLLCELPHPAFLLIPTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED

AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDSPILLTCPTISILSFFSV
ALLVILACVLWKKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVD
DIQARDEVEGFLQDTPPQQLLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAG
NVSACDAPILSSSRSLDCRESGKNPHVYQDLLLSLGTNSTLPPPSLQSGILTLNPVAQ
GQPILTSLSGNQEEAYVTMSSFYQNN (SEQ ID NO: 243).

[0340] The present disclosure contemplates, the expression of polynucleotides encoding the anti-PSMA binding domains disclosed herein and the anti-PSMA CARs disclosed herein, co-expression of polynucleotides comprising the engineered DN TGF- β Receptors with constitutively expressed anti-PSMA CARs, conditionally expressed anti-PSMA CARs and anti-PSCA synNotch receptor described herein, fragments thereof, cells and compositions comprising the same, and vectors that express polypeptides. "Polypeptide," "polypeptide fragment," "peptide" and "protein" are, unless specified to the contrary, and according to conventional meaning, i.e., as a sequence of amino acids. Polypeptides are not limited to a specific length, e.g., they may comprise a full length protein sequence or a fragment of a full length protein, and may include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. In various embodiments, the polypeptides contemplated herein comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein.

[0341] Polypeptides include "polypeptide variants." Polypeptide variants may differ from a naturally occurring polypeptide in one or more substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated, for example, by modifying one or more of the above polypeptide sequences. For example, in some embodiments, it may be desirable to improve the binding affinity and/or other biological properties of the engineered DN TGF- β Receptors, engineered anti-PSMA CARs and engineered anti-PSCA synNotch Receptors by introducing one or more substitutions, deletions, additions and/or insertions. Preferably, polypeptides of the disclosure include polypeptides having at least about 50%, 60%, 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% amino acid identity thereto. Polypeptides of the disclosure include variants having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any of the reference sequences described herein (see, e.g., Sequence Listing), typically where the variant maintains at least one biological activity of the reference sequence. Polypeptides include "polypeptide fragments." Polypeptide fragments refer to a polypeptide, which may be monomeric or multi-meric that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion or substitution of a naturally-occurring or recombinantly-produced polypeptide.

In certain embodiments, a polypeptide fragment may comprise an amino acid chain at least 5 to about 500 amino acids long. It will be appreciated that in certain embodiments, fragments are at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 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, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 150, 200, 250, 300, 350, 400, or 450 amino acids long.

[0342] The polypeptide may also be fused in-frame or conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support.

[0343] As noted above, polypeptides of the present disclosure may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a reference polypeptide may be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985, *Proc. Natl. Acad. Sci. USA*, 82: 488–492), Kunkel *et al.*, (1987, *Methods in Enzymol*, 154: 367–382), U.S. Pat. No. 4,873,192, Watson, J. D. *et al.*, (*Molecular Biology of the Gene*, Fourth Edition, Benjamin/Cummings, Menlo Park, Calif., 1987) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.*, (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.).

[0344] In certain embodiments, a variant will contain conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Modifications may be made in the structure of the polynucleotides and polypeptides of the present disclosure and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics.

[0345] Polypeptide variants further include glycosylated forms, aggregative conjugates with other molecules, and covalent conjugates with unrelated chemical moieties (e.g., pegylated molecules). Covalent variants may be prepared by linking functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue, as is known in the art. Variants also include allelic variants, species variants, and muteins. Truncations or deletions of regions which do not affect functional activity of the proteins are also variants.

[0346] Where expression of two or more polypeptides is desired, the polynucleotide sequences encoding them may be separated by an IRES sequence as discussed elsewhere herein. In another embodiment, two or more polypeptides may be expressed as a fusion protein that comprises one

or more self-cleaving polypeptide sequences. In other embodiments, they are expressed from different promoters and can be in two or more (such as three) vectors. In some embodiments, an anti-PSMA CARs encoded in the same vector as an engineered DN TGF- β Receptor and is operably linked to the same promoter as the engineered DN TGF- β Receptor where the sequences are separated by an IRES sequence. In some embodiments, an anti-PSMA CARs encoded in the same vector as an engineered DN TGF- β Receptor is operably linked to a different promoter than the promoter the engineered DN TGF- β Receptor. In some embodiments, an anti-PSMA CARs encoded in the same vector as an engineered DN TGF- β Receptor is operably linked to a conditionally active promoter and the engineered DN TGF- β Receptor is operably linked to a constitutively active promoter. In some embodiments, an anti-PSCA synNotch receptor is encoded in a different vector than either an anti-PSMA CARs or an engineered DN TGF- β Receptor, which could be on the same or different vectors. In some embodiments, a nucleic acid encoding an anti-PSCA synNotch receptor is operably connected to a constitutively active promoter. In some embodiments, a nucleic acid encoding an anti-PSMA CAR is operably connected to a constitutively active promoter. In some embodiments, a nucleic acid encoding an anti-PSMA CAR is operably connected to a conditionally active promoter, for example active upon binding of the transcriptional activator domain of an anti-PSCA synNotch receptor. In some embodiments, a nucleic acid encoding an DN TGF- β Receptor is operably connected to a constitutively active promoter. In embodiments the transcriptional activator domain of an anti-PSCA synNotch receptor comprises GAL4-VP64 and the conditionally active promoter comprises one or more GAL4 binding sites, such as 1, 2, 3, 4, 5, 6, or 7 GAL4 binding sites. In an embodiment a GAL4 binding site has the nucleic acid sequence according to.

[0347] Polypeptides of the present disclosure include fusion polypeptides. In some embodiments, fusion polypeptides and polynucleotides encoding fusion polypeptides are provided. Fusion polypeptides and fusion proteins refer to a polypeptide having at least two, three, four, five, six, seven, eight, nine, or ten or more polypeptide segments. Fusion polypeptides are typically linked C-terminus to N-terminus, although they may also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. The polypeptides of the fusion protein may be in any order or a specified order. Fusion polypeptides or fusion proteins may also include conservatively modified variants, polymorphic variants, alleles, mutants, subsequences, and interspecies homologs, so long as the desired transcriptional activity of the fusion polypeptide is preserved. Fusion polypeptides may be produced by chemical synthetic methods or by chemical linkage between the two moieties or may generally be prepared using other common techniques. Ligated DNA sequences comprising the fusion polypeptide are operably linked to suitable transcriptional or translational control elements as discussed elsewhere herein.

[0348] In one embodiment, a fusion partner comprises a sequence that assists in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments or to facilitate transport of the fusion protein through the cell membrane.

[0349] Fusion polypeptides may further comprise a polypeptide cleavage signal between each of the polypeptide domains described herein. In addition, polypeptide site may be put into any linker peptide sequence. Exemplary polypeptide cleavage signals include polypeptide cleavage recognition sites such as protease cleavage sites, nuclease cleavage sites (e.g., rare restriction enzyme recognition sites, self-cleaving ribozyme recognition sites), and self-cleaving viral oligopeptides (see deFelipe and Ryan, 2004. *Traffic*, 5(8); 616–26).

[0350] Suitable protease cleavages sites and self-cleaving peptides are known to the skilled person (see, e.g., in Ryan *et al.*, 1997. *J Gener. Viral.* 78, 699–722; Scymczak *et al.* (2004) *Nature Biotech.* 5, 589–594). Exemplary protease cleavage sites include, but are not limited to the cleavage sites of potyvirus Nia proteases (e.g., tobacco etch virus protease), potyvirus HC proteases, potyvirus PI (P35) proteases, byovirus Nia proteases, byovirus RNA–2–encoded proteases, aphthovirus L proteases, enterovirus 2A proteases, rhinovirus 2A proteases, picorna 3C proteases, comovirus 24K proteases, nepovirus 24K proteases, RTSV (rice tungro spherical virus) 3C–like protease, PYVF (parsnip yellow fleck virus) 3C–like protease, heparin, thrombin, factor Xa and enterokinase. Due to its high cleavage stringency, TEV (tobacco etch virus) protease cleavage sites may be used. In other embodiments, self-cleaving peptides may include those polypeptide sequences obtained from potyvirus and cardiovirus 2A peptides, FMDV (foot–and–mouth disease virus), equine rhinitis A virus, Thosea asigna virus and porcine teschovirus. In other embodiments, the self-cleaving polypeptide site comprises a 2A or 2A–like site, sequence or domain (Donnelly *et al.*, 2001. *J Gen. Viral.* 82:1027–1041).

[0351] Generally, it is understood that any appropriate viral vector or vectors may be used for transduction of the engineered constructs described herein. In one embodiment described herein, a cell (e.g., T cell or NK cell) is transduced with a retroviral vector, e.g., a lentiviral vector, encoding an engineered DN TGF- β Receptor construct and an engineered anti-PSMA CARs and/or engineered anti-PSCA synNotch receptors as described herein. The transduced T cells elicits a stable, long–term, and persistent response.

[0352] As used herein, the term "retrovirus" refers to an RNA virus that reverse transcribes its genomic RNA into a linear double–stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. Illustrative retroviruses suitable for use in some embodiments, include, but are not limited to: Moloney murine leukemia virus (M–MuLV), Moloney murine

sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV) and lentivirus.

[0353] As used herein, the term "lentivirus" refers to a group (or genus) of complex retroviruses. Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

[0354] The term "vector" is used herein to refer to a nucleic acid molecule capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, e.g., inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. Useful vectors include, for example, plasmids (e.g., DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, e.g., replication defective retroviruses and lentiviruses.

[0355] As will be evident to one of skill in the art, the term "viral vector" is widely used to refer either to a nucleic acid molecule (e.g., a transfer plasmid) that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also host cell components in addition to nucleic acid(s).

[0356] The term viral vector may refer either to a virus or viral particle capable of transferring a nucleic acid into a cell or to the transferred nucleic acid itself. Viral vectors and transfer plasmids contain structural and/or functional genetic elements that are primarily derived from a virus. The term "retroviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus. The term "lentiviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus. The term "hybrid vector" refers to a vector, LTR or other nucleic acid containing both retroviral, e.g., lentiviral, sequences and non-retroviral viral sequences. In one embodiment, a hybrid vector refers to a vector or transfer plasmid comprising retroviral e.g., lentiviral, sequences for reverse transcription, replication, integration and/or packaging.

[0357] In some embodiments, the terms "lentiviral vector," "lentiviral expression vector" may be used to refer to lentiviral transfer plasmids and/or infectious lentiviral particles. Where reference

is made herein to elements such as cloning sites, promoters, regulatory elements, heterologous nucleic acids, etc., it is to be understood that the sequences of these elements are present in RNA form in the lentiviral particles of the disclosure and are present in DNA form in the DNA plasmids of the disclosure. In one embodiment described herein, the expression vector is a lentivirus expression vector.

[0358] At each end of the provirus are structures called "long terminal repeats" or "LTRs." The term "long terminal repeat (LTR)" refers to domains of base pairs located at the ends of retroviral DNAs which, in their natural sequence context, are direct repeats and contain U3, R and U5 regions. LTRs generally provide functions fundamental to the expression of retroviral genes (e.g., promotion, initiation and polyadenylation of gene transcripts) and to viral replication. The LTR contains numerous regulatory signals including transcriptional control elements, polyadenylation signals and sequences needed for replication and integration of the viral genome. The viral LTR is divided into three regions called U3, R, and U5. The U3 region contains the enhancer and promoter elements. The U5 region is the sequence between the primer binding site and the R region and contains the polyadenylation sequence. The R (repeat) region is flanked by the U3 and U5 regions. The LTR is composed of U3, R and U5 regions and appears at both the 5' and 3' ends of the viral genome. Adjacent to the 5' LTR are sequences necessary for reverse transcription of the genome (the tRNA primer binding site) and for efficient packaging of viral RNA into particles (the Psi site).

[0359] As used herein, the term "packaging signal" or "packaging sequence" refers to sequences located within the retroviral genome which are required for insertion of the viral RNA into the viral capsid or particle, see e.g., Clever et al., 1995. J of Virology, Vol. 69, No. 4; pp. 2101–2109. Several retroviral vectors use the minimal packaging signal (also referred to as the psi ['P] sequence) needed for encapsidation of the viral genome. Thus, as used herein, the terms "packaging sequence," "packaging signal," "psi" and the symbol "P," are used in reference to the non-coding sequence required for encapsidation of retroviral RNA strands during viral particle formation.

[0360] In various embodiments, vectors comprise modified 5' LTR and/or 3' LTRs. Either or both of the LTR may comprise one or more modifications including, but not limited to, one or more deletions, insertions, or substitutions. Modifications of the 3' LTR are often made to improve the safety of lentiviral or retroviral systems by rendering viruses replication-defective. As used herein, the term "replication-defective" refers to virus that is not capable of complete, effective replication such that infective virions are not produced (e.g., replication-defective lentiviral progeny). The term "replication-competent" refers to wild-type virus or mutant virus that is

capable of replication, such that viral replication of the virus is capable of producing infective virions (e.g., replication-competent lentiviral progeny).

[0361] "Self-inactivating" (SIN) vectors refers to replication-defective vectors, e.g., retroviral or lentiviral vectors, in which the right (3') LTR enhancer-promoter region, known as the U3 region, has been modified (e.g., by deletion or substitution) to prevent viral transcription beyond the first round of viral replication. This is because the right (3') LTR U3 region is used as a template for the left (5') LTR U3 region during viral replication and, thus, the viral transcript cannot be made without the U3 enhancer-promoter. In a further embodiment of the disclosure, the 3'LTR is modified such that the U5 region is replaced, for example, with an ideal poly(A) sequence. It should be noted that modifications to the LTRs such as modifications to the 3'LTR, the 5'LTR, or both 3' and 5'LTRs, are also contemplated herein.

[0362] An additional safety enhancement is provided by replacing the U3 region of the 5'LTR with a heterologous promoter to drive transcription of the viral genome during production of viral particles. Examples of heterologous promoters which may be used include, for example, viral simian virus 40 (SV40) (e.g., early or late), cytomegalovirus (CMV) (e.g., immediate early), Moloney murine leukemia virus (MoMLV), Rous sarcoma virus (RSV), and herpes simplex virus (HSV) (thymidine kinase) promoters. Typical promoters are able to drive high levels of transcription in a Tat-independent manner. This replacement reduces the possibility of recombination to generate replication-competent virus because there is no complete U3 sequence in the virus production system. In certain embodiments, the heterologous promoter has additional advantages in controlling the manner in which the viral genome is transcribed. For example, the heterologous promoter may be inducible, such that transcription of all or part of the viral genome will occur only when the induction factors are present. Induction factors include, but are not limited to, one or more chemical compounds or the physiological conditions such as temperature or pH, in which the host cells are cultured.

[0363] In some embodiments, viral vectors comprise a TAR element. The term "TAR" refers to the "trans-activation response" genetic element located in the R region of lentiviral (e.g., HIV) LTRs. This element interacts with the lentiviral trans-activator (tat) genetic element to enhance viral replication.

[0364] The "R region" refers to the region within retroviral LTRs beginning at the start of the capping group (i.e., the start of transcription) and ending immediately prior to the start of the poly A tract. The R region is also defined as being flanked by the U3 and U5 regions. The R region plays a role during reverse transcription in permitting the transfer of nascent DNA from one end of the genome to the other.

[0365] As used herein, the term "FLAP element" refers to a nucleic acid whose sequence includes the central polypurine tract and central termination sequences (cPPT and CTS) of a includes the central polypurine tract and central termination sequences (cPPT and CTS) of a retrovirus, e.g., HIV-I or HIV-2. Suitable FLAP elements are described in U.S. Pat. No. 6,682,907 and in Zennou, et al., 2000, *Cell*, 101: 173. During HIV-I reverse transcription, central initiation of the plus-strand DNA at the central polypurine tract (cPPT) and central termination at the central termination sequence (CTS) lead to the formation of a three-stranded DNA structure: the HIV-I central DNA flap. While not wishing to be bound by any theory, the DNA flap may act as a cis-active determinant of lentiviral genome nuclear import and/or may increase the titer of the virus.

[0366] In one embodiment, retroviral or lentiviral transfer vectors comprise one or more export elements. The term "export element" refers to a cis-acting post-transcriptional regulatory element which regulates the transport of an RNA transcript from the nucleus to the cytoplasm of a cell. Examples of RNA export elements include, but are not limited to, the human immunodeficiency virus (HIV) rev response element (RRE) (see e.g., Cullen et al., 1991. *J Virol.* 65: 1053; and Cullen et al., 1991. *Cell* 58: 423), and the hepatitis B virus post-transcriptional regulatory element (HPRE). Generally, the RNA export element is placed within the 3' UTR of a gene, and may be inserted as one or multiple copies.

[0367] In other embodiments, expression of heterologous sequences in viral vectors is increased by incorporating post-transcriptional regulatory elements, efficient polyadenylation sites, and optionally, transcription termination signals into the vectors. A variety of posttranscriptional regulatory elements may increase expression of a heterologous nucleic acid at the protein, e.g., woodchuck hepatitis virus post-transcriptional regulatory element (WPRE; Zufferey et al., 1999, *J Virol.*, 73:2886); the post-transcriptional regulatory element present in hepatitis B virus (HPRE) (Huang et al., *Mol. Cell. Biol.*, 5:3864); and the like (Liu et al., 1995, *Genes Dev.*, 9:1766).

[0368] In some embodiments, vectors may include regulatory oligonucleotides having transcriptional or translational regulatory activity. Such an oligonucleotide can be used in a variety of gene expression configurations for regulating control of expression. A transcriptional regulatory oligonucleotide, can increase (enhance) or decrease (silence) the level of expression of a recombinant expression construct. Regulatory oligonucleotides may selectively regulate expression in a context specific manner, including, for example, for conferring tissue specific, developmental stage specific, or the like expression of the polynucleotide, including constitutive or inducible expression. A regulatory oligonucleotide of the disclosure also can be a component of an expression vector or of a recombinant nucleic acid molecule comprising the regulatory oligonucleotide operatively linked to an expressible polynucleotide. A regulatory element can be of various lengths from a few nucleotides to several hundred nucleotides.

[0369] Elements directing the efficient termination and polyadenylation of the heterologous nucleic acid transcripts increases heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. In some embodiments, vectors comprise a polyadenylation sequence 3' of a polynucleotide encoding a polypeptide to be expressed. The term "poly A site" or "poly A sequence" as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation sequences may promote mRNA stability by addition of a poly A tail to the 3' end of the coding sequence and thus, contribute to increased translational efficiency. Efficient polyadenylation of the recombinant transcript is desirable as transcripts lacking a poly A tail are unstable and are rapidly degraded. Illustrative examples of poly A signals that may be used in a vector of the disclosure, includes an ideal poly A sequence (e.g., AATAAA, ATTAAA, AGTAAA), a bovine growth hormone poly A sequence (BGHpA), a rabbit β -globin poly A sequence (r β gpA), or another suitable heterologous or endogenous poly A sequence known in the art.

[0370] Also described herein are "codon-optimized" nucleic acids. A "codon-optimized" nucleic acid refers to a nucleic acid sequence that has been altered such that the codons are optimal for expression in a particular system (such as a particular species or group of species). For example, a nucleic acid sequence can be optimized for expression in mammalian cells or in a particular mammalian species (such as human cells) by replacing at least one, more than one, or a significant number, of codons of the native sequence with codons that are more frequently or most frequently used in the genes of that species. Codon optimization does not alter the amino acid sequence of the encoded protein.

[0371] The codon-optimized nucleotide sequences presented in the instant disclosure can present improved properties related to expression efficacy. In some embodiments, the DNA sequence to be transcribed may be optimized to facilitate more efficient transcription and/or translation. In some embodiments, the DNA sequence may be optimized regarding cis-regulatory elements (e.g., TATA box, termination signals, and protein binding sites), artificial recombination sites, chi sites, CpG dinucleotide content, negative CpG islands, GC content, polymerase slippage sites, and/or other elements relevant to transcription; the DNA sequence may be optimized regarding cryptic splice sites, mRNA secondary structure, stable free energy of mRNA, repetitive sequences, RNA instability motif, and/or other elements relevant to mRNA processing and stability; the DNA sequence may be optimized regarding codon usage bias, codon adaptability, internal chi sites, ribosomal binding sites (e.g., IRES), premature polyA sites, Shine-Dalgarno (SD) sequences, and/or other elements relevant to translation; and/or the DNA sequence may be optimized

regarding codon context, codon-anticodon interaction, translational pause sites, and/or other elements relevant to protein folding.

[0372] The vectors may have one or more LTRs, wherein any LTR comprises one or more modifications, such as one or more nucleotide substitutions, additions, or deletions. The vectors may further comprise one or more accessory elements to increase transduction efficiency (e.g., a cPPT /FLAP), viral packaging (e.g., a Psi (Ψ) packaging signal, RRE), and/or other elements that increase therapeutic gene expression (e.g., poly (A) sequences), and may optionally comprise a WPRE or HPRE. The skilled artisan would appreciate that many other different embodiments may be fashioned from the existing embodiments of the disclosure.

[0373] A "host cell" includes cells transfected, infected, or transduced in vivo, ex vivo, or in vitro with a recombinant vector or a polynucleotide of the disclosure. Host cells may include packaging cells, producer cells, and cells infected with viral vectors. In some embodiments, host cells infected with viral vector of the disclosure are administered to a subject in need of therapy. In certain embodiments, the term "target cell" is used interchangeably with host cell and refers to transfected, infected, or transduced cells of a desired cell type. In some embodiments, the target cell is a T cell.

[0374] Large scale viral particle production is often necessary to achieve a reasonable viral titer. Viral particles are produced by transfecting a transfer vector into a packaging cell line that comprises viral structural and/or accessory genes, e.g., gag, pol, env, tat, rev, vif, vpr, vpu, vpx, or nef genes or other retroviral genes.

[0375] As used herein, the term "packaging vector" refers to an expression vector or viral vector that lacks a packaging signal and comprises a polynucleotide encoding one, two, three, four or more viral structural and/or accessory genes. Typically, the packaging vectors are included in a packaging cell, and are introduced into the cell via transfection, transduction or infection. Methods for transfection, transduction or infection are well known by those of skill in the art. A retroviral/lentiviral transfer vector of the present disclosure may be introduced into a packaging cell line, via transfection, transduction or infection, to generate a producer cell or cell line. The packaging vectors of the present disclosure may be introduced into human cells or cell lines by common methods including, e.g., calcium phosphate transfection, lipofection or electroporation. In some embodiments, the packaging vectors are introduced into the cells together with a dominant selectable marker, such as neomycin, hygromycin, puromycin, blastocidin, zeocin, thymidine kinase, DHFR, Gln synthetase or ADA, followed by selection in the presence of the appropriate drug and isolation of clones. A selectable marker gene may be linked physically to genes encoding by the packaging vector, e.g., by IRES or self-cleaving viral peptides.

[0376] Viral envelope proteins (env) determine the range of host cells which may ultimately be infected and transformed by recombinant retroviruses generated from the cell lines. In the case of lentiviruses, such as HIV-1, HIV-2, SIV, FIV and EIV, the env proteins include gp41 and gp120. In some embodiments, the viral env proteins expressed by packaging cells of the disclosure are encoded on a separate vector from the viral gag and pol genes, as has been previously described.

[0377] Illustrative examples of retroviral-derived env genes which may be employed in the embodiments described herein include, but are not limited to: MLV envelopes, IOAI envelope, BAEV, FeLV-B, RDI 14, SSAV, Ebola, Sendai, FPV (Fowl plague virus), and influenza virus envelopes. Similarly, genes encoding envelopes from RNA viruses (e.g., RNA virus families of Picomaviridae, Calciviridae, Astroviridae, Togaviridae, Flaviviridae, Coronaviridae, Paramyxoviridae, Rhabdoviridae, Filoviridae, Orthomyxoviridae, Bunyaviridae, Arenaviridae, Reoviridae, Bimaviridae, Retroviridae) as well as from the DNA viruses (families of Hepadnaviridae, Circoviridae, Parvoviridae, Papovaviridae, Adenoviridae, Herpesviridae, Poxviridae, and Iridoviridae) may be utilized. Representative examples include, FeLV, VEE, HFVW, WDSV, SFV, Rabies, ALV, BIV, BL V, EBV, CAEV, SNV, ChTL V, STLV, MPMV, SMRV, RAV, FuSV, MH2, AEV, AMV, CTIO, and EIAV.

[0378] In other embodiments, envelope proteins for pseudotyping a virus of present disclosure include, but are not limited to any of the following virus: Influenza A such as H1N1, H1N2, H3N2 and H5N1 (bird flu), Influenza B, Influenza C virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rotavirus, any virus of the Norwalk virus group, enteric adenoviruses, parvovirus, Dengue fever virus, Monkey pox, Mononegavirales, Lyssavirus such as rabies virus, Lagos bat virus, Mokola virus, Duvenhage virus, European bat virus 1 & 2 and Australian bat virus, Ephemerovirus, Vesiculovirus, Vesicular Stomatitis Virus (VSV), Herpes viruses such as Herpes simplex virus types 1 and 2, varicella zoster, cytomegalovirus, Epstein-Barr virus (EBV), human herpesviruses (HHV), human herpesvirus type 6 and 8, Human immunodeficiency virus (HIV), papilloma virus, murine gamma herpes virus, Arenaviruses such as Argentine hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Sabia-associated hemorrhagic fever virus, Venezuelan hemorrhagic fever virus, Lassa fever virus, Machupo virus, Lymphocytic choriomeningitis virus (LCMV), Bunyaviridae such as Crimean-Congo hemorrhagic fever virus, Hantavirus, hemorrhagic fever with renal syndrome causing virus, Rift Valley fever virus, Filoviridae (filovirus) including Ebola hemorrhagic fever and Marburg hemorrhagic fever, Flaviviridae including Kaysanur Forest disease virus, Omsk hemorrhagic fever virus, Tick-borne encephalitis causing virus and Paramyxoviridae such as Hendra virus and Nipah virus, variola major and variola minor (smallpox), alphaviruses such as Venezuelan equine

encephalitis virus, eastern equine encephalitis virus, western equine encephalitis virus, SARS-associated coronavirus (SARS-Co V), West Nile virus, or any encephalitis causing virus.

[0379] The terms "pseudotype" or "pseudotyping" as used herein, refer to a virus whose viral envelope proteins have been substituted with those of another virus possessing other characteristics. For example, HIV may be pseudotyped with vesicular stomatitis virus G-protein (VSV-G) envelope proteins, which allows HIV to infect a wider range of cells because HIV envelope proteins (encoded by the env gene) normally target the virus to CD4+ presenting cells.

[0380] As used herein, the term "packaging cell lines" is used in reference to cell lines that do not contain a packaging signal, but do stably or transiently express viral structural proteins and replication enzymes (e.g., gag, pol and env) which are necessary for the correct packaging of viral particles. Any suitable cell line may be employed to prepare packaging cells of the disclosure. Generally, the cells are mammalian cells. In another embodiment, the cells used to produce the packaging cell line are human cells. Suitable cell lines which may be used to produce the packaging cell line include, for example, CHO cells, BHK cells, MDCK cells, C3H 10T1/2 cells, FLY cells, Psi-2 cells, BOSC 23 cells, P A317 cells, WEHI cells, COS cells, BSC 1 cells, BSC 40 cells, BMT 10 cells, VERO cells, W138 cells, MRC5 cells, A549 cells, HT1080 cells, 293 cells, 293T cells, B-50 cells, 3T3 cells, NIH3T3 cells, HepG2 cells, Saos-2 cells, Huh7 cells, HeLa cells, W163 cells, 211 cells, and 211A cells.

[0381] As used herein, the term "producer cell line" refers to a cell line which is capable of producing recombinant retroviral particles, comprising a packaging cell line and a transfer vector construct comprising a packaging signal. The production of infectious viral particles and viral stock solutions may be carried out using conventional techniques. Methods of preparing viral stock solutions are known in the art and are illustrated by, e.g., Y. Soneoka et al. (1995) Nucl. Acids Res. 23:628-633, and N. R. Landau et al. (1992) J Virol. 66:5110-5113. Infectious virus particles may be collected from the packaging cells using conventional techniques. For example, the infectious particles may be collected by cell lysis, or collection of the supernatant of the cell culture, as is known in the art. Optionally, the collected virus particles may be purified if desired. Suitable purification techniques are well known to those skilled in the art.

[0382] The delivery of a gene(s) or other polynucleotide sequence using a retroviral or lentiviral vector by means of viral infection rather than by transfection is referred to as "transduction." In one embodiment, retroviral vectors are transduced into a cell through infection and provirus integration. In certain embodiments, a target cell, e.g., a T cell or NK cell, is "transduced" if it comprises a gene or other polynucleotide sequence delivered to the cell by infection using a viral or retroviral vector. In some embodiments, a transduced cell comprises one or more genes or other polynucleotide sequences delivered by a retroviral or lentiviral vector in its cellular genome.

[0383] In some embodiments, host cells expressing one or more of the constructs of the disclosure (anti-PSMA CAR, anti-PSCA synNotch receptor, and/or). The host cells may be transduced with one or more viral vectors comprising nucleic acid sequences encoding one or more polypeptides expressing a DN TGF- β receptor construct of the disclosure and an engineered TCR and/or a CAR. The host cells may be administered to a subject to treat and/or prevent T cell malignancies. Other methods relating to the use of viral vectors in gene therapy, which may be utilized according to certain embodiments of the present disclosure, may be found in, e.g., Kay, M.A. (1997) *Chest* 111(6 Supp.): 138S–142S; Ferry, N. and Heard, J.M. (1998) *Hum. Gene Ther.* 9:1975–81; Shiratory, Y. et al., (1999) *Liver* 19:265–74; Oka, K. et al., (2000) *Curr. Opin. Lipidol.* 11:179–86; Thule, P. M. and Liu, J.M. (2000) *Gene Ther.* 7:1744–52; Yang, N. S. (1992) *Crit. Rev. Biotechnol.* 12:335–56; Alt, M. (1995) *J Hepatol.* 23:746–58; Brody, S. L. and Crystal, R.G. (1994) *Ann. NY Acad. Sci.* 716:90–101; Strayer, D.S. (1999) *Expert Opin. Investig. Drugs* 8:2159–2172; Smith–Arica, J. R. and Bartlett, J. S. (2001) *Curr. Cardiol. Rep.* 3:43–49; and Lee, H. C. et al., (2000) *Nature* 408:483–8.

[0384] The compositions described herein may comprise one or more polynucleotides, polypeptides, vectors comprising same, and T cell compositions, as contemplated herein. One embodiment described herein is a composition comprising a modified T cell that co-expresses one or more engineered DN TGF- β Receptors described herein with an engineered TCR and/or CAR. Compositions include, but are not limited to pharmaceutical compositions. A "pharmaceutical composition" refers to a composition formulated in pharmaceutically-acceptable or physiologically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions of the present disclosure may be administered in combination with other agents as well, such as, e.g., cytokines, growth factors, hormones, small molecules, chemotherapeutics, pro-drugs, drugs, antibodies, or other various pharmaceutically-active agents. There is virtually no limit to other components that may also be included in the compositions, provided that the additional agents do not adversely affect the ability of the composition to deliver the intended therapy.

[0385] The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0386] As used herein "pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative,

dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl

[0387] cellulose and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter, waxes, animal and vegetable fats, paraffins, silicones, bentonites, silicic acid, zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations.

[0388] In one embodiment described herein, compositions of the present disclosure comprise an amount of modified T cells contemplated herein. It may generally be stated that a pharmaceutical composition comprising the T cells contemplated herein may be administered at a dosage of 10^2 to 10^{10} cells/kg body weight, 10^5 to 10^9 cells/kg body weight, 10^5 to 10^8 cells/kg body weight, 10^5 to 10^7 cells/kg body weight, 10^7 to 10^9 cells/kg body weight, or 10^7 to 10^8 cells/kg body weight, including all integer values within those ranges. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. T cells modified to express an engineered TCR or CAR may be administered multiple times at dosages within these ranges. The cells may be allogeneic, syngeneic, xenogeneic, or autologous to the patient undergoing therapy. If desired, the treatment may also include administration of mitogens (e.g., PHA) or lymphokines, cytokines, and/or chemokines (e.g., IFN- γ , IL-2, IL-7, IL-15, IL-12, TNF- α , IL-18, and TNF- β , GM-CSF, IL-4, IL-13, Flt3-L, RANTES, MIP1 α , etc.) as described herein to enhance engraftment and function of infused T cells.

[0389] Generally, compositions comprising the cells activated and expanded as described herein may be utilized in the treatment and prevention of diseases that arise in individuals who are immunocompromised or immunosuppressed. In some, compositions comprising the modified T cells contemplated herein are used in the treatment of cancers. The modified T cells described herein may be administered either alone, or as a pharmaceutical composition in combination with carriers, diluents, excipients, and/or with other components such as IL-2, IL-7, and/or IL-15 or other cytokines or cell populations. In some embodiments, pharmaceutical compositions

contemplated herein comprise an amount of genetically modified T cells, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients.

[0390] Pharmaceutical compositions comprising modified T cells contemplated herein may further comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present disclosure may be formulated for parenteral administration, e.g., intravascular (intravenous or intra-arterial), intraperitoneal or intramuscular administration.

[0391] The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following: sterile diluents such as water for injection, saline solution, such as physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Sterile injectable pharmaceutical composition are also included.

[0392] In some embodiments, compositions contemplated herein comprise an effective amount of an expanded modified T cell composition, alone or in combination with one or more therapeutic agents. Thus, the T cell compositions may be administered alone or in combination with other known cancer treatments, such as radiation therapy, chemotherapy, transplantation, immunotherapy, hormone therapy, photodynamic therapy, etc. The compositions may also be administered in combination with antibiotics and anti-viral agents. Such therapeutic agents may be accepted in the art as a treatment for a disease state as described herein, such as a cancer. In one embodiment the compositions contemplated herein may also be administered with inhibitors of TGF- β , for example the small molecule inhibitor LY55299. Exemplary therapeutic agents contemplated include cytokines, growth factors, steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, therapeutic antibodies, or other active and ancillary agents.

[0393] In certain embodiments, compositions comprising T cells contemplated herein may be administered in conjunction with any number of chemotherapeutic agents. Illustrative examples of chemotherapeutic agents include but are not limited to alkylating agents such as thiotepa and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and

piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine resins; nitrogen mustards such as chlorambucil, chlornaphazine, chlorthosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabacin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitostane, testolactone; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxorubicin (TAXOTERE®, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RPS 2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™ (alitretinoin); ONTAK™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen,

raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

[0394] A variety of other therapeutic agents may be used in conjunction with the compositions described herein. In one embodiment, the composition comprising T cells is administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

[0395] In some embodiments, NSAIDs are chosen from the group consisting of ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors such as VIOXX® (rofecoxib) and CELEBREX® (celecoxib), and sialylates. Exemplary analgesics are chosen from the group consisting of acetaminophen, oxycodone, tramadol or propoxyphene hydrochloride. Exemplary glucocorticoids are chosen from the group consisting of cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface markers (e.g., CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists (e.g., etanercept (ENBREL®), adalimumab (HUMIRA®) and infliximab (REMICADE®), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary disease-modifying anti-rheumatic drugs (DMARDs) include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular) and minocycline.

[0396] In other embodiments, the therapeutic antibodies suitable for combination with the CAR modified T cells contemplated herein, include but are not limited to, abagovomab, adcatumumab, afutuzumab, alemtuzumab, altumomab, amatuximab, anatumomab, arcitumomab, bavituximab, bectumomab, bevacizumab, bivatuzumab, blinatumomab, brentuximab, cantuzumab, catumaxomab, cetuximab, citatuzumab, cixutumumab, clivatuzumab, conatumumab, daratumumab, drozitumab, duligotumab, dusigitumab, detumomab, dacetuzumab, dalotuzumab, ecromeximab, elotuzumab, ensituximab, ertumaxomab, etaracizumab, farietuzumab, ficlatuzumab, figitumumab, flinvotumab, futuximab, ganitumab, gentuzumab, girentuximab, glembatumumab, ibritumomab, igovomab, imgatuzumab, indatuximab, inotuzumab, intetumumab, ipilimumab, iratumumab, labetuzumab, lexatumumab, lintuzumab, lorvotuzumab,

lucatumumab, mapatumumab, matuzumab, milatuzumab, minretumomab, mitumomab, moxetumomab, namatumab, naptumomab, necitumumab, nimotuzumab, nofetumomab, ocaratuzumab, ofatumumab, olaratumab, onartuzumab, oportuzumab, oregovomab, panitumumab, parsatuzumab, patritumab, pentumomab, pertuzumab, pintumomab, pritumumab, racotumomab, radretumab, rilotumumab, rituximab, robatumumab, satumomab, sibrotuzumab, siltuximab, simtuzumab, solitomab, tacatuzumab, taplitumomab, tenatumomab, teprotumumab, tigatuzumab, tositumomab, trastuzumab, tucotuzumab, ublituximab, veltuzumab, vorsetuzumab, votumumab, zalutumumab, CC49 and 3F8.

[0397] In some embodiments, the compositions described herein are administered in conjunction with a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, chemokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prolaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β , and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF- α or TNF- β ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

[0398] The present disclosure contemplates, in part, genetically modified T cell redirected to a target cell, e.g., a tumor or cancer cell, and that comprises the engineered DN TGF- β Receptors described herein and an engineered TCR or CAR having a binding domain that binds to target antigens on the cells, including the CAR- DN TGF- β Receptor constructs described herein. Cancer cells may also spread to other parts of the body through the blood and lymph systems. There are several main types of cancer. Carcinoma is a cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is a cancer that begins in bone, cartilage, fat, muscle,

blood vessels, or other connective or supportive tissue. Leukemia is a cancer that starts in blood forming tissue such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the blood. Lymphoma and multiple myeloma are cancers that begin in the cells of the immune system. Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord.

[0399] In one embodiment, the target cell expresses an antigen, e.g., target antigen. In one embodiment, the target cell is a pancreatic parenchymal cell, pancreatic duct cell, hepatic cell, cardiac muscle cell, skeletal muscle cell, osteoblast, skeletal myoblast, neuron, vascular endothelial cell, pigment cell, smooth muscle cell, glial cell, fat cell, bone cell, chondrocyte, pancreatic islet cell, CNS cell, PNS cell, liver cell, adipose cell, hepatic cell, renal cell, lung cell, skin cell, ovary cell, follicular cell, epithelial cell, immune cell, or an endothelial cell.

[0400] In certain embodiments, the target cell is part of a pancreatic tissue, neural tissue, cardiac tissue, bone marrow, muscle tissue, bone tissue, skin tissue, liver tissue, hair follicles, vascular tissue, adipose tissue, lung tissue, and kidney tissue.

[0401] In a one embodiment, the target cell is a tumor cell. In another embodiment, the target cell is a cancer cell, such as a cell in a patient with cancer. Exemplary cells that may be killed with the disclosed methods include cells of prostate tumors. In one embodiment, the target cell is a malignant cell of the prostate.

[0402] The modified T cells and NK cells contemplated herein provide improved adoptive immunotherapy for use in the treatment of various conditions including, without limitation, cancer, infectious disease, autoimmune disease, inflammatory disease, and immunodeficiency. In some embodiments, the specificity of a primary T cell is redirected to tumor or cancer cells by genetically modifying the primary T cell engineered to co-express a TCR or CAR contemplated herein with an engineered DN TGF- β Receptor. For example, with the CAR-DN TGF- β Receptor constructs described herein, the antigen binding domain of the scFv of the CAR directs the T cell to the cells expressing the tumor antigen, and the TGF- β Receptor constructs described herein protect the population of such T cells by inhibiting the suppressive effect of TGF- β .

[0403] In one embodiment of the present disclosure includes a type of cellular therapy where T cells are modified to co-express an engineered DN TGF- β Receptor with an engineered TCR or CAR, including the CAR-DN TGF- β Receptor constructs described herein, that target cancer cells expressing a target antigen, and the modified T cell is infused to a recipient in need thereof. The infused cell is thus able to kill tumor cells in the recipient with minimal impact from TGF- β suppression.

[0404] Any cell may be used as a host cell for the polynucleotides, the vectors, or the polypeptides of the present disclosure. In some embodiments, the cell can be a prokaryotic cell, fungal cell,

yeast cell, or higher eukaryotic cells such as a mammalian cell. Suitable prokaryotic cells include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*; *Enterobacter*; *Erwinia*; *Klebsiella*; *Proteus*; *Salmonella*, e.g., *Salmonella typhimurium*; *Serratia*, e.g., *Serratia marcescens*, and *Shigella*; Bacilli such as *B. subtilis* and *B. licheniformis*; *Pseudomonas* such as *P. aeruginosa*; and *Streptomyces*. In some embodiments, the cell is a human cell. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell is selected from the group consisting of a T cell, a B cell, a tumor infiltrating lymphocyte (TIL), a TCR expressing cell, a natural killer (NK) cell, a dendritic cell, a granulocyte, an innate lymphoid cell, a megakaryocyte, a monocyte, a macrophage, a platelet, a thymocyte, and a myeloid cell. In one embodiment, the immune cell is a T cell. In another embodiment, the immune cell is an NK cell. In certain embodiments, the T cell is a tumor-infiltrating lymphocyte (TIL), autologous T cell, engineered autologous T cell (eACT™), an allogeneic T cell, a heterologous T cell, or any combination thereof. Unlike antibody therapies or standalone TCR or CAR modified T cells, T cells (or any cells as described above) modified to co-express the engineered DN TGF- β Receptors described herein with an engineered TCR or CAR are able to not only replicate in vivo, and thus contribute to long-term persistence that may lead to sustained cancer therapy, but have the added advantage of avoiding the suppressive impact of TGF- β . Thus, in one embodiment described herein is a method of inhibiting the activity of TGF- β comprising administering to a subject in need thereof a therapeutically effective amount of the modified T cell co-expressing the DN TGF- β Receptors described herein and a TCR or CAR as described herein.

[0405] In another embodiment, T cells co-expressing the DN TGF- β Receptors with an engineered TCR or CAR as described herein may undergo T cell expansion such that a population of therapeutic T cells may remain or persist for an extended period. Thus, another embodiment described herein is a method of expanding a population of T cells comprising administering to a subject in need thereof a therapeutically effective amount of the T cells described herein.

[0406] In one embodiment described herein, the population of T cells remains between at between about 50% to about 100% after 7 days, at between about 60% to about 90% after 7 days, or at between about 70% to about 80% after 7 days. In another embodiment described herein, the population of T cells remains at about 50% after 7 days, at about 60% after 7 days, at about 70% after 7 days, at about 80% after 7 days, at about 90% after 7 days or at about 100% after 7 days.

[0407] In another embodiment described herein, administration of the modified T cells described herein results in an expansion of transduced T cells in the presence of TGF- β 1 by about 10% to about 100%, about 20% to about 90%, or about 30% to about 80%. In another embodiment described herein, administration of the modified T cells described herein results in an expansion

of transduced T cells in the presence of TGF- β 1 by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

[0408] In one embodiment described herein, administration of a modified T cell from a codon-optimized sequence as described herein results in an increase in expression efficiency by about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%. In another embodiment described herein, administration of a modified T cell from a codon-optimized sequence as described herein results in an increase in transduction efficiency by about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

[0409] Without being bound by any theory, it is believed that despite the promising use of CAR T cell therapy, constitutive tonic signaling in the absence of tumor antigen can result in reduced efficacy, poor CAR T cell survival and toxicity (Ajina et al., Mol Cancer Ther., 17(9):1795-1815(2018)). Thus, a CAR T cell therapy with reduced tonic signaling results superior performance. In one embodiment described herein, administration of a modified T cell from a codon-optimized sequence as described herein in the absence of tumor antigen results in a reduction of cytokine release by about 10%, about 20%, about 30%, about 40%, about 50%, 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%. In one embodiment described herein, administration of a modified T cell from a codon-optimized sequence in the absence of tumor antigen results in a reduction of cytokine release by about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

[0410] In another embodiment, administration of the modified T cell from a codon-optimized sequence as described herein results in a reduction in tumor volume of between about 50% to about 10% to about 100%, about 20% to about 90%, or about 30% to about 80%. In another embodiment the reduction in tumor volume is about 10%, about 20%, about 30%, about 40%, about 50%, 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%.

[0411] Another embodiment described herein is a method of treating a cancer in a subject in need thereof comprising administering an effective amount, e.g., therapeutically effective amount of a composition comprising T cells co-expressing the DN TGF- β Receptors and a TCR or CAR as described herein. The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

[0412] Another embodiment described herein is a method of treating a hepatic cancer in a subject in need thereof comprising administering an effective amount, e.g., therapeutically effective amount of a composition comprising T cells co-expressing the DN TGF- β Receptors and a TCR or CAR as described herein, including the CAR-DN TGF- β Receptor constructs described herein. The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

[0413] In other embodiments, compositions comprising T cell genetically modified with a vector comprising a promoter operably linked to a polynucleotide encoding a DN TGF- β Receptor and a polynucleotide encoding a TCR or CAR, including the CAR-DN TGF- β Receptor constructs described herein, are used in the treatment of solid tumors or cancers including, without limitation, liver cancer, pancreatic cancer, lung cancer, breast cancer, bladder cancer, brain cancer, bone cancer, thyroid cancer, kidney cancer, skin cancer or virus induced cancers.

[0414] In some embodiments, compositions comprising T cell genetically modified with a vector comprising a promoter operably linked to a polynucleotide encoding a DN TGF- β Receptor and a polynucleotide encoding a CAR and/or a synNotch molecule, including the CAR-DN TGF- β Receptor constructs described herein, comprises an antigen-specific binding domain that binds an epitope of PSCA or PSMA are used in the treatment of various cancers.

[0415] In other embodiments, methods comprising administering a therapeutically effective amount of modified T cells contemplated herein or a composition comprising the same, to a patient in need thereof, alone or in combination with one or more therapeutic agents, are provided. In certain embodiments, the cells of the disclosure are used in the treatment of patients at risk for developing a cancer. Thus, the present disclosure provides methods for the treatment or prevention of a cancer comprising administering to a subject in need thereof, a therapeutically effective amount of the modified T cells of the disclosure.

[0416] One of ordinary skill in the art would recognize that multiple administrations of the compositions of the disclosure may be required to effect the desired therapy. For example a composition may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more times over a span of 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 1 year, 2 years, 5, years, 10 years, or more.

[0417] In certain embodiments, it may be desirable to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom according to the present disclosure, and reinfuse the patient with these activated and expanded T cells. This process may be carried out multiple times every few weeks. In certain embodiments, T cells may be activated from blood draws of from 10cc to 400cc. Not to be bound by theory, using

this multiple blood draw/multiple reinfusion protocol may serve to select out certain populations of T cells.

[0418] The administration of the compositions contemplated herein may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. In some embodiments, compositions are administered parenterally. The phrases "parenteral administration" and "administered parenterally" as used herein refers to modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravascular, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intratumoral, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion. In one embodiment, the compositions contemplated herein are administered to a subject by direct injection into a tumor, lymph node, or site of infection.

[0419] In one embodiment, a subject in need thereof is administered an effective amount of a composition to increase a cellular immune response to a cancer in the subject. The immune response may include cellular immune responses mediated by cytotoxic T cells capable of killing infected cells, regulatory T cells, and helper T cell responses. Humoral immune responses, mediated primarily by helper T cells capable of activating B cells thus leading to antibody production, may also be induced. A variety of techniques may be used for analyzing the type of immune responses induced by the compositions of the present disclosure, which are well described in the art; e.g., Current Protocols in Immunology, Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober (2001) John Wiley & Sons, NY, N.Y.

[0420] In the case of T cell-mediated killing, CAR-ligand binding initiates CAR signaling to the T cell, resulting in activation of a variety of T cell signaling pathways that induce the T cell to produce or release proteins capable of inducing target cell apoptosis by various mechanisms. These T cell-mediated mechanisms include (but are not limited to) the transfer of intracellular cytotoxic granules from the T cell into the target cell, T cell secretion of proinflammatory cytokines that may induce target cell killing directly (or indirectly via recruitment of other killer effector cells), and up regulation of death receptor ligands (e.g. FasL) on the T cell surface that induce target cell apoptosis following binding to their cognate death receptor (e.g. Fas) on the target cell.

[0421] In embodiments described herein is a method of treating a subject diagnosed with a cancer, comprising removing T cells from the subject, genetically modifying said T cells with a vector comprising a nucleic acid encoding an engineered DN TGF- β Receptor and an anti-PSMA CAR as contemplated herein, including the anti-PSMA CAR-DN TGF- β Receptor constructs described herein, thereby producing a population of modified T cells, and administering the population of

modified T cells to the same subject. In embodiments described herein is a method of treating a subject diagnosed with a cancer, comprising removing T cells from the subject, genetically modifying said T cells with a vector comprising a nucleic acid encoding an engineered DN TGF- β Receptor and an anti-PSMA CAR as contemplated herein, including the anti-PSMA CAR-DN TGF- β Receptor constructs described herein, thereby producing a population of modified T cells, and administering the population of modified T cells to the same subject nucleic acid construct encoding an anti-PSCA synNotch molecule.

[0422] In certain embodiments, the present disclosure also provides methods for stimulating an effector cell mediated immune modulator response to a target cell population in a subject comprising the steps of administering to the subject an immune effector cell population expressing a nucleic acid construct encoding an engineered DN TGF- β Receptor and an anti-PSMA CAR molecule, including the CAR-DN TGF- β Receptor constructs described herein. In some example the immune effector cell population further express a nucleic acid construct encoding an anti-PSCA synNotch molecule.

[0423] The methods for administering the cell compositions described herein includes any method which is effective to result in reintroduction of ex vivo genetically modified immune effector cells that either directly express an engineered CAR in the subject or on reintroduction of the genetically modified progenitors of immune effector cells that on introduction into a subject differentiate into mature immune effector cells that express the engineered CAR, engineered DN TGF- β Receptor and an anti-PSMA CAR molecule, including the CAR-DN TGF- β Receptor constructs described herein. In some example the immune effector cell population further express a nucleic acid construct encoding an anti-PSCA synNotch molecule. One method comprises transducing peripheral blood T cells ex vivo with a nucleic acid construct in accordance with the present disclosure and returning the transduced cells into the subject.

[0424] Although the foregoing disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this disclosure that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those skilled in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

EXAMPLES

Example 1

[0425] CD3⁺ cells obtained from ALLCells® (Alameda, California) were isolated from peripheral blood mononuclear cells obtained from healthy donors and frozen down in CryoStor® cell cryopreservation media (Sigma Aldrich®). Pan CD3⁺ T cells were isolated from leukopaks containing peripheral blood mononuclear cells (PBMCs) by negative selection using a commercially available kit from STEMCELL Technologies™ (Vancouver, Canada) according to manufacturer's directions and then frozen in liquid nitrogen. Chimeric antigen receptor (CAR) T cells were generated from frozen human Pan CD3⁺ T cells. Before lentivirus transduction, the CD3⁺ pan T cells were thawed, and activated *ex vivo* using anti-CD3/CD28 Dynabeads®, (ThermoFisher Scientific) and 100IU/ml exogenous interleukin-2 (IL-2) according to manufacturer recommendations. The activated cells were rested overnight. One day after anti-CD3/CD28 bead activation, T cells were plated and transduced with a lentiviral vector (with anti-PSMA CAR). Canonical CARs (CARs without synNotch receptor transcriptional activation) were transduced with a multiplicity of infection (MOI) of 6-9. Synthetic notch (synNotch) constructs were dually transduced with a synNotch lentivirus vector MOI of 6-9 and a CAR payload lentivirus vector MOI of 6-9. T cells were de-beaded 3 days after transduction and evaluated for expression and cytotoxicity assays between days 8 and 11 following transduction.

[0426] Canonicals CAR vectors included an EF1a promoter to drive constitutive expression of the anti-PSMA CAR. synNotch vectors included an EF1a promoter to drive constitutive expression of an anti-PSCA synNotch receptor. The CAR payload vectors (for synNotch receptor activated conditional expression) included 5x gal4 binding sites-minimal CMV promoter to drive conditional expression of the anti-PSMA CAR. synNotch constructs were transduced with synNotch and CAR payload vectors simultaneously as described above.

[0427] The anti-PSMA CARs used in the following examples included a CSF2RA signal sequence, an anti-PSMA scFv, CD8α hinge domain, an CD8α transmembrane domain, a 4-1BB co-stimulatory domain, and a CD3zeta signaling domain. The synNotch receptor included an anti-PSCA scFv, a murine Notch core, a gal4 DNA binding domain, and a vp64 transactivation domain. A myc tag was included to simulate ligand induced activation of the synNotch receptors.

[0428] Human prostate cell line 22RV1 and human leukemia cell line K562 were obtained from the American Type Culture Collection (ATCC). Cells were cultured at 37°C, 5% CO₂, in either Roswell Park Memorial Institute (RPMI; 22RV1) or Iscove's Modified Dulbecco's Medium (IMDM; K562), supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS). 22RV1 and K562 cells were engineered to knockout (KO) or overexpress PSCA and PSMA as described in Table 10.

Table 10

Cell Line	PSCA	PSMA
22RV1	-	+
22RV1-PSMA KO	-	-
22RV1-PSMA KO-PSCA	+	-
22RV1-PSCA	+	+
K562	-	-
K562-PSCA	+	-
K562-PSMA	-	+
K562-PSCA-PSMA	+	+

Example 2

[0429] Seven days after transduction, T cells transduced as described in example 1 were harvested to determine transduction efficiencies. 1.5×10^5 T cells were plated in 96 well plates in duplicate. For canonical CAR T cells, T cells were cultured overnight in hTCM (human T cell media), that included of x-vivo 15 (Lonza (Basel, Switzerland)), 5% human serum (Valley Biomedical (Winchester, Virginia)), and 1% Glutamax (Gibco). For synNotch T receptor expressing cells, the T cells were cultured in the presence and absence of myc beads overnight to stimulate the synNotch receptor and thereby induce CAR expression. After overnight culture, CAR expression was measured using recombinant PSMA (rPSMA) containing a 6X His tag (SEQ ID NO: 260). The cells were stained with an anti-His antibody and the stained cells were detected via flow cytometry. Only cells bound by the rPSMA (and anti-His) are detected to measure expression. Table 11 shows canonical CAR expression. Table 12 shows synNotch and CAR payload expression.

Table 11

Donor ID	Construct	scFv	%CAR+	
#1	104	Ab4	91.5%	90.0%
	101	Ab1	95.4%	95.4%
	103	Ab3	90.0%	91.0%
	105	Ab5	84.4%	83.9%
	UTD		0.9%	1.1%
#2	104	Ab4	90.7%	92.0%
	101	Ab1	95.6%	96.3%
	103	Ab3	93.2%	92.7%

#3	105	Ab5	88.4%	88.1%
	UTD		0.9%	1.0%
	104	Ab4	91.0%	92.1%
	101	Ab1	95.6%	96.5%
	103	Ab3	88.4%	87.9%
#3	105	Ab5	83.2%	83.6%
	UTD		0.7%	0.7%

Table 12

Donor ID	Construct	scFv	%synNotch		%CAR+ (Unstimulated)		%CAR+ (Stimulated)	
#1	Ab6 SynNotch/201	Ab1	61.9%	61.6%	3.7%	3.7%	41.5%	41.9%
	Ab6 SynNotch/202	Ab2	71.1%	71.2%	1.2%	1.5%	26.1%	24.2%
	Ab6 SynNotch/203	Ab3	64.7%	65.0%	3.2%	3.2%	50.5%	50.3%
	Ab6 SynNotch/205	Ab5	59.8%	59.6%	2.5%	2.8%	52.4%	52.6%
	UTD		1.0%	1.0%	0.9%	1.1%	0.6%	0.8%
#2	Ab6 SynNotch/201	Ab1	59.3%	58.8%	6.5%	6.1%	49.1%	51.8%
	Ab6 SynNotch/202	Ab2	67.5%	67.4%	1.4%	1.6%	24.5%	24.6%
	Ab6 SynNotch/203	Ab3	62.8%	62.5%	5.6%	5.5%	57.5%	57.4%
	Ab6 SynNotch/205	Ab5	58.7%	56.8%	4.8%	4.2%	58.7%	60.5%
	UTD		0.8%	0.9%	0.9%	1.0%	0.9%	1.3%
#3	Ab6 SynNotch/201	Ab1	66.7%	66.5%	6.2%	6.2%	44.0%	43.3%
	Ab6 SynNotch/202	Ab2	77.7%	77.8%	1.4%	1.4%	42.5%	40.9%

	Ab6							
	SynNotch/203	Ab3	70.0%	70.4%	4.6%	4.6%	52.3%	51.5%
	Ab6							
	SynNotch/205	Ab5	66.5%	66.3%	5.5%	5.3%	57.1%	56.9%
	UTD		1.1%	1.0%	0.7%	0.7%	1.1%	1.1%

Example 3

[0430] For T cell-dependent cytotoxicity assays, T cells cytotoxicity of T cells transduce as described in example 1 was measured in real time at an effector (T cell)-to-target cell (as described in Table 10) ratio (E:T) of 1:1. Target cells were plated on xCELLigence plates overnight with impedance measured every 15min. Briefly, 50µl of hTCM is added to the xCELLigence plate and a measurement is taken to create a baseline for the plate. Then 3×10^4 of each of the 4 different 22RV1 cell lines were plated on xCELLigence plates in 50µl and impedance was measured every 15min overnight (16-18hrs). Lastly, 3×10^4 CAR+ effectors are added in 100µl and impedance is measured every 15min for an additional 72-96hrs. The instrument takes each of the readings, called “Cell Index” and calculates a “% Cytotoxicity” based on how the Tumor cells alone have grown on the same plate. Target cells for canonical CAR T cells were 22RV1-PSMA knockout (KO) (Figure 1) and 22RV1 (Figure 2). Target cells for synNotch T cells were 22RV1-PSMA KO (Figure 3), 22RV1-PSMA KO-PSCA (Figure 4), 22RV1 (Figure 5), and 22RV1-PSCA (Figure 6).

Example 4

[0431] As described in Table 10 5×10^4 target cells were co-cultured with 5×10^4 CAR+ T cells in a 96well plate in hTCM media in a total volume of 200µl for 72hrs. Supernatants were then harvested and cytokine release (IFNγ and IL-2) was determined via Ella with IFNγ or IL-2 cartridges per manufactures recommendations (Protein Simple, San Jose CA).

Table 13: IFNγ release from canonical CARs co-cultured with 22RV1 cells.

DONER #1: IFNγ	4211	4212	4215	1550	UTD
22RV1-PSMA KO	11.1	12.4	5.89	3.62	5.69
	7.31	20.5	6.06	118	6.48
22RV1	3919	2053	1132	899	6.28
	3678	1963	1364	820	5.65

22RV1-PSCA	4699	2548	1673	1160	7.28
	3857	1973	1597	1103	6.6
22RV1-PSMA KO-PSCA	9.08	11.7	4.51	1.01	5.39
	11.5	16	5.45	3.44	6.26

Table 14: IL-2 release from canonical CARs co-cultured with 22RV1 cells.

DONER #1: IL-2	4211	4212	4215	1550	UTD
22RV1-PSMA KO	3.99	0.455	2.22	0.529	2.62
	0.822	25.6	0	0.549	0.099
22RV1	12.6	7.18	6.53	6.18	2.06
	14.3	6.11	8.97	4.7	0.993
22RV1-PSCA	13.8	7.14	8.69	7.98	10.2
	13.5	8.96	7.01	7.33	1.09
22RV1-PSMA KO-PSCA	2.27	0.087	0.96	0.123	0.404
	1.95	0.35	0.567	0.621	0.576

Table 15: IFN γ release from canonical CARs co-cultured with K562 cells.

DONER #1: IFN γ	4211	4212	4215	1550	UTD
K562	3.35	4.2	3.12	1.28	2.15
	3.15	4.78	2.29	2.55	2.6
K562-PSCA	2.39	5.13	0.832	1.24	1.3
	3.19	4.86	1.4	0.047	0.536
K562-PSMA	21043	13396	12236	13666	1.16
	19426	18943	10892	15000	1.61
K562-PSCA-PSMA	22344	16388	15661	16464	5.85
	28231	25309	16407	22367	3.19

Table 16: IL-2 release from canonical CARs co-cultured with K562 cells.

DONER #1: IL-2	4211	4212	4215	1550	UTD
K562	1.63	0.531	1.36	0.555	0.691
	0	0.448	7.33	0.58	0.644
K562-PSCA	0.822	0.384	1.42	0	0.273

	0	0	0	0.062	0
K562-PSMA	16661	17413	25446	21569	0
	20680	21031	25759	23822	0
K562-PSCA-PSMA	23093	20216	29370	28190	0.063
	24756	22018	33154	25095	0.028

Table 17: IFN γ release from synNotch T cells co-cultured with 22RV1 cells.

DONER #1: IFN γ	1912/4204	1912/4206	1912/4207	1912/4209	UTD
22RV1-PSMA KO	1.74	6.31	2.89	2.47	5.69
	2.97	6.13	2.61	3.16	6.48
22RV1	31.6	48.7	21.4	18.3	6.28
	13.2	84.9	21.1	18.8	5.65
22RV1-PSCA	13081	16970	11493	11273	7.28
	14236	12507	11823	8820	6.6
22RV1-PSMA KO-PSCA	11	37.8	7.31	5.11	5.39
	11.4	30.5	6.57	4.78	6.26

Table 18: IL-2 release from synNotch T cells co-cultured with 22RV1 cells.

DONER #1: IL-2	1912/4204	1912/4206	1912/4207	1912/4209	UTD
22RV1-PSMA KO	5.08	0.638	3.53	0	2.62
	1.86	1.51	1.11	0	0.099
22RV1	27.4	32.7	31.4	26.3	2.06
	20	27.2	27.3	32.8	0.993
22RV1-PSCA	2204	1022	937	715	10.2
	2544	966	835	829	1.09
22RV1-PSMA KO-PSCA	24.7	9.39	4.94	10.7	0.404
	23.7	2.64	2.33	10.3	0.576

Table 19: IFN γ release from synNotch T cells co-cultured with K562 cells.

DONER #1: IFN γ	1912/4204	1912/4206	1912/4207	1912/4209	UTD
K562	1.93	2.4	1.99	0.317	2.15
	1.03	5.36	1.35	2.66	2.6

K562-PSCA	7.83	41.1	4.31	0	1.3
	8.63	45.3	3.15	4.16	0.536
K562-PSMA	31.2	17.5	18.2	2.56	1.16
	28.9	32.4	15.6	10	1.61
K562-PSCA-PSMA	39889	37494	38511	29710	5.85
	40127	47355	42126	35489	3.19

Table 20: IL-2 release from synNotch T cells co-cultured with K562 cells.

DONER #1: IL-2	1912/4204	1912/4206	1912/4207	1912/4209	UTD
K562	1.59	1.54	2.16	0	0.691
	1.16	0.655	0.355	0.615	0.644
K562-PSCA	3.22	0.819	3.02	2.37	0.273
	5.65	0.187	0.45	2.23	0
K562-PSMA	9.42	8.49	9.77	2.5	0
	5.22	5.99	7.43	4.26	0
K562-PSCA-PSMA	44348	85959	29228	37515	0.063
	35870	55799	25689	25276	0.028

Table 21: IFN γ release from canonical CARs co-cultured with 22RV1 cells.

DONER #2: IFN γ	4211	4212	4215	1550	UTD
22RV1-PSMA KO	16	15.3	5.81	2.19	0.787
	14.5	19.5	4.77	2.21	4.4
22RV1	4537	4056	3063	1891	2.19
	4417	3047	2301	1408	1.38
22RV1-PSCA	4928	4636	2657	2080	1.44
	5795	3771	3106	1993	0.591
22RV1-PSMA KO-PSCA	13.2	15.8	5.5	0.645	1.3
	11.9	9.08	4.42	0.933	1.53

Table 22: IL-2 release from canonical CARs co-cultured with 22RV1 cells.

DONER #2: IL-2	4211	4212	4215	1550	UTD
22RV1-PSMA KO	3.07	1.79	2.32	0	0.655
	0.192	0	1.78	3.1	1.6
22RV1	9.61	4.28	6.86	7.59	0.956
	7.49	3.96	3.97	0.849	0.704
22RV1-PSCA	8.1	3.93	6.32	5	0.486
	8.81	3.15	3.92	4.08	0.501
22RV1-PSMA KO-PSCA	0.143	1.4	2.57	0	0.481
	0.427	0	1.55	0	0

Table 23: IFN γ release from Canonical CARs co-cultured with K562 cells.

DONER #2: IFN γ	4211	4212	4215	1550	UTD
K562	11	10.2	3.68	4.93	1.06
	11.1	7.76	5.76	4.7	2.95
K562-PSCA	12.8	11.4	7.68	4.95	2.74
	11.3	8.26	3.69	2.04	2.33
K562-PSMA	29454	32708	29759	25791	2.63
	27437	26993	29358	25893	1.85
K562-PSCA-PSMA	38792	37657	25578	36122	3.21
	33466	28020	34273	33250	1.63

Table 24: IL-2 release from Canonical CARs co-cultured with K562 cells.

DONER #2: IL-2	4211	4212	4215	1550	UTD
K562	0	0.342	0.416	1.06	0.113
	0	0	0.146	0	0
K562-PSCA	0.108	0	0.498	0.162	0
	0.041	0	0	0.578	0
K562-PSMA	23311	17945	29384	32289	0.336
	23012	18898	32330	35611	0
K562-PSCA-PSMA	30215	29211	40977	43314	0
	26986	24065	43911	47153	0.016

Table 25: IFN γ release from synNotch T cells co-cultured with 22RV1 cells.

DONER #2: IFN γ	1912/4204	1912/4206	1912/4207	1912/4209	UTD
22RV1-PSMA KO	1.56	13.6	2.94	2.22	0.787
	2.75	16.2	3.96	1.62	4.4
22RV1	68.9	101	42.7	21.4	2.19
	50.4	66.6	37.5	22.4	1.38
22RV1-PSCA	16212	15199	12526	8831	1.44
	15016	17913	13830	9328	0.591
22RV1-PSMA KO-PSCA	27.4	84.1	16	4.5	1.3
	24.6	117	14.7	6.81	1.53

Table 26: IL-2 release from synNotch T cells co-cultured with 22RV1 cells.

DONER #2: IL-2	1912/4204	1912/4206	1912/4207	1912/4209	UTD
22RV1-PSMA KO	0.368	0	1.92	0.506	0.655
	0.121	0.748	0.134	0.709	1.6
22RV1	34.8	20	26.3	17	0.956
	20.4	9.11	16.9	12	0.704
22RV1-PSCA	1241	94.6	287	440	0.486
	1307	115	258	556	0.501
22RV1-PSMA KO-PSCA	24.6	3.84	2.11	3.46	0.481
	12.3	2.01	3.11	3.57	0

Table 27: IFN γ release from synNotch T cells co-cultured with K562 cells.

DONER #2: IFN γ	1912/4204	1912/4206	1912/4207	1912/4209	UTD
K562	1.93	11.9	3.58	2.4	1.06
	0.916	11.7	2.52	1.71	2.95
K562-PSCA	13.8	230	10.2	11.8	2.74
	7.68	213	8.92	9.95	2.33
K562-PSMA	37.7	63.4	92.1	11.6	2.63
	48.4	38.7	43	12.7	1.85
K562-PSCA-PSMA	36941	49398	49600	40575	3.21
	43708	43476	49110	36315	1.63

Table 28: IL-2 release from synNotch T cells co-cultured with K562 cells.

DONER #2: IL-2	1912/4204	1912/4206	1912/4207	1912/4209	UTD
K562	0	0	0.618	0	0.113
	0	2.03	0	0.104	0
K562-PSCA	2.28	0.907	1.19	4.97	0
	0	0.522	1.11	0	0
K562-PSMA	9.73	10.1	12.4	15.7	0.336
	9.4	5.04	6.94	6.17	0
K562-PSCA-PSMA	56844	53309	41059	33992	0
	56812	56739	28879	26926	0.016

Table 29: IFN γ release from Canonical CARs co-cultured with 22RV1 cells.

DONER #3: IFN γ	4211	4212	4215	1550	UTD
22RV1-PSMA KO	15.7	31.8	2.89	0	2.91
	14.9	23.1	3.11	0	3.52
22RV1	963	1087	333	266	8.43
	1095	1233	406	248	3.77
22RV1-PSCA	1453	1509	392	313	5.41
	1352	1270	431	299	2.42
22RV1-PSMA KO-PSCA	14.4	42.4	1.19	0	2.15
	12.1	25.6	1.31	0	2.67

Table 30: IL-2 release Canonical CARs co-cultured with 22RV1 cells.

DONER #3: IL-2	4211	4212	4215	1550	UTD
22RV1-PSMA KO	1.93	3.46	0.137	0.13	0
	0	3.64	0	1.35	1.16
22RV1	7.01	6.67	4.83	6.16	21.4
	9.46	5.1	3.25	4.59	6.83
22RV1-PSCA	6.85	2.77	3.55	4.78	8.2
	9.16	2.82	2.74	4.36	1.88
22RV1-PSMA KO-PSCA	0	0.907	2.11	0	0
	0.022	0.465	0.147	0	0.948

Table 31: IFN γ release from Canonical CARs co-cultured with K562 cells.

DONER #3: IFN γ	4211	4212	4215	1550	UTD
K562	3.95	12.1	2.44	0.122	1.74
	7.59	10.9	2.57	1.56	2.7
K562-PSCA	5.17	20	1.64	2.16	1.14
	4.76	24.4	0	0.684	1.43
K562-PSMA	20686	23162	10258	13894	37.9
	24434	22309	14384	19840	33.7
K562-PSCA-PSMA	26446	18179	18075	17588	50.9
	21796	27232	15897	17508	52.4

Table 32: IL-2 release from synNotch T cells co-cultured with K562 cells.

DONER #3: IL-2	4211	4212	4215	1550	UTD
K562	4.15	5.44	5.44	5.65	8.79
	3.01	5.5	4.1	5.66	4.97
K562-PSCA	3.84	6.64	3.18	6.29	5.75
	3.84	4.89	2.78	4.32	4.46
K562-PSMA	16547	11304	17363	14695	16.7
	15454	12690	17380	16003	27.4
K562-PSCA-PSMA	22756	17217	29446	21885	29.9
	19618	16775	22623	21235	32.5

Table 33: IFN γ release from synNotch T cells co-cultured with 22RV1 cells.

DONER #3: IFN γ	1912/4204	1912/4206	1912/4207	1912/4209	UTD
22RV1-PSMA KO	1.85	2.4	0.691	2.57	2.91
	1.97	1.52	2.05	0.436	3.52
22RV1	33.2	58.6	26.4	12.8	8.43
	16.6	47.4	24	10.9	3.77
22RV1-PSCA	6124	9040	8200	5535	5.41
	6378	9062	6616	5078	2.42
22RV1-PSMA KO-PSCA		49.2	6.68	20.5	2.15
	38.3	36.8	11.3	15.6	2.67

Table 34: IL-2 release from synNotch T cells co-cultured with 22RV1 cells.

DONER #3: IL-2	1912/4204	1912/4206	1912/4207	1912/4209	UTD
22RV1-PSMA KO	1.39	0	1.4	1.25	0
	0.439	0	0	0.338	1.16
22RV1	44.1	43.8	45.3	10.4	21.4
	24.8	40	39.2	17.6	6.83
22RV1-PSCA	1031	1179	503	225	8.2
	1120	1243	609	323	1.88
22RV1-PSMA KO-PSCA	93	13.7	12.2	45.9	0
	71.4	10.1	12.1	40.3	0.948

Table 35: IFN γ release from synNotch T cells co-cultured with K562 cells.

DONER #3: IFN γ	1912/4204	1912/4206	1912/4207	1912/4209	UTD
K562	1.64	3.65	1.73	0.13	1.74
	1.44	3.53	0.594	0.654	2.7
K562-PSCA	16.9	59.4	10.7	3.56	1.14
	38.1	75.6	7.93	10.6	1.43
K562-PSMA	18.5	14.7	20.3	3.97	37.9
	34.2	10.5	23.1	2.94	33.7
K562-PSCA-PSMA	33444	46610	38118	25258	50.9
	31062	37241	30922	25004	52.4

Table 36: IL-2 release from synNotch T cells co-cultured with K562 cells.

DONER #3: IL-2	1912/4204	1912/4206	1912/4207	1912/4209	UTD
K562	5.42	4.53	4.4	4.98	8.79
	4.72	4.39	5.03	4.97	4.97
K562-PSCA	18.1	8.88	13.9	9.25	5.75
	21.1	6.29	11.7	12.9	4.46
K562-PSMA	12.3	17.5	17.5	13.3	16.7
	14.7	16.1	13.1	8.45	27.4
K562-PSCA-PSMA	35691	57642	28190	33381	29.9
	33075	44658	22130	26937	32.5

Example 5

[0432] 22RV1 or mitomycin C treated-K562 cells were co-cultured with Cell Trace Violet (CTV) labeled-T cells in a 96 well plate for 96hrs. Following incubation, plates were analyzed via flow cytometry for dilution of CTV.

Table 37: % Divided canonical CAR T cells following K562 co-culture.

Donor ID	Construct	scFv	K562	K562-PSCA	K562-PSMA	K562-PSCA-PSMA	T cells only
#1	104	Ab4	1.54	1.62	87.9	89.15	1.86
	101	Ab1	2.73	3.41	93.85	94.5	4.26
	103	Ab3	1.99	2.23	92.5	93.85	3.1
	105	Ab5	4.34	5.12	88.95	87.1	6.19
	UTD						
#2	104	Ab4	0.28	0.56	89.1	90.45	0.41
	101	Ab1	0.58	0.47	91	93.3	0.54
	103	Ab3	0.31	0.92	90.95	92.95	0.49
	105	Ab5	0.17	1.3	92.65	93.55	0.15
	UTD		5.83	3.01	7.35	5.91	8.31
#3	104	Ab4	0.5	0.77	93.4	94.8	2.21
	101	Ab1	1.47	1.22	92.9	94	4.07
	103	Ab3	0.96	1.36	95.6	96.6	3.11
	105	Ab5	1.85	2.28	96.3	96.3	7.5
	UTD		3.57	4.99	5.57	3.99	6.58

Table 38: % Divided canonical CAR T cells following 22RV1 co-culture.

Donor ID	Construct	scFv	22RV1-PSMA KO	22RV1	22RV1-PSCA	22RV1-PSMA KO-PSCA	T cells only
#1	104	Ab4	0.89	89	89.3	0.67	1.86
	101	Ab1	3.76	94.25	94.15	3.27	4.26
	103	Ab3	1.03	87.7	89.75	0.88	3.1
	105	Ab5	4.63	78.1	81.35	3.72	6.19
	UTD						
#2	104	Ab4		91.55	90.75	0.14	0.41

	101	Ab1	0.61	91.6	92.3	0.73	0.54
	103	Ab3	0.08	90.1	90.9	0.21	0.49
	105	Ab5	0	90.7	91.55	0.05	0.15
	UTD		2.98	3.08	2.95	2.33	8.31
#3	104	Ab4	0.16	87.9	90.3	0.2	2.21
	101	Ab1	0.7	91	92.95	0.47	4.07
	103	Ab3	0.41	93.75	94.65	0.33	3.11
	105	Ab5	2.35	84.35	87.6	1.17	7.5
	UTD		7.03	7.02	5.72	5.76	6.58

Table 39: % Divided synNotch T cells following K562 co-culture.

Donor ID	Construct	scFv	K562	K562-PSCA	K562-PSMA	K562-PSCA-PSMA	T cells only
#1	Ab6 SynNotch/201	Ab1	1.75	1.79	7.46	31.15	0.93
	Ab6 SynNotch/202	Ab2	25.8	33.35	35.8	68.65	31.25
	Ab6 SynNotch/203	Ab3	1.01	2.22	6.49	37.65	1.41
	Ab6 SynNotch/205	Ab5	7.14	6.3	11.26	43.05	8.09
	UTD						
#2	Ab6 SynNotch/201	Ab1	1.82	3.47	7.78	16.6	0.98
	Ab6 SynNotch/202	Ab2	29.6	31.4	40.55	59.5	35.9
	Ab6 SynNotch/203	Ab3	0.34	2.93	7.92	15.95	
	Ab6 SynNotch/205	Ab5	1.62	3.12	10.34	16.15	2.49
	UTD		5.83	3.01	7.35	5.91	8.31
#3	Ab6 SynNotch/201	Ab1	3.82	17.15	12.55	56.65	5.66

	Ab6 SynNotch/202	Ab2	6.32	26.6	17	61.35	7.45
	Ab6 SynNotch/203	Ab3	7.47	24.74	21.95	67.85	8.34
	Ab6 SynNotch/205	Ab5	7.67	35.9	18.45	75.65	12.3
	UTD		3.57	4.99	5.57	3.99	6.58

Table 40: % Divided synNotch T cells following 22RV1 co-culture.

Donor ID	Construct	scFv	22RV1- PSMA KO	22RV1	22RV1- PSCA	22RV1- PSMA KO-PSCA	T cells only
#1	Ab6 SynNotch/201	Ab1	0.88	1.56	28.2	1.75	0.93
	Ab6 SynNotch/202	Ab2	29.45	31.45	77.75	23.9	31.25
	Ab6 SynNotch/203	Ab3	1.11	2.29	48.1	1.17	1.41
	Ab6 SynNotch/205	Ab5	2.68	3.25	52.1	2.52	8.09
	UTD						
#2	Ab6 SynNotch/201	Ab1		0.69	15.85	0.52	0.98
	Ab6 SynNotch/202	Ab2		18.65	47.95	16.55	35.9
	Ab6 SynNotch/203	Ab3		0.42	16.8	0.59	
	Ab6 SynNotch/205	Ab5		1.83	22.1	0.91	2.49
	UTD		2.98	3.08	2.95	2.33	8.31
#3	Ab6 SynNotch/201	Ab1	7.08	10.58	53.7	10.62	5.66

	Ab6 SynNotch/202	Ab2	9.39	13.55	62.25	18.5	7.45
	Ab6 SynNotch/203	Ab3	10.45	18.5	65.35	12.65	8.34
	Ab6 SynNotch/205	Ab5	11.6	14.75	75.95	14.6	12.3
	UTD		7.03	7.02	5.72	5.76	6.58

Example 6

[0433] Female NSG mice were implanted with 5×10^5 K562-PSCA-PSMA subcutaneously in the dorsal flank in 50% Matrigel. On day 14, mice were dosed with either 2×10^6 or 5×10^6 CAR+ T cells. Tumors were measured via calipers.

Table 41

Canonical CAR								
Days post tumor implantation	Vehicle							
14	108	108	108	126	126	126	126	144
17	365	365	288	405	405	405	405	405
21	1183	864	787	847	1183	1080	1008	1268
24	1666	1470	1437	1437	1666	1764	1764	1862
27	2560	2025	2138	2432	2560	2890	2560	2890
Days post tumor implantation	UT							
14	108	108	108	126	126	126	126	144
17	365	196	405	320	405	405	405	405
21	726	666	864	908	1183	936	1099	1183
24	1470	1183	1913	1296	1913	1568	1913	1666
27		2025	2746	2250	2560		2746	2304
Days post tumor implantation	Ab1-17 canonical 2e6							

14	108	108	108	126	126	126	126	144
17	288	172	172	288	256	288	256	288
21	726	550	500	1008	936	847	726	1099
24	1099	936	666	1268	1268	1080	936	1568
27		1080	726	1352	1521	1437	1008	2025
30		1008	550	1352	1521	1862	1268	
34		726	365	1080	1437	1437	1268	
37		550	256	600	1080	847	936	
41		500	196	405	726	650	550	
44		500	196	320		405	405	
48		405				288	365	
51		288				172	288	
55		196				108	256	
Days post tumor implantation	Ab1-17 canonical 5e6							
14	108	108	108	126	126	126	126	144
17	405	172	256	365	256	320	320	405
21	936	550	666	864	936	787	847	1268
24	1268	864	936	1008	1268	1080	1080	1764
27	726	550	405		1470	1080	1152	1960
30	320	365	172		1470	550	1080	1666
34	126	108	63		500	365	486	908
37	32	63	14		288	256	486	600
41	4	14	1		288	172	320	
44	1	4			288	172		
48		1				63		
51		1				32		
55		1				32		
Days post tumor implantation	Ab4 canonical 2e6							
14	108	108	108	126	126	126	126	144
17	256	256	196	320	320	288	365	500

21	864	726	600	1008	908	847	1008	1470
24	1183	936	726	1666	1152	1080	1268	1764
27	1568	1008	936		1224	1764	2025	2432
30	1352	1008	1183		1606	1764		
34	1666	1080	1568		1606			
37	1666	1080	1568		1960			
41	2025	1080	1568		2250			
44		1080	2025					
Days post tumor implantation	Ab4 canonical 5e6							
14	108	108	108	126	126	126	126	144
17	196	288	196	405	320	288	405	320
21	787	936	666	1008	936	1099	1268	1008
24	1008	1268	726	1352	1268	1764	1666	1352
27	1152	1568	864	1666	1268	1862	2025	1764
30	1152	1568	936	1666	1268	2025		1764
34	1008	1183	726	936				1183
37	288	446	288	405				550
41	63	320	172	365				320
44	4	288	108	288				
48	1	172						
51		63						
Days post tumor implantation	Ab3-21 canonical 2e6							
14	108	108	108	108	126	126	126	144
17	196	196	196	288	365	288	288	288
21	666	666	864	1099	864	1008	936	847
24	787	726	1268	1568	1183	1268	1470	1268
27	1008		1666	2304	2176	2025	1913	1666
30	1080		2138				2304	2025
34	1080							
37	1268							

Days post tumor implantation	Ab3-21 canonical 5e6							
14	108	108	108	108	126	126	126	144
17	256	256	256	365	256	320	365	320
21	666	936	864	1183	847	1008	1099	1080
24	1008	1183	1008	1372	1268	1352	1470	1666
27	1008	1183	1183	1372	1268	1437	1688	
30	1080	1183	1470	1372		1666	2048	
34	1080	864	1800	1099		1352		
37	847	666	1800			1352		
41	847	666						
44		365						
48		288						
51		172						
Days post tumor implantation	Ab5 canonical 2e6							
14	108	108	108	108	126	126	126	144
17	172	172	256	196	405	365	256	288
21	500	726	726	600	936	1099	787	1008
24	864	1152	1183	1099	1470		1183	1470
27	864	1352	1666	1372			1800	1666
30	936	1568	2025	1800				2025
34	1008	1470		2176				
37	1008	1470						
41	1268	1183						
44	2025	1183						
48		1183						
51		1183						
Days post tumor implantation	Ab5 canonical 5e6							
14	108	108	108	108	126	126	126	144

17	196	288	196	256	256	365	405	320
21	550	726	600	600	864	847	1008	700
24	726	936	600	787		1268	1568	847
27	500	936	365	550		1352		847
30	365	726	365	365				847
34	172	320	172	256				288
37	63	288	63	172				172
41	63	256	32	172				48
44	32	108	14	108				32
48	4	63	4	63				
51	4	32	4	14				
55	4	4	1	4				

Table 41

synNotch								
Days post tumor implantation	Vehicle							
14	108	108	108	126	126	126	126	144
17	365	365	288	405	405	405	405	405
21	1183	864	787	847	1183	1080	1008	1268
24	1666	1470	1437	1437	1666	1764	1764	1862
27	2560	2025	2138	2432	2560	2890	2560	2890
Days post tumor implantation	UT							
14	108	108	108	126	126	126	126	144
17	365	196	405	320	405	405	405	405
21	726	666	864	908	1183	936	1099	1183
24	1470	1183	1913	1296	1913	1568	1913	1666
27		2025	2746	2250	2560		2746	2304

Days post tumor implantation	Ab1-17 synNotch 2e6							
14	108	108	108	126	126	126	126	144
17	172	288	245	256	320	405	256	446
21	666	726	700	787	650	1268	864	847
24	1008	1268	908	1183	847		1183	1080
27	1080	2025	908	1268	1183		1568	
30	1080		908	1268	1470		1568	
34	288		908	1268	1470		1268	
37	288		600	1268	1268		1268	
41	288		600		1268		1268	
44	196		320		1352		1470	
48	172		126		1437		1688	
51			108					
55			108					
Days post tumor implantation	Ab1-17 synNotch 5e6							
14	108	108	108	126	126	126	126	144
17	256	256	196	365	256	446	365	405
21	726	936	600	936	666	1089	1089	864
24	936	936	787	1568	666	1368	1368	1183
27	1008	726	600	2025	666	1368	1368	
30	666	550	550		405	1368	1080	
34	365	500	288		172	567	550	
37	365	365	172		108	416	256	
41	256	172	108		32	245	196	
44	172	108	63		32	144	172	
48	126	108	32		32	88	108	
51	63	32	32		32	88	32	
55	63	32	32		32	88	32	

Days post tumor implantation	Ab2-7 synNotch 2e6							
14	108	108	108	126	126	126	126	144
17	256	288	196	320	365	365	405	320
21	666	787	666	936	864	1183	1080	1080
24	864	1080	726	1080	1268	1268	1352	1521
27	864		864	1152	1352	1268	1521	1862
30	864		864	1152	1666	1800	1521	2156
34	666		726	847	1568	2176	1080	
37	288		405	446	1268		847	
41	172		288	405	1268		787	
44	172		288	365	1268		446	
48	108		88	256	1268		446	
51	108		88	172	1268		405	
55	108		75	172	1268			
Days post tumor implantation	Ab2-7 synNotch 5e6							
14	108	108	108	126	126	126	126	144
17	172	172	256	245	365	288	320	288
21	600	550	936	800	1008	787	787	787
24	600	600	936	1029	1183	787	1008	847
27	365	365	726	968	1183	500	1008	527
30	256	256	365	650	550	365	650	
34	108	75	172	320	288	256	352	
37	63	32	63	221	172	172	320	
41	63	14	63		108	108	221	
44	14	14	63		108	108	221	
48	4	4	32		63	63	126	
51		4	32		32	32	63	
55		4	32		32	32	32	

Days post tumor implantation	Ab3-21 synNotch 2e6							
14	108	108	108	108	126	126	126	144
17	288	365	256	196	365	256	405	320
21	936	936	666	600	1008	726	1268	1008
24	1470	1099	1099	936	1568	1268	1913	
27	1913	1372	1183	1183	2025	1666	2432	
30	2138	1800	1268			2138		
34		2025	2025					
Days post tumor implantation	Ab3-21 synNotch 5e6							
14	108	108	108	108	126	126	126	144
17	256	288	256	196	196	288	288	365
21	550	847	666	550	486	936	864	787
24	936	847	864	787	908		1470	1080
27	1099	1008	726	1008	968		1800	1960
30	1099	1268	726	1437	1029		2025	
34	1688	1470	726		1089			
37	1800		726		1440			
41	2176		726		2058			
Days post tumor implantation	Ab5 synNotch 2e6							
14	108	108	108	108	126	126	126	144
17	365	288	320	256	365	320	405	405
21	864	650	1183	550	936	726	787	1008
24	1372	1183	1913	1099	1568	1268	1183	1666
27	1800	1800	2890		2304	2304	1568	2025
30	2025	2304					2025	

Days post tumor implantation	Ab5 synNotch 5e6							
14	108	108	108	108	126	126	126	144
17	288	196	221	288	365	196	365	288
21	726	500	650	936	1183	726	1099	1080
24	936	550	650	1183		726	1372	1268
27	666	405	600	1183		726	1470	1690
30	500	288	446	1372		486		1960
34	500	196	288	1470		486		2250
37	365	63	108	1470		352		
41		32	75	1913		352		
44		14	14	2025		352		
48		14	14			352		
51		14	14			256		
55		14	14					

Example 7

[0434] Female NSG mice were implanted with 5×10^5 K562-PSCA-PSMA subcutaneously in the dorsal flank in 50% Matrigel. On day 13, mice were dosed with either 2×10^6 or 5×10^6 CAR+ T cells. Tumors were measured via calipers.

Day post inoculation	Vehicle							
13	108	108	108	126	126	172	172	172
16	172	172	256	365	221	288	256	256
20	550	500	726	787	650	600	726	936
23	1008	787	1268	1352	968	1080	1568	1568
27	2138	2048	2304		1606	2025	2304	2890
30					2058			
Day post inoculation	Nontransduced							
13	108	108	126	126	126	144	172	172
16	172	288	256	256	196	320	256	288

20	500	726	666	726	787	1008	650	864
23	1099	1183	1268	1268	1183	1764	1152	1470
27	2048	2890	2457	2601	2432	2890	2432	2916
Day post inoculation	synNotch/mAb1 anti-PSMA CAR (2e6)							
13	108	108	126	126	126	144	172	172
16	172	172	256	365	196	365	405	256
20	446	365	650	864	446	787	1099	550
23	486	405	1080	1372	700	1080	1688	864
27	650	288	1437	1470	527	1470	2176	1099
30	700	196	1666	1470	486	1800		787
34	700	172	2025	1268	245	1568		726
38	486	108		936	196	1268		500
41	196	108		787	126	787		500
44	172	75		486	108	550		500
48	172	75		550	32	550		500
Day post inoculation	synNotch/mAb1 anti-PSMA CAR+DNR (2e6)							
13	108	108	126	126	126	144	172	172
16	256	108	256	320	365	196	365	256
20	446	256	550	666	600	666	864	726
23	666	320	726	666	787	936	1183	1099
27	726	352	787	726	650	1470	1470	1183
30	666	245	726	666	446	1470	1470	1080
34	446	126	550	365	320	1568	1470	1080
38	320	126	405	288	196	1372	787	787
41	144	108	256	256	172	936	666	600
44	144	108	288	256	126	864	550	550
48	75	32	196	75	63	666	550	446
Day post inoculation	synNotch/mAb1 anti-PSMA CAR (5e6)							
13	108	108	126	126	126	144	172	172
16	172	196	172	196	256	365	365	405
20	288	245	288	405	405	936	1099	936

23	288	352	288	405	405	1080	1470	1268
27	108	221	172	196	196	1152	1800	1183
30	63	172	144	172	126	787	1470	726
34	63	126	75	108	108	486	600	666
38	14	108	88	108	108	352	550	500
41	14	88	88	88	63	320	500	405
44	14	108	126	63	63	320	365	320
48	14	108	32	32	32	288	288	288
Day post inoculation	synNotch/mAb1 anti-PSMA CAR+DNR (5e6)							
13	108	108	126	126	126	126	172	172
16	172	172	256	288	256	256	288	405
20	256	446	550	726	500	666	666	1008
23	196	787	666	864	550	600	726	1568
27	126	787	550	787	405	500	864	1666
30	108	405	288	550	256	288	726	1470
34	75	320	256	446	126	196	405	550
38	32	221	196	320	75	172	256	365
41	18	221	196	288	63	172	196	196
44	14	221	196	288		196	256	196
48	0	172	126	256		75		172

[0435] In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. An antibody, or antigen binding fragment thereof comprising an anti-PSMA binding domain, wherein the anti-PSMA binding domain comprise sequences of three heavy chain complementarity determining regions (HCDRs) of any one of the heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NOs: 1, 25, 49, and 73, and sequences of three light chain CDRs (LCDRs) of the light chain variable region (LCVR) selected from the group consisting of SEQ ID NOs: 12, 36, 60, and 84.

2. The antibody, or antigen binding fragment thereof of claim 1, wherein the antibody, or antigen binding fragment thereof comprises a first domain comprising three heavy chain complementarity determining regions (HCDRs) and a second domain comprising three light chain complementarity determining regions (LCDRs), wherein:

the HCDRs and LCDRs comprise:

(i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22;

(ii) an HCDR1 according to any of SEQ ID NOs: 27-29; an HCDR2 according to any of SEQ ID NOs: 30-32; an HCDR3 according to any one of SEQ ID NOs: 33-35; an LCDR1 according to any of SEQ ID NOs: 38-40; an LCDR2 according to any of SEQ ID NOs: 41-43; an LCDR3 according to any one of SEQ ID NOs: 44-46;

(iii) an HCDR1 according to any of SEQ ID NOs: 51-53; an HCDR2 according to any of SEQ ID NOs: 54-56; an HCDR3 according to any one of SEQ ID NOs: 57-59; an LCDR1 according to any of SEQ ID NOs: 62-64; an LCDR2 according to any of SEQ ID NOs: 65-67; an LCDR3 according to any one of SEQ ID NOs: 68-70; or

(iv) an HCDR1 according to any of SEQ ID NOs: 75-77; an HCDR2 according to any of SEQ ID NOs: 78-80; an HCDR3 according to any one of SEQ ID NOs: 81-83; an LCDR1 according to any of SEQ ID NOs: 86-88; an LCDR2 according to any of SEQ ID NOs: 89-91; an LCDR3 according to any one of SEQ ID NOs: 92-94.

3. The antibody, or antigen binding fragment thereof of claim 1 or 2, wherein the antibody, or antigen binding fragment thereof comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein:

- (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1 and the light chain variable domain is at least 80% identical to SEQ ID NO: 12;
- (ii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 25 and the light chain variable domain is at least 80% identical to SEQ ID NO: 36;
- (iii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 49 and the light chain variable domain is at least 80% identical to SEQ ID NO: 60; or
- (iv) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 73 and the light chain variable domain is at least 80% identical to SEQ ID NO: 84.

4. The antibody, or antigen binding fragment thereof of any one of claims 1-3, wherein the three HCDRs and the three LCDRs are comprised by a single polypeptide.

5. The antibody, or antigen binding fragment thereof of any one of claims 1-4, wherein the antigen binding fragment thereof comprises an scFv.

6. A nucleic acid encoding the antibody, or antigen binding fragment thereof of any one of claims 1-5.

7. A chimeric antigen receptor, comprising the antibody, or antigen binding fragment thereof of any one of claims 1-5.

8. The chimeric antigen receptor of claim 7, further comprising a transmembrane domain of 4-1BB/CD137, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a zeta chain of a T cell receptor, or any combination thereof.

9. A nucleic acid comprising the chimeric antigen receptor of any one of claims 7 or 8.

10. A recombinant vector comprising the nucleic acid of claim 6 or 9.

11. The recombinant vector of claim 10, wherein the nucleic acid encoding the antibody, antigen binding fragment thereof or chimeric antigen receptor is operatively connected to a constitutively active promoter or a conditionally activated promoter.

12. The recombinant vector of claim 11, wherein the conditionally activated promoter comprises at least one transcriptional activator binding site.
13. The recombinant vector of claim 12, wherein the transcriptional activator binding site comprises one or more GAL4 binding sites.
14. The recombinant vector or nucleic acid of any one of claims 9-13 wherein the recombinant vector further comprises a nucleic acid encoding a dominant negative TGF β receptor (DN TGF β R), comprising:
an extracellular domain (ECD) from a TGF- β receptor
and a transmembrane domain (TMD), wherein the recombinant polypeptide lacks amino acid residues responsible for signaling and phosphorylation present in a wild-type TGF- β receptor.
15. A synthetic notch (synNotch) receptor polypeptide comprising from N to C terminus:
an extracellular anti-PSCA binding domain,
a Notch core domain comprising one or more proteolytic cleavage sites, and
an intracellular domain comprising a transcriptional activator comprising a DNA binding domain and a transactivation domain, wherein binding of the binding extracellular anti-PSCA binding domain to PSCA induces cleavage of the Notch core domain at the one or more proteolytic cleavage sites, thereby releasing the intracellular domain and the transcriptional regulator.
16. The synNotch receptor polypeptide of claim 15, wherein the anti-PSCA binding domain comprising a first domain comprising three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and a second domain comprising three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein
(i) the HCDR1 has a sequence according to any one of SEQ ID NOs: 152-154,
(ii) the HCDR2 has a sequence according to any one of SEQ ID NOs: 155-157;
(iii) the HCDR3 has a sequence according to any one of SEQ ID NOs: 158-160;
(iv) the LCDR1 has a sequence according to any one of SEQ ID NOs: 163-165;
(v) the LCDR2 has a sequence according to any one of SEQ ID NOs: 166-168; and
(vi) the LCDR3 has a sequence according to any one of SEQ ID NOs: 169-171.

17. The synNotch receptor polypeptide of any one of claims 15-16, wherein the transcriptional regulator is a transcriptional activator.
18. The synNotch receptor polypeptide of claim 17, wherein the transcriptional activator comprises GAL4, HNF1 alpha or HNF1 beta.
19. The synNotch receptor polypeptide of claim 17, wherein the transcriptional activator comprises a transactivation domain selected from the group consisting of VP64, RelA (p65), YAP, WWTR1(TAZ), CREB3(LZIP), and MyoD.
20. A nucleic encoding the synNotch receptor polypeptide of any one of claims 15-19.
21. A recombinant vector comprising the nucleic acid of claim 20.
22. A host cell transformed with the nucleic acid of any one of claims 6, 9 or 20 or the recombinant vector of any one of claims 10-14 and/or 21.
23. The host cell of claim 22, where the host cell comprises a T cell or an NK cell.
24. A pharmaceutical composition comprising the T cell and/or an NK cell of claim 23.
25. A method of treating disease in a patient in need of thereof, comprising administering the T cell and/or an NK cell of claim 23 or the pharmaceutical composition of claim 24 to the patient.
26. The method of claim 25, where the disease is prostate cancer.
27. A method of inducing an immune response in a subject or immunizing a subject against a prostate cancer, the method comprising administering the T cell and/or an NK cell of claim 23 or the pharmaceutical composition of claim 24 to the patient.

28. The method of any one of claims 25-27 where in the T cell and/or an NK cell s allogeneic to the patient.