



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

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2022/06/01
(87) Date publication PCT/PCT Publication Date: 2022/12/01
(85) Entrée phase nationale/National Entry: 2022/09/28
(86) N° demande PCT/PCT Application No.: US 2022/031836
(30) Priorités/Priorities: 2021/06/01 (US63/202,211);
2021/11/09 (US63/263,809); 2022/04/06 (US63/362,594)

(51) Cl.Int./Int.Cl. *C12N 15/62* (2006.01),
A61K 35/17 (2015.01), *A61K 39/00* (2006.01),
A61P 35/00 (2006.01), *C07K 14/725* (2006.01),
C07K 16/28 (2006.01), *C07K 19/00* (2006.01),
C12N 15/12 (2006.01), *C12N 15/13* (2006.01),
C12N 5/0783 (2010.01), *C12N 5/10* (2006.01)
(71) Demandeur/Applicant:
TRIUMVIRA IMMUNOLOGICS USA, INC., US
(72) Inventeurs/Inventors:
BADER, ANDREAS, US;
HELSEN, CHRISTOPHER W., US;
IP, PHILBERT, US;
BENATAR, TANIA, US; ...

(54) Titre : COUPLEURS D'ANTIGENE DE LYMPHOCYTES T DE CLAUDINE 18.2 ET LEURS UTILISATIONS
(54) Title: CLAUDIN 18.2 T CELL-ANTIGEN COUPLERS AND USES THEREOF

(57) Abrégé/Abstract:

Claudin 18.2 T cell antigen couplers (TACs) polypeptides having (i) an antigen-binding domain that binds Claudin 18.2, (ii) an antigen-binding domain that binds a protein associated with a TCR complex, and (iii) a T cell receptor signaling domain polypeptide are provided.



(72) Inventeurs(suite)/Inventors(continued): WANG, LING, CA

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

CLAUDIN 18.2 T CELL-ANTIGEN COUPLERS AND USES THEREOF

Claudin 18.2 T cell antigen couplers (TACs) polypeptides having (i) an antigen-binding domain that binds Claudin 18.2, (ii) an antigen-binding domain that binds a protein associated with a TCR complex, and (iii) a T cell receptor signaling domain polypeptide are provided.

CLAUDIN 18.2 T CELL-ANTIGEN COUPLERS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/202,211, filed June 1, 2021; U.S. Provisional Patent Application No. 63/263,809, filed November 9, 2021; and U.S. Provisional Patent Application No. 63/362,594, filed April 6, 2022, the disclosures of each of which are hereby incorporated by reference in their entireties for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 31, 2022, is named TMV-006WO_SL.txt and is 87,860 bytes in size.

SUMMARY

[0003] Disclosed herein, in certain embodiments, are nucleic acids encoding a Claudin 18.2 T cell-antigen coupler (TAC). In some embodiments, the nucleic acid comprises: (a) a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (b) a second polynucleotide encoding an antigen-binding domain that binds a protein associated with a TCR complex; and (c) a third polynucleotide encoding a TCR co-receptor cytosolic domain and transmembrane domain. In some embodiments, the nucleic acid comprises, in order (*e.g.*, from 5' to 3'): (a) a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (b) a second polynucleotide encoding an antigen-binding domain that binds a protein associated with a TCR complex; and (c) a third polynucleotide encoding a TCR co-receptor cytosolic domain and transmembrane domain. In some embodiments, the antigen-binding domain that binds Claudin 18.2 is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer, fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from an antibody selected from IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, and aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding

domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or GC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A,

EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR

(e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362

(claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having

at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide encodes an antigen-binding

domain comprising an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the

first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence

having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide encodes an antigen-binding

domain comprising an amino acid sequence having at least 95% sequence identity with the

amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence

having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide encodes an antigen-binding

domain comprising an amino acid sequence having at least 97% sequence identity with the

amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence

having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide encodes an antigen-binding

domain comprising an amino acid sequence having at least 99% sequence identity with the

amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising the amino acid sequence of

SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the first polynucleotide

encodes an antigen-binding domain comprising an amino acid sequence comprising a CDR1

comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 1 Kabat CDR1), a CDR2 comprising SEQ ID NO: 58 (Claudin 18.2 Nanobody 1 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59

(Claudin 18.2 Nanobody 1 Kabat CDR3). In some embodiments, the first polynucleotide

encodes an antigen-binding domain comprising an amino acid sequence comprising a CDR1

comprising SEQ ID NO: 60 (Claudin 18.2 Nanobody 1 IMGT CDR1), a CDR2 comprising SEQ ID NO: 61 (Claudin 18.2 Nanobody 1 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62

(Claudin 18.2 Nanobody 1 IMGT CDR3). In some embodiments, the CDR sequences of the

antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of

Claudin 18.2 Nanobody 1 (*i.e.*, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence comprising a CDR1, CDR2, and CDR3 each having 100% identity to the corresponding CDR in Claudin 18.2 Nanobody 1). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the

antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 2 Kabat CDR1), a CDR2 comprising SEQ ID NO: 63 (Claudin 18.2 Nanobody 2 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 2 Kabat CDR3). In some embodiments, the first polynucleotide encodes an antigen-binding domain comprising an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 64 (Claudin 18.2 Nanobody 2 IMGT CDR1), a CDR2 comprising SEQ ID NO: 65 (Claudin 18.2 Nanobody 2 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 2 IMGT CDR3). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2 (*i.e.*, the first polynucleotide encodes an antigen-binding domain

comprising an amino acid sequence comprising a CDR1, CDR2, and CDR3 each having 100% identity to the corresponding CDR in Claudin 18.2 Nanobody 2). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the

non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the protein associated with the TCR complex is a CD3 protein, for example, a CD3 protein of a TCR complex on a T cell expressing the TAC. In some embodiments, the CD3 protein is a CD3 γ protein, CD3 δ protein and/or CD3 ϵ protein. In some embodiments, the CD3 protein is a CD3 ϵ protein. In some embodiments, binding of the CD3 protein induces activation of the T cell. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer; fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is derived from an antibody selected from UCHT1, OKT3, F6A, and L2K. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a UCHT1 antigen-binding domain, for example, an scFv derived from UCHT1. In some embodiments, the UCHT1 antigen-binding domain comprises a Y to T mutation at a position corresponding to amino acid 182 of SEQ ID NO: 8 (Y182T). In some embodiments, the UCHT1 antigen-binding domain is a humanized variant of UCHT1 (huUCHT1), for example, a humanized variant of UCHT1 comprising a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at

least 97% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T))). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*,

framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein

associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is an OKT3 antigen-binding domain. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO:

16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 16 (OKT3)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least

96% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a F6A antigen-binding domain. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98%

sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 18 (F6A)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein

associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a L2K antigen-binding domain. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino

acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some

5 embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-

10 binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 20

15 (L2K)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid

20 sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*,

25 framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least

30 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein

associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the cytosolic domain is a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain. In some embodiments, the cytosolic domain is a CD8 cytosolic domain and the transmembrane domain is a CD8 transmembrane domain. In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4

transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the components encoded by the first, second, and/or third polynucleotides are connected in any suitable manner, such as in any suitable order and/or comprising any suitable linker(s). In some embodiments, the components encoded by (a), components encoded by (b), and components encoded by (c) are fused directly to each other, or joined by at least one linker. In some embodiments, the components encoded by (a) and the components encoded by (c) are fused to the components encoded by (b). In some embodiments, the components encoded by (b) and the components encoded by (c) are fused to the components encoded by (a). In some embodiments, at least one linker joins the components encoded by (a) to the components encoded by (b). In some embodiments, the at least one linker is a glycine and/or serine-rich linker, a large protein domain, a long helix structure, or a short helix structure. In some embodiments, the at least one linker comprises an amino acid sequence having at least 80% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)₄-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 85% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)₄-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 90% identity with the amino acid sequence of

SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 95% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 96% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 97% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 98% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 80% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector),

SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the nucleic acid sequence does not encode a co-stimulatory domain and/or an activation domain. In some embodiments, the nucleic acid sequence further encodes a leader sequence. In some embodiments, the leader sequence

5 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some

10 embodiments, the leader sequence comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ

15 ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ

20 ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID

25 NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the nucleic acid comprises a sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence

30 identity with the nucleic acid sequence of SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, or SEQ ID NO: 72. In some embodiments, the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, or SEQ ID NO: 73.

[0004] Disclosed herein, in certain embodiments, are expression vectors comprising a nucleic acid disclosed herein (e.g., a nucleic acid encoding a Claudin 18.2-TAC disclosed herein). In some embodiments, the expression vector comprises a promoter functional in a mammalian cell. In some embodiments, the expression vector is a lentiviral vector, for example, a VSV-G pseudotyped lentiviral vector. In some embodiments, the expression vector is a γ retroviral vector, for example, a GALV pseudotyped γ - retroviral vector.

[0005] Disclosed herein, in certain embodiments, are Claudin 18.2 T cell-antigen couplers (TACs). In some embodiments, the Claudin 18.2-TAC comprises: (a) an antigen-binding domain that binds Claudin 18.2; (b) an antigen-binding domain that binds a protein associated with a TCR complex; and (c) a TCR co-receptor cytosolic domain and transmembrane domain. In some embodiments, the Claudin 18.2-TAC comprises, in order (e.g., from N-terminus to C-terminus): (a) an antigen-binding domain that binds Claudin 18.2; (b) an antigen-binding domain that binds a protein associated with a TCR complex; and (c) a TCR co-receptor cytosolic domain and transmembrane domain. In some embodiments, the antigen-binding domain that binds Claudin 18.2 is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer; fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from an antibody selected from IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, and aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or GC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of the antigen-binding domain(s) of

IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182.

5 In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of
10 the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences
15 of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182). In some embodiments, the CDR sequences of the antigen-binding domain that binds
20 Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding
25 domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of
30 the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR

sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino

acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some

5 embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the

10 antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some

15 embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 1 Kabat CDR1), a CDR2 comprising SEQ ID NO: 58 (Claudin 18.2 Nanobody 1 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 1 Kabat CDR3). In some

20 embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 60 (Claudin 18.2 Nanobody 1 IMGT CDR1), a CDR2 comprising SEQ ID NO: 61 (Claudin 18.2 Nanobody 1 IMGT CDR1), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 1 IMGT CDR3). In some

25 embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1, CDR2, and CDR3 each having 100% identity to the corresponding CDR in Claudin 18.2 Nanobody 1). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2

30 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding

domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the

antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 2 Kabat CDR1), a CDR2 comprising SEQ ID NO: 63 (Claudin 18.2 Nanobody 2 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 2 Kabat CDR3). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 64 (Claudin 18.2 Nanobody 2 IMGT CDR1), a CDR2 comprising SEQ ID NO: 65 (Claudin 18.2 Nanobody 2 IMGT CDR1), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 2 IMGT CDR3). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1, CDR2, and CDR3 each having 100% identity to the corresponding CDR in Claudin 18.2 Nanobody 2). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding

domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the protein associated with the TCR complex is a CD3 protein, for example, a CD3 protein of a TCR complex on a T cell expressing the TAC. In some embodiments, the CD3 protein is a CD3 γ protein, CD3 δ protein and/or CD3 ϵ protein. In some embodiments, the CD3 protein is a CD3 ϵ protein. In some embodiments, binding of the CD3 protein induces activation of the T cell. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer; fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is derived from an antibody selected from UCHT1, OKT3, F6A, and L2K. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a UCHT1 antigen-binding domain, for example, a UCHT1 single chain

antibody. In some embodiments, the UCHT1 antigen-binding domain comprises a Y to T mutation at a position corresponding to amino acid 182 of SEQ ID NO: 8 (Y182T). In some embodiments, the UCHT1 antigen-binding domain is a humanized variant of UCHT1 (huUCHT1), for example, a humanized variant of UCHT1 comprising a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the

CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T))). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)).

(huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (*e.g.*, framework) sequences of

the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is an OKT3 antigen-binding domain. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 16 (OKT3)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of

the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-

binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a F6A antigen-binding domain. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A).

In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 18 (F6A)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the

amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the

antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex is a L2K antigen-binding domain. In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each

having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 20 (L2K)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some

embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the cytosolic domain is a CD4 cytosolic domain and the transmembrane domain is a CD4 transmembrane domain. In some embodiments, the cytosolic domain is a CD8 cytosolic domain and the transmembrane domain is a CD8 transmembrane domain. In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 95% sequence identity with SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 96% sequence identity with SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 97% sequence identity with SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 98% sequence identity with SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 99% sequence identity with SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the components of the Claudin 18.2-TAC are connected in any suitable manner, such as in any suitable order and/or comprising any suitable linker(s). In some embodiments, component (a), component (b), and component (c) are fused directly to each

other, or joined by at least one linker. In some embodiments, component (a) and component (c) are fused to component (b). In some embodiments, component (b) and component (c) are fused to component (a). In some embodiments, at least one linker joins component (a) to component (b). In some embodiments, the at least one linker is a glycine and/or serine-rich linker, a large protein domain, a long helix structure, or a short helix structure. In some embodiments, the at least one linker comprises an amino acid sequence having at least 80% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 85% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 90% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 95% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 96% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 97% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID

NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 98% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 80% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the Claudin 18.2-TAC does not comprise a co-stimulatory domain and/or an activation domain. In some embodiments, the Claudin 18.2-TAC further comprises a leader sequence. In some embodiments, the leader sequence comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an

amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader),
5 SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ
10 ID NO: 40 (huCD8a leader). In some embodiments, the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 67. In some embodiments, the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%,
15 at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 69. In some embodiments, the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 71. In some embodiments, the Claudin 18.2-TAC polypeptide
20 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 73.

[0006] Disclosed herein, in certain embodiments, are T cells comprising a nucleic acid disclosed herein, an expression vector disclosed herein, or a Claudin 18.2-TAC disclosed herein. In some
25 embodiments, the T cell is a $\gamma\delta$ T cell, e.g., a $\delta 2$ T cell, a $\delta 1s$ T cell, or a $\gamma 9\delta 2$ T cell.

[0007] Disclosed herein, in certain embodiments, are pharmaceutical compositions comprising a T cell disclosed herein, and a pharmaceutically acceptable excipient.

[0008] Disclosed herein, in certain embodiments, are methods of treating a Claudin 18.2-expressing cancer in an individual in need thereof, comprising administering to the individual a
30 T cell or a pharmaceutical composition disclosed herein. In some embodiments, the cancer is a solid cancer or a liquid cancer. In some embodiments, the cancer is a pancreatic cancer (e.g., pancreatic adenocarcinoma, a gastric cancer (e.g., gastric adenocarcinoma), a signet ring cell carcinoma, a mucinous gastric cancer, a gastroesophageal cancer (e.g., gastroesophageal

junction (GEJ) adenocarcinoma), an esophageal cancer, a cancer of the digestive system, an ovarian cancer, a mucinous ovarian cancer cell, or a lung cancer (e.g., non-small cell lung cancer).

5 [0009] Disclosed herein in certain embodiments, are conjugates comprising a T cell disclosed herein and a Claudin 18.2-expressing cancer cell. In some embodiments, the Claudin 18.2-TAC polypeptide is present on the surface of the T cell and the Claudin 18.2 is present on the surface of the cancer cell. In some embodiments, the Claudin 18.2-TAC polypeptide is bound to the Claudin 18.2. In some embodiments, the cancer cell is a cell of a solid cancer. In some
10 embodiments, the cancer cell is a pancreatic cancer cell, a gastric cancer cell, a signet ring cell carcinoma cell, a cancer of the digestive system, a mucinous gastric cancer cell, a gastroesophageal cancer cell, an esophageal cancer cell, an ovarian cancer cell, a mucinous ovarian cancer cell, a non-small cell lung cancer cell, or a lung cancer cell. In some embodiments, the cancer cell is a gastric cancer cell or a gastroesophageal cancer cell.

BRIEF DESCRIPTION OF THE DRAWINGS

15 [0010] The invention can be more completely understood with reference to the following drawings.

[0011] **Figure 1** depicts cell surface expression of the indicated TAC and an mStrawberry red fluorescence protein transduction marker in T cells. NTD: non-transduced control cells.

[0012] **Figures 2A-2C** depict the assessment of Claudin 18.2-TAC T cell manufacturing.

20 **Figure 2A** depicts the overall cell count over time of T cells engineered to express indicated Claudin 18.2-TACs. **Figure 2B** depicts the number of cell doublings over time of T cells engineered to express indicated Claudin 18.2-TACs. **Figure 2C** depicts the percent viability over time of T cells engineered to express indicated Claudin 18.2-TACs.

25 [0013] **Figure 3** depicts histograms of expression of indicated Claudin 18.2-TACs in T cells from indicated donors as measured by flow cytometry.

[0014] **Figure 4** depicts the relative expression of indicated Claudin 18.2-TACs in T cells as measured by flow cytometry.

30 [0015] **Figures 5A and B** depict binding of GFP-labeled Claudin 18.2 Nanobody 2 to indicated cells engineered to express Claudin 18.2 or control (**Figure 5A**), binding of GFP- Claudin 18.2 Nanobody 2 fusion protein to a library of known membrane-anchored human proteins (**Figure 5B**).

[0016] **Figures 6A-6C** depict assays demonstrating the activation of Claudin 18.2-TAC T cells after co-culture with target cells expressing Claudin 18.2 (top panels) or Claudin 18.1 (bottom panels). **Figure 6A** shows confirmation of Claudin 18.2-TAC expression as measured by flow cytometry. **Figure 6B** shows scatterplots of CD69 (vertical axes) staining vs TAC expression (horizontal axes) in engineered T cells exposed to cells expressing the indicated antigen. **Figure 6C** shows normalized CD69 % positivity in T cells expressing the indicated TACs and exposed to target cells expressing the indicated antigens.

[0017] **Figure 7** depicts cytotoxicity induced by T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 69 or 73 following co-culture with KATO III cells engineered to express enhanced Luciferase (KATO IIIeLuc). NTD: non-transduced control cells. Effector cell:target cell (E:T) ratios that induced 50 percent killing of target cells (IC50) were calculated and the E:T ratio needed to reach half maximal cytotoxicity is shown.

[0018] **Figure 8** is a schematic of an *in vitro* cytotoxicity assay described in Example 3.

[0019] **Figure 9** depicts representative images of co-cultures of the indicated effector cell (TAC T cell) and target cell (NALM6CLDN18.2/eGFP or NALM6eGFP cells) at different E:T ratios after 5 days of co-culture.

[0020] **Figure 10** depicts a quantitative analysis of tumor cell growth as measured by eGFP area for co-cultures of the indicated effector cell (TAC T cell) and target cell (NALM6CLDN18.2/eGFP or NALM6eGFP cells) at different E:T ratios. NTD: non-transduced control cells.

[0021] **Figure 11** depicts an area under the curve (AUC) analysis of the cytotoxicity results shown in **Figure 10**.

[0022] **Figure 12** depicts IFN γ , TNF α , and IL2 production, as measured by flow cytometry, following co-culture of the indicated effector cell (TAC T cell) and target cell (NALM6CLDN18.2 or NALM6eGFP cells). Indicated effector cell (TAC T cell) cytokine production in the absence of any target cells is shown as a control.

[0023] **Figure 13** depicts markers that allow for an assessment of T cell activation, exhaustion and memory phenotype.

[0024] **Figure 14** depicts the results of a 16 parameter UMAP analysis highlighting CD8 and CD69 expression following co-culture of Claudin 18.2-TAC T cells or non-transduced (NTD) control cells with NALM6eGFP or NALM6CLDN18.2/eGFP cells. Colors indicate the relative abundance of the illustrated markers.

[0025] Figure 15 depicts flow cytometry plots of CD69 staining (vertical axis) and Claudin 18.2-TAC transduction (horizontal axis) of CLDN18.9-TAC T cells co-cultured with indicated Claudin 18.2-expressing cell lines.

[0026] Figures 16A and 16B depict an *in vitro* assay showing cytotoxicity of target cells by T cells expressing the indicated Claudin 18.2-TACs. **Figure 16A** depicts the normalized cytotoxicity at indicated effector:target (E:T) ratios. **Figure 16B** depicts the measured IC₅₀ values of T cells expressing the indicated TACs.

[0027] Figures 17A and 17B depict an assessment of the proliferation of Claudin 18.2-TAC-expressing T cells. **Figure 17A** shows proliferation of cells as measured by flow cytometry-based detection of a cell tracking dye. **Figure 17B** shows the measured division index (DI) of T cells expressing the indicated TACs.

[0028] Figures 18A and 18B depict an assessment of the activation of T cells expressing the indicated Claudin 18.2-TACs. **Figure 18A** shows scatterplots of CD69 (vertical axes) staining vs TAC expression (horizontal axes) in engineered T cells exposed to indicated cell lines.

Figure 18B shows normalized CD69 % positivity in T cells expressing the indicated TACs and exposed to indicated target cells.

[0029] Figures 19A and 19B depict an assay testing repeated stimulation of TAC-expressing T cells. **Figure 19A** depicts a schematic of the assay. **Figure 19B** depicts graphs showing viability of indicated target cells following incubation with T cells expressing the indicated TACs.

[0030] Figure 20 depicts a schematic of *in vivo* murine experiments described in Example 9 (top) and resulting total flux (photons/second) as the sum of the dorsal and ventral reads for the indicated treatment groups (bottom).

[0031] Figures 21A-21C depict luminescence (total flux; **Figure 21A**), overall survival (**Figure 21B**), and relative change in body weight (**Figure 21C**) for the indicated treatment groups. NT: Non treated animals (square); NTD: mice administered non-transduced control T cells (circle).

[0032] Figure 22 depicts a schematic of *in vivo* murine model of the antitumor activity of Claudin 18.2-TAC T cells and tumor volumes over time in response to indicated dosages.

[0033] Figure 23 depicts a schematic of an *in vivo* murine model of the antitumor activity of Claudin 18.2-TAC T cells (top panel), luminescence (total flux; center-left panel), overall survival (bottom-left panel), and whole body scans of mice in the indicated treatment groups over the treatment course (right panel).

[0034] **Figure 24** depict a schematic of an *in vivo* murine model of the antitumor activity of Claudin 18.2-TAC T cells (top panel), tumor volumes over time in response to indicated dosages (center panels), and overall survival (bottom panels).

[0035] **Figures 25A-25B** depict an *in vivo* murine model of the antitumor activity of Claudin 18.2-TAC T cells and different dosage levels. **Figure 25A** depicts a schematic of the model. **Figure 25B** depicts measurement of tumor volumes over the course of the experiment following treatment with T cell expressing indicated TACs at indicated dosage levels.

[0036] **Figures 26A-26B** depict an *in vivo* murine model of the antitumor activity of Claudin 18.2-TAC T cells and different dosage levels. **Figure 26A** depicts a schematic of the model. **Figure 26B** depicts measurement of tumor volumes over the course of the experiment following treatment with T cell expressing indicated TACs at indicated dosage levels.

[0037] **Figures 27A-27B** depict an *in vivo* murine model of the antitumor activity of Claudin 18.2-TAC T cells and different dosage levels. **Figure 27A** depicts a schematic of the model. **Figure 27B** depicts measurement of tumor flux over the course of the experiment following treatment with T cell expressing indicated TACs at indicated dosage levels.

[0038] **Figures 28A-28B** depict an *in vivo* murine re-challenge model of the antitumor activity of Claudin 18.2-TAC T cells in MHC-knockout mice. **Figure 28A** depicts a schematic of the model. **Figure 28B** depicts measurement of tumor volumes over the course of the experiment following treatment with T cell expressing indicated TACs over the course of the experiments, with rechallenge timepoints indicated.

DETAILED DESCRIPTION

[0039] Cancer is a major health challenge. According to the American Cancer Society, more than one million people in the United States are diagnosed with cancer each year. While patients with early stage disease are sometimes treated effectively by conventional therapies (surgery, radiation, chemotherapy), few options are available to patients with advanced disease, and those options are typically palliative in nature.

[0040] Active immunotherapy seeks to employ the patient's immune system to clear tumors and offers an option to patients who have failed conventional therapies. Generally, this treatment involves infusing patients with large numbers of tumor-specific T cells. This approach has proven to be successful in early phase clinical trials for a number of diseases, including melanoma, myeloma, leukemia, lymphoma and synovial sarcoma. As a specific example, several clinical studies have demonstrated that immunotherapy with T cells are curative in patients with advanced melanoma, confirming the utility of this approach. Additionally, patients

suffering from chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL) have also been effectively treated and cured with T cell immunotherapy.

[0041] To this point, most engineered T cell therapies involving genetic modification of the T cells yield: (i) forced expression of T cell receptor (TCR); or (ii) a chimeric antigen receptor (CAR) specific for antigen targets on the tumor. To date, the chimeric antigen receptors used for engineering T cells consist of: (i) a targeting domain, usually a single-chain fragment variable (scFv); (ii) a transmembrane domain; and (iii) a cytosolic domain that contains signaling elements from the T cell receptor and associated proteins. Such chimeric antigen receptors have also been referred to as “T-body” or “Chimeric Immune Receptor” (CIR), but currently, most researchers use the term “CAR”. One advantage of the CAR approach is that it allows any patient’s immune cells to be targeted against any desirable target in a major histocompatibility complex (MHC) independent manner. This is appealing as MHC presentation is often defective in tumor cells.

[0042] CARs are considered in modular terms and scientists have spent considerable time investigating the influence of different cytoplasmic signaling domains on CAR function. Conventional CARs generally share two main components: (i) the CD3 zeta cytoplasmic domain, which contains immunotyrosine activation motifs (ITAMs) critical for T cell activation; and (ii) components of costimulatory receptors that trigger important survival pathways such as the Akt pathway.

[0043] The first-generation CARs employed a single signaling domain from either CD3 ζ or Fc ϵ RI γ . Second-generation CARs combined the signaling domain of CD3 ζ with the cytoplasmic domain of costimulatory receptors from either the CD28 or TNFR family of receptors. Most CAR-engineered T cells that are currently being tested in the clinic employ second-generation CARs where CD3 ζ is coupled to the cytoplasmic domain of either CD28 or CD137. These second generation CARs have demonstrated anti-tumor activity in CD19-positive tumors. Third-generation CARs combined multiple costimulatory domains, but there is concern that third-generation CARs may lose antigen-specificity.

[0044] While CAR-engineered T cells have shown considerable promise in clinical application, they rely on a synthetic method for replacing the native activation signal that is provided by the T cell receptor (TCR). Since this synthetic receptor does not deliver all of the signaling components associated with the TCR (ex. ITAMs on CD3 γ , CD3 δ , CD3 ϵ), it remains unclear whether the T cells are optimally activated by the CAR or how the CAR activation affects T cell differentiation (ex. progression to memory). Furthermore, since the CAR signaling domains are

disconnected from their natural regulatory partners by the very nature of the CAR structure, there is an inherent risk that CARs may lead to a low-level of constitutive activation, which could result in off-target toxicities. Therefore, the synthetic nature of the prototypic CAR may disrupt canonical mechanisms that limit TCR activation, and may underpin the severe toxicity often associated with therapeutic doses of conventional CAR T cells.

[0045] Given these limitations, it is preferable to re-direct T cells to attack tumors via their natural TCR. An alternate chimeric receptor, termed a T cell Antigen Coupler (TAC or TAC) receptor, has been developed which employs a distinct biology to direct the T cell to attack tumors. While the CAR is a fully synthetic receptor that stitches together components of T cell receptor (TCR) signaling complex, the TAC receptor re-directs the TCR towards tumor targets and recapitulates the native TCR signaling structure. For example, in some embodiments, the TACs disclosed herein activate natural Major Histocompatibility complex (MHC) signaling through the T cell receptor (TCR), while retaining MHC-unrestricted targeting. Further, the TACs disclosed herein recruit the T Cell Receptor (TCR) in combination with co-receptor stimulation. Moreover, in some embodiments, TACs disclosed herein show enhanced activity and safety.

[0046] TACs are distinct from traditional CAR technology in that they include a second extracellular ligand that binds a protein associated with the TCR complex. Furthermore, TACs contain TCR co-receptor cytosolic domains as opposed to the TCR signaling domains used in CARs. The TCR co-receptor domains can facilitate T cell activation through endogenous TCR signaling as opposed to CARs, which function independently of the endogenous TCR. Instead, CARs typically function via their own synthetic activation domain (e.g., CD3z). This tonic signaling induced by CARs can lead to effector cell exhaustion. The structural differences between TACs and CARs results in molecules with different structural features and different functionality. For example, antigen-binding domains that are not functional in CARs may have satisfactory manufacturability or function in the context of a TAC.

[0047] Claudin 18.1 and 18.2 (CLDN18.1 and CLDN18.2) are highly conserved multi-membrane spanning proteins found in tight junctions. The extracellular domains of Claudin 18.1 and 18.2 include two prominent loops. Claudin 18.1 and 18.2 show significant divergence in the first extracellular loop but are otherwise identical. While Claudin 18.1 is broadly expressed in lung tissue, Claudin 18.2 is predominantly expressed in gastric tissues and therefore a target for treating gastric cancers.

Certain terminology

[0048] The term “antigen-binding domain,” refers to any substance or molecule that binds, directly or indirectly, to a target (e.g., Claudin 18.2). Antigen-binding domains include antibodies or fragments thereof, peptides, peptidomimetics, proteins, glycoproteins, proteoglycans, carbohydrates, lipids, nucleic acids, or small molecules that bind to a target.

5 **[0049]** As used herein, unless otherwise indicated, the term “antibody” is understood to mean an intact antibody (e.g., an intact monoclonal antibody), or a fragment thereof, such as a Fc fragment of an antibody (e.g., an Fc fragment of a monoclonal antibody), or an antigen-binding fragment of an antibody (e.g., an antigen-binding fragment of a monoclonal antibody), including an intact antibody, antigen-binding fragment, or Fc fragment that has been modified, engineered,
10 or chemically conjugated. In general, antibodies are multimeric proteins that contain four polypeptide chains. Two of the polypeptide chains are called immunoglobulin heavy chains (H chains), and two of the polypeptide chains are called immunoglobulin light chains (L chains). The immunoglobulin heavy and light chains are connected by an interchain disulfide bond. The immunoglobulin heavy chains are connected by interchain disulfide bonds. A light chain
15 consists of one variable region (VL) and one constant region (CL). The heavy chain consists of one variable region (VH) and at least three constant regions (CH1, CH2 and CH3). The variable regions determine the binding specificity of the antibody. Each variable region contains three hypervariable regions known as complementarity determining regions (CDRs) flanked by four relatively conserved regions known as framework regions (FRs). The extent of the FRs and
20 CDRs has been defined (Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917). The three CDRs, referred to as CDR1, CDR2, and CDR3, contribute to the antibody binding specificity. Naturally occurring antibodies have been used as starting material for engineered antibodies, such as chimeric
25 antibodies and humanized antibodies. Examples of antibody-based antigen-binding fragments include Fab, Fab’, (Fab’)2, Fv, single chain antibodies (e.g., scFv), minibodies, and diabodies. Examples of antibodies that have been modified or engineered include chimeric antibodies, humanized antibodies, and multispecific antibodies (e.g., bispecific antibodies). An example of a chemically conjugated antibody is an antibody conjugated to a toxin moiety.

30 **[0050]** The term “T cell” as used herein refers to a type of lymphocyte that plays a central role in cell-mediated immunity. T cells, also referred to as T lymphocytes, are distinguished from other lymphocytes, such as B cells and natural killer cells, by the presence of a T-cell receptor (TCR) on the cell surface. There are several subsets of T cells with distinct functions, including

but not limited to, T helper cells, cytotoxic T cells, memory T cells, regulatory T cells and natural killer T cells.

[0051] The term “ $\gamma\delta$ T cell” or “gamma delta T cell” or “gd T cell” as used herein refers to any lymphocyte having a $\gamma\delta$ T cell receptor (TCR) on its surface, including one γ -chain and one δ -chain.

[0052] The term “T cell antigen coupler” or TAC is used interchangeably with “trifunctional T cell antigen coupler” or Tri-TAC and refers to an engineered nucleic acid construct or polypeptide comprising (a) an antigen-binding domain that binds a target, (b) an antigen-binding domain that binds a protein associated with a T cell receptor (TCR) complex, and (c) a T cell receptor signaling domain.

[0053] The term “polynucleotide” and/or “nucleic acid sequence” and/or “nucleic acid” as used herein refers to a sequence of nucleoside or nucleotide monomers consisting of bases, sugars and intersugar (backbone) linkages. The term also includes modified or substituted sequences comprising non-naturally occurring monomers or portions thereof. The nucleic acid sequences of the present application may be deoxyribonucleic acid sequences (DNA) or ribonucleic acid sequences (RNA) and may include naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The sequences may also contain modified bases. Examples of such modified bases include aza and deaza adenine, guanine, cytosine, thymidine and uracil; and xanthine and hypoxanthine. The nucleic acids of the present disclosure may be isolated from biological organisms, formed by laboratory methods of genetic recombination or obtained by chemical synthesis or other known protocols for creating nucleic acids.

[0054] The term “isolated polynucleotide” or “isolated nucleic acid sequence” as used herein refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. An isolated nucleic acid is also substantially free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) from which the nucleic acid is derived. The term “nucleic acid” is intended to include DNA and RNA and is either double stranded or single stranded, and represents the sense or antisense strand. Further, the term “nucleic acid” includes the complementary nucleic acid sequences.

[0055] The term “recombinant nucleic acid” or “engineered nucleic acid” as used herein refers to a nucleic acid or polynucleotide that is not found in a biological organism. For example, recombinant nucleic acids may be formed by laboratory methods of genetic recombination (such as molecular cloning) to create sequences that would not otherwise be found in nature.

Recombinant nucleic acids may also be created by chemical synthesis or other known protocols for creating nucleic acids.

[0056] The terms “peptide”, “polypeptide,” and “protein” as used herein mean a chain of amino acids. The term protein as used herein further means a large molecule comprising one or more chains of amino acids and, in some embodiments, is a fragment or domain of a protein or a full length protein. Furthermore, as used herein, the term protein either refers to a linear chain of amino acids or to a chain of amino acids that has been processed and folded into a functional protein. The protein structure is divided into four distinct levels: (1) primary structure – referring to the sequence of amino acids in the polypeptide chain, (2) secondary structure – referring to the regular local sub-structures on the polypeptide backbone chain, such as α -helix and β -sheets, (3) tertiary structure – referring to the three-dimensional structure of monomeric and multimeric protein molecules, and (4) quaternary structure – referring to the three-dimensional structure comprising the aggregation of two or more individual polypeptide chains that operate as a single functional unit. The use of peptide or polypeptide herein does not mean that the chain of amino acids is not also a protein (*i.e.*, a chain of amino acids having a secondary, tertiary or quaternary structure).

[0057] The term “isolated polypeptide” refers to a polypeptide substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

[0058] The term “vector” as used herein refers to a polynucleotide that is used to deliver a nucleic acid to the inside of a cell. In some embodiments, a vector is an expression vector comprising expression control sequences (for example, a promoter) operatively linked to a nucleic acid to be expressed in a cell. Vectors known in the art include, but are not limited to, plasmids, phages, cosmids and viruses.

[0059] The term “tumor antigen” or “tumor associated antigen” as used herein refers to an antigenic substance produced in tumor cells that triggers an immune response in a host (*e.g.*, which is presented by MHC complexes). In some embodiments, a tumor antigen is on the surface of a tumor cell.

[0060] As used herein, the term “transmembrane and cytosolic domain” refers to a polypeptide that comprises a transmembrane domain and a cytosolic domain of a protein associated with the T cell receptor (TCR) complex. In some embodiments, such transmembrane and cytosolic domain may include, but is not limited to, protein domains that (a) associate with the lipid raft and/or (b) bind Lck.

[0061] A “TCR co-receptor” as used herein, refers to a molecule that assists the T cell receptor (TCR) in communicating with an antigen-presenting cell and may be considered part of the first signal that leads to the activation of the TCR. Examples of TCR co-receptors include, but are not limited to, CD4, LAG3, and CD8.

5 **[0062]** A “TCR co-stimulator” or “co-stimulatory domain” as used herein, refers to a molecule that enhances the response of a T cell to an antigen and may be considered as the second signal that leads to the activation of the TCR. Examples of TCR co-stimulators include, but are not limited to, ICOS, CD27, CD28, 4-1BB (CD 137), OX40 (CD134), CD30, CD40, lymphocyte
10 fiction-associated antigen 1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds CD83.

[0063] The terms “recipient”, “individual”, “subject”, “host”, and “patient”, are used interchangeably herein and in some embodiments, refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. “Mammal” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm
15 animals, and laboratory, zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, mice, rats, rabbits, guinea pigs, monkeys etc. In some embodiments, the mammal is human. None of these terms require the supervision of medical personnel.

[0064] As used herein, the terms “treatment,” “treating,” and the like, in some embodiments, refer to administering an agent, or carrying out a procedure, for the purposes of obtaining an
20 effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of affecting a partial or complete cure for a disease and/or symptoms of the disease. “Treatment,” as used herein, may include treatment of a disease or disorder (e.g., cancer) in a mammal, particularly in a human, and includes: (a) preventing the disease or a symptom of a disease from occurring in a subject which may be
25 predisposed to the disease but has not yet been diagnosed as having it (e.g., including diseases that may be associated with or caused by a primary disease; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease, *i.e.*, causing regression of the disease. Treating may refer to any indicia of success in the treatment or amelioration or prevention of a cancer, including any objective or subjective parameter such as abatement; remission;
30 diminishing of symptoms; or making the disease condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms is based on one or more objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term

"treating" includes the administration of the compounds or agents of the present invention to prevent, delay, alleviate, arrest or inhibit development of the symptoms or conditions associated with diseases (e.g., cancer). The term "therapeutic effect" refers to the reduction, elimination, or prevention of the disease, symptoms of the disease, or side effects of the disease in the subject.

5 **[0065]** As used herein, singular forms “a”, “and,” and “the” include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to “an antibody” includes a plurality of antibodies and reference to “an antibody” in some embodiments includes multiple antibodies, and so forth.

10 **[0066]** As used herein, all numerical values or numerical ranges include whole integers within or encompassing such ranges and fractions of the values or the integers within or encompassing ranges unless the context clearly indicates otherwise. Thus, for example, reference to a range of 90-100%, includes 91%, 92%, 93%, 94%, 95%, 95%, 96%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth. In another example, reference to a range of 1-5,000 fold includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,
15 15, 16, 17, 18, 19, 20, fold, etc., as well as 1.1, 1.2, 1.3, 1.4, 1.5, fold, etc., 2.1, 2.2, 2.3, 2.4, 2.5, fold, etc., and so forth.

[0067] “About” a number, as used herein, refers to range including the number and ranging from 10% below that number to 10% above that number. “About” a range refers to 10% below the lower limit of the range, spanning to 10% above the upper limit of the range.

20 **[0068]** “Percent (%) identity” refers to the extent to which two sequences (nucleotide or amino acid) have the same residue at the same positions in an alignment. For example, “an amino acid sequence is X% identical to SEQ ID NO: Y” refers to % identity of the amino acid sequence to SEQ ID NO: Y and is elaborated as X% of residues in the amino acid sequence are identical to the residues of sequence disclosed in SEQ ID NO: Y. Generally, computer programs are
25 employed for such calculations. Exemplary programs that compare and align pairs of sequences, include ALIGN (Myers and Miller, 1988), FASTA (Pearson and Lipman, 1988; Pearson, 1990) and gapped BLAST (Altschul et al., 1997), BLASTP, BLASTN, or GCG (Devereux et al., 1984).

30 **[0069]** As used herein, the term “selective binding” refers to the higher affinity with which a molecule (e.g., protein such as an antigen-binding domain of TAC) binds its target molecule (e.g., target antigen such as Claudin 18.2) over other molecules. Unless indicated otherwise, the terms “selective binding” and “specific binding” are used interchangeably herein.

T cell-antigen couplers (TACs)

[0070] Disclosed herein, in certain embodiments, are nucleic acids encoding Claudin 18.2 T cell-antigen coupler (TAC) polypeptides. In some embodiments, the nucleic acids encoding the Claudin 18.2-TAC comprise: (a) a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (b) a second polynucleotide encoding an antigen-binding domain that binds the TCR complex; and (c) a third polynucleotide encoding a transmembrane domain and cytosolic domain. In some embodiments, the nucleic acids comprise, in order (e.g., from 5' to 3'): (a) the first polynucleotide; (b) the second polynucleotide; and (c) the third polynucleotide encoding a TCR co-receptor cytosolic domain and transmembrane domain. In some embodiments, the nucleic acids encoding the Claudin 18.2-TAC do not encode a co-stimulatory domain. In some embodiments, the nucleic acids encoding the Claudin 18.2-TAC do not encode a co-activation domain.

[0071] Further disclosed herein, in certain embodiments, are Claudin 18.2 T cell-antigen coupler (TAC) polypeptides. In some embodiments, the Claudin 18.2-TAC polypeptides comprise: (a) an antigen-binding domain that binds Claudin 18.2; (b) an antigen-binding domain that binds the TCR complex; and (c) a transmembrane domain and cytosolic domain. In some embodiments, the Claudin 18.2-TAC polypeptides comprise, in order (e.g., from N-terminus to C-terminus) (a) the antigen-binding domain that binds Claudin 18.2; (b) the antigen-binding domain that binds the TCR complex; and (c) the transmembrane domain and cytosolic domain. In some embodiments, the Claudin 18.2-TAC polypeptides do not include a co-stimulatory domain. In some embodiments, the Claudin 18.2-TAC polypeptides do not include a co-activation domain.

[0072] Further disclosed herein, in certain embodiments, are expression vectors comprising a nucleic acid encoding a Claudin 18.2-TAC polypeptide as described herein.

[0073] Further disclosed herein, in certain embodiments, are T cells comprising a nucleic acid encoding a Claudin 18.2-TAC polypeptide as described herein, T cells comprising an expression vector encoding a Claudin 18.2-TAC polypeptide as described herein, or T cells comprising a Claudin 18.2-TAC polypeptide as described herein.

[0074] Further disclosed herein, in certain embodiments, are methods of treating a cancer in an individual in need thereof, comprising administering to the individual a T cell comprising a Claudin 18.2 T cell-antigen coupler (TAC) polypeptide as described herein.

Claudin 18.2 Antigen-Binding Domain

[0075] In certain embodiments, the Claudin 18.2-TAC polypeptide comprises a Claudin 18.2 antigen-binding domain. In some embodiments, the Claudin 18.2 antigen-binding domain selectively binds Claudin 18.2. In some embodiments, the Claudin 18.2 antigen-binding domain

binds to Claudin 18.2 on a target cell. In some embodiments, a target cell is a cell associated with a disease state, including, but not limited to, cancer. In some embodiments, a target cell is a tumor cell.

[0076] In some embodiments, the Claudin 18.2 antigen-binding domain is an antibody or a fragment thereof. In some embodiments, the Claudin 18.2 antigen-binding domain is selected from single chain antibodies (e.g., single-chain fragment variable antibodies (scFvs)), single domain antibodies (e.g., heavy-chain-only antibodies (VHH), shark heavy-chain-only antibodies (VNAR)), nanobodies, diabodies, minibodies, Fab fragments, Fab' fragments, F(ab')₂ fragments, or Fv fragments that bind to Claudin 18.2.

[0077] In some embodiments, the Claudin 18.2 antigen-binding domain is selected from ankyrin repeat proteins (DARPs), affibodies, adnectins, affilins, phylomers, fynomers, affimers, peptide aptamers, lectins, knottins, centyrins, anticalins, peptides, peptidomimetics, proteins, glycoproteins, or proteoglycans that bind to Claudin 18.2, or naturally occurring ligands for Claudin 18.2. In some embodiments, the Claudin 18.2 antigen-binding domain is a non-protein compound that binds to Claudin 18.2, including but not limited to carbohydrates, lipids, nucleic acids, or small molecules.

[0078] In some embodiments, the Claudin 18.2 antigen-binding domain is a designed ankyrin repeat (DARPin) targeted to Claudin 18.2. In some embodiments, the Claudin 18.2 antigen-binding domain is a single-chain variable fragment (ScFv) targeted to Claudin 18.2. In some embodiments, the Claudin 18.2 antigen-binding domain is a nanobody targeted to Claudin 18.2.

[0079] In some embodiments, the Claudin 18.2 antigen-binding domain is of a nanobody referred to herein as Claudin 18.2 Nanobody 1 (having an amino acid sequence depicted in SEQ ID NO: 55). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid

sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of SEQ ID NO: 55 (Claudin 18.2 Nanobody 1). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 1 Kabat CDR1), a CDR2 comprising SEQ ID NO: 58 (Claudin 18.2 Nanobody 1 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 1 Kabat CDR3). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 60 (Claudin 18.2 Nanobody 1 IMGT CDR1), a CDR2 comprising SEQ ID NO: 61 (Claudin 18.2 Nanobody 1 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 1 IMGT CDR3). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1, CDR2, and CDR3 each having 100% identity to the corresponding CDR in Claudin 18.2 Nanobody 1). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (*e.g.*,

framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 1, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 1.

[0080] In some embodiments, the Claudin 18.2 antigen-binding domain is of a nanobody referred to herein as Claudin 18.2 Nanobody 2 (having an amino acid sequence depicted in SEQ ID NO: 56). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some

embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of SEQ ID NO: 56 (Claudin 18.2 Nanobody 2). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 2 Kabat CDR1), a CDR2 comprising SEQ ID NO: 63 (Claudin 18.2 Nanobody 2 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 2 Kabat CDR3). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 64 (Claudin 18.2 Nanobody 2 IMGT CDR1), a CDR2 comprising SEQ ID NO: 65 (Claudin 18.2 Nanobody 2 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 2 IMGT CDR3). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1, CDR2, and CDR3 each having 100% identity to the corresponding CDR in Claudin 18.2 Nanobody 2). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding

domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of Claudin 18.2 Nanobody 2, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of Claudin 18.2 Nanobody 2.

[0081] In some embodiments, the Claudin 18.2 antigen-binding domain is of an antibody selected from IMAB362 (also known as claudiximab and zolbetuximab; having a heavy chain variable region depicted in SEQ ID NO: 48 and a light chain variable region depicted in SEQ ID NO: 49), 43-14A (available from Creative Biolabs, catalog no. HPAB-0120-YJ), EPR 19202 (available from Abcam, catalog no. ab222512), and aGC182. In some embodiments, the Claudin 18.2 antigen-binding domain comprises a humanized antigen-binding domain of an antibody selected from 43-14A and EPR 19202.

[0082] In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from IMAB362 (claudiximab, zolbetuximab). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO:

48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of SEQ ID NO: 48 (IMAB362 heavy chain variable region) and the amino acid

sequence of SEQ ID NO: 49 (IMAB362 light chain variable region). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab) (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (*e.g.*, framework) sequences of IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab,

zolbetuximab), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab). In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of IMAB362 (claudiximab, zolbetuximab), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of IMAB362 (claudiximab, zolbetuximab).

[0083] In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from mouse anti-Claudin 18.2 recombinant antibody clone 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of the antigen-binding domain(s) of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3,

CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of 43-14A, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of 43-14A.

[0084] In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from rabbit monoclonal antibody to Claudin 18.2 EPR 19202.

In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of the antigen-binding domain(s) of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds

Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of EPR 19202. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of EPR 19202, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of EPR 19202.

[0085] In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from monoclonal mouse anti-Claudin 18.2 antibody aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 95% sequence identity with the

amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the antigen-binding domain that binds Claudin 18.2 comprises the amino acid sequence of the antigen-binding domain(s) of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182 (*i.e.*, the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at

least 96% sequence identity with the non-CDR (e.g., framework) sequences of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of aGC182. In some embodiments, the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of aGC182, and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds Claudin 18.2 have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of aGC182.

[0086] Amino acid sequences of exemplary antigen-binding domains that bind Claudin 18.2 are provided in Table 1.

Table 1. Table of Sequences

SEQ ID NO	Description	Nucleotide/Amino Acid
SEQ ID NO: 48	IMAB362 Heavy Chain Variable Region	Amino Acid
SEQ ID NO: 49	IMAB362 Light Chain Variable Region	Amino Acid
SEQ ID NO: 55	Claudin 18.2 Nanobody 1	Amino Acid
SEQ ID NO: 56	Claudin 18.2 Nanobody 2	Amino Acid
SEQ ID NO: 57	Claudin 18.2 Nanobody 1 CDR1 (Kabat); Claudin 18.2 Nanobody 2 CDR1 (Kabat)	Amino Acid
SEQ ID NO: 58	Claudin 18.2 Nanobody 1 CDR2 (Kabat)	Amino Acid
SEQ ID NO: 59	Claudin 18.2 Nanobody 1 CDR3 (Kabat); Claudin 18.2 Nanobody 2 CDR3 (Kabat)	Amino Acid
SEQ ID NO: 60	Claudin 18.2 Nanobody 1 CDR1 (IMGT)	Amino Acid
SEQ ID NO: 61	Claudin 18.2 Nanobody 1 CDR2 (IMGT)	Amino Acid
SEQ ID NO: 62	Claudin 18.2 Nanobody 1 CDR3 (IMGT); Claudin 18.2 Nanobody 2 CDR3 (IMGT)	Amino Acid
SEQ ID NO: 63	Claudin 18.2 Nanobody 2 CDR2 (Kabat)	Amino Acid
SEQ ID NO: 64	Claudin 18.2 Nanobody 2 CDR1 (IMGT)	Amino Acid
SEQ ID NO: 65	Claudin 18.2 Nanobody 2 CDR2 (IMGT)	Amino Acid

TCR Complex Protein Antigen-Binding Domain

[0087] In certain embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds a protein associated with the TCR complex. A “TCR complex protein antigen-binding domain,” also referred to as a “TCR complex antigen-binding domain,” “antigen-binding

domain that binds the TCR complex,” or “antigen-binding domain that binds a protein associated with the TCR complex,” refers to any substance or molecule that binds, directly or indirectly, to a protein associated with a TCR complex. In some embodiments, the antigen-binding domain that binds a protein associated with a TCR complex selectively binds to a protein of the TCR. In some embodiments, the antigen-binding domain that binds a protein associated with a TCR complex comprises a substance that specifically binds to a protein of the TCR.

[0088] In some embodiments, the TCR complex protein antigen-binding domain is selected from antibodies or fragments thereof, for example, single chain antibodies (e.g., single-chain fragment variable antibodies (scFvs)), single domain antibodies (e.g., heavy-chain-only antibodies (VHH), shark heavy-chain-only antibodies (VNAR)), nanobodies, diabodies, minibodies, Fab fragments, Fab' fragments, F(ab')₂ fragments, or Fv fragments that bind to a protein of the TCR. In some embodiments, the TCR complex protein antigen-binding domain is selected from ankyrin repeat proteins (DARPin), affibodies, adnectins, affilins, phylomers; fynomers, affimers, peptide aptamers, lectins, knottins, centyrins, anticalins, peptides, peptidomimetics, proteins, glycoproteins, or proteoglycans that bind to a protein of the TCR, or naturally occurring ligands for a protein of the TCR. In some embodiments, the TCR complex protein antigen-binding domain is a non-protein compound that binds to a protein of the TCR, including but not limited to carbohydrates, lipids, nucleic acids, or small molecules. In some embodiments, the TCR complex protein antigen-binding domain is a designed ankyrin repeat (DARPin) targeted to a protein of the TCR. In some embodiments, the TCR complex protein antigen-binding domain is a single-chain variable fragment (ScFv) targeted to a protein of the TCR. In some embodiments, the TCR complex protein antigen-binding domain is a nanobody targeted to a protein of the TCR.

[0089] Proteins associated with the TCR include, but are not limited, to the TCR alpha (α) chain, TCR beta (β) chain, TCR gamma (γ) chain, TCR delta (δ) chain, CD3 γ chain, CD3 δ chain and CD3 ϵ chains. In some embodiments, an antigen-binding domain that binds a protein associated with the TCR complex is an antibody to the TCR alpha (α) chain, TCR beta (β) chain, TCR gamma (γ) chain, TCR delta (δ) chain, CD3 γ chain, CD3 δ chain and/or CD3 ϵ chain. In some embodiments, the protein associated with a TCR complex is CD3. In some embodiments, the protein associated with a TCR complex is CD3 ϵ . In some embodiments, the antigen-binding domain that binds CD3 is an antibody, for example, a single chain antibody, for example a single-chain variable fragment (scFv). Examples of CD3 antibodies, include, but are not limited

to, UCHT1, OKT3, F6A, L2K, muromonab, oteixizumab, teplizumab, visilizumab, CD3-12, MEM-57, 4D10A6, CD3D, or TR66.

[0090] In some embodiments, the antigen-binding domain that binds the TCR complex is UCHT1, or a variant thereof. In some embodiments, the UCHT1 antigen-binding domain is encoded by SEQ ID NO: 7. In some embodiments, the UCHT1 antigen-binding domain comprises SEQ ID NO: 8. In some embodiments, the UCHT1 antigen-binding domain is mutated. In some embodiments, the UCHT1 antigen-binding domain comprises a Y to T mutation at a position corresponding to amino acid 182 of SEQ ID NO: 8 (Y182T). In some embodiments, the UCHT1 (Y182T) antigen-binding domain is encoded by SEQ ID NO: 43. In some embodiments, the UCHT1 (Y182T) antigen-binding domain comprises SEQ ID NO: 44. In some embodiments, the antigen-binding domain that binds the TCR complex is a humanized UCHT1 (huUCHT1). In some embodiments, the huUCHT1 antigen-binding domain is encoded by SEQ ID NO: 33. In some embodiments, the huUCHT1 antigen-binding domain comprises SEQ ID NO: 34. In some embodiments, the huUCHT1 has a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (Y177T). In some embodiments, the huUCHT1 (Y177T) antigen-binding domain is encoded by SEQ ID NO: 35. In some embodiments, the huUCHT1 antigen-binding domain comprises SEQ ID NO: 36.

[0091] In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 95%

sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some
embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein
associated with the TCR complex comprises a nucleotide sequence having at least 96%
sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some
5 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein
associated with the TCR complex comprises a nucleotide sequence having at least 97%
sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some
embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein
associated with the TCR complex comprises a nucleotide sequence having at least 98%
10 sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some
embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein
associated with the TCR complex comprises a nucleotide sequence having at least 99%
sequence identity with the nucleotide sequence of SEQ ID NO: 7 (UCHT1). In some
embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein
15 associated with the TCR complex comprises the nucleotide sequence of SEQ ID NO: 7
(UCHT1).

[0092] In some embodiments, the antigen-binding domain that binds the protein associated with
the TCR complex comprises an amino acid sequence having at least 70% sequence identity with
the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the antigen-
20 binding domain that binds the protein associated with the TCR complex comprises an amino
acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID
NO: 8 (UCHT1). In some embodiments, the antigen-binding domain that binds the protein
associated with the TCR complex comprises an amino acid sequence having at least 80%
sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some
25 embodiments, the antigen-binding domain that binds the protein associated with the TCR
complex comprises an amino acid sequence having at least 85% sequence identity with the
amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the antigen-binding
domain that binds the protein associated with the TCR complex comprises an amino acid
sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 8
30 (UCHT1). In some embodiments, the antigen-binding domain that binds the protein associated
with the TCR complex comprises an amino acid sequence having at least 95% sequence identity
with the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the antigen-
binding domain that binds the protein associated with the TCR complex comprises an amino
acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID

NO: 8 (UCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity

with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some
5 embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least
10 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least
15 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least
20 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least
25 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1).

[0093] In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1
30 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at

least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises the nucleotide sequence of SEQ ID NO: 43 (UCHT1 (Y182T)).

[0094] In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated

with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and

the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)), and

the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 44 (UCHT1 (Y182T)).

[0095] In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 33 (huUCHT1). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein

associated with the TCR complex comprises the nucleotide sequence of SEQ ID NO: 33 (huUCHT1).

[0096] In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex

comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least

97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 34 (huUCHT1).

[0097] In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1

(Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises the nucleotide sequence of SEQ ID NO: 35 (huUCHT1 (Y177T)).

[0098] In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the antigen-binding domain that

complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 36 (huUCHT1 (Y177T)).

[0099] In some embodiments, the antigen-binding domain that binds to the protein associated with the TCR complex is OKT3. In some embodiments, the murine OKT3 antigen-binding domain is encoded by SEQ ID NO: 15. In some embodiments, the OKT3 antigen-binding domain comprises SEQ ID NO: 16.

[0100] In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 75%

sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

5 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

10 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

15 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

20 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

25 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 15 (OKT3). In some

embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises the nucleotide sequence of SEQ ID NO: 15 (OKT3).

[0101] In some embodiments, the antigen-binding domain that binds the protein associated with

30 the TCR complex comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated

with the TCR complex comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein

associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino

acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3).

5 **[0102]** In some embodiments, the antigen-binding domain that binds to the protein associated with the TCR complex is F6A. In some embodiments, the murine F6A antigen-binding domain is encoded by SEQ ID NO: 17. In some embodiments, the F6A antigen-binding domain comprises SEQ ID NO: 18.

10 **[0103]** In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some
15 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 85%
20 sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some
25 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some
30 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 98%

sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 17 (F6A). In some

embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises the nucleotide sequence of SEQ ID NO: 17 (F6A).

[0104] In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding

domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-

binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some

embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO:

18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID

NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments,

the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein

associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 18 (F6A).

[0105] In some embodiments, the antigen-binding domain that binds to the protein associated with the TCR complex is L2K. In some embodiments, the murine L2K antigen-binding domain is encoded by SEQ ID NO: 19. In some embodiments, the L2K antigen-binding domain comprises SEQ ID NO: 20.

[0106] In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 90%

sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some

5 embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 97%

10 sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some

embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 19 (L2K). In some embodiments, the polynucleotide encoding the antigen-binding domain that binds the protein associated with the TCR complex comprises the nucleotide sequence of SEQ ID NO: 19 (L2K).

[0107] In some embodiments, the antigen-binding domain that binds the protein associated with

20 the TCR complex comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated

25 with the TCR complex comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein

30 associated with the TCR complex comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding

domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the antigen-binding domain that binds the protein associated with the TCR complex comprises the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K) (*i.e.*, the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence comprising a CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, each having 100% identity to the corresponding CDR in the amino acid sequence of SEQ ID NO: 20 (L2K)). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 85% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (*e.g.*, framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 90% sequence identity with the non-CDR (*e.g.*, framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some

embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 95% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 96% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 97% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 98% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K). In some embodiments, the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K), and the non-CDR (e.g., framework) sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 99% sequence identity with the non-CDR (e.g., framework) sequences of the amino acid sequence of SEQ ID NO: 20 (L2K).

[0108] Amino acid and nucleotide sequences of exemplary antigen-binding domains that bind a protein associated with the TCR complex are provided in Table 2.

Table 2. Table of Sequences

SEQ ID NO	Description	Nucleotide/Amino Acid
SEQ ID NO: 7	UCHT1 ¹	Nucleotide
SEQ ID NO: 8	UCHT1 ²	Amino Acid
SEQ ID NO: 15	OKT3	Nucleotide

SEQ ID NO: 16	OKT3	Amino Acid
SEQ ID NO: 17	F6A	Nucleotide
SEQ ID NO: 18	F6A	Amino Acid
SEQ ID NO: 19	L2K	Nucleotide
SEQ ID NO: 20	L2K	Amino Acid
SEQ ID NO: 33	huUCHT1	Nucleotide
SEQ ID NO: 34	huUCHT1	Amino Acid
SEQ ID NO: 35	huUCHT1 (Y177T)	Nucleotide
SEQ ID NO: 36	huUCHT1 (Y177T)	Amino Acid
SEQ ID NO: 43	UCHT1 (Y182T)	Nucleotide
SEQ ID NO: 44	UCHT1 (Y182T)	Amino Acid

¹ Light chain, nucleotides 1-324; Linker, nucleotides 325-387; Heavy chain, nucleotides 388-750

² Light chain, amino acids 1-108; Linker, amino acids 109-128; Heavy chain, amino acids 129-250

5 **Transmembrane domain and Cytosolic domain**

[0109] In some embodiments, a Claudin 18.2 T cell antigen coupler polypeptide comprises a T cell receptor signaling domain polypeptide. In some embodiments, a Claudin 18.2 T cell antigen coupler polypeptide comprises a transmembrane domain of a TCR signaling domain. In some embodiments, a Claudin 18.2 T cell antigen coupler polypeptide comprises a cytosolic domain of a TCR signaling domain polypeptide. In some embodiments, a Claudin 18.2 T cell antigen coupler polypeptide comprises a transmembrane domain and a cytosolic domain of a TCR signaling domain polypeptide.

[0110] In some embodiments, the T cell receptor signaling domain polypeptide comprises a TCR co-receptor domain. In some embodiments, the TCR signaling domain polypeptide comprises a transmembrane domain and/or a cytosolic domain of a TCR co-receptor. In some embodiments, the TCR co-receptor is CD4, CD8, LAG3, or a chimeric variation thereof.

[0111] In some embodiments, the TCR co-receptor is CD4. In some embodiments, the Claudin 18.2-TAC comprises a transmembrane domain and a cytosolic domain of a CD4 co-receptor. In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide

encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises the nucleotide sequence of SEQ ID NO: 11 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain).

In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain).

[0112] In some embodiments, the TCR co-receptor is CD8. In some embodiments, the TCR co-receptor is CD8 α . In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and

transmembrane domain comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises the nucleotide sequence of SEQ ID NO: 27 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 98% sequence identity

with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain). In some embodiments, the cytosolic and transmembrane domain comprise the amino acid sequence of SEQ ID NO: 28 (CD8 transmembrane and cytosolic domain).

[0113] In some embodiments, the TCR signaling domain polypeptide comprises a chimera of sequences or domains from co-receptors. In some embodiments, the TCR signaling domain polypeptide comprises a chimera of CD8 α and CD8 β , wherein the CD8 α arginine rich region is replaced with the CD8 β arginine rich region (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 99% sequence identity

with the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises the nucleotide sequence of SEQ ID NO: 29 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera). In some embodiments, the cytosolic and transmembrane domain comprise the amino acid sequence of SEQ ID NO: 30 (CD8 α +R(β) chimera).

[0114] In some embodiments, the TCR signaling domain polypeptide comprises a chimera of CD8 α and CD8 β , where the CD8 α CXCP domain, which contains an Lck binding motif, is appended to the C-terminus of the CD8 β cytosolic domain (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 75%

sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the polynucleotide encoding the cytosolic and transmembrane domain comprises the nucleotide sequence of SEQ ID NO: 31 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 32

(CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera). In some embodiments, the cytosolic and transmembrane domain comprise the amino acid sequence of SEQ ID NO: 32 (CD8 β +Lck chimera).

[0115] In some embodiments, the TCR signaling domain polypeptide includes both a cytosolic domain and a transmembrane domain of a TCR co-receptor protein. In some embodiments, the cytosolic domain and transmembrane domain are from the same co-receptor or from different co-receptors.

[0116] Amino acid and nucleotide sequences of exemplary transmembrane and cytosolic domains are provided in Table 3.

Table 3. Table of Sequences

SEQ ID NO	Description	Nucleotide/Amino Acid
SEQ ID NO: 11	CD4 Domain ¹	Nucleotide
SEQ ID NO: 12	CD4 Domain ²	Amino Acid
SEQ ID NO: 27	CD8 α Domain	Nucleotide
SEQ ID NO: 28	CD8 α Domain	Amino Acid
SEQ ID NO: 29	CD8 α +R(β) Domain	Nucleotide
SEQ ID NO: 30	CD8 α +R(β) Domain	Amino Acid
SEQ ID NO: 31	CD8 α +Lck Domain	Nucleotide
SEQ ID NO: 32	CD8 α +Lck Domain	Amino Acid

¹ Extracellular linker, nucleotides 1-66; Transmembrane domain, nucleotides 67-132; Cytosolic domain, nucleotides 133-254

² Extracellular linker, amino acids 1-22; Transmembrane domain, amino acids 23-44; Cytosolic domain, amino acids 45-84

Configurations, Linkers, and Connectors

[0117] In some embodiments, a nucleic acid disclosed herein is in an order of (1) a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (2) a second polynucleotide encoding an antigen-binding domain that binds a TCR complex; (3) a third polynucleotide encoding a transmembrane domain and a cytosolic domain. In some

embodiments, a nucleic acid disclosed herein is in an order of (1) a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (2) a second polynucleotide encoding an antigen-binding domain that binds a TCR complex; (3) a third polynucleotide encoding a transmembrane domain and a cytosolic domain, wherein the order is 5' end to 3' end. In some embodiments, a nucleic acid disclosed herein is in an order of (1) a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (2) a second polynucleotide encoding an antigen-binding domain that binds a TCR complex; (3) a third polynucleotide encoding a transmembrane domain and a cytosolic domain, wherein the order is 3' end to 5' end. In some embodiments, a nucleic acid described herein is in an order of (1) a first polynucleotide encoding an antigen-binding domain that binds a TCR complex; (2) a second polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (3) a third polynucleotide encoding a transmembrane domain and a cytosolic domain. In some embodiments, a nucleic acid described herein is in an order of (1) a first polynucleotide encoding an antigen-binding domain that binds a TCR complex; (2) a second polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (3) a third polynucleotide encoding a transmembrane domain and a cytosolic domain, wherein the order is 5' end to 3' end. In some embodiments, a nucleic acid described herein is in an order of (1) a first polynucleotide encoding an antigen-binding domain that binds a TCR complex; (2) a second polynucleotide encoding an antigen-binding domain that binds Claudin 18.2; (3) a third polynucleotide encoding a transmembrane domain and a cytosolic domain, wherein the order is 3' end to 5' end.

[0118] In some embodiments, a Claudin 18.2-TAC polypeptide disclosed herein is in an order of (1) an antigen-binding domain that binds Claudin 18.2; (2) an antigen-binding domain that binds a TCR complex; (3) a transmembrane domain and a cytosolic domain, wherein the order is N-terminus to C-terminus. In some embodiments, a Claudin 18.2-TAC polypeptide disclosed herein is in an order of (1) an antigen-binding domain that binds Claudin 18.2; (2) an antigen-binding domain that binds a TCR complex; (3) a transmembrane domain and a cytosolic domain, wherein the order is C-terminus to N-terminus. In some embodiments, a Claudin 18.2-TAC polypeptide described herein is in an order of (1) an antigen-binding domain that binds a TCR complex; (2) an antigen-binding domain that binds Claudin 18.2; (3) a transmembrane domain and a cytosolic domain, wherein the order is N-terminus to C-terminus. In some

embodiments, a Claudin 18.2-TAC polypeptide described herein is in an order of (1) an antigen-binding domain that binds a TCR complex; (2) an antigen-binding domain that binds Claudin 18.2; (3) a transmembrane domain and a cytosolic domain, wherein the order is C-terminus to N-terminus.

5 **[0119]** In some embodiments, the antigen-binding domain that binds Claudin 18.2, the antigen-binding domain that binds the TCR complex, and/or the transmembrane domain and cytosolic domain are directly fused. For example, the antigen-binding domain that binds Claudin 18.2 and the transmembrane domain and cytosolic domain are both fused to the antigen-binding domain that binds the TCR complex. In some embodiments, the antigen-binding domain that binds
10 Claudin 18.2, the antigen-binding domain that binds the TCR complex, and/or the transmembrane domain and cytosolic domain are joined by at least one linker. In some embodiments, the antigen-binding domain that binds Claudin 18.2 and the antigen-binding domain that binds the TCR complex are directly fused, and joined to the transmembrane domain and cytosolic domain by a linker. In some embodiments, the antigen-binding domain that binds
15 the TCR complex and the transmembrane domain and cytosolic domain are directly fused, and joined to the antigen-binding domain that binds Claudin 18.2 by a linker.

[0120] In some embodiments, the linker is a peptide linker. In some embodiments, the peptide linker comprises 1 to 40 amino acids. In some embodiments, the peptide linker comprises 1 to 30 amino acids. In some embodiments, the peptide linker comprises 1 to 15 amino acids. In
20 some embodiments, the peptide linker comprises 1 to 10 amino acids. In some embodiments, the peptide linker comprises 1 to 6 amino acids. In some embodiments, the peptide linker comprises 30 to 40 amino acids. In some embodiments, the peptide linker comprises 32 to 36 amino acids. In some embodiments, the peptide linker comprises 5 to 30 amino acids. In some embodiments, the peptide linker comprises 5 amino acids. In some embodiments, the peptide linker comprises
25 10 amino acids. In some embodiments, the peptide linker comprises 15 amino acids. In some embodiments, the peptide linker comprises 20 amino acids. In some embodiments, the peptide linker comprises 25 amino acids. In some embodiments, the peptide linker comprises 30 amino acids. In some embodiments, the peptide linker comprises a glycine and/or serine-rich linker.

[0121] In some embodiments, the at least one linker comprises an amino acid sequence having
30 at least 80% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)₄-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ

ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 85% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 90% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 95% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 96% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 97% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 98% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker comprises an amino acid sequence having at least 80% identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-

based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker). In some embodiments, the at least one linker

5 comprises the amino acid sequence of SEQ ID NO: 6 ((G4S)₄-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker).

10 **[0122]** In some embodiments, the peptide linker that joins the antigen-binding domain that binds Claudin 18.2 to the antigen-binding domain that binds a TCR complex (e.g., UCHT1) is known as the connector to distinguish this protein domain from other linkers in the TAC. The connector may be of any size. In some embodiments, the connector between the antigen-binding domain that binds a TCR complex and the antigen-binding domain that binds Claudin 18.2 is a short

15 helix comprising SEQ ID NO: 22. In some embodiments, the connector between the antigen-binding domain that binds a TCR complex and the antigen-binding domain that binds Claudin 18.2 is a short helix encoded by SEQ ID NO: 21. In some embodiments, the connector between the antigen-binding domain that binds a TCR complex and the antigen-binding domain that binds Claudin 18.2 is a long helix comprising SEQ ID NO: 24. In some embodiments, the

20 connector between the antigen-binding domain that binds a TCR complex and the antigen-binding domain that binds Claudin 18.2 is a long helix encoded by SEQ ID NO: 23. In some embodiments, the connector between the antigen-binding domain that binds a TCR complex and the antigen-binding domain that binds Claudin 18.2 is a large domain comprising SEQ ID NO: 26. In some embodiments, the connector between the antigen-binding domain that binds a TCR

25 complex and the antigen-binding domain that binds Claudin 18.2 is a large domain encoded by SEQ ID NO: 25.

[0123] In some embodiments, a nucleic acid or TAC disclosed herein comprises a leader sequence. In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 1 (mIgG

30 leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader). In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 1 (mIgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader). In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 90% sequence identity with the

nucleotide sequence of SEQ ID NO: 1 (mulgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader). In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 1 (mulgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader).

5 In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 1 (mulgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader). In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 1 (mulgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader). In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 1 (mulgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader). In some embodiments, the leader sequence is encoded by a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 1 (mulgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader). In some embodiments, the leader sequence comprises the nucleotide sequence of SEQ ID NO: 1 (mulgG leader), SEQ ID NO: 37 (hulgG leader), or SEQ ID NO: 39 (huCD8a leader).

[0124] In some embodiments, a nucleic acid or TAC disclosed herein comprises a leader sequence. In some embodiments, the leader sequence comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader).

leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader). In some embodiments, the leader sequence comprises the amino acid sequence of SEQ ID NO: 2 (mulgG leader), SEQ ID NO: 38 (hulgG leader), or SEQ ID NO: 40 (huCD8a leader).

[0125] Amino acid and nucleotide sequences of exemplary linkers, connectors, and leader sequences are provided in Table 4.

Table 4. Table of Sequences

SEQ ID NO	Description	Nucleotide/Amino Acid
SEQ ID NO: 1	mulgG leader (secretion signal)	Nucleotide
SEQ ID NO: 2	mulgG leader (secretion signal)	Amino Acid
SEQ ID NO: 3	Myc Tag	Nucleotide
SEQ ID NO: 4	Myc Tag	Amino Acid
SEQ ID NO: 5	(G4S)4-based linker	Nucleotide
SEQ ID NO: 6	(G4S)4-based linker	Amino Acid
SEQ ID NO: 9	G4S-based linker	Nucleotide
SEQ ID NO: 10	G4S-based linker	Amino Acid
SEQ ID NO: 13	CD4 based linker	Nucleotide
SEQ ID NO: 14	CD4 based linker	Amino Acid
SEQ ID NO: 21	Short Helix connector	Nucleotide
SEQ ID NO: 22	Short Helix connector	Amino Acid
SEQ ID NO: 23	Long Helix connector	Nucleotide
SEQ ID NO: 24	Long Helix connector	Amino Acid
SEQ ID NO: 25	Large domain connector	Nucleotide
SEQ ID NO: 26	Large domain connector	Amino Acid
SEQ ID NO: 37	hulgG leader	Nucleotide
SEQ ID NO: 38	hulgG leader	Amino Acid
SEQ ID NO: 39	huCD8a leader	Nucleotide
SEQ ID NO: 40	huCD8a leader	Amino Acid
SEQ ID NO: 41	Flexible Connector	Amino Acid
SEQ ID NO: 42	Flexible Connector	Nucleotide
SEQ ID NO: 45	G4S flexible linker	Amino Acid
SEQ ID NO: 54	G4S flexible linker	Nucleotide
SEQ ID NO: 46	G4S3 flexible linker	Amino Acid
SEQ ID NO: 47	G4S3 flexible linker	Nucleotide

Specific TACs

[0126] Disclosed herein, in certain embodiments, are Claudin 18.2-TAC polypeptides comprising (a) an antigen-binding domain that binds Claudin 18.2, (b) a single-chain antibody

(scFv) that binds CD3ε, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor.

[0127] Disclosed herein, in certain embodiments, are Claudin 18.2-TAC polypeptides comprising (a) a DARPin that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor.

[0128] Disclosed herein, in certain embodiments, are Claudin 18.2-TACs comprising (a) a scFv that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD4

co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor.

[0129] Disclosed herein, in certain embodiments, are Claudin 18.2-TACs comprising (a) a nanobody that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor.

[0130] Disclosed herein, in certain embodiments, are Claudin 18.2-TACs comprising (a) an antigen-binding domain that binds Claudin 18.2, (b) a single-chain antibody (scFv) that binds CD3ε, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-

binding domain that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) an antigen-binding domain that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor.

[0131] Disclosed herein, in certain embodiments, are Claudin 18.2-TACs comprising (a) a DARPin that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD4 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a DARPin that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor.

[0132] Disclosed herein, in certain embodiments, are Claudin 18.2-TACs comprising (a) a scFv that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of

the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a scFv that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor.

[0133] Disclosed herein, in certain embodiments, are Claudin 18.2-TACs comprising (a) a nanobody that binds Claudin 18.2, (b) UCHT1, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) UCHT1 (Y182T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) huUCHT1, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) huUCHT1 (Y177T), and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) OKT3, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) F6A, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor. In some embodiments, the Claudin 18.2-TAC polypeptides comprise (a) a nanobody that binds Claudin 18.2, (b) L2K, and (c) a transmembrane and cytosolic domain of the CD8 co-receptor.

[0134] In certain instances, the Claudin 18.2-TAC polypeptides draw CD3 and TCR into lipid raft regions of the membrane, and brings Lck into the proximity of the TCR, similar to natural MHC binding.

[0135] In some embodiments, the Claudin 18.2-TAC comprises (from N- to C-terminus): (a) a myc tag, (b) a (G4S)₄-based linker, (c) a humanized variant of UCHT1 (huUCHT1), (d) a G4S-based linker, and (e) a CD4 transmembrane and cytosolic domain.

[0136] For example, in some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of

SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and the nucleotide sequence of SEQ ID NO: 50 (Scaffold 1).

[0137] In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 70% sequence identity with the

amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 51 (Scaffold 1). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and the amino acid sequence of SEQ ID NO: 51 (Scaffold 1).

[0138] In some embodiments, the Claudin 18.2-TAC comprises (from N- to C-terminus): (a) a myc tag, (b) a (G4S)₄-based linker, (c) a humanized variant of UCHT1 comprising a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (huUCHT1 (Y177T)), (d) a G4S-based linker, and (e) a CD4 transmembrane and cytosolic domain.

[0139] For example, in some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin

18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence encoding an antigen-binding domain that binds Claudin 18.2 and the nucleotide sequence of SEQ ID NO: 52 (Scaffold 2).

[0140] In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds

Claudin 18.2 and an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 53 (Scaffold 2). In some embodiments, the Claudin 18.2-TAC comprises an antigen-binding domain that binds Claudin 18.2 and the amino acid sequence of SEQ ID NO: 53 (Scaffold 2).

[0141] Amino acid and nucleotide sequences of exemplary TACs scaffolds are provided in Table 5.

Table 5. Table of Sequences

SEQ ID NO	Description	Nucleotide/Amino Acid
SEQ ID NO: 50	Scaffold 1 (Myc tag – (G4S)4-based linker – huUCHT1 – G4S-based linker – CD4)	Nucleotide
SEQ ID NO: 51	Scaffold 1 (Myc tag – (G4S)4-based linker – huUCHT1 – G4S-based linker – CD4)	Amino Acid
SEQ ID NO: 52	Scaffold 2 (Myc tag – (G4S)4-based linker – huUCHT1 (Y177T) – G4S-based linker – CD4)	Nucleotide
SEQ ID NO: 53	Scaffold 2 (Myc tag – (G4S)4-based linker – huUCHT1 (Y177T) – G4S-based linker – CD4)	Amino Acid

[0142] In some embodiments, the Claudin 18.2-TAC comprises (from N- to C-terminus): (a) a CD8a leader, (b) Claudin 18.2 Nanobody 1 (SEQ ID NO: 55), (c) a myc tag, (d) a (G4S)4-based linker, (e) a humanized variant of UCHT1, (d) a G4S-based linker, and (e) a CD4 transmembrane and cytosolic domain.

- 5 **[0143]** In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In
- 10 some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some
- 15 embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some
- 20 embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some
- 25 embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the

Claudin 18.2-TAC comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises the nucleotide sequence of SEQ ID NO: 66 (Nanobody 1/huUCHT1 Scaffold).

5 **[0144]** In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises
10 an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 90% sequence identity
15 with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 67
20 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the
25 Claudin 18.2-TAC comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises the amino acid sequence of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold).

[0145] In some embodiments, the Claudin 18.2-TAC comprises (from N- to C-terminus): (a) a
30 CD8a leader, (b) Claudin 18.2 Nanobody 1 (SEQ ID NO: 55), (c) a myc tag, (d) a (G4S)₄-based linker, (e) a humanized variant of UCHT1 comprising a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (huUCHT1 (Y177T)), (d) a G4S-based linker, and (e) a CD4 transmembrane and cytosolic domain.

[0146] In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises the nucleotide sequence of SEQ ID NO: 68 (Nanobody 1/huUCHT1 Y177T Scaffold).

[0147] In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises

a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises the nucleotide sequence of SEQ ID NO: 74 (Nanobody 1/huUCHT1 Y177T Scaffold).

[0148] In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC

comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises the amino acid sequence of SEQ ID NO: 69 (Nanobody 1/huUCHT1 Y177T Scaffold).

[0149] In some embodiments, the Claudin 18.2-TAC comprises from N- to C-terminus): (a) a CD8a leader, (b) Claudin 18.2 Nanobody 2 (SEQ ID NO: 56), (c) a myc tag, (d) a (G4S)₄-based linker, (e) a humanized variant of UCHT1, (d) a G4S-based linker, and (e) a CD4 transmembrane and cytosolic domain.

[0150] In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 70

(Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises the nucleotide sequence of SEQ ID NO: 70 (Nanobody 2/huUCHT1 Scaffold).

[0151] In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold). In some embodiments, the Claudin 18.2-TAC comprises the amino acid sequence of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold).

[0152] In some embodiments, the Claudin 18.2-TAC comprises (from N- to C-terminus): (a) a CD8a leader, (b) Claudin 18.2 Nanobody 2 (SEQ ID NO: 56), (c) a myc tag, (d) a (G4S)₄-based linker, (e) a humanized variant of UCHT1 comprising a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (huUCHT1 (Y177T)), (d) a G4S-based linker, and (e) a CD4 transmembrane and cytosolic domain.

[0153] In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises the nucleotide sequence of SEQ ID NO: 72 (Nanobody 2/huUCHT1 Y177T Scaffold).

[0154] In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 75% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 80% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 85% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 90% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 95% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 96% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 97% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 98% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises a nucleotide sequence having at least 99% sequence identity with the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the polynucleotide encoding the Claudin 18.2-TAC comprises the nucleotide sequence of SEQ ID NO: 75 (Nanobody 2/huUCHT1 Y177T Scaffold).

[0155] In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 70% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 75% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 80% sequence identity with the

amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 85% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 95% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 96% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 97% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises an amino acid sequence having at least 99% sequence identity with the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold). In some embodiments, the Claudin 18.2-TAC comprises the amino acid sequence of SEQ ID NO: 73 (Nanobody 2/huUCHT1 Y177T Scaffold).

[0156] Amino acid and nucleotide sequences of exemplary Claudin 18.2-TACs are provided in Table 6

Table 6. Table of Sequences

SEQ ID NO	Description	Nucleotide/Amino Acid
SEQ ID NO: 66	Nanobody 1/huUCHT1 Scaffold (huCD8a leader – Claudin 18.2 Nanobody 1 – Myc tag – (G4S)4-based linker – huUCHT1 – G4S-based linker – CD4)	Nucleotide
SEQ ID NO: 67	Nanobody 1/huUCHT1 Scaffold (huCD8a leader – Claudin 18.2 Nanobody 1 – Myc tag – (G4S)4-based linker – huUCHT1 – G4S-based linker – CD4)	Amino Acid
SEQ ID NO: 68	Nanobody 1/huUCHT1 Y177T Scaffold (huCD8a leader – Claudin 18.2 Nanobody 1 – Myc tag – (G4S)4-based linker – huUCHT1 (Y177T) – G4S-based linker – CD4)	Nucleotide
SEQ ID NO: 69	Nanobody 1/huUCHT1 Y177T Scaffold (huCD8a leader – Claudin 18.2 Nanobody 1 – Myc tag – (G4S)4-based linker –	Amino Acid

	huUCHT1 (Y177T) – G4S-based linker – CD4)	
SEQ ID NO: 70	Nanobody 2/huUCHT1 Scaffold (huCD8a leader – Claudin 18.2 Nanobody 2 – Myc tag – (G4S)4-based linker – huUCHT1 – G4S-based linker – CD4)	Nucleotide
SEQ ID NO: 71	Nanobody 2/huUCHT1 Scaffold (huCD8a leader – Claudin 18.2 Nanobody 2 – Myc tag – (G4S)4-based linker – huUCHT1 – G4S-based linker – CD4)	Amino Acid
SEQ ID NO: 72	Nanobody 2/huUCHT1 Y177T Scaffold (huCD8a leader – Claudin 18.2 Nanobody 2 – Myc tag – (G4S)4-based linker – huUCHT1 (Y177T) – G4S-based linker – CD4)	Nucleotide
SEQ ID NO: 73	Nanobody 2/huUCHT1 Y177T Scaffold (huCD8a leader – Claudin 18.2 Nanobody 2 – Myc tag – (G4S)4-based linker – huUCHT1 (Y177T) – G4S-based linker – CD4)	Amino Acid
SEQ ID NO: 74	Nanobody 1/huUCHT1 Y177T Scaffold (huCD8a leader – Claudin 18.2 Nanobody 1 – Myc tag – (G4S)4-based linker – huUCHT1 (Y177T) – G4S-based linker – CD4)	Nucleotide
SEQ ID NO: 75	Nanobody 2/huUCHT1 Y177T Scaffold (huCD8a leader – Claudin 18.2 Nanobody 2 – Myc tag – (G4S)4-based linker – huUCHT1 (Y177T) – G4S-based linker – CD4)	Nucleotide

Vector Constructs

[0157] Disclosed herein, in certain embodiments, are vectors comprising a Claudin 18.2-TAC nucleic acid sequence as disclosed herein. In some embodiments, the vectors further comprise a promoter. In some embodiments, the promoter is functional in a mammalian cell. Promoters, regions of DNA that initiate transcription of a particular nucleic acid sequence, are well known in the art. A “promoter functional in a mammalian cell” refers to a promoter that drives expression of the associated nucleic acid sequence in a mammalian cell. A promoter that drives expression of a nucleic acid sequence is referred to as being “operably connected” to the nucleic acid sequence.

[0158] A variety of delivery vectors and expression vehicles are employed to introduce nucleic acids described herein into a cell.

[0159] Disclosed herein, in certain embodiments, are vectors comprising:

- a. a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2;

- b. a second polynucleotide encoding an antigen-binding domain that binds a protein associated with a TCR complex;
- c. a third polynucleotide encoding a T cell receptor signaling domain polypeptide; and
- d. a promoter that is functional in a mammalian cell.

5 **[0160]** In some embodiments, the first polynucleotide and third polynucleotide are fused to the second polynucleotide and the coding sequence is operably connected to the promoter. In some embodiments, the second polynucleotide and third polynucleotide are fused to the first polynucleotide and the coding sequence is operably connected to the promoter. In some
10 embodiments, the vector is designed for expression in mammalian cells. In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is a retroviral vector.

[0161] In some embodiments, vectors that are useful comprise vectors derived from retroviruses, lentiviruses, Murine Stem Cell Viruses (MSCV), pox viruses, adenoviruses, and adeno-associated viruses. Other delivery vectors that are useful comprise vectors derived from herpes simplex viruses, transposons, vaccinia viruses, human papilloma virus, Simian
15 immunodeficiency viruses, HTLV, human foamy virus and variants thereof. Further vectors that are useful comprise vectors derived from spumaviruses, mammalian type B retroviruses, mammalian type C retroviruses, avian type C retroviruses, mammalian type D retroviruses and HTLV/BLV type retroviruses. Examples of lentiviral vectors useful in the disclosed compositions and methods are a pCCL4 vector, a pLVX vector (e.g., pLVX-IRES-ZsGreen1
20 Vector, Cat. No. 632187, Takara Bio) and a pCDH vector (e.g., pCDH-CMV-MCS cDNA Single Promoter Cloning and Expression Lentivector, Cat. No. CD500B-1, System Bio). One example of a γ -retroviral vector useful in the disclosed compositions and methods is a pRetroQ vector (e.g., pRetroQ-DsRed Monomer-N1 Vector, Cat. No. 632507, Takara Bio).

Pharmaceutical Compositions

25 **[0162]** Disclosed herein, in certain embodiments, are pharmaceutical compositions comprising an engineered T cell disclosed herein (transduced with and/or expressing a Claudin 18.2-TAC polypeptide), and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, but are not limited to, buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins;
30 polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); or preservatives. In some embodiments, the engineered T cells are formulated for intravenous administration.

[0163] Pharmaceutical compositions are administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration is determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages are determined by clinical trials. When "an immunologically effective amount," "an anti-tumor effective amount," "a tumor-inhibiting effective amount," or
5 "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered is determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject).

[0164] In some embodiments, the engineered T cells and/or pharmaceutical compositions described herein are administered at a dosage of 10^1 to 10^{15} cells per kg body weight, 10^4 to 10^9 cells per kg body weight, optionally 10^5 to 10^8 cells per kg body weight, 10^6 to 10^7 cells per kg body weight or 10^5 to 10^6 cells per kg body weight, including all integer values within those ranges. In some embodiments, the modified T cells and/or pharmaceutical compositions
15 described herein are administered at a dosage of greater than 10^1 cells per kg body weight. In some embodiments, the modified T cells and/or pharmaceutical compositions described herein are administered at a dosage of less than 10^{15} cells per kg body weight.

[0165] In some embodiments, the engineered T cells and/or pharmaceutical compositions described herein are administered at a dosage of 0.5×10^6 cells, 2×10^6 cells, 4×10^6 cells, 5×10^6 cells, 1.2×10^7 cells, 2×10^7 cells, 5×10^7 cells, 2×10^8 cells, 5×10^8 cells, 2×10^9 cells, 0.5 - 2000×10^6 cells, 0.5 - 2×10^6 cells, 0.5 - 2×10^7 cells, 0.5 - 2×10^8 cells, or 0.5 - 2×10^9 cells, including all integer values within those ranges.
20

[0166] Also disclosed herein are pharmaceutical compositions comprising engineered/modified and unmodified T cells, or comprising different populations of engineered/modified T cells with or without unmodified T cells. One of ordinary skill in the art would understand that a
25 therapeutic quantity of engineered/modified T cells need not be homogenous in nature.

[0167] In some embodiments, T cell compositions are administered multiple times at these dosages. In some embodiments, the dosage is administered a single time or multiple times, for example daily, weekly, biweekly, or monthly, hourly, or is administered upon recurrence, relapse or progression of the cancer being treated. The cells, in some embodiments, are
30 administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. of Med. 319:1676, 1988).

[0168] In some embodiments, the pharmaceutical composition is substantially free of, *e.g.*, there are no detectable levels of a contaminant, *e.g.*, selected from the group consisting of endotoxin, mycoplasma, replication competent lentivirus (RCL), p24, VSV-G nucleic acid, HIV gag, residual anti-CD3/anti-CD28 coated beads, mouse antibodies, pooled human serum, bovine serum albumin, bovine serum, culture media components, vector packaging cell or plasmid components, a bacterium a fungus, mycoplasma, IL-2, and IL-7.

[0169] In some embodiments, the modified/engineered T cells and/or pharmaceutical compositions are administered by methods including, but not limited to, aerosol inhalation, injection, infusion, ingestion, transfusion, implantation or transplantation. The modified T cells and/or pharmaceutical compositions may be administered to a subject transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, by intravenous (i.v.) infusion, or intraperitoneally. The modified/engineered T cells and/or pharmaceutical compositions thereof may be administered to a patient by intradermal or subcutaneous injection. The modified/engineered T cells and/or pharmaceutical compositions thereof may be administered by i.v. injection. The modified/engineered T cells and/or pharmaceutical compositions thereof may be injected directly into a tumor, lymph node, or site of infection.

[0170] A pharmaceutical composition may be prepared by known methods for the preparation of pharmaceutically acceptable compositions that are administered to subjects, such that an effective quantity of the T cells is combined in a mixture with a pharmaceutically acceptable carrier. Suitable carriers are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing Company, Easton, Pa., USA, 2000). On this basis, the compositions may include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable carriers or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

[0171] Suitable pharmaceutically acceptable carriers include essentially chemically inert and nontoxic compositions that do not interfere with the effectiveness of the biological activity of the pharmaceutical composition. Examples of suitable pharmaceutical carriers include, but are not limited to, water, saline solutions, glycerol solutions, N-(1(2,3-dioleoyloxy)propyl)N,N,N-trimethylammonium chloride (DOTMA), diolesylphosphatidyl-ethanolamine (DOPE), and liposomes. In some embodiments, such compositions contain a therapeutically effective amount of the compound, together with a suitable amount of carrier so as to provide the form for direct administration to the patient.

[0172] Pharmaceutical compositions include, without limitation, lyophilized powders or aqueous or non-aqueous sterile injectable solutions or suspensions, which may further contain antioxidants, buffers, bacteriostats and solutes that render the compositions substantially compatible with the tissues or the blood of an intended recipient. Other components that may be present in such compositions include water, surfactants (such as Tween), alcohols, polyols, glycerin and vegetable oils, for example. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, tablets, or concentrated solutions or suspensions.

[0173] A pharmaceutical composition disclosed herein may be formulated into a variety of forms and administered by a number of different means. A pharmaceutical formulation may be administered orally, rectally, or parenterally, in formulations containing conventionally acceptable carriers, adjuvants, and vehicles as desired. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection and infusion techniques. Administration includes injection or infusion, including intra-arterial, intracardiac, intracerebroventricular, intradermal, intraduodenal, intramedullary, intramuscular, intraosseous, intraperitoneal, intrathecal, intravascular, intravenous, intravitreal, epidural and subcutaneous), inhalational, transdermal, transmucosal, sublingual, buccal and topical (including epicutaneous, dermal, enema, eye drops, ear drops, intranasal, vaginal) administration. In some exemplary embodiments, a route of administration is via an injection such as an intramuscular, intravenous, subcutaneous, or intraperitoneal injection.

[0174] Liquid formulations include an oral formulation, an intravenous formulation, an intranasal formulation, an ocular formulation, an otic formulation, an aerosol, and the like. In certain embodiments, a combination of various formulations is administered. In certain embodiments a composition is formulated for an extended release profile.

Methods of Treatment and Use

[0175] Disclosed herein, in certain embodiments, are methods of using engineered T cells disclosed herein in the treatment of a Claudin 18.2-expressing cancer in an individual in need thereof.

[0176] In some embodiments, an antigen-binding domain that binds Claudin 18.2 of a TAC polypeptide disclosed herein binds to Claudin 18.2 on a tumor cell. In some embodiments, an antigen-binding domain that binds Claudin 18.2 of a TAC polypeptide disclosed herein selectively binds to Claudin 18.2 on a tumor cell. In some embodiments, an antigen-binding

domain that binds Claudin 18.2 of a TAC polypeptide disclosed herein specifically binds to Claudin 18.2 on a tumor cell.

[0177] Disclosed herein, in certain embodiments, are methods of treating a cancer expressing Claudin 18.2 in an individual in need thereof, comprising administering to the individual an engineered T cell disclosed herein or a pharmaceutical composition comprising an engineered T cell disclosed herein.

[0178] Further disclosed herein is use of an engineered T cell disclosed herein in the preparation of a medicament to treat cancer expressing Claudin 18.2 in an individual in need thereof.

Additionally disclosed herein in certain embodiments is the use of an engineered T cell disclosed herein or a pharmaceutical composition disclosed herein to treat a cancer expressing Claudin 18.2 in an individual in need thereof.

[0179] In some embodiments, the engineered T cells disclosed herein are part of a combination therapy. In some embodiments, effectiveness of a therapy disclosed herein is assessed multiple times. In some embodiments, patients are stratified based on a response to a treatment disclosed herein. In some embodiments, an effectiveness of treatment determines entrance into a trial.

[0180] In some embodiments, the engineered T cells disclosed herein are administered in combination with a lymphodepleting therapy, or are administered to a subject who has received a lymphodepleting therapy. Examples of lymphodepleting therapies include nonmyeloablative lymphodepleting chemotherapy, myeloablative lymphodepleting chemotherapy, fludarabine, cyclophosphamide, corticosteroids, alemtuzumab, total body irradiation (TBI), and any combination thereof.

[0181] Cancers that may be treated with engineered T cells disclosed herein include any form of neoplastic disease. In some embodiments, cancers that are treated include, but are not limited to, a pancreatic cancer (e.g., pancreatic adenocarcinoma, a gastric cancer (e.g., gastric adenocarcinoma), a gastroesophageal cancer (e.g., gastroesophageal junction (GEJ) adenocarcinoma), an esophageal cancer, an ovarian cancer, or a lung cancer (e.g., non-small cell lung cancer).

EXAMPLES

[0182] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1 – Manufacturing of Claudin 18.2-TAC T cells

[0183] This example was designed to test the manufacturability of nanobody-based Claudin 18.2-TACs. Previous versions of TACs have used either scFv or DARPIn antigen-binding domains.

[0184] T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73).

[0185] In one experiment, two of the Claudin 18.2-TACs (SEQ ID NOs: 67 or 71) were expressed by a lentiviral vector, which co-expressed mStrawberry red fluorescence protein as a transduction marker. Results are shown in **Figure 1** and depict surface expression of the Claudin 18.2-TACs. Non-transduced control cells (NTD) were used as a negative control.

[0186] In another experiment, Claudin 18.2-TAC T cells were produced using a commercial pilot grade lentivector and. Claudin 18.2-TAC T cells were produced from CD4/CD8+ selected peripheral blood lymphocytes which were activated with CD3/CD28/CD2 and IL-2 and IL-7, followed by pilot grade lentiviral transduction.

[0187] During the transduction and growth period, viable cells were counted at days 4, 7 and 9. The number of cells (**Figure 2A**), cell doublings (**Figure 2B**), and viability (**Figure 2C**) are shown. Claudin 18.2-TAC Nanobody 1 (SEQ ID NO:67) (open diamonds) Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) (closed diamonds), Claudin 18.2-TAC Nanobody 2 (SEQ ID NO:71) (open triangles), and Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:73) (closed triangles) T cells are shown. T cells engineered with Nanobody 1 and 2 in the context of the Claudin 18.2-TAC huUCHT1 Y177T scaffold (SEQ ID NOs: 69 and 73, respectively) showed the preferred manufacturing properties, showing higher levels of viability and cell expansion relative to T cells engineered with the Nanobody 1 and 2 in the context of the Claudin 18.2-TAC scaffold (SEQ ID NOs: 67 and 71, respectively).

[0188] In another experiment, Claudin 18.2-TAC T cells were produced using a research grade lentivector. CLDN18.2-TAC T cells were produced from CD4/CD8+ selected peripheral blood lymphocytes which were activated overnight with CD3/CD28/CD2 and IL-2 and IL-7, followed by research grade lentiviral transduction at MOI 10 in G-Rex well plates for 8 days.

5 [0189] T cells were produced from 3 separate donors. After 8 days, all T cells were collected and stained for surface TAC expression. Histograms showing the TAC expression are overlaid with each of the T cell constructs per donor. Median fluorescent intensity (MFI) values for the Claudin 18.2-TAC constructs are shown alongside their respective histograms (**Figure 3**). T cells engineered with Nanobody 1 and 2 in the context of the huUCHT1 Y177T scaffold (SEQ ID NOs: 69 and 73, respectively) showed higher TAC surface expression compared to T cells engineered with Nanobody 1 and 2 in the context of the huUCHT1 scaffold (SEQ ID NOs: 67 and 71, respectively). This effect was consistent across all three donors tested.

10 [0190] In another experiment, the TAC surface expression of T cells engineered with either Claudin 18.2 T cell-antigen coupler (TAC) (SEQ ID NOs: 69 and 73) or its next generation scaffold (huUCHT1 Y177T) (SEQ ID NOs: 67 and 71), including Claudin 18.2 Nanobody 1 (SEQ ID NOs: 67 and 69) or Nanobody 2 (SEQ ID NOs: 71 and 73) produced using either of the above-described manufacturing methods were compared (**Figure 4**). The Claudin 18.2-TAC MFI was calculated as a fraction of a relevant positive control TAC. The relative MFI values demonstrated that the Y177T scaffold of both Nanobody 1 and 2 showed enhanced surface expression, with Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) T cells significantly showing the highest TAC surface expression.

20 [0191] These results show that T cells can be engineered to express any of the four tested Claudin 18.2-TACs. Furthermore, these results show for the first time that TACs comprising a nanobody-based antigen-binding domain can be effectively manufactured.

25 **Example 2 – Specificity of Claudin 18.2-TAC T cells for Claudin 18.2 relative to Claudin 18.1**

30 [0192] The binding specificity of the Claudin 18.2 binder (Claudin Nanobody 2, SEQ ID NO: 56) used in exemplary Claudin 18.2-TACs was assessed using a recombinant protein comprising the Claudin 18.2 binding domain fused to GFP. The purified protein was then tested for its ability to bind to Claudin 18.2-expressing human HEK or avian QT6 cells. As shown in **Figure 5A**, the Claudin 18.2-binding domain of SEQ ID NO:56 was able to bind the Claudin 18.2-engineered target cells but not to non-engineered control HEK and QT6 cells. The fusion

protein showed concentration dependent binding to Claudin 18.2-engineered cells but not vector only control cells.

[0193] Claudin 18.2 Nanobody 2 (SEQ ID NO: 56) was further tested for potential binding against other proteins using a protein array encompassing over 6000 human membrane proteins, collectively representing ~94% of membrane-anchored human proteins. The Claudin 18.2 binder-GFP fusion protein bound Claudin 18.2 and no other surface protein. This demonstrates that the binding domain (SEQ ID NO: 56) used in Claudin 18.2-TAC is specific for Claudin 18.2 (**Figure 5B**).

[0194] To further assess the specificity of the Claudin 18.2 Nanobody 2 when used in the TAC molecule expressed by T cells, Claudin 18.2 T cell-antigen coupler (TAC) T cells were engineered as described in Example 1 using a Claudin 18.2-TAC including Nanobody 2/huUCHT1 Y177T Scaffold (SEQ ID NO: 73) and assayed for specificity for Claudin 18.2 positive target cells. Specificity was also evaluated in target cells expressing Claudin 18.1, a close homolog of Claudin 18.2 and result of alternative splicing of the Claudin 18 transcript.

[0195] In another experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73).

[0196] N87 gastric cancer cells engineered to overexpress either Claudin 18.1 or 18.2 T cells expressing the Claudin 18.2-TAC. The expression of both Claudin 18.2 and Claudin 18.1 was tested via flow cytometry, and the respective histograms are shown (**Figure 6A**). To assess the activity of T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73, T cells were co-cultured at a 1:1 ratio with N87-CLDN18.1 and N87-CLDN18.2 target cells. Following the 4 hour co-culture, T cells were harvested and analyzed by flow cytometry for the expression of CD69 in T cells expressing Claudin 18.2-TAC (SEQ ID NO: 73) (identified by fluorescent protein transduction marker).

[0197] As shown in **Figure 6B**, T cells expressing Claudin 18.2-TAC (SEQ ID NO: 73) were successfully activated when co-cultured with Claudin 18.2 but not Claudin 18.1 positive target

cells, indicating that Claudin 18.2-TAC T cells do not cross-react with Claudin 18.1 expressing cells. Neither T cells alone nor NTD negative controls lead to CD69 up regulation (data not shown). When stimulated with Claudin 18.2-expressing tumor cells, non-transduced T cells in the Claudin 18.2-TAC T cell product also show upregulation of CD69.

[0198] Figure 6C shows the combined results of Claudin 18.2-TAC Nanobody 1/huUCHT1 (SEQ ID NO: 67) (open diamonds), Claudin 18.2-TAC Nanobody 1/huUCHT1 Y177T (SEQ ID NO: 69) (closed diamonds), Claudin 18.2-TAC Nanobody 2/huUCHT1 (SEQ ID NO: 71) (open triangles), and Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO: 73) (closed triangles) in co-culture with either Claudin 18.1 or 18.2-engineered N87 cells. Since N87 cells naturally express the HER2 oncoprotein, CD69 upregulation was normalized to CD69 expression observed with the HER2-TAC T cell positive control co-cultured with N87 cells. Data represents the average of 3 different experiments with TAC-T cells derived from three different donors. Positive control HER2-TAC T cells are shown as closed grey circles, while NTD (non-transduced) negative control T cells are shown as closed black circles. T cells expressing Claudin 18.2-TAC were successfully activated when co-cultured with Claudin 18.2 but not Claudin 18.1-positive target cells or non-engineered N87 tumor cells, indicating that Claudin 18.2-TAC T cells do not cross-react with Claudin-18.1-expressing cells. NTD negative controls do not show upregulation of CD69. When stimulated with Claudin 18.2-expressing tumor cells, non-transduced T cells in the Claudin 18.2-TAC T cell product also show upregulation of CD69.

Example 3 – Cytokine release by Claudin 18.2-TAC T cells following antigen-specific activation

[0199] Claudin 18.2 T cell-antigen coupler (TAC) T cells are engineered as described in Example 1 and assayed for *in vitro* activity.

[0200] The Claudin 18.2-TAC T cells are assayed for pro-inflammatory cytokine release following co-culture with Claudin 18.2 and Claudin 18.1 positive target cells. T cells and target cells are co-cultured for 4-24 hours. It is expected that pro-inflammatory cytokines, such as TNF α and INF γ , are detected following co-culture of Claudin 18.2-TAC T cells with Claudin 18.2 positive target cells, but not with Claudin 18.1 positive target cells.

[0201] The Claudin 18.2-TAC T cells are also assayed for *in vitro* proliferation following co-culture with Claudin 18.2 and Claudin 18.1 positive target cells. Claudin 18.2-TAC cells are labeled with a fluorescent marker and then co-cultured with mitomycin C treated target cells. After several days of culture, T cell proliferation is analyzed. It is expected that Claudin 18.2-

TAC T cells only show proliferation when cultured with Claudin 18.2 positive target cells, and not with Claudin 18.1 positive target cells.

Example 4 – *In vitro* cytotoxicity of Claudin 18.2-TAC T cells

[0202] T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) a UCHT1 antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67) or Nanobody 2/huUCHT1 Y177T Scaffold (SEQ ID NO: 71). KATO III cells, naturally expressing Claudin 18.2, were engineered to express enhanced Luciferase (eLuc) to yield a KATO III^{eLuc} cell line. T cells expressing either Claudin 18.2-TAC (SEQ ID NO: 67 or 71) were co-cultured with KATO III^{eLuc} for 30 hours. At the end of the co-culture the viability of the KATO III^{eLuc} cells was assessed by measuring luminescence relative to an untreated control. Results are shown in **Figure 7**. As depicted, Claudin 18.2-TAC T cells engineered to express either the Claudin 18.2-TAC of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold); or the Claudin 18.2-TAC of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold), but not non-transduced control T cells (NTD), were able to efficiently engage and kill KATO III^{eLuc} cells.

Example 5 – *In vitro* cytotoxicity of Claudin 18.2-TAC T cells

[0203] T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) a UCHT1 antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67) or Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71). NALM6 cells were engineered to express eGFP (NALM6^{eGFP}) or eGFP and Claudin 18.2 (NALM6^{CLDN18.2/eGFP}). T cells expressing either Claudin 18.2-TAC (SEQ ID NO: 67 or 71) were co-cultured with NALM6^{CLDN18.2/eGFP} or NALM6^{eGFP} cells. Non-transduced (NTD) T cells were used as negative control. Since NALM6 tumor cells naturally express the CD19 antigen, CD19-TAC T cells were used as a positive control. The cell culture was monitored using a Cytation instrument. Cells were cultured for 5 days, and each well was imaged every 6 hours. All experiments were performed in triplicate. A schematic of the experiment is depicted in **Figure 8**.

[0204] **Figure 9** depicts images of co-cultures at different E:T ratios after 5 days of co-culture. T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 71, when co-cultured with NALM6^{eGFP} cells, did not control tumor cell growth. However, when T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 71 were co-cultured with NALM6^{CLDN18.2/eGFP} cells, tumor cell growth was controlled at or above an E:T ratio of 1:20 (500 Claudin 18.2-TAC T cells:10,000 target cells). By comparison, CD19-TAC T cells, which are known to be cytotoxic towards NALM6 cells, controlled tumor cell growth at or above an E:T ratio of 1:5 (2000 CD19-TAC T cells:10,000 target cells).

[0205] **Figure 10** depicts a quantitative analysis of tumor cell growth as a function of eGFP area. Tumor cells alone grew exponentially. NTD T cells, used at a 1:1 E:T ratio, caused a brief delay in NALM6^{eGFP} /NALM6^{CLDN18.2/eGFP} growth, but ultimately but did not control tumor cell growth. T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 67 or 71 did not control NALM6^{eGFP} cell growth. However, when T cells engineered to express either the Claudin 18.2-TAC of SEQ ID NO: 67 (Nanobody 1/huUCHT Scaffold) or the TAC of SEQ ID NO:71 (Nanobody 2/huUCHT1 Scaffold TAC) were co-cultured with NALM6^{CLDN18.2/eGFP} cells, there was significant control of tumor cell growth that was superior to that of CD19-TAC T cells.

[0206] **Figure 11** depicts an area under the curve (AUC) analysis of the cytotoxicity results shown in **Figure 10**. AUC was plotted against E:T ratio. The time course of target cells alone was used to normalize all data to 100%. When the TAC of SEQ ID NO: 67 (Nanobody 1/huUCHT1 Scaffold); or the TAC of SEQ ID NO: 71 (Nanobody 2/huUCHT1 Scaffold) were co-cultured with NALM6^{eGFP}, a limited effect on tumor cell growth was observed at high E:T ratios, comparable to the NTD control. However, in cultures with NALM6^{CLDN18.2/eGFP} both Claudin 18.2-TAC constructs showed significant tumor control with roughly equal efficiency, which was superior to that of CD19-TAC T cells

Example 6 – *In vitro* cytokine production by Claudin 18.2-TAC T cells

[0207] T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) a UCHT1 antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67) or Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71). NALM6 cells were engineered to express Claudin 18.2 (NALM6^{CLDN18.2}). T cells expressing either Claudin 18.2-TAC (SEQ ID NO: 67 or 71) were co-

cultured with NALM6^{CLDN18.2} cells, NALM6 cells, or no target cells. Non-transduced (NTD) T cells were used as negative control. CD19-TAC T cells were used as a positive control. Cells were stained for IFN γ , TNF α , and IL2, and analyzed by flow cytometry. Results are shown in **Figure 12**. Both Claudin 18.2-TAC T cell variants showed cytokine production in the presence but not in the absence of Claudin 18.2-expressing target cells, with qualitatively superior cytokine production observed in the presence of the Claudin 18.2-TAC of SEQ ID NO: 71 compared to the Claudin 18.2-TAC of SEQ ID NO: 67. Activation of Claudin 18.2-TAC T cell variants was similar or superior to that of CD19-TAC T cells. There was no indication of auto-activation.

Example 7 – Phenotypic Characterization of Claudin 18.2-TAC T cells

[0208] T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) a UCHT1 antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 2/huUCHT1 Y177T Scaffold (SEQ ID NO: 73). NALM6 cells were engineered to express eGFP (NALM6^{eGFP}) or eGFP and Claudin 18.2 (NALM6^{CLDN18.2/eGFP}). T cells expressing the Claudin 18.2-TAC or non-transduced (NTD) control cells were co-cultured with NALM6^{eGFP} or NALM6^{CLDN18.2/eGFP} cells and analyzed for the 16 markers depicted in **Figure 13**. These markers allow an assessment of T cell activation, exhaustion and memory phenotype. Cells were analyzed and clustered using the UMAP algorithm. Results are shown in **Figure 14**. Expression of CD8 and CD69 is shown, with shades indicating the relative abundance of the illustrated markers. The data shows similar patterns of expression of all surface markers analyzed on Claudin 18.2-TAC T cell products and NTD cells cultured with NALM6 cells. However, when cultured with Claudin 18.2-expressing NALM6 cells, Claudin 18.2-TAC T cell products (but not NTD cells) become activated as indicated by upregulation of CD69. Claudin 18.2-TAC T cell products retain similar memory phenotypes to the NTD controls even post activation (data not shown). Together, this data demonstrates that Claudin 18.2-TAC T cell products do not exhibit tonic signaling and are activated only in the presence of the Claudin 18.2 antigen.

Example 8 – *In vitro* activity of Claudin 18.2-TAC T cells

[0209] In one experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) a UCHT1 antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain:

Nanobody 2/huUCHT1 Y177T Scaffold (SEQ ID NO: 73). T cells expressing the Claudin 18.2-TAC (SEQ ID NO: 73) were co-cultured at a 1:1 ratio with a variety of tumor cell lines naturally expressing Claudin 18.2 (OE19, NUGC-4 [gastric adenocarcinoma], KATO III [gastric carcinoma], DAN-G [pancreatic adenocarcinoma]). Following the 4 hour co-culture, Claudin 18.2-TAC-expressing T cells (identified by fluorescent protein transduction marker) were harvested and analyzed for CD69 surface expression (early activation marker) by flow cytometry. As shown in **Figure 15**, T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73 were successfully activated when co-cultured by all Claudin 18.2 positive target cells but not with Claudin 18.2 negative control cells (data not shown). Neither NTD negative controls (data not shown) nor T cells alone showed activation. When stimulated with Claudin 18.2-expressing tumor cells, non-transduced T cells in the Claudin 18.2-TAC (SEQ ID NO: 73) T cell product also show upregulation of CD69.

[0210] In another experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). Claudin 18.2-TAC T cells were co-cultured at E:T ratios 1:1, 1:2.5, 1:5, 1:10, 1:20, 1:50 and 1:100 with 1×10^4 NALM6^{CLDN18.2GFPeLuc} target cells/well in a cell imaging reader. Photos were captured every 8 hours for 5 days. The area of GFP-expressing cells is calculated for each time point and E:T ratio. From these values, the area under the curve (AUC) for each of the Claudin 18.2-TAC T cells was calculated and plotted, representing target cell killing at each E:T ratio.

[0211] **Figure 16A** shows exemplary results of Claudin 18.2-TAC Nanobody 1/huUCHT1 (SEQ ID NO:67) (open diamonds), Claudin 18.2-TAC NA3SH1/huUCHT1 Y177T (SEQ ID NO:69) (closed diamonds), Claudin 18.2-TAC Nanobody 2/huUCHT1 (SEQ ID NO:71) (open triangles), and Claudin 18.2-TAC Nanobody 2/huUCHT1 Y177T (SEQ ID NO:73) (closed triangles) in co-culture with NALM6^{CLDN18.2eGFP}. Data shown demonstrate the different levels of target cell killing dependent on the used E:T ratios. NTD and target cells alone are shown as negative controls. The IC₅₀ was calculated from individual AUC curves derived from the averaged values of 2 different cytotoxicity assays with product manufactured from two different

donors. IC₅₀ value represents the E:T ratio at which 50% of target killing is observed after a 5-day co-culture (**Figure 16B**). The graph shows that the TAC constructs containing Nanobody 1 (SEQ ID NO:67) have a higher level of cytotoxicity compared to Nanobody 2 (hVH6) variants.

5 **[0212]** In another experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding
10 domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). Proliferation of T cells engineered with Claudin 18.2-TAC variants, co-cultured in a 1:3 E:T ratio for 4 days with either OE19, NALM6^{CLDN18.2} or NALM6 target cell lines, was
15 evaluated. NALM6^{CLDN18.2} is a leukemic cell line that was engineered to overexpress Claudin 18.2, and OE19 is a gastroesophageal cancer cell line naturally expressing Claudin 18.2. The parental NALM6 cell line lacks any Claudin 18.2 expression and was used as a negative control. Target cells were preincubated with mitomycin, whereas T cells were loaded with cell tracing (CTV) dye prior to co-culture. After 4 days, T cells were analyzed by flow cytometry for CTV
20 staining, which can track T cell divisions. Representative histogram flow plots showing CTV dye dilution in Claudin 18.2-TAC Nanobody 2 (SEQ ID NO:71) and Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:73) T cells after co-culture with the various target cells (**Figure 17A**). The data show that both Claudin 18.2-TAC T cell cultures proliferate upon co-culture with Claudin 18.2 target cell lines, OE19 and NALM6^{CLDN18.2}, but not the Claudin 18.2
25 negative NALM6 cell line. The division index (DI), a measure of proliferation, from all Claudin 18.2-TAC T cells was averaged across 3 separate donors, and normalized to their respective positive controls (*i.e.*, HER2-TAC T for OE19 and CD19-TAC T for NALM6^{CLDN18.2} and NALM6) (**Figure 17B**). When co-cultured with OE19, T cells engineered with the TAC huUCHT1 Y177T scaffold comprising either Nanobody 1 or 2 showed enhanced proliferation
30 when compared to T cells engineered with the TAC scaffold of either Nanobody 1 or 2. When co-cultured with NALM6^{CLDN18.2}, T cells engineered with the huUCHT1 Y177T scaffold of either Nanobody 1 or 2 showed similar proliferation when compared to T cells engineered with the TAC scaffold of either Nanobody 1 or 2. No proliferation was observed against Claudin 18.2 negative control cells.

[0213] In another example, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). T cell activation was measured as a function of the upregulation of the early T cell activation marker CD69. T cells engineered with Claudin 18.2-TAC variants were co-cultured at a 1:1 ratio with a variety of tumor cell lines naturally expressing Claudin 18.2, such as OE19 (gastroesophageal carcinoma), NUGC-4 and KATO III (both gastric adenocarcinoma), and DAN-G (pancreatic adenocarcinoma). In addition, a co-culture with NALM6 cells engineered to overexpress Claudin 18.2 was used. Following a 4-hour co-culture, Claudin 18.2-TAC-expressing T cells were harvested and analyzed for CD69 surface expression by flow cytometry. **Figure 18A** shows representative flow plots of T cells engineered with Claudin 18.2-TAC Nanobody 2/huUCHT1 Y177T (SEQ ID NO:73). CD69 expression is shown relative to the fluorescent protein transduction marker. T cell activation is evident for each Claudin 18.2-TAC T cell product as shown by CD69 upregulation, which was also observed for a fraction of non-transduced T cells in Claudin 18.2-TAC T cell products. No CD69 expression was observed in Claudin 18.2-TAC T cell products cultured in the absence of target cells (T cells alone). **Figure 18B** shows the average of 3 independent co-culture experiments. The cultures included T cells engineered with Claudin 18.2 T cell-antigen coupler (TAC) or its next-generation scaffold (huUCHT1 Y177T) comprising Claudin 18.2 Nanobody 1 or Nanobody 2, derived from the manufacturing of 3 different donors. The resulting CD69 expression was normalized to the HER2-TAC (OE19, NUGC-4, KATO III, DAN-G) or CD19-TAC (NALM6) positive controls. T cells engineered with Claudin 18.2 T cell-antigen coupler (TAC) or its next generation scaffold (huUCHT1 Y177T) including Claudin 18.2 Nanobody 1 and 2 were activated by all Claudin 18.2 expressing cell lines, the engineered NALM6^{CLDN18.2} control, but not the NALM6 negative control. This data demonstrates that T cells engineered with Claudin 18.2-TAC are able to identify and react against a broad range of target cells, including tumor cells naturally expressing Claudin 18.2.

[0214] In another experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat

CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). The ability of the Claudin 18.2-TAC to retain sustained cytotoxicity was evaluated. The experimental schematic is shown in

Figure 19A. Claudin 18.2-TAC T cells were co-cultured with NALM6^{CLDN18.2/eGFP} or N87^{CLDN18.2/eGFP} cells at E:T ratios of 3:1 and 5:1 (total T cells: target cells) for 4 days. Target cell fluorescence was monitored using a Cytation instrument on D0 and D4. Each assay condition was set up in triplicates in a 48-well tissue culture dish. On D4, total cell counts were performed on the T cells which were carried forward into fresh co-cultures with new target cells while maintaining the same E:T ratios. Surplus T cells were subjected to flow cytometry analysis. Twelve successive 4-day rounds of co-culture were performed. Non-transduced (NTD) T cells were included in the first round as negative controls. Quantitative analysis of tumor cell growth as a function of eGFP integrated intensity is shown. The fluorescence signal captured on D4 of each round was normalized to D0 of that round (**Figure 19B**). Tumor cells alone grew out uncontrolled in each round as expected. NALM6^{CLDN18.2/eGFP} cells co-cultured with Claudin 18.2-TAC (Nanobody 2/Y177T) T cells were successfully cleared in each of the 12 rounds. Growth of N87^{CLDN18.2/eGFP} cells co-cultured with Claudin 18.2-TAC T cells was controlled in the first 5 rounds, with the degree of control lessening in later rounds. Asterisks indicate time points when some of the 48 wells needed to be combined to retain the desired E:T ratios.

Example 9 – *In vivo* activity of Claudin 18.2-TAC T cells

[0215] T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) a UCHT1 antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 2/huUCHT1 Y177T Scaffold (SEQ ID NO: 73).

[0216] In one experiment, NSG mice were inoculated with 5×10^5 NALM6^{CLDN18.2/eLuc} tumor cells. Four days after engraftment, mice were treated with a single intravenous dose of T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73. Non-treated (NT) mice and mice treated with non-transduced T cells (NTD) were used as negative controls. Mice were dosed with 4×10^6 TAC T cells or an equivalent number of NTD cells that matches the total T cell dose used for TAC T cells. Total luminescence was measured weekly.

[0217] The resulting total flux (photons/second) as the sum of the dorsal and ventral reads is shown in **Figure 20**. Complete tumor rejection was achieved in all animals treated with T cells

expressing the Claudin 18.2-TAC of SEQ ID NO: 73 during the 30 day study duration. Negative controls did not show any tumor control. No TAC associated toxicity was observed.

[0218] The amount of luminescence presented as total flux is shown in **Figure 21A**. Overall survival is shown in **Figure 21B**. Relative change in body weight, as a means to assess toxicity, is shown in **Figure 21C**. Non treated animals (NT) or mice administered non-transduced control T cells (NTD) showed no tumor control. All of these animals reached endpoint within the 30-day period and showed tumor induced weight drops as early as day 20 post tumor inoculation. In contrast, all animals treated with T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73 showed complete tumor control, survived the 30-day study period, and lacked signs of toxicity.

[0219] In another experiment, NSG mice were inoculated with 1×10^7 N87^{CLDN18.2} tumor cells (**Figure 22**, top panel). Fourteen days after engraftment, mice were treated with a single dose of either 3 or 6×10^6 T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73. The negative controls include non-treated (NT) mice or mice treated with non-transduced T cells (NTD). Tumor volume was measured bi-weekly, and the resulting tumor volume is shown in **Figure 22**, bottom panels. Complete tumor rejection was achieved in all animals treated with T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73. Neither NTD nor NT controls showed tumor regression.

[0220] In another experiment, mice were inoculated with 5×10^5 NALM6^{CLDN18.2/eLuc} tumor cells (**Figure 23**, top panel). Seven days after engraftment, mice were treated with a single dose of T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73. Non-treated (NT) mice are shown. At this dose level non-transduced T cells (NTD) were not included as negative control as they showed no response when given at a 4×10^6 dose (data not shown). Mice were dosed with 2×10^6 TAC T cells. Total luminescence was measured weekly, and the resulting total flux (photons/second) as the sum of the dorsal and ventral reads is shown in **Figure 23**, center-left panel. The luminescence images are shown (**Figure 23**, right panel). T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73 were able to induce complete tumor regression, while NTD controls had no impact (data not shown). Survival curves are shown, demonstrating the survival of mice treated with T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73 (**Figure 23**, bottom-left panel).

[0221] In another experiment, NSG mice were inoculated with 1×10^6 OE19 tumor cells which naturally expresses Claudin 18.2 (**Figure 24**, top panel). Nineteen days after engraftment of tumor cells, mice were treated with a single dose of either 1 or 2×10^6 T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73. The negative controls include non-treated (NT) mice or

mice treated with non-transduced T cells (NTD). Tumor volume was measured bi-weekly, and the resulting tumor volume is shown in **Figure 24**, center panels. Complete tumor rejection was achieved in all animals treated T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73, whereas control animals showed tumor outgrowth. Survival curves of treated and control mice are shown in **Figure 24**, bottom panels, demonstrating the survival mice treated with T cells expressing the Claudin 18.2-TAC of SEQ ID NO: 73.

[0222] In one example, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). NSG mice were inoculated with 1×10^7 N87^{CLDN18.2} tumor cells and then treated with CLDN18.2 TAC T cells. The experimental schematic is shown in **Figure 25A**. Fourteen days after engraftment, mice were treated with a single dose of either 3 or 6×10^6 T cells engineered with Claudin 18.2 T cell-antigen coupler (TAC) and its next-generation scaffold (huUCHT1 Y177T) comprising Claudin 18.2 Nanobody 1 or Nanobody 2. The negative controls include non-treated (NT) mice or mice treated with non-transduced T cells (NTD). HER2-TAC T cells were used as positive control. Tumor volume was measured bi-weekly. Tumor volumes of mice treated with Claudin 18.2-TAC Nanobody (SEQ ID NO: 67) (closed inverted triangle), Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO: 69) (open inverted triangle), Claudin 18.2-TAC Nanobody 2 (SEQ ID NO:71) (closed triangles), and Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO: 73) (open triangles) are shown (**Figure 25B**). Non-treated control mice are shown (closed square). As negative controls, mice treated with non-transduced (open circles) and, as positive controls, mice treated with HER2-TAC (closed circle) are shown. Complete tumor rejection was achieved in all animals treated with T cells expressing the Claudin 18.2-TAC receptors. Neither NTD nor NT controls showed tumor regression. The HER2-Tac positive control induced tumor regression similar to the Claudin 18.2-TAC variants.

[0223] In another experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and

SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). NSG mice were inoculated with 1×10^6 OE19 tumor cells subcutaneously and then treated with subtherapeutic doses of Claudin 18.2-TAC T cells. The experimental schematic is shown in **Figure 26A**. Nineteen days after engraftment, mice were treated with a single intravenous dose of either 1 or 2×10^6 T cells engineered with Claudin 18.2 T cell-antigen coupler (TAC) and its next-generation scaffold (huUCHT1 Y177T) comprising Claudin 18.2 Nanobody 1 and 2. Non-treated (NT) mice and mice treated with non-transduced T cells (NTD) were used as negative controls. As additional negative controls, mice were treated with 2×10^6 CD19-TAC T cells and, as positive controls, mice were treated with 6×10^6 HER2-TAC T cells. Tumor volume was measured biweekly. Tumor volume of mice treated with Claudin 18.2-TAC Nanobody 1 (SEQ ID NO:67) (closed inverted triangle), Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) (open inverted triangle), Claudin 18.2-TAC Nanobody 2 (SEQ ID NO:71) (closed triangles), and Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:71) (open triangles) are shown (**Figure 26B**). Control groups are shown. NTD are represented by open circles; NT are represented by closed black squares. Mice treated with CD19-TAC T cells are represented by grey closed circles, and those treated with HER2-TAC T cells are represented by small light grey closed circles. Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:71) T cells showed complete tumor clearance or significant tumor regression at both 1×10^6 and 2×10^6 doses. Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) showed complete tumor clearance at the 2×10^6 dose. With the 1×10^6 dose, Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) showed total tumor clearance or significant tumor regression in 60% of treated mice, and tumor control in the remaining 40%. At the 2×10^6 dose level Claudin 18.2-TAC Nanobody 2 (SEQ ID NO:71) showed tumor clearance in 40% of mice and some level of tumor control in the remaining 60%. At the 2×10^6 dose level Claudin 18.2-TAC Nanobody 1 (SEQ ID NO:67) showed complete tumor clearance or significant tumor regression in 40% mice, while the remaining 60% showed tumor outgrowth. At the 1×10^6 treatment dose level, Claudin 18.2-TAC Nanobody 1 (SEQ ID NO:67) treated mice failed to show any tumor control. Mice treated with the HER2-TAC T cell positive control achieved complete tumor clearance.

[0224] In another experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2

Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69),

5 Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). NSG mice were inoculated with 5×10^5 NALM6^{CLND18.2/eLuc} tumor cells intravenously and then treated with Claudin 18.2-TAC T cells. The experimental schematic is shown in **Figure 27A**. Seven days after engraftment, mice were treated with a single intravenous dose of 1 or 0.5 , 1 or 2×10^6 T cells engineered with Claudin 18.2 T cell-antigen coupler (TAC)

10 and its next-generation scaffold (TAC, Y177T) comprising Claudin 18.2 Nanobody 1 and 2. Non-treated (NT) mice and mice treated with non-transduced T cells (NTD) were used as negative controls. As negative controls, mice were treated with 2×10^6 HER2-TAC T cells and, as positive controls, mice were treated with 4×10^6 CD19-TAC T cells. Tumor growth was measured weekly by total luminescence. The resulting total flux (photons/second) as the sum of

15 the dorsal and ventral reads are shown. Mice were treated with Claudin 18.2-TAC Nanobody 1 (SEQ ID NO:67) (closed inverted triangle), Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) (open inverted triangle), Claudin 18.2-TAC Nanobody 2 (SEQ ID NO:71) (closed triangles), and Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:71) (open triangles) are shown (**Figure 27B**). NTD are represented by open circles; NT are represented by closed

20 squares. Mice treated with CD19-TAC T cells are represented by grey closed circles, and animals treated with HER2-TAC T cells are represented by closed hexagon. At the 2×10^6 and 1×10^6 dose levels, both Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) and Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:71) T treated groups showed complete tumor clearance, whereas Claudin 18.2-TAC Nanobody 1 (SEQ ID NO:67) and Claudin 18.2-TAC

25 Nanobody 2 (SEQ ID NO:71) T cells showed tumor outgrowth. At the 0.5×10^6 dose level, Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) treated mice showed complete tumor clearance or significant tumor regression. Claudin 18.2-TAC Nanobody 1/Y177T (SEQ ID NO:69) treated animals showed some tumor regression and control, but ultimately tumors did grow out. In Claudin 18.2-TAC Nanobody 1 (SEQ ID NO:67) and Claudin 18.2-TAC Nanobody

30 2 (SEQ ID NO:71) treated animals tumors grew out. Animals treated with CD19-TAC T cells as positive control showed complete tumor clearance. Neither NTD, NT nor HER2-TAC T negative controls showed tumor any regression.

[0225] In another experiment, T cells were engineered to express a Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 1 (including SEQ ID NO: 57 (Kabat

CDR1), SEQ ID NO: 58 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), or Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 or huUCHT1 (Y177T) antigen-binding domain that binds CD3, and (iii) a CD4 cytosolic and transmembrane domain: Nanobody 1/huUCHT1 Scaffold (SEQ ID NO: 67), Nanobody 1/huUCHT1 (Y177T) (SEQ ID NO:69), Nanobody 2/huUCHT1 Scaffold (SEQ ID NO: 71), or Nanobody 2/huUCHT1 (Y177T) (SEQ ID NO: 73). NSG-MHC I/II double knock-out (DKO) mutant mice were inoculated with 1×10^7 NCI-N87^{CLDN18.2} tumor cells subcutaneously and then treated with Claudin 18.2-TAC T cells (Figure 14). NSG-MHC I/II DKO were chosen as they lack MHC I/II and, thus, are less likely to cause any T cell driven xenoreactivity against mouse tissue which can result in GvHD and make persistence data challenging to interpret. The experimental schematic is shown in **Figure 28A**. Ten to twenty-one days after engraftment, mice were treated with a single intravenous dose of 6×10^6 Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:71) T cells. Non-treated (NT) mice and mice treated with non-transduced T cells (NTD) were used as negative controls. Tumor volume was monitored biweekly. After 30 days post-ACT or about 14 days post initial tumor clearance, mice were re-challenged with 1×10^7 NCI-N87^{CLDN18.2} tumor cells injected subcutaneously in the opposite flank. Tumors were monitored biweekly. The resulting tumor volumes are shown in **Figure 28B**. Non-treated negative control mice challenged with either the first (group 1) or second (group 2) N87^{CLDN18.2} tumor challenge showed complete outgrowth in both cases. The NTD control group showed initial tumor outgrowth. Two out of 4 mice showed tumor regression 20 days post ACT. However, only one mouse ultimately cleared the tumor while the second mice showed tumor outgrowth. Upon rechallenge of these mice, only 1 of 4 mice was resistant to the N87^{CLDN18.2} re-challenge, in agreement with some level of alloreactivity. In contrast, mice treated with Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:71) T cells showed rapid and complete tumor clearance following ACT. Following the rechallenge, all mice remained protected and were able to clear all tumors. Thus, the experiment demonstrates that Claudin 18.2-TAC Nanobody 2/Y177T (SEQ ID NO:71)-expressing T cells can persist *in vivo*.

Example 10 – Comparison of *in vitro* activities of T cells expressing different Claudin 18.2-TACs

[0226] A first population of T cells is engineered to express a first Claudin 18.2 T cell-antigen coupler (TAC) including (i) Claudin 18.2 Nanobody 2 (including SEQ ID NO: 57 (Kabat CDR1), SEQ ID NO: 63 (Kabat CDR2), and SEQ ID NO: 59 (Kabat CDR3)), (ii) an huUCHT1 (Y177T) antigen-binding domain that binds CD3 (SEQ ID NO: 36), and (iii) a CD4 cytosolic

and transmembrane domain (SEQ ID NO: 12). A second population of T cells is engineered to express a second Claudin 18.2 T cell-antigen coupler (TAC) including (i) a scFv-based Claudin 18.2-binding domain, (ii) an huUCHT1 (Y177T) antigen-binding domain that binds CD3 (SEQ ID NO: 36), and (iii) a CD4 cytosolic and transmembrane domain (SEQ ID NO: 12).

- 5 **[0227]** Target cells that naturally express Claudin 18.2 are engineered to express enhanced Luciferase (eLuc).

- 10 **[0228]** T cells expressing the first Claudin 18.2-TAC are compared to those expressing the second Claudin 18.2 TAC. TAC T cells are co-cultured with the eLuc-expressing target cells for 30 hours. At the end of the co-culture the viability of the eLuc-expressing target cells is assessed by measuring luminescence relative to an untreated control. Results are analyzed to compare the relative activities of the two populations of T cells.

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid encoding a Claudin 18.2 T cell-antigen coupler (TAC) polypeptide, the nucleic acid comprising:
 - (a) a first polynucleotide encoding an antigen-binding domain that binds Claudin 18.2;
 - (b) a second polynucleotide encoding an antigen-binding domain that binds a protein associated with a TCR complex; and
 - (c) a third polynucleotide encoding a TCR co-receptor cytosolic domain and transmembrane domain;wherein components encoded by (a), components encoded by (b), and components encoded by (c) are fused directly to each other, or joined by at least one linker.
2. The nucleic acid of claim 1, wherein the first polynucleotide, the second polynucleotide, and the third polynucleotide are in order.
3. The nucleic acid of claim 1 or 2, wherein the antigen-binding domain that binds Claudin 18.2 is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer; fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody.
4. The nucleic acid of any one of claims 1-3, wherein the antigen-binding domain that binds Claudin 18.2 is a designed ankyrin repeat (DARPin) polypeptide, a single chain variable fragment (scFv), or a nanobody.
5. The nucleic acid of any one of claims 1-4, wherein the antigen-binding domain that binds Claudin 18.2 is a nanobody.
6. The nucleic acid of any one of claims 1-5, wherein the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from an antibody selected from IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, and aGC182.
7. The nucleic acid of any one of claims 1-6, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182.
8. The nucleic acid of any one of claims 1-7, wherein the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of the amino acid sequence of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182.

9. The nucleic acid of any one of claims 1-8, wherein the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of the amino acid sequence of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182.
10. The nucleic acid of any one of claims 1-9, wherein the first polynucleotide encodes an antigen-binding domain comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 1 Kabat CDR1), a CDR2 comprising SEQ ID NO: 58 (Claudin 18.2 Nanobody 1 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 1 Kabat CDR3).
11. The nucleic acid of any one of claims 1-9, wherein the first polynucleotide encodes an antigen-binding domain comprising a CDR1 comprising SEQ ID NO: 60 (Claudin 18.2 Nanobody 1 IMGT CDR1), a CDR2 comprising SEQ ID NO: 61 (Claudin 18.2 Nanobody 1 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 1 IMGT CDR3).
12. The nucleic acid of any one of claims 1-11, wherein the first polynucleotide encodes an antigen-binding domain having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 55 (Claudin 18.2 Nanobody 1).
13. The nucleic acid of any one of claims 1-9, wherein the first polynucleotide encodes an antigen-binding domain comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 2 Kabat CDR1), a CDR2 comprising SEQ ID NO: 63 (Claudin 18.2 Nanobody 2 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 2 Kabat CDR3).
14. The nucleic acid of any one of claims 1-9, wherein the first polynucleotide encodes an antigen-binding domain comprising a CDR1 comprising SEQ ID NO: 64 (Claudin 18.2 Nanobody 2 IMGT CDR1), a CDR2 comprising SEQ ID NO: 65 (Claudin 18.2 Nanobody 2 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 2 IMGT CDR3).
15. The nucleic acid of any one of claims 1-9, 13, or 14, wherein the first polynucleotide encodes an antigen-binding domain having at least 80%, at least 85%, at least 90%, at least 95%,

at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 56 (Claudin 18.2 Nanobody 2).

16. The nucleic acid of any one of claims 1-15, wherein the protein associated with the TCR complex is a CD3 protein.

17. The nucleic acid of claim 16, wherein the CD3 protein is a CD3 γ protein, CD3 δ protein and/or CD3 ϵ protein. In some embodiments, the CD3 protein is a CD3 ϵ protein.

18. The nucleic acid of claim 17, wherein the CD3 protein is a CD3 ϵ protein.

19. The nucleic acid of any one of claims 1-18, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer; fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody.

20. The nucleic acid of any one of claims 1-19, wherein the antigen-binding domain that binds the protein associated with the TCR complex is derived from an antibody selected from UCHT1 OKT3, F6A, and L2K.

21. The nucleic acid of claim 20, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a UCHT1 antigen-binding domain.

22. The nucleic acid of claim 21, wherein the UCHT1 antigen-binding domain is an scFv of UCHT1.

23. The nucleic acid of claim 21 or 22, wherein the UCHT1 antigen-binding domain comprises a Y to T mutation at a position corresponding to amino acid 182 of SEQ ID NO: 8 (Y182T).

24. The nucleic acid of any one of claims 21-23, wherein the UCHT1 antigen-binding domain comprises a humanized variant of UCHT1 (huUCHT1).

25. The nucleic acid of claim 24, wherein the UCHT1 antigen-binding domain comprises a humanized variant of UCHT1 comprising a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (huUCHT1 (Y177T)).

26. The nucleic acid of any one of claims 21-25, wherein antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)).

27. The nucleic acid of any one of claims 21-26, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity

with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)).

28. The nucleic acid of any one of claims 21-27, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)).

29. The nucleic acid of claim 20, wherein the antigen-binding domain that binds the protein associated with the TCR complex is an OKT3 antigen-binding domain.

30. The nucleic acid of claim 29, wherein the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 16 (OKT3).

31. The nucleic acid of claim 29 or 30, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3).

32. The nucleic acid of any one of claims 29-31, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3).

33. The nucleic acid of claim 20, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a F6A antigen-binding domain.

34. The nucleic acid of claim 33, wherein the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 18 (F6A).

35. The nucleic acid of claim 33 or 34, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A).
36. The nucleic acid of any one of claims 33-35, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A).
37. The nucleic acid of claim 20, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a L2K antigen-binding domain.
38. The nucleic acid of claim 37, wherein the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 20 (L2K).
39. The nucleic acid of claim 37 or 38, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K).
40. The nucleic acid of any one of claims 37-39, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K).
41. The nucleic acid of any one of claims 1-40, wherein the transmembrane domain is a CD4 transmembrane domain and the cytosolic domain is a CD4 cytosolic domain.
42. The nucleic acid of claim 41, wherein the transmembrane and cytosolic domain comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain).
43. The nucleic acid of any one of claims 1-42, wherein the transmembrane domain is a CD8 transmembrane domain and the cytosolic domain is a CD8 cytosolic domain.

44. The nucleic acid of any one of claims 1-43, wherein the component encoded by (a) and the component encoded by (c) are fused to the component encoded by (b).
45. The nucleic acid of any one of claims 1-43, wherein the component encoded by (b) and the component encoded by (c) are fused to the component encoded by (a).
46. The nucleic acid of any one of claims 1-45, wherein at least one linker joins the component encoded by (a) to the component encoded by (b).
47. The nucleic acid of claim 46, wherein the at least one linker is a glycine and/or serine-rich linker, a large protein domain, a long helix structure, or a short helix structure.
48. The nucleic acid of claim 46 or 47, wherein the at least one linker comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker).
49. The nucleic acid of any one of claims 1-48, wherein the nucleic acid sequence does not encode a co-stimulatory domain.
50. The nucleic acid of any one of claims 1-49, wherein the nucleic acid sequence does not encode an activation domain.
51. The nucleic acid of any one of claims 1-50, wherein the nucleic acid sequence further encodes a leader sequence.
52. The nucleic acid of claim 51, wherein the leader sequence comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 2 (mIgG leader), SEQ ID NO: 38 (hIgG leader), or SEQ ID NO: 40 (huCD8a leader).
53. A nucleic acid encoding a Claudin 18.2-TAC polypeptide, wherein the nucleic acid comprises a sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the nucleic acid sequence of SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, or SEQ ID NO: 75.
54. The nucleic acid of claim 53, wherein the Claudin 18.2-TAC comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least

97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, or SEQ ID NO: 73.

55. An expression vector comprising the nucleic acid of any one of claims 1-54.

56. The expression vector of claim 55, further comprising a promoter functional in a mammalian cell.

57. The expression vector of claim 55 or 56, wherein the expression vector is a lentiviral vector.

58. The expression vector of claim 57, wherein the lentiviral vector is a VSV-G pseudotyped lentivirus.

59. The expression vector of claim 57 or 58, wherein the expression vector is a γ retroviral vector.

60. The expression vector of claim 59, wherein the γ retroviral vector is a GALV pseudotyped γ -retrovirus.

61. A Claudin 18.2 T cell-antigen coupler (TAC) polypeptide, comprising:

(a) an antigen-binding domain that binds Claudin 18.2;

(b) an antigen-binding domain that binds a protein associated with a TCR complex; and

(c) a TCR co-receptor cytosolic domain and transmembrane domain;

wherein component (a), component (b), and component (c) are fused directly to each other, or joined by at least one linker.

62. The Claudin 18.2-TAC polypeptide of claim 61, wherein the first polynucleotide, the second polynucleotide, and the third polynucleotide are in order.

63. The Claudin 18.2-TAC polypeptide of claim 61 or 62, wherein the antigen-binding domain that binds Claudin 18.2 is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer; fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody.

64. The Claudin 18.2-TAC polypeptide of claim 63, wherein the antigen-binding domain that binds Claudin 18.2 is a designed ankyrin repeat (DARPin) polypeptide, a single chain variable fragment (scFv), or a nanobody.

65. The Claudin 18.2-TAC polypeptide of claim 63 or 64, wherein the antigen-binding domain that binds Claudin 18.2 is a nanobody.

66. The Claudin 18.2-TAC polypeptide of any one of claims 61-65, wherein the antigen-binding domain that binds Claudin 18.2 comprises an antigen-binding domain derived from an antibody selected from IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, and aGC182.

67. The Claudin 18.2-TAC polypeptide of any one of claims 61-66, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of the antigen-binding domain(s) of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182.
68. The Claudin 18.2-TAC polypeptide of any one of claims 61-67, wherein the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of the amino acid sequence of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182.
69. The Claudin 18.2-TAC polypeptide of any one of claims 61-68, wherein the CDR sequences of the antigen-binding domain that binds Claudin 18.2 have 100% identity with the CDR sequences of the amino acid sequence of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182, and the non-CDR sequences of the antigen-binding domain that binds Claudin 18.2 have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of IMAB362 (claudiximab, zolbetuximab), 43-14A, EPR 19202, or aGC182.
70. The Claudin 18.2-TAC polypeptide of any one of claims 61-69, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 1 Kabat CDR1), a CDR2 comprising SEQ ID NO: 58 (Claudin 18.2 Nanobody 1 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 1 Kabat CDR3).
71. The Claudin 18.2-TAC polypeptide of any one of claims 61-69, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 60 (Claudin 18.2 Nanobody 1 IMGT CDR1), a CDR2 comprising SEQ ID NO: 61 (Claudin 18.2 Nanobody 1 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 1 IMGT CDR3).
72. The Claudin 18.2-TAC polypeptide of any one of claims 61-71, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 55 (Claudin 18.2 Nanobody 1).
73. The Claudin 18.2-TAC polypeptide of any one of claims 61-69, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 57 (Claudin 18.2 Nanobody 2 Kabat CDR1), a CDR2 comprising SEQ

ID NO: 63 (Claudin 18.2 Nanobody 2 Kabat CDR2), and a CDR3 comprising SEQ ID NO: 59 (Claudin 18.2 Nanobody 2 Kabat CDR3).

74. The Claudin 18.2-TAC polypeptide of any one of claims 61-69, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence comprising a CDR1 comprising SEQ ID NO: 64 (Claudin 18.2 Nanobody 2 IMGT CDR1), a CDR2 comprising SEQ ID NO: 65 (Claudin 18.2 Nanobody 2 IMGT CDR2), and a CDR3 comprising SEQ ID NO: 62 (Claudin 18.2 Nanobody 2 IMGT CDR3).

75. The Claudin 18.2-TAC polypeptide of any one of claims 61-69, 73, or 74, wherein the antigen-binding domain that binds Claudin 18.2 comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 56 (Claudin 18.2 Nanobody 2).

76. The Claudin 18.2-TAC polypeptide of any one of claims 61-75, wherein the protein associated with the TCR complex is a CD3 protein.

77. The Claudin 18.2-TAC polypeptide of claim 76, wherein the CD3 protein is a CD3 γ protein, CD3 δ protein and/or CD3 ϵ protein.

78. The Claudin 18.2-TAC polypeptide of claim 76 or 77, wherein the CD3 protein is a CD3 ϵ protein.

79. The Claudin 18.2-TAC polypeptide of any one of claims 61-78, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a designed ankyrin repeat (DARPin) polypeptide, single chain variable fragment (scFv), single domain antibody, diabody, affibody, adnectin, affilin, phylomer; fynomer, affimer, peptide aptamer, knottin, centyrin, anticalin, or nanobody.

80. The Claudin 18.2-TAC polypeptide of any one of claims 61-79, wherein the antigen-binding domain that binds the protein associated with the TCR complex is derived from an antibody selected from UCHT1, OKT3, F6A, and L2K.

81. The Claudin 18.2-TAC polypeptide of claim 80, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a UCHT1 antigen-binding domain.

82. The Claudin 18.2-TAC polypeptide of claim 81, wherein the UCHT1 antigen-binding domain is an scFv of UCHT1.

83. The Claudin 18.2-TAC polypeptide of claim 81 or 82, wherein the UCHT1 antigen-binding domain comprises a Y to T mutation at a position corresponding to amino acid 182 of SEQ ID NO: 8 (Y182T).

84. The Claudin 18.2-TAC polypeptide of any one of claims 81-83, wherein the UCHT1 antigen-binding domain comprises a humanized variant of UCHT1 (huUCHT1).

85. The Claudin 18.2-TAC polypeptide of claim 84, wherein the UCHT1 antigen-binding domain comprises a humanized variant of UCHT1 comprising a Y to T mutation at a position corresponding to amino acid 177 of SEQ ID NO: 34 (huUCHT1 (Y177T)).
86. The Claudin 18.2-TAC polypeptide of any one of claims 81-85, wherein antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)).
87. The Claudin 18.2-TAC polypeptide of any one of claims 81-86, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)).
88. The Claudin 18.2-TAC polypeptide of any one of claims 81-87, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)), and the non-CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 8 (UCHT1), SEQ ID NO: 44 (UCHT1 (Y182T)), SEQ ID NO: 34 (huUCHT1), or SEQ ID NO: 36 (huUCHT1 (Y177T)).
89. The Claudin 18.2-TAC polypeptide of claim 80, wherein the antigen-binding domain that binds the protein associated with the TCR complex is an OKT3 antigen-binding domain.
90. The Claudin 18.2-TAC polypeptide of claim 89, wherein the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 16 (OKT3).
91. The Claudin 18.2-TAC polypeptide of claim 89 or 90, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3).
92. The Claudin 18.2-TAC polypeptide of any one of claims 89-91, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR

complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3).

93. The Claudin 18.2-TAC polypeptide of claim 80, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a F6A antigen-binding domain.

94. The Claudin 18.2-TAC polypeptide of claim 93, wherein the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 18 (F6A).

95. The Claudin 18.2-TAC polypeptide of claim 93 or 94, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A).

96. The Claudin 18.2-TAC polypeptide of any one of claims 93-95, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 18 (F6A).

97. The Claudin 18.2-TAC polypeptide of claim 80, wherein the antigen-binding domain that binds the protein associated with the TCR complex is a L2K antigen-binding domain.

98. The Claudin 18.2-TAC polypeptide of claim 96, wherein the antigen-binding domain that binds the protein associated with the TCR complex comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with SEQ ID NO: 20 (L2K).

99. The Claudin 18.2-TAC polypeptide of claim 97 or 98, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K).

100. The Claudin 18.2-TAC polypeptide of any one of claims 97-99, wherein the CDR sequences of the antigen-binding domain that binds the protein associated with the TCR complex have 100% identity with the CDR sequences of the amino acid sequence of SEQ ID NO: 16 (OKT3), and the non-CDR sequences of the antigen-binding domain that binds the

protein associated with the TCR complex have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with the non-CDR sequences of the amino acid sequence of SEQ ID NO: 20 (L2K).

101. The Claudin 18.2-TAC polypeptide of any one of claims 61-100, wherein the transmembrane domain is a CD4 transmembrane domain and the cytosolic domain is a CD4 cytosolic domain.

102. The Claudin 18.2-TAC polypeptide of claim 101, wherein the transmembrane and cytosolic domain comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 12 (CD4 transmembrane and cytosolic domain).

103. The Claudin 18.2-TAC polypeptide of claim 61-100, wherein the transmembrane domain is a CD8 transmembrane domain and the cytosolic domain is a CD8 cytosolic domain.

104. The Claudin 18.2-TAC polypeptide of any one of claims 61-103, wherein component (a) and component (c) are fused to component (b).

105. The Claudin 18.2-TAC polypeptide of any one of claims 61-104, wherein component (b) and component (c) are fused to component (a).

106. The Claudin 18.2-TAC polypeptide of any one of claims 61-105, wherein at least one linker joins component (a) to component (b).

107. The Claudin 18.2-TAC polypeptide of claim 106, wherein the at least one linker is a glycine and/or serine-rich linker, a large protein domain, a long helix structure, or a short helix structure.

108. The Claudin 18.2-TAC polypeptide of claim 107, wherein the at least one linker comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 6 ((G4S)4-based linker), SEQ ID NO: 10 (G4S-based linker), SEQ ID NO: 14 (CD4 based linker), SEQ ID NO: 22 (short helix connector), SEQ ID NO: 24 (long helix connector), SEQ ID NO: 26 (large domain connector), SEQ ID NO: 41 (flexible connector), SEQ ID NO: 45 (G4S flexible linker), or SEQ ID NO: 46 (G4S3 flexible linker).

109. The Claudin 18.2-TAC polypeptide of any one of claims 62-108, wherein the Claudin 18.2-TAC does not comprise a co-stimulatory domain.

110. The Claudin 18.2-TAC polypeptide of any one of claims 62-109, wherein the Claudin 18.2-TAC does not comprise an activation domain.

111. A Claudin 18.2-TAC polypeptide, wherein the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 67.
112. A Claudin 18.2-TAC polypeptide, wherein the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 69.
113. A Claudin 18.2-TAC polypeptide, wherein the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 71.
114. A Claudin 18.2-TAC polypeptide, wherein the Claudin 18.2-TAC polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with the amino acid sequence of SEQ ID NO: 73.
115. A T cell comprising the nucleic acid of any one of claims 1-54.
116. A T cell comprising the expression vector of any one of claims 54-60.
117. A T cell comprising the Claudin 18.2-TAC polypeptide of any one of claims 61-114.
118. A pharmaceutical composition comprising the T cell of any one of claims 115-117, and a pharmaceutically acceptable excipient.
119. A method of treating a Claudin 18.2-expressing cancer in an individual in need thereof, comprising administering to the individual the pharmaceutical composition of claim 118.
120. The method of claim 119, wherein the cancer is a solid cancer.
121. The method of claim 119 or 120, wherein the cancer is a pancreatic cancer, a gastric cancer, a signet ring cell carcinoma, a mucinous gastric cancer, a gastroesophageal cancer, an esophageal cancer, a digestive system cancer, an ovarian cancer, a mucinous ovarian cancer, a non-small cell lung cancer, or a lung cancer.
122. The method of claim 121, wherein the cancer is a gastric cancer or a gastroesophageal cancer.
123. A conjugate comprising a T cell comprising the Claudin 18.2-TAC polypeptide of any one of claims 61-114 bound to Claudin 18.2 expressed on a cancer cell.
124. The conjugate of claim 123, wherein the cancer cell is a cell of a solid cancer.

125. The conjugate of claim 123 or 124, wherein the cancer cell is a pancreatic cancer cell, a gastric cancer cell, a signet ring cell carcinoma cell, a mucinous gastric cancer cell, a gastroesophageal cancer cell, an esophageal cancer cell, a digestive system cancer cell, an ovarian cancer cell, a mucinous ovarian cancer cell, a non-small cell lung cancer cell, or a lung cancer cell.

126. The conjugate of any one of claims 123-125, the cancer cell is a gastric cancer cell or a gastroesophageal cancer cell.

Figure 1

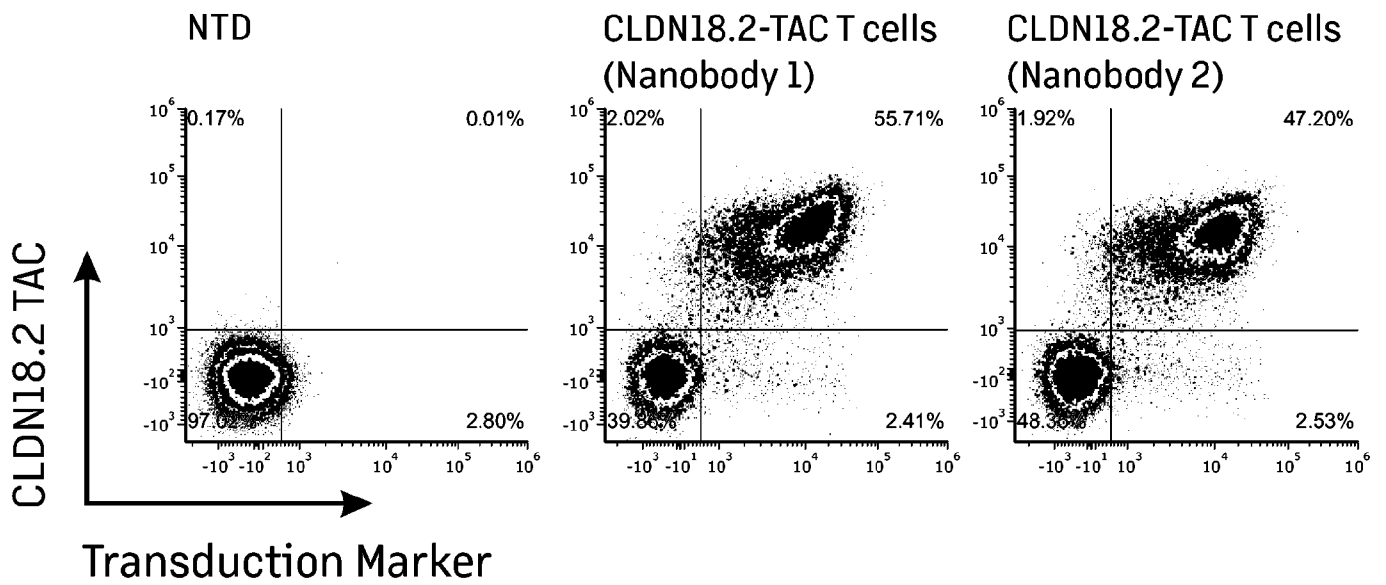


Figure 2A

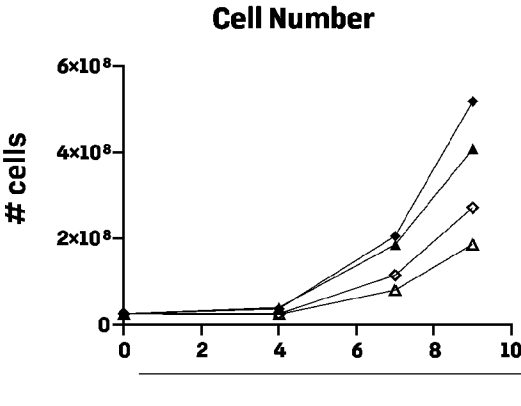


Figure 2B

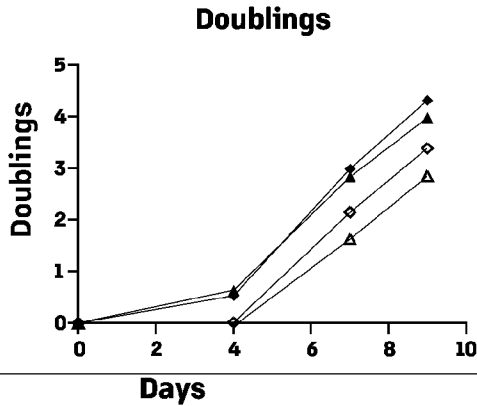
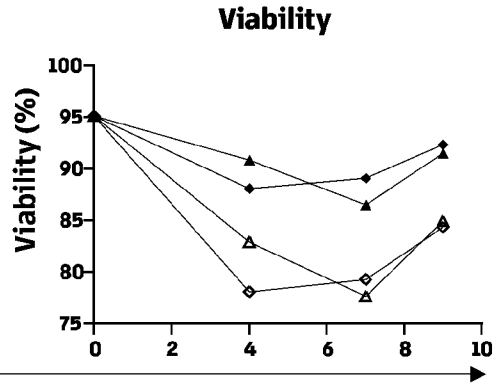


Figure 2C



◇ CLDN18.2 TAC (Nanobody 1) ◆ CLDN18.2 TAC (Nanobody 1/Y177T)
△ CLDN18.2 TAC (Nanobody 2) ▲ CLDN18.2 TAC (Nanobody 2/Y177T)

CD3+TAC+				
	Nano1/Y177T MOI = 30	Nano2/Y177T MOI = 30	Nano1 MOI = 60	Nano2 MOI = 60
Day 9	54.9 %	58.7 %	53.5 %	59.1 %

Figure 3

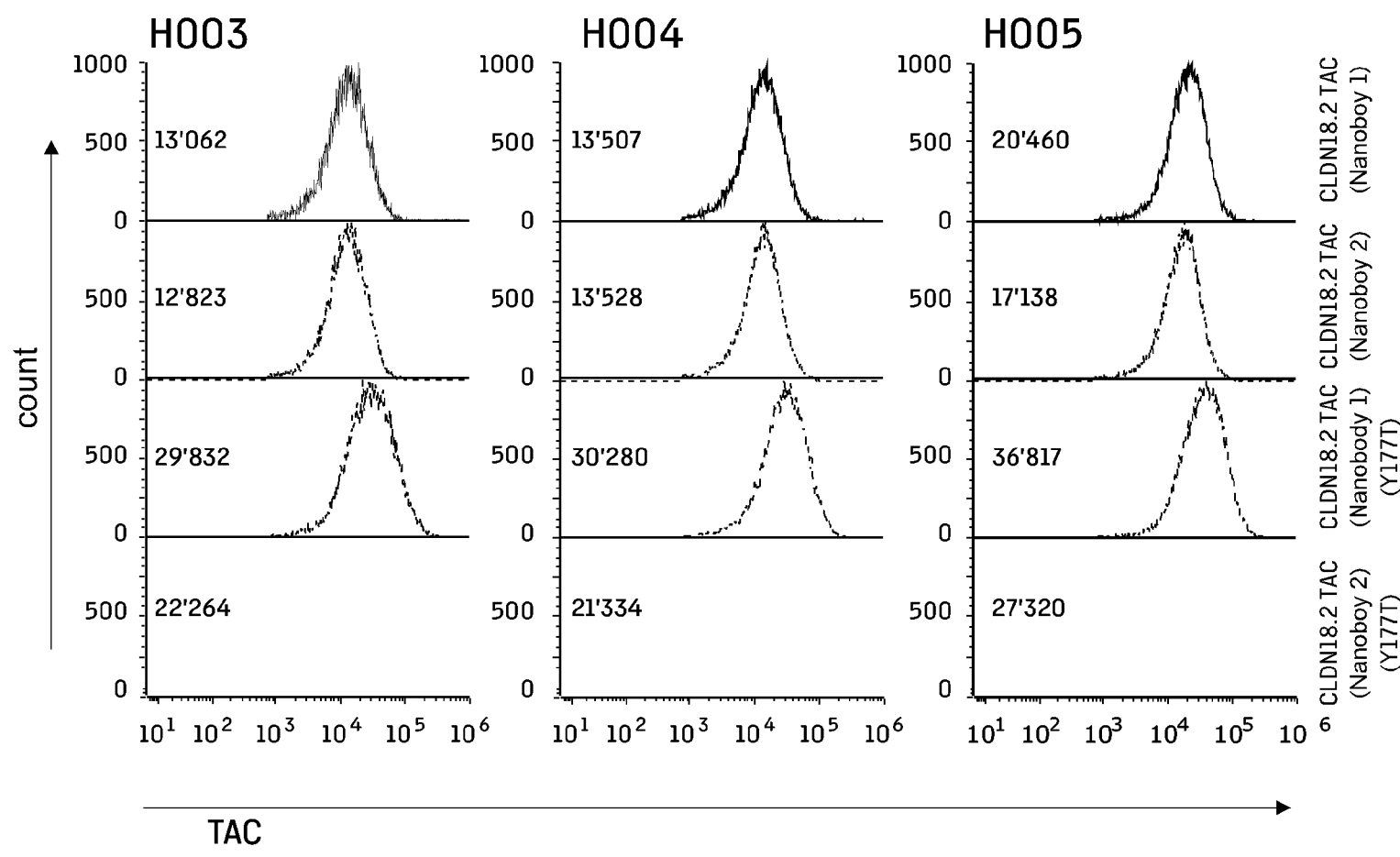


Figure 4

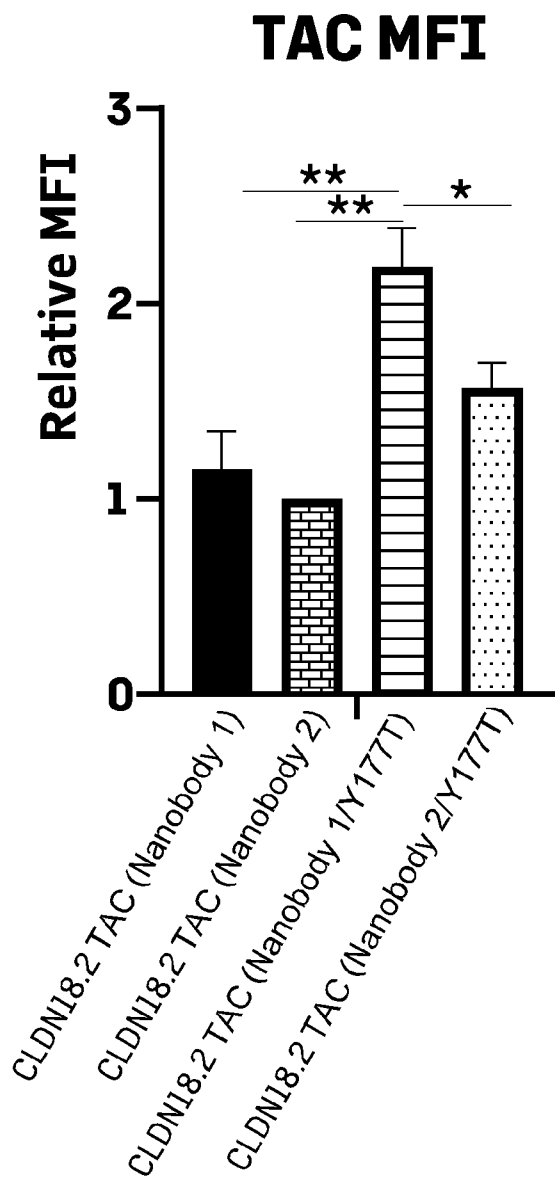


Figure 5A

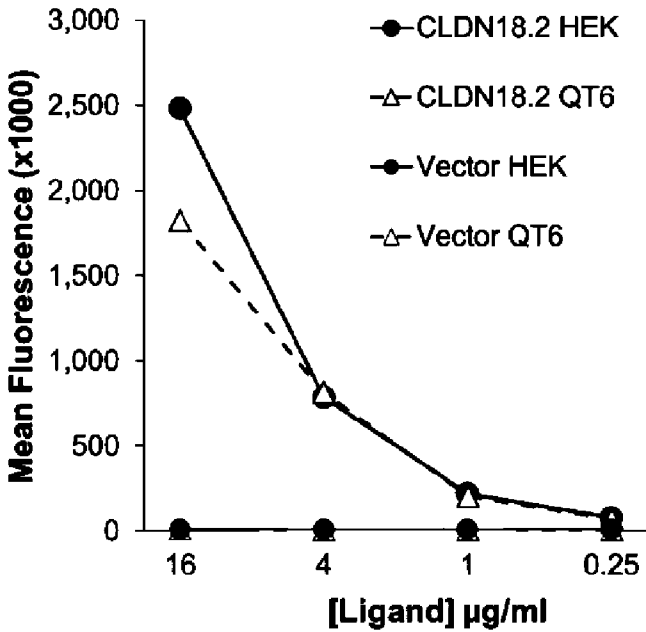


Figure 5B

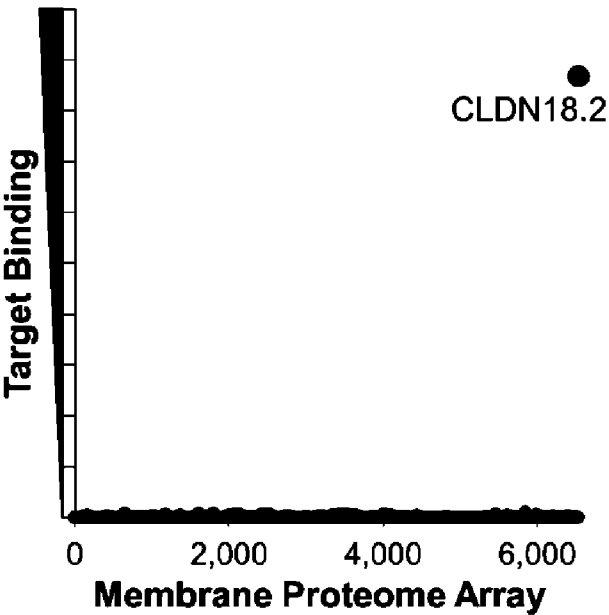


Figure 6A

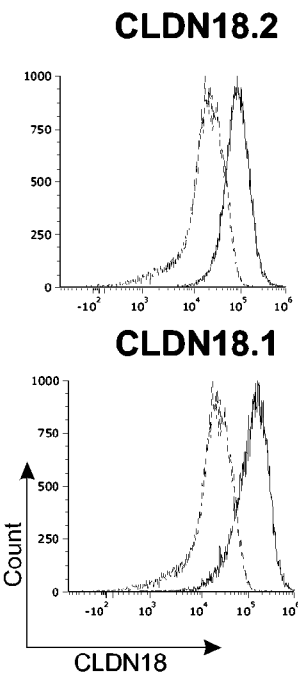


Figure 6B

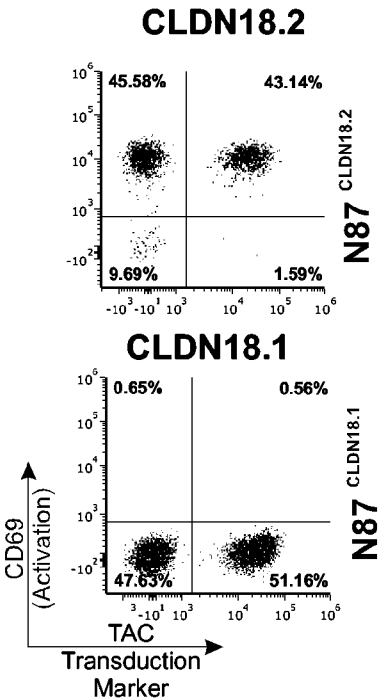


Figure 6C

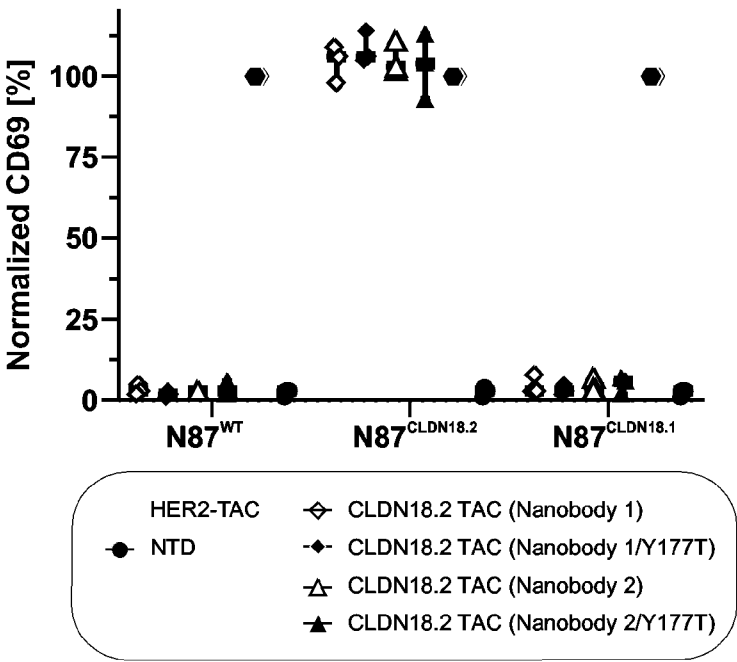


Figure 7

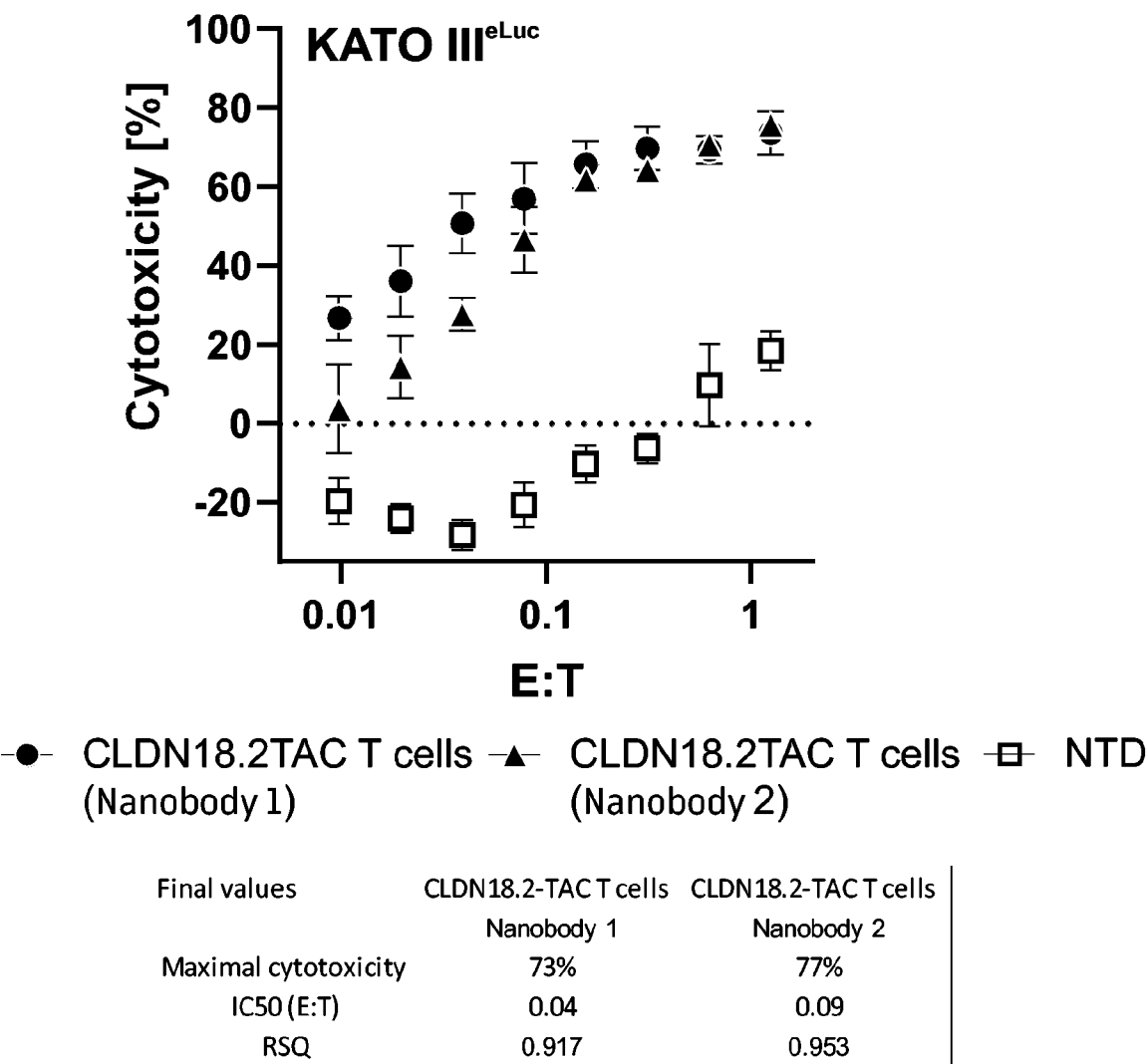


Figure 8

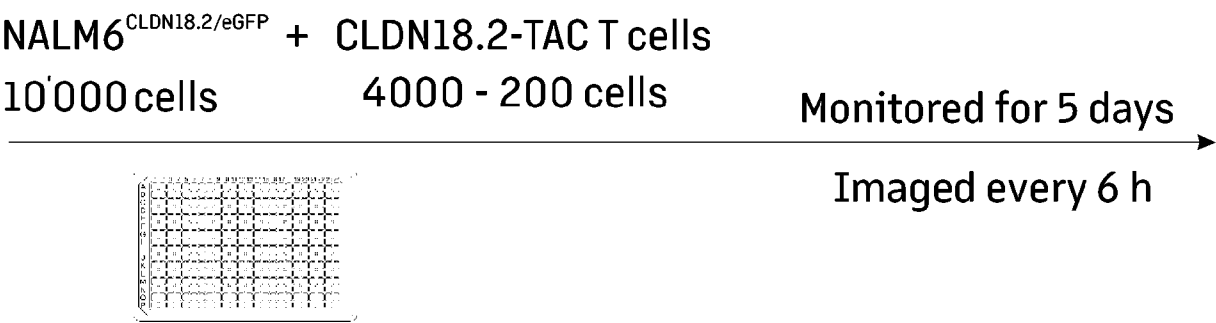


Figure 9

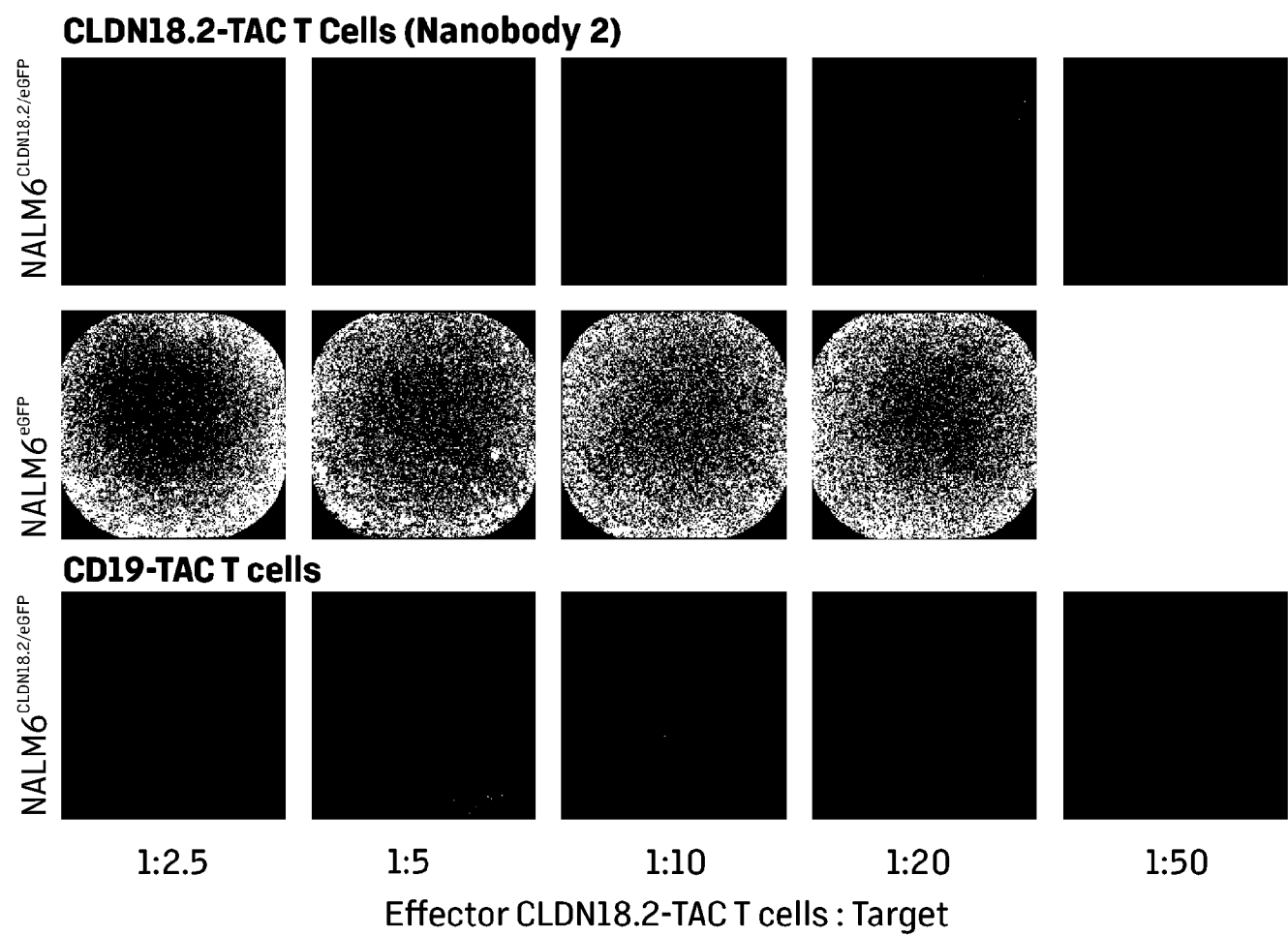


Figure 10

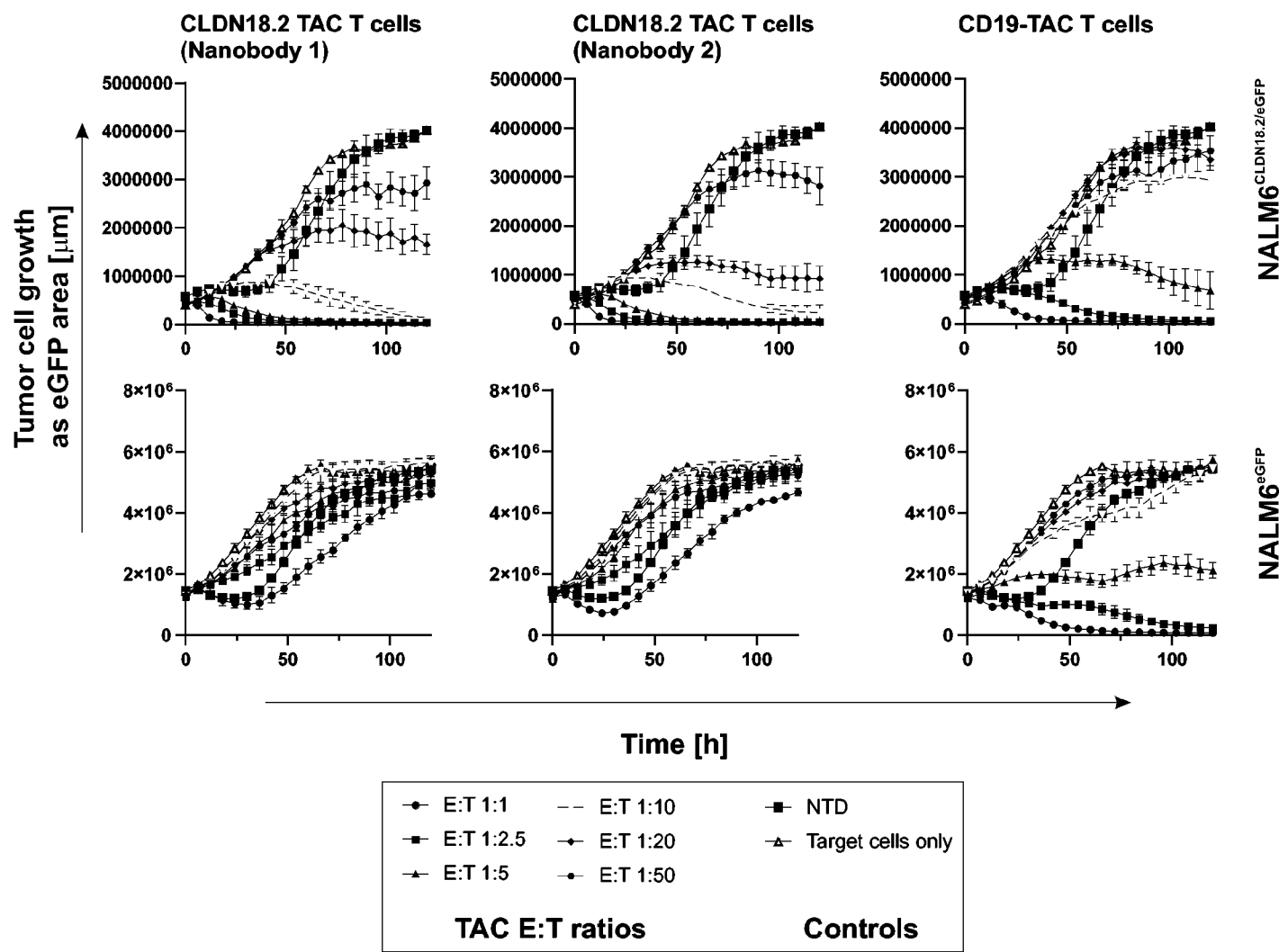


Figure 11

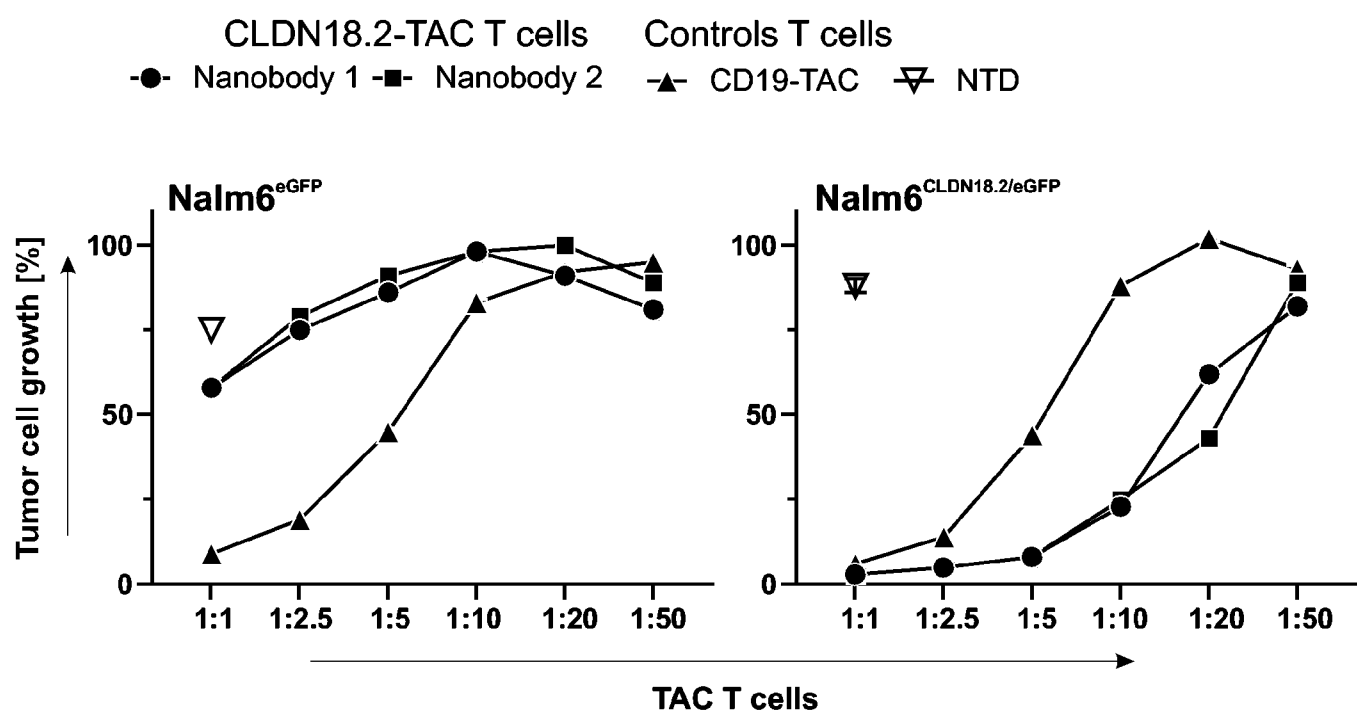


Figure 12

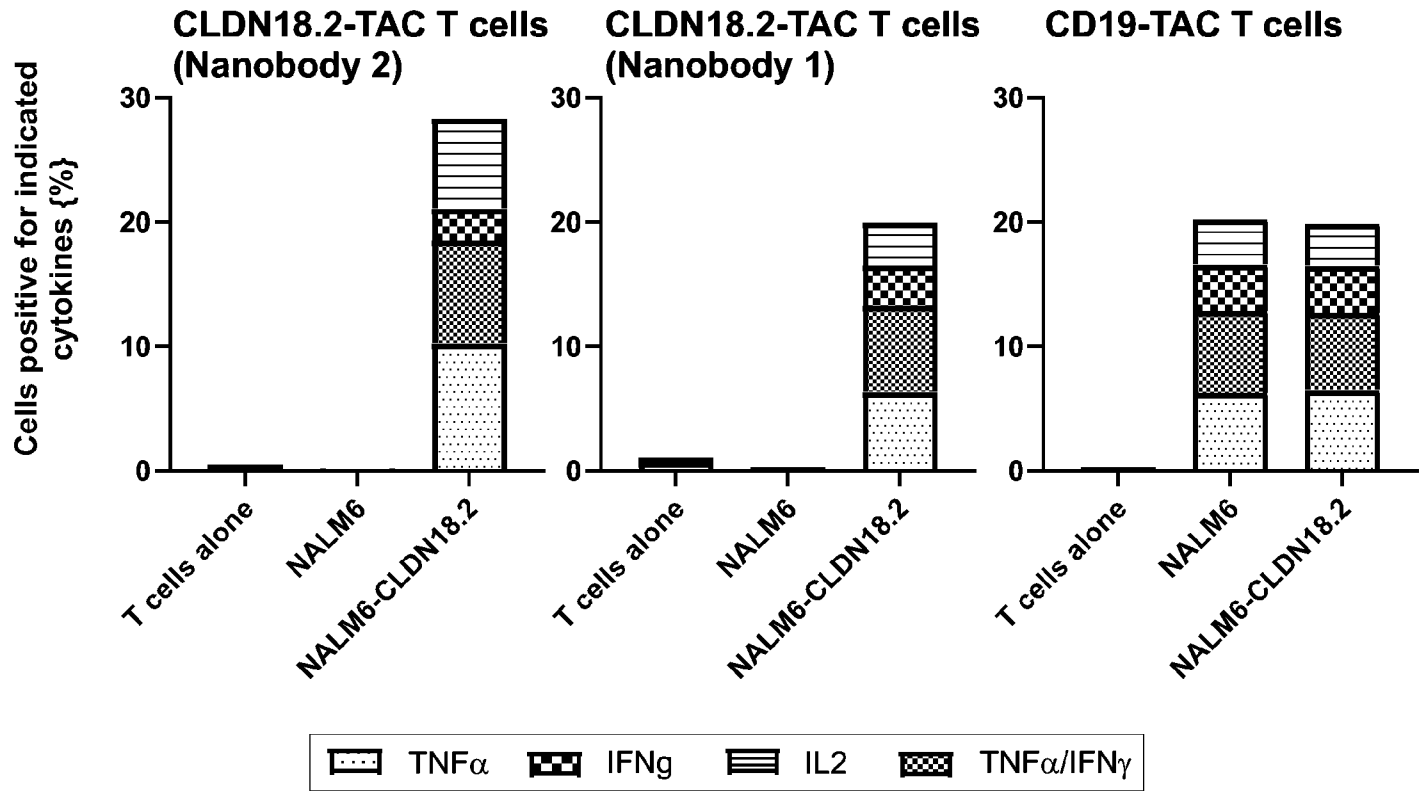


Figure 13

	M a r k e r	F l u o r o p h o r e
Exhaustion/ Activation + M e m o r y	T C R a b	A P C
	C D 4	B V 6 0 5
	C D 8	P E C y 7
	P D 1	S B 6 4 5
	T I M 3	B V 4 2 1
	C D 6 9	P a c i f i c B l u e
	L A G 3	P E e F l u o r 6 1 0
	C D 2 7	B V 5 7 0
	C D 4 4	A F 7 0 0
	T I G I T	PerCPeFluor710
	C D 2 5	B V 7 1 1
	C C R 7	F I T C o r A F 4 8 8
	C D 4 5 R A	P E F i r e 6 4 0
	C D 4 5 R O	B V 5 1 0
	C D 6 2 L	B V 7 8 5
	C D 1 6 2	P E
	V i a b i l i t y	N e a t R

Figure 14

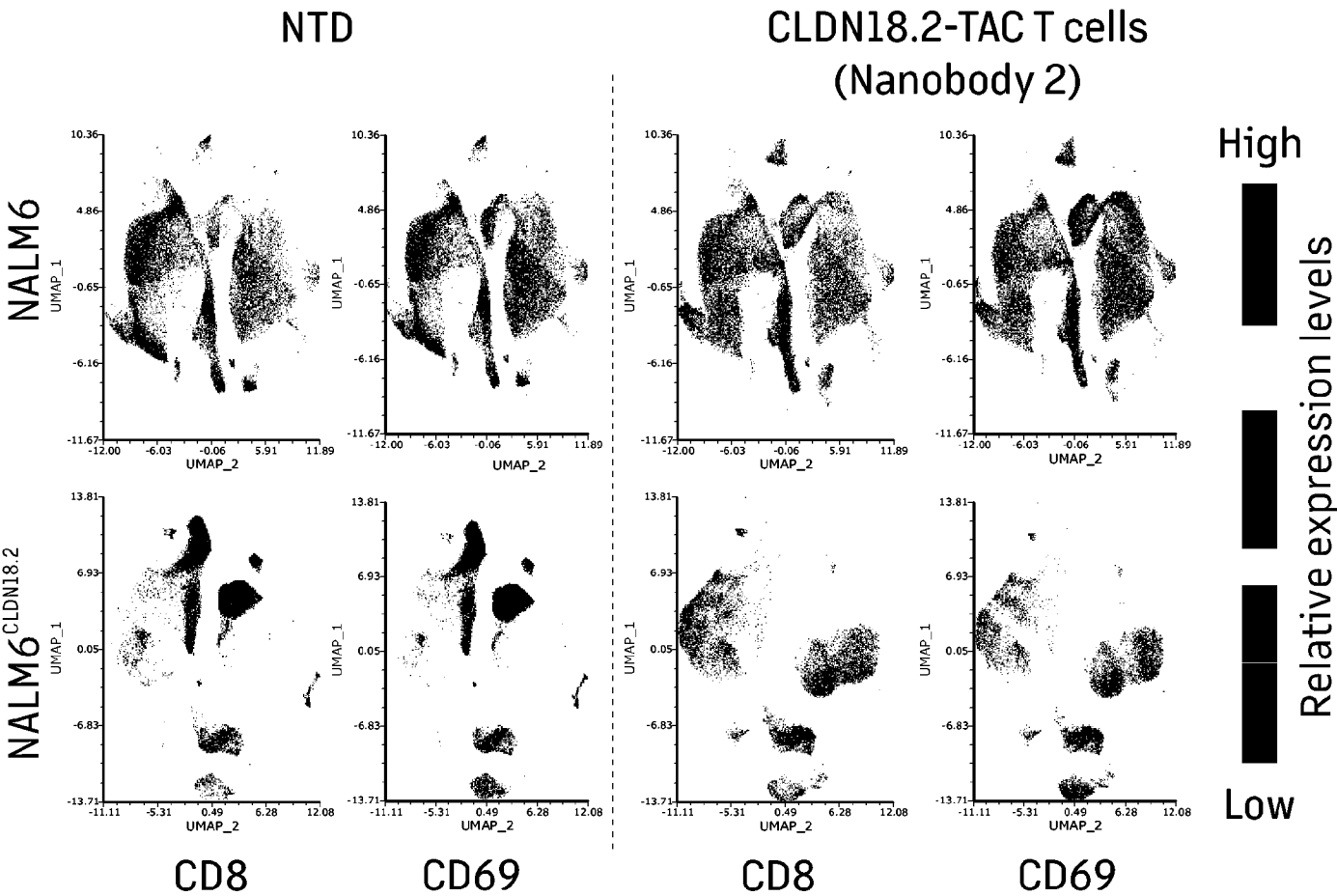


Figure 15

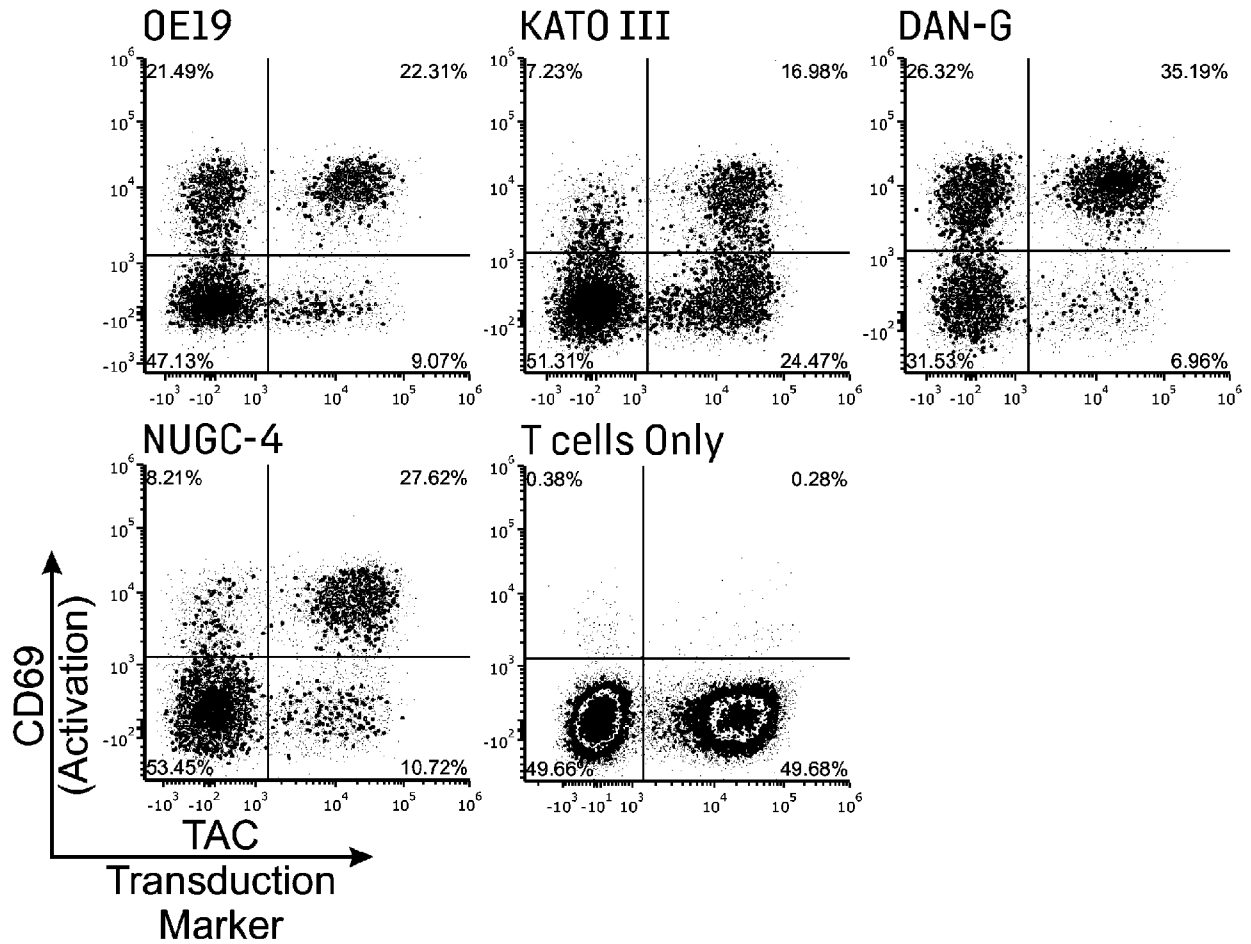


Figure 16A

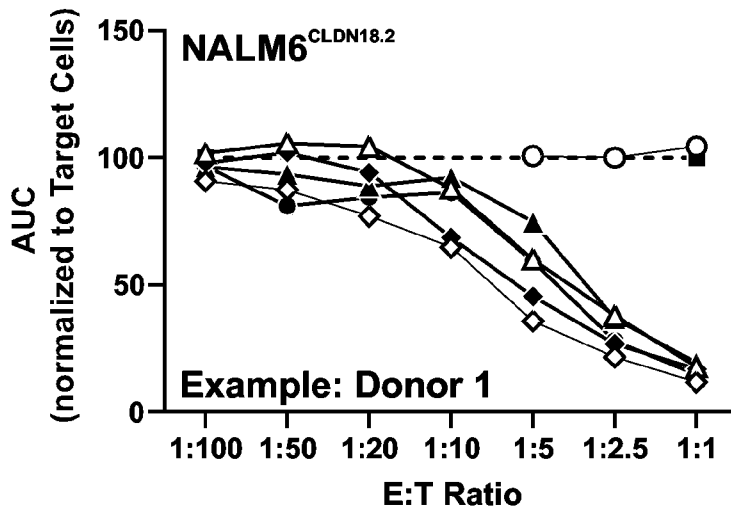
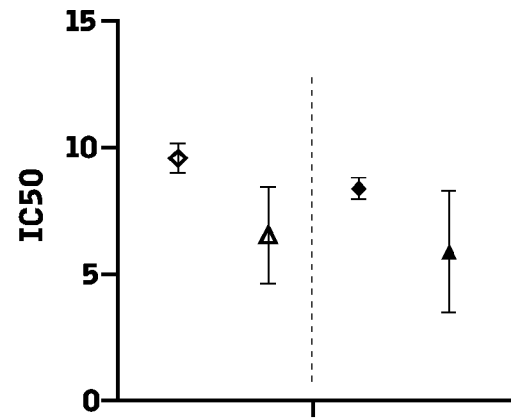
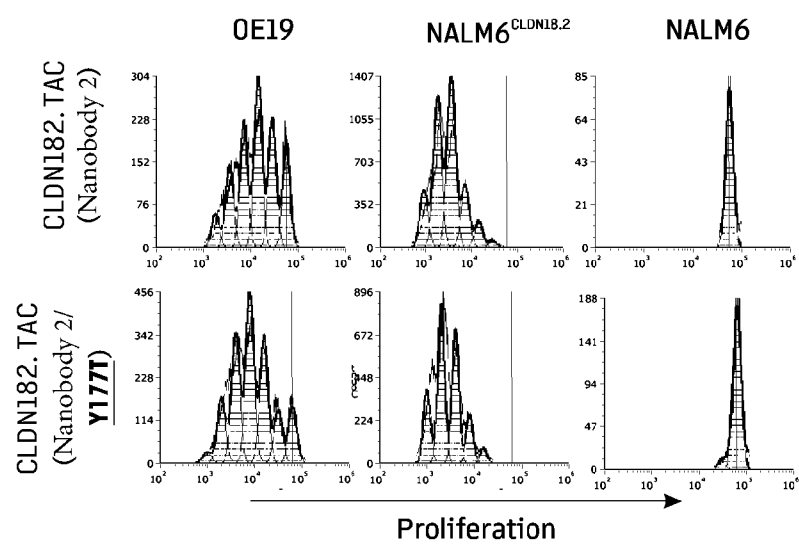


Figure 16B



- ◇ CLDN18.2-TAC (Nanobody 1) ◆ CLDN18.2-TAC (Nanobody 1/Y177T)
- △ CLDN18.2-TAC (Nanobody 2) ▲ CLDN18.2-TAC (Nanobody 2/Y177T)
- CD19-TAC
- NTD
- Target

Figure 17A



Example Donor 1

Figure 17B

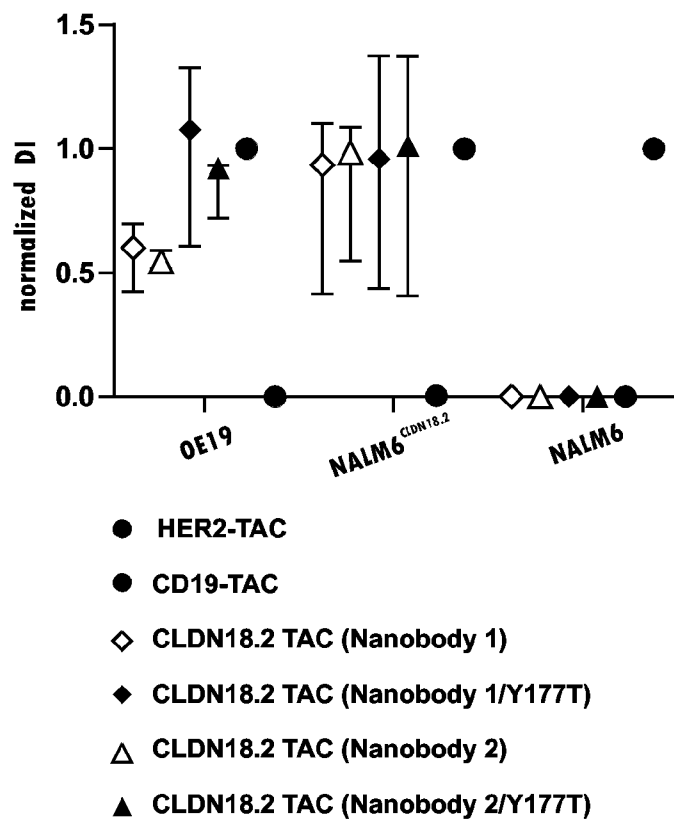


Figure 18A

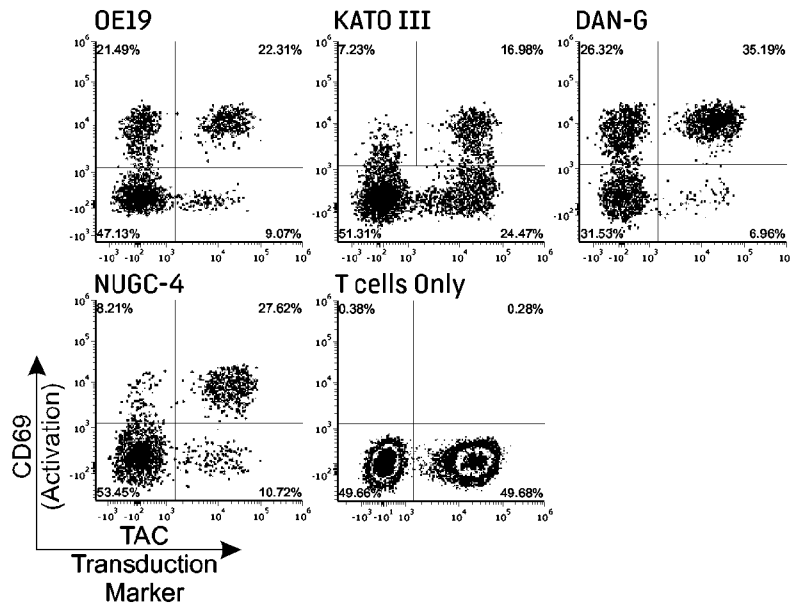


Figure 18B

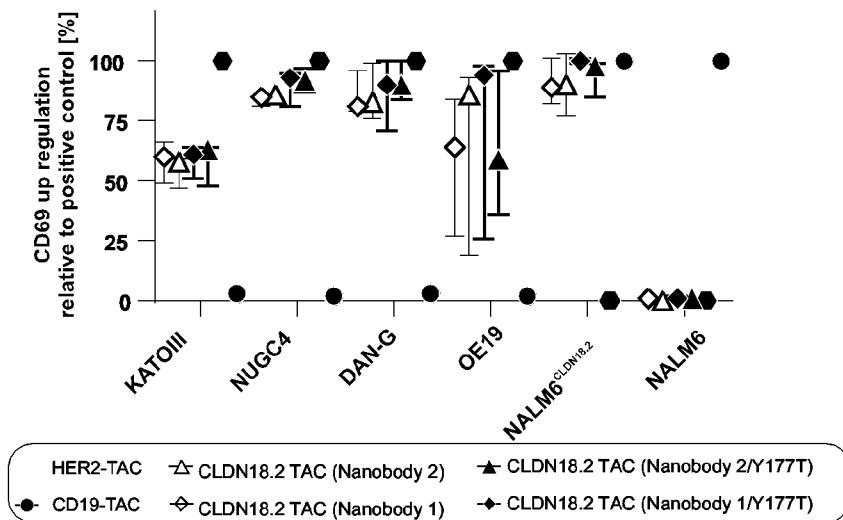


Figure 19A

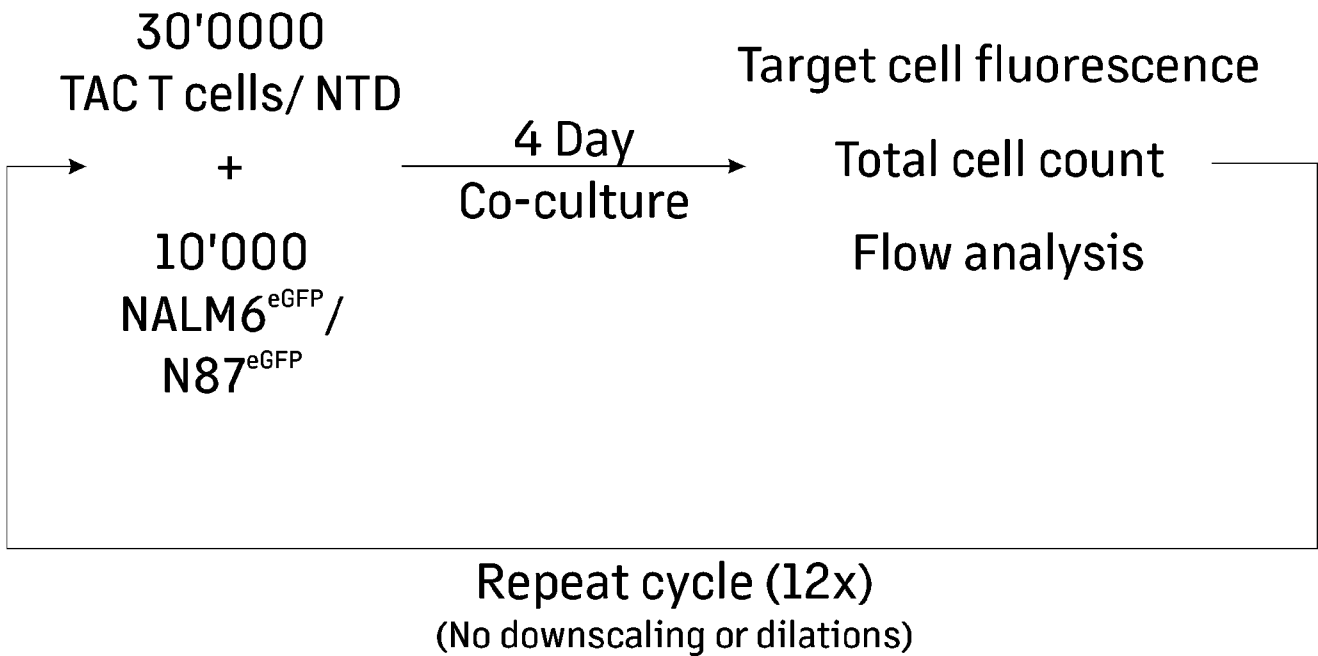
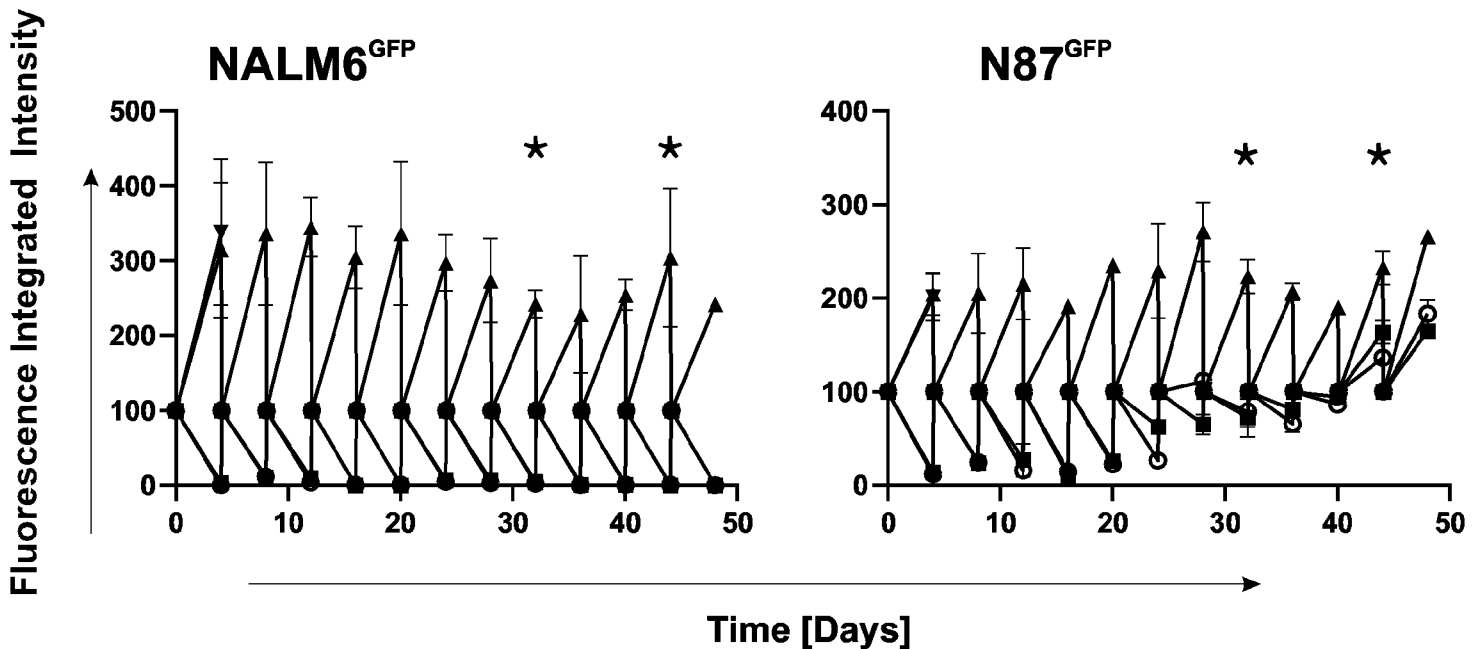


Figure 19B



○ 5:1 CLDN18.2 TAC (Nanobody 2/Y177T)

■ 3:1 CLDN18.2 TAC (Nanobody 2/Y177T)

▲ Target

▼ NTD

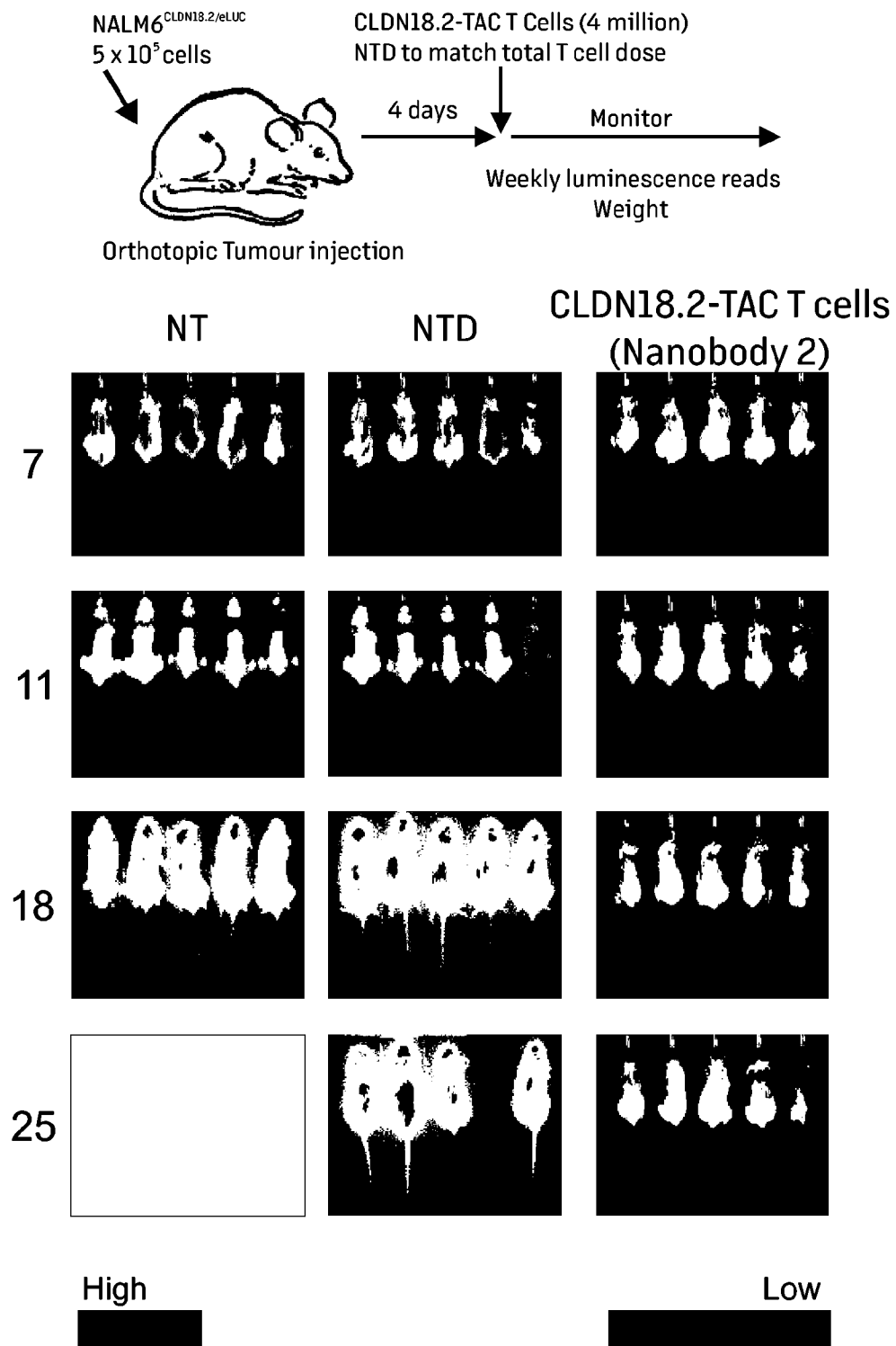
Figure 20

Figure 21A

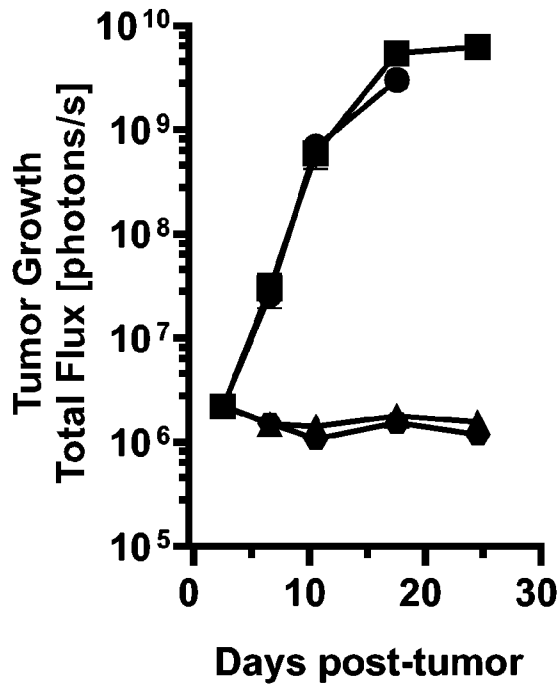


Figure 21B

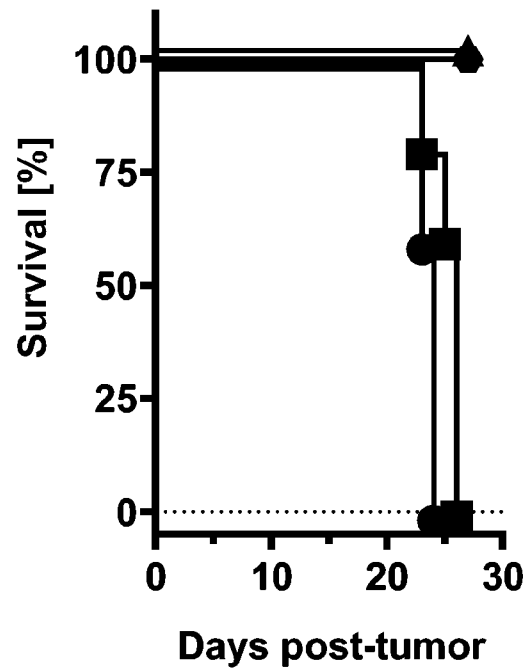
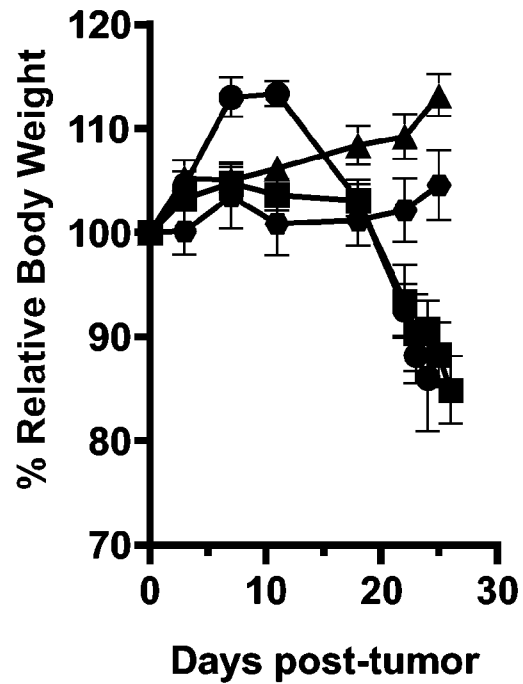


Figure 21C



- NT
- ▲ CLDN18.2-TAC T cells (Nanobody 2)
- NTD
- ◆ CLDN18.2-TAC T cells (Nanobody 1)

Figure 22

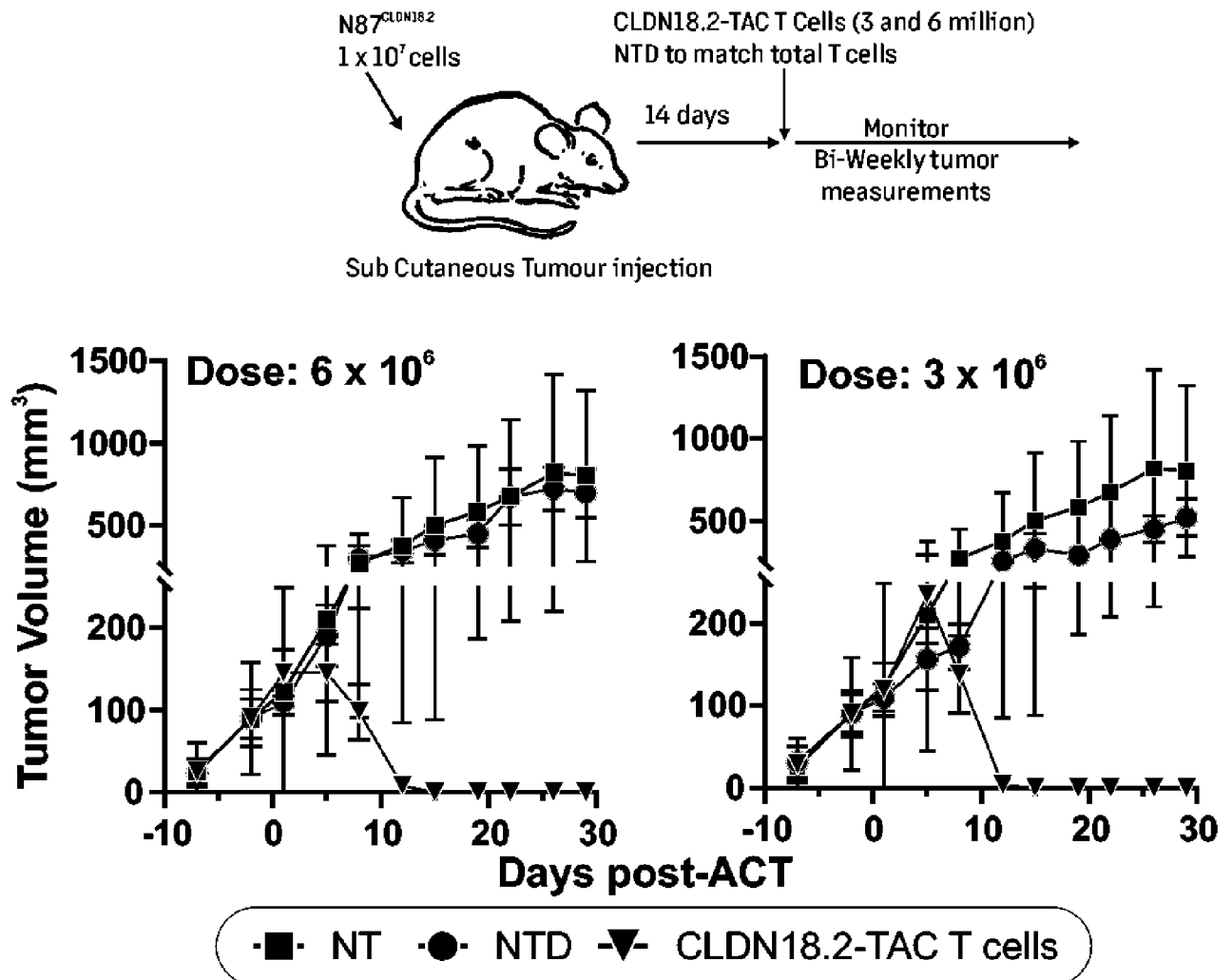


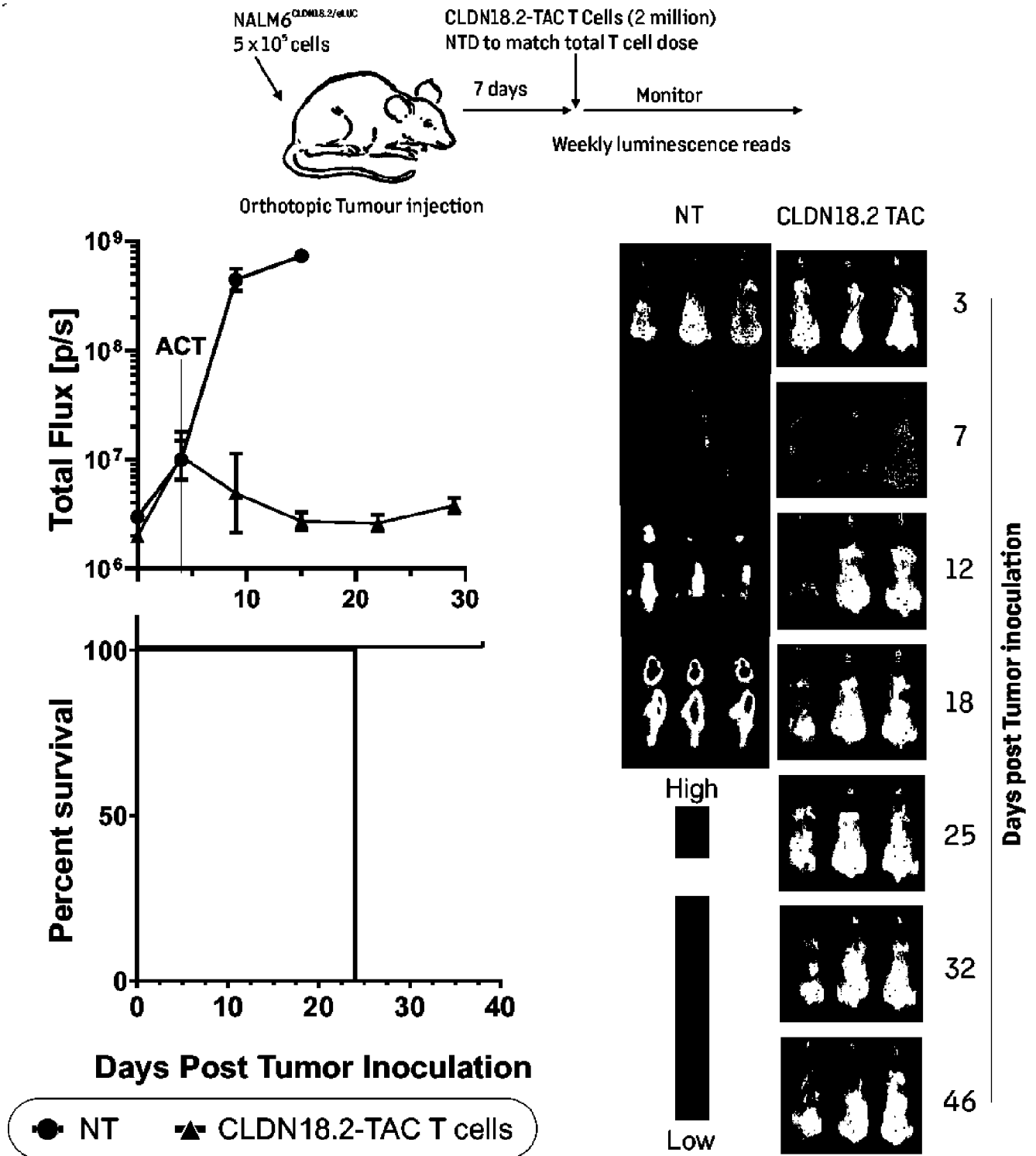
Figure 23

Figure 24

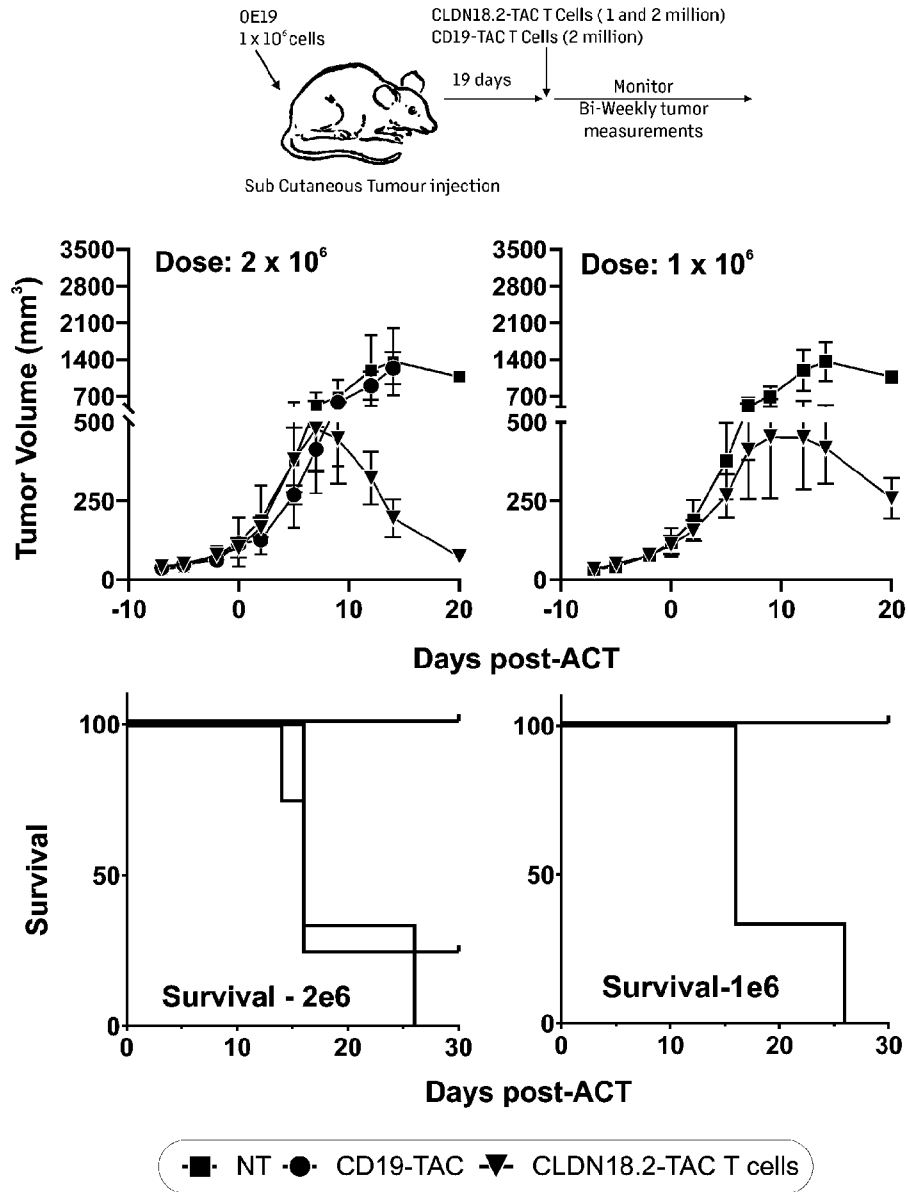


Figure 25A**Test article Dose: (3 and 6 million)**

CLDN18.2-TAC T Cells (Nanobody 2)

CLDN18.2-TAC T Cells (Nanobody 1)

CLDN18.2-TAC T Cells (Nanobody 2/Y177T)

CLDN18.2-TAC T Cells (Nanobody 1/Y177T)

Controls:NTD (match total CDLN18.2 T cell dose)(negative) HER2-TAC T cells (6 million) (positive)N87
 1×10^7 cells

Sub Cutaneous Tumour injection

14 days

Monitor
Bi-Weekly tumor
measurements

Figure 25B

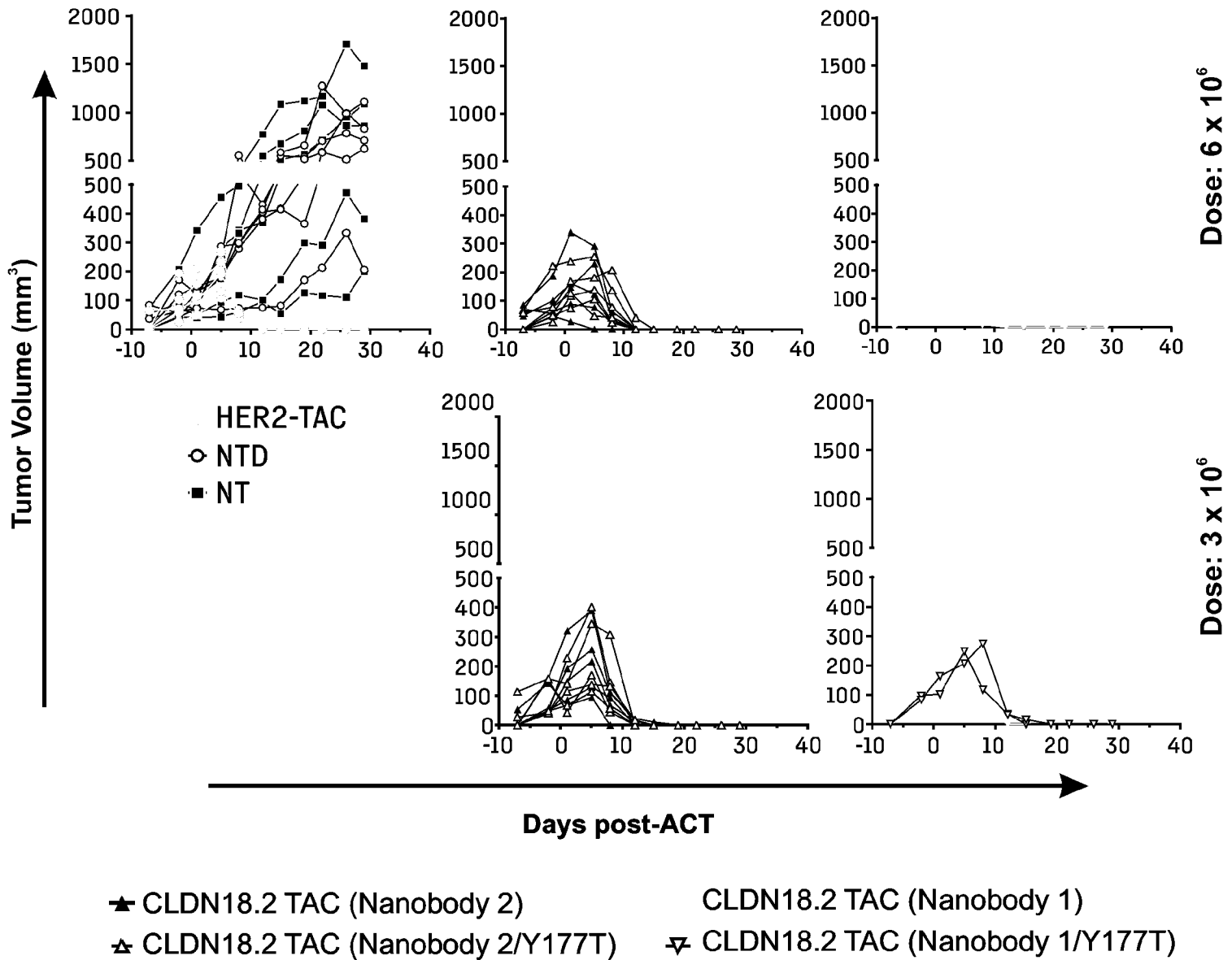


Figure 26A**Test article Dose: (1 and 2 million)**

CLDN18.2-TAC T Cells (Nanobody 2)

CLDN18.2-TAC T Cells (Nanobody 1)

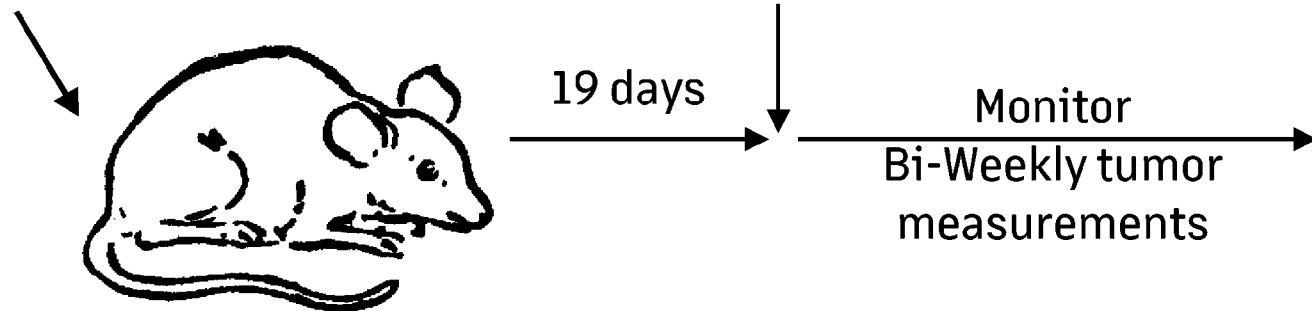
CLDN18.2-TAC T Cells (Nanobody 2/Y177T)

CLDN18.2-TAC T Cells (Nanobody 1/Y177T)

Controls:

CD19-TAC T Cells (2 million) (negative) NTD (2 million) (negative)

HER2-TAC T cells (6 million) (positive)

OE19
 1×10^6 cells

Sub Cutaneous Tumour injection

Figure 26B

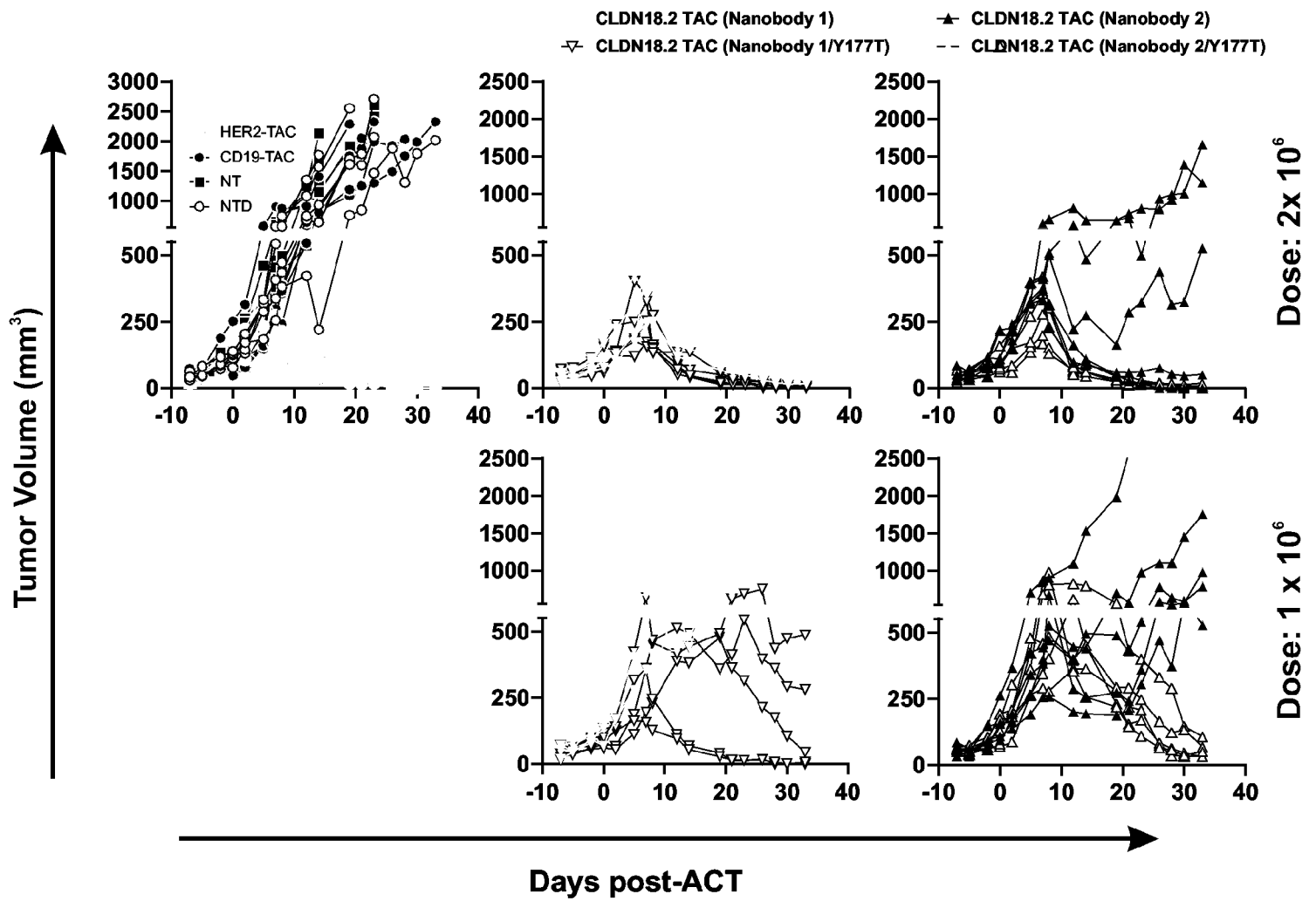


Figure 27A

Test Articles: Dose: (0.5, 1 and 2 million)

CLDN18.2-TAC T Cells (Nanobody 2)

CLDN18.2-TAC T Cells (Nanobody 1)

CLDN18.2-TAC T Cells (Nanobody 2/Y177T)

CLDN18.2-TAC T Cells (Nanobody 1/Y177T)

Controls:

CD19-TAC T Cells, Dose: (4 million), positive

HER2-TAC T Cells, Dose: (2 million), negative

NTD T Cells, Dose: (Total T cells matching 2 million TAC),
negative

NALM6^{CLDN18.2/eLUC}

5×10^5 cells



Orthotopic Tumour injection

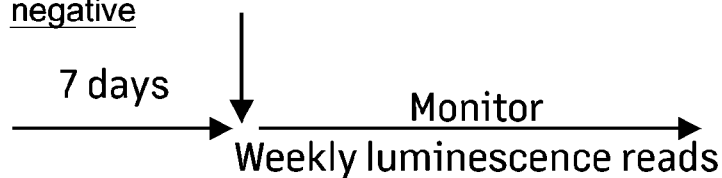


Figure 27B

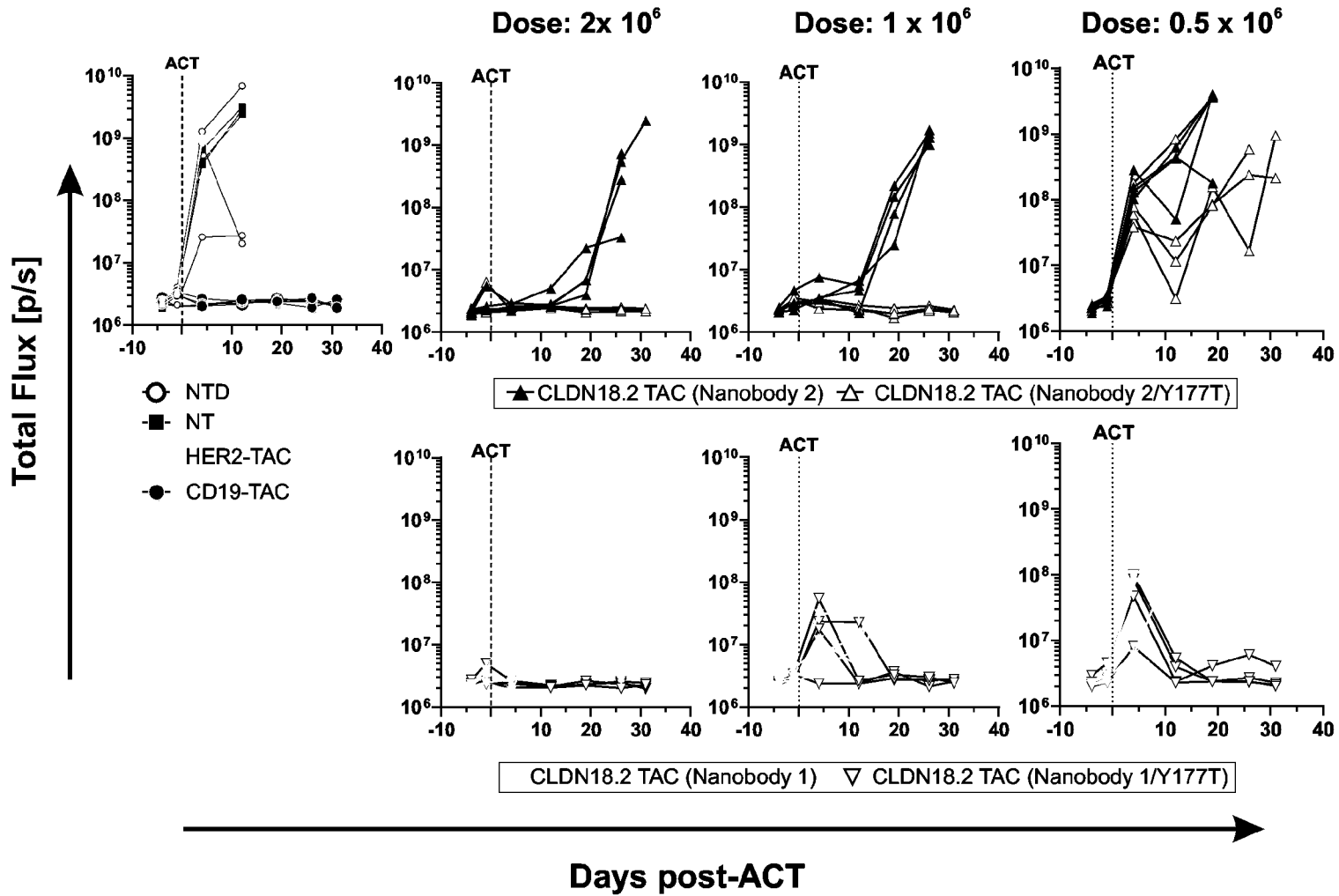


Figure 28A

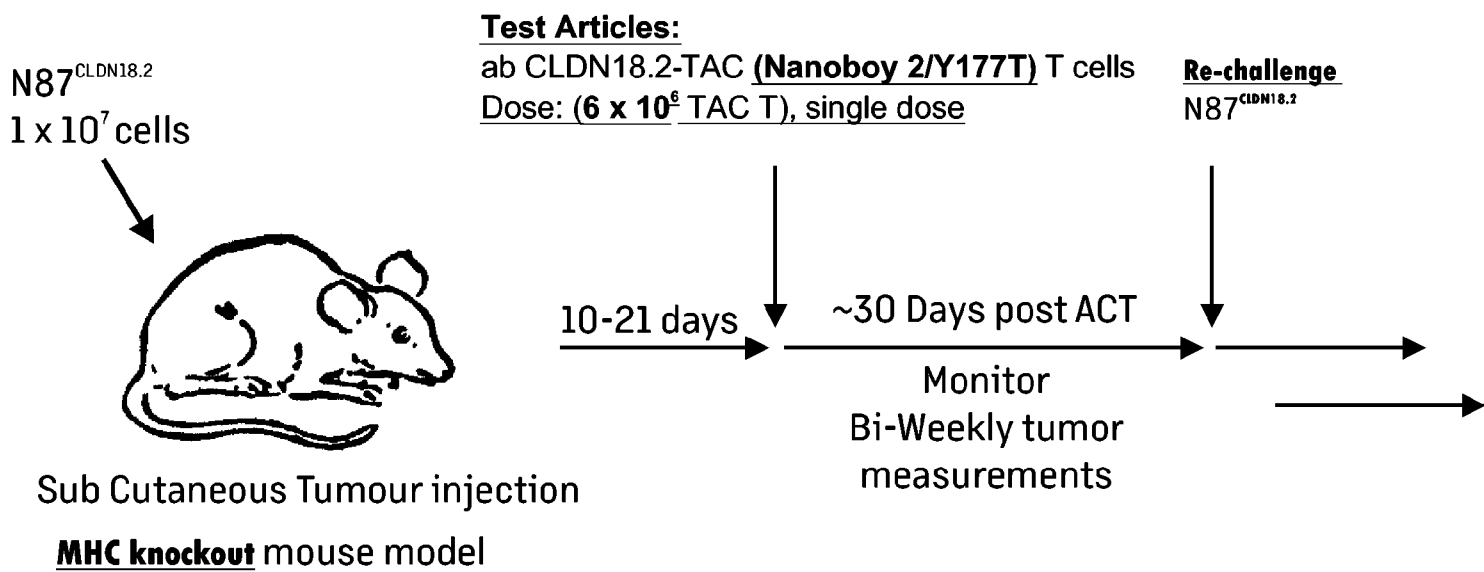


Figure 28B

