[0004] In another aspect, provided herein is a composition comprising a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises: (i) an MHC-peptide complex engager comprising: (a) a TCR alpha variable (TRAV) domain, and (b) a TCR beta variable (TRBV) domain; wherein the peptide in the MHC-peptide complex comprises a RAS peptide sequence; and (ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the T cell engager comprises an anti-CD3 binding domain comprising a heavy chain variable region (VH) that comprises a heavy chain complementarity determining region 3 (HC CDR3) sequence of ARGIYSDSSDFIGNL (SEQ ID NO: 713) or GIYSDSSDFIGNL (SEQ ID NO: 707). In some embodiments, the multispecific molecule comprises a post-translational modification. In some embodiments, the multispecific molecule comprises a mammalian glycosylation signature. In some embodiments, the multispecific molecule is soluble. In some embodiments, the multispecific molecule lacks a trans-membrane domain. In some embodiments, the multispecific molecule consists of two polypeptides, a first polypeptide and a second polypeptide. In some embodiments, the first polypeptide and the second polypeptide comprise one or more disulfide bonds between the two polypeptides. In some embodiments, the multispecific molecule comprises a first polypeptide and a second polypeptide, wherein the first polypeptide comprises the TRAV domain and the second polypeptide comprises the TRBV domain. In some embodiments, the peptide of the MHC-peptide complex is a peptide from a cancer antigen. In some embodiments, the peptide of the MHC-peptide complex is a mutant peptide, and wherein the MHC of the MHC-peptide complex binds the mutant peptide with a higher affinity compared to a corresponding wild-type peptide. In some embodiments, the peptide of the MHC-peptide complex does not comprise an amino acid sequence YLEPGPVTA (SEQ ID NO: 885). In some embodiments, the MHC of the MHC-peptide complex comprises a class I MHC. In some embodiments, the MHC of the MHC-peptide complex is a class I MHC polypeptide. In some embodiments, the MHC of the MHC-peptide complex is encoded by an HLA that is represented in less than 1% of a human population. In some embodiments, the MHC of the MHC-peptide complex has a peptide binding affinity greater than at least 50 nM. In some embodiments, the one or more binding domains of the T cell engager bind to an extracellular domain of an endogenous receptor expressed by a T cell. In some embodiments, the one or more binding domains of the T cell engager bind to an extracellular domain of a receptor selected from a group consisting of CD3, CD2, CD7, CD5, CD4, CD28, ICAM-1 and CD8. In some embodiments, the one or more binding domains of the T cell engager binds to CD3 delta, CD3 gamma or CD3 epsilon. In some embodiments, the one or more binding domains of the T cell engager comprises an antibody domain or antigen binding fragment thereof. In some embodiments, the binding domain of the T cell engager comprises an scFv or an sdAb. In some embodiments, the one or more binding domains of the T cell engager comprises a VHH.

[0005] In some embodiments, the one or more binding domains of the T cell engager comprises a first binding domain that binds to an extracellular domain of a first receptor expressed by a T cell and a second binding domain that binds to an extracellular domain of a second receptor expressed by a T cell. In some embodiments, the first binding domain of the T cell engager that binds to an extracellular domain of a first receptor expressed by a T cell comprises a first VHH, and the second binding domain of the T cell engager that binds to an extracellular domain of a second receptor expressed by a T cell comprises a second VHH. In one embodiment, the C-terminus of the first binding domain of the T cell engager is connected to the N-terminus of the second binding domain of the T cell engager; or the C-terminus of the first binding domain of the T cell engager is connected to the C-terminus of the second binding domain of the T cell engager. In some embodiments, the N-terminus of the first binding domain of the T cell engager is connected to the N-terminus of the second binding domain of the T cell engager; or the N-terminus of the first binding domain of the T cell engager is connected to the C-terminus of the second binding domain of the T cell engager. In some embodiments, the first binding domain of the T cell engager that binds to an extracellular domain of a first receptor expressed by a T cell comprises a scFv, and the second binding domain of the T cell engager that binds to an extracellular domain of a second receptor expressed by a T cell comprises a VHH. In some embodiments, the binding domain that binds to an extracellular domain of the first receptor expressed by a T cell and the second binding domain that binds to an extracellular domain of the second receptor expressed by a T cell are connected by a peptide linker. In some embodiments, the first binding domain binds to an extracellular domain of a first receptor expressed by a T cell selected from a group consisting of CD3, CD2, CD7, CD5, CD4, CD28, ICOS and CD8; and the second binding domain binds to an extracellular domain of a second receptor expressed by a T cell selected from a group consisting of CD3, CD2, CD7, CD5, CD4, CD28, ICOS and CD8. In some embodiments, the first receptor expressed by a T cell and the second receptor expressed by a T cell are different. In some embodiments, the first receptor expressed by a T cell is CD3 and the second receptor expressed by a T cell is CD2. In some embodiments, the first receptor expressed by a T cell is CD2 and the second receptor expressed by a T cell is CD3. In some embodiments, the first binding domain and the second binding domain of the T cell engager are configured to bind to the same receptor expressed by a T cell. In some embodiments, the VH of the anti-CD3 binding domain comprises a HC CDR2 sequence of IYTNNIGST (SEQ ID NO: 712) or CIYTNNIGSTWYASWAKG (SEQ ID NO: 706). In some embodiments, the VH of the anti-CD3 binding domain comprises a HC CDR1 sequence of GFSFSNNYY (SEQ ID NO: 711) or NNYYMC (SEQ ID NO: 705). In some embodiments, the anti-CD3 binding domain comprises a light chain variable region (VL) that comprises a light chain complementarity determining region 3 (LC CDR3) sequence of QQYNTIINVDRT (SEQ ID NO: 716). In some embodiments, the VL of the anti-CD3 binding domain comprises a LC CDR2 sequence of KAS (SEQ ID NO: 715) or KASTLAS (SEQ ID NO: 709). In some embodiments, the VL of the

[0010] In some embodiments, the T cell engager comprises an additional anti-CD3 binding domain. In some embodiments, the T cell engager further comprises an anti-TRBC1 binding domain. In some embodiments, the T cell engager further comprises an anti-CD2 binding domain.

[0011] In some embodiments, the T cell engager comprises an additional anti-CD3 binding domain comprising a heavy chain variable region (VH) that comprises a heavy chain complementarity determining region 3 (HC CDR3) sequence of ARYYDDHYCLDY (SEQ ID NO: 720), ARYYDDHYSLDY (SEQ ID NO: 721), YYDDHYCLDY (SEQ ID NO: 675) or YYDDHYSLDY (SEQ ID NO: 717). In some embodiments, the VH of the additional anti-CD3 binding domain comprises a HC CDR2 sequence of INPSRGYT (SEQ ID NO: 719) or YINPSRGYTNYNQKFKD (SEQ ID NO: 674).

[0012] In some embodiments, the VH of the additional anti-CD3 binding domain comprises a HC CDR1 sequence of GYTFTRYT (SEQ ID NO: 718) or RYTMH (SEQ ID NO: 673). In some embodiments, the additional anti-CD3 binding domain comprises a light chain variable region (VL) that comprises a light chain complementarity determining region 3 (LC CDR3) sequence of QQWSSNPLT (SEQ ID NO: 678). In some embodiments, the VL of the additional anti-CD3 binding domain comp In some embodiments, the VL of the additional anti-CD3 binding domain comprises a LC CDR1 sequence of SSVSY (SEQ ID NO: 722) or RASSSVSYMN (SEQ ID NO: 676). In some embodiments, the additional anti-CD3 binding domain comprises: a HC CDR1 sequence of GYTFTRYT (IMGT) (SEQ ID NO: 718), a HC CDR2 sequence of INPSRGYT (IMGT) (SEQ ID NO: 719), a HC CDR3 sequence of ARYYDDHYCLDY(IMGT) (SEQ ID NO: 720) or ARYYDDHYSLDY (SEQ ID NO: 721), a LC CDR1 sequence of SSVSY (SEQ ID NO: 722), a LC CDR2 sequence of DTS (SEQ ID NO: 881), and a LC CDR3 sequence of QQWSSNPLT (SEQ ID NO: 724).

[0013] In one example, the additional anti-CD3 binding domain comprises: a HC CDR1 sequence of RYTMH (SEQ ID NO: 673), a HC CDR2 sequence of YINPSRGYTNYNQKFKD (SEQ ID NO: 674), a HC CDR3 sequence of YYDDHYCLDY (SEQ ID NO: 675) or YYDDHYSLDY (SEQ ID NO: 717), a LC CDR1 sequence of RASSSVSYMN (SEQ ID NO: 676), a LC CDR2 sequence of DTSKVAS (SEQ ID NO: 677), and a LC CDR3 sequence of QQWSSNPLT (SEQ ID NO: 678). In some embodiments the VH of the additional anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to the sequence QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNY NQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSS (SEQ ID NO: 671) or QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNY NQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS (SEQ ID NO: 726). In some embodiments, the VL of the additional anti-CD3 binding domain

comprises a sequence with at least 80% sequence identity to the sequence QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRF SGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK (SEQ ID NO: 672). In some embodiments, the additional anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNY NQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSG GGGSGGGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGT SPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKL ELK (SEQ ID NO: 727) or QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRF SGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSGGGGSGGGGSG GGGSGGGGSQVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGY INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWG QGTTLTVSS (SEQ ID NO: 670). In some embodiments, the additional anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNY NQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSG GGGSGGGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGT SPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKL ELK (SEQ ID NO: 725) or QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRF SGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSGGGGSGGGGSG GGGSGGGGSQVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGY INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWG QGTTLTVSS (SEQ ID NO: 728).

[0014] In one example, the T cell engager comprises an additional anti-CD3 binding domain comprising a heavy chain variable region (VH) that comprises a heavy chain complementarity determining region 3 (HC CDR3) sequence of ARSGYYGDSDWYFDV (SEQ ID NO: 731) or SGYYGDSDWYFDV (SEQ ID NO: 607). In some embodiments, the VH of the additional anti-CD3 binding domain comprises a HC CDR2 sequence of INPYKGVS (SEQ ID NO: 730) or LINPYKGVSTYNQKFKD (SEQ ID NO: 606). In some embodiments, the VH of the additional anti-CD3 binding domain comprises a HC CDR1 sequence of GYSFTGYT (SEQ ID NO: 729) or GYTMN (SEQ ID NO: 605). In some embodiments, the additional anti-CD3 binding domain comprises a light chain variable region (VL) that comprises a light chain complementarity determining region 3 (LC CDR3) sequence of QQGNTLPWT (SEQ ID NO: 610). In some embodiments, the VL of the additional anti-CD3 binding

determining region 3 (HC CDR3) sequence of AAKIRPYIFKIAGQYDY (SEQ ID NO: 736) or KIRPYIFKIAGQYDY (SEQ ID NO: 618). In some embodiments, the VH of the additional anti-CD3 binding domain comprises a HC CDR2 sequence of IVWSDGNT (SEQ ID NO: 735) or AIVWSDGNTYYEDFVKG (SEQ ID NO: 617). In some embodiments, the VH of the additional anti-CD3 binding domain comprises a HC CDR1 sequence of GRTYRGYS (SEQ ID NO: 734) or GYSMA (SEQ ID NO: 616). In some embodiments, the additional anti-CD3 binding domain is a VHH domain. In some embodiments, the additional anti-CD3 binding domain comprises: a HC CDR1 sequence of GRTYRGYS (SEQ ID NO: 734), a HC CDR2 sequence of IVWSDGNT (SEQ ID NO: 735), and a HC CDR3 sequence of AAKIRPYIFKIAGQYDY (SEQ ID NO: 736). In some embodiments, the additional anti-CD3 binding domain comprises: a HC CDR1 sequence of GYSMA (SEQ ID NO: 616), a HC CDR2 sequence of AIVWSDGNTYYEDFVKG (SEQ ID NO: 617), and a HC CDR3 sequence of KIRPYIFKIAGQYDY (SEQ ID NO: 618). In some embodiments, the VH of the additional anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to the sequence EVQLVESGGGPVQAGGSLRLSCAASGRTYRGYSMAWFRQSPGKEREFVAAIVWSDGNTYY EDFVKGRFTISRDSAKNTLYLQMTNLKPEDTALYYCAAKIRPYIFKIAGQYDYWGQGTQVT VSS (SEQ ID NO: 615).

[0018] In one example, the T cell engager further comprises an anti-TRBC1 binding domain comprising a heavy chain variable region (VH) that comprises a heavy chain complementarity determining region 3 (HC CDR3) sequence of ARGAGYNFDGAYRFEDF (SEQ ID NO: 742) or GAGYNFDGAYRFEDF (SEQ ID NO: 739). In some embodiments, the VH of the anti-TRBC1 binding domain comprises a HC CDR2 sequence of INPYNDDI (SEQ ID NO: 741) or FINPYNDDIQSNERFRG (SEQ ID NO: 738). In some embodiments, the VH of the anti-TRBC1 binding domain comprises a HC CDR1 sequence of GYTFTGYV (SEQ ID NO: 740) or GYVMH (SEQ ID NO: 737). In some embodiments, the anti-TRBC1 binding domain comprises a light chain variable region (VL) that comprises a light chain complementarity determining region 3 (LC CDR3) sequence of SQSTHVPYT (SEQ ID NO: 745). In some embodiments, the VL of the anti-TRBC1 binding domain comprises a LC CDR2 sequence of RVS (SEQ ID NO: 747) or RVSNRFP (SEQ ID NO: 744). In some embodiments, the VL of the anti-TRBC1 binding domain comprises a LC CDR1 sequence of QRLVHSNGNTY (SEQ ID NO: 746) or RSSQRLVHSNGNTYLH (SEQ ID NO: 743).

[0019] In some embodiments, the anti-TRBC1 binding domain comprises: a HC CDR1 sequence of GYTFTGYV (SEQ ID NO: 740), a HC CDR2 sequence of INPYNDDI (SEQ ID NO: 741), a HC CDR3 sequence of ARGAGYNFDGAYRFEDF (SEQ ID NO: 742), a LC CDR1 sequence of QRLVHSNGNTY (SEQ ID NO: 746), a LC CDR2 sequence of RVS (SEQ ID NO: 747), and a LC CDR3 sequence of SQSTHVPYT (SEQ ID NO: 745). In one embodiment, the anti-TRBC1 binding domain comprises: a HC CDR1 sequence of GYVMH (SEQ ID NO: 737), a HC CDR2 sequence of FINPYNDDIQSNERFRG (SEQ ID NO: 738), a HC CDR3 sequence of GAGYNFDGAYRFEDF

(SEQ ID NO: 739), a LC CDR1 sequence of RSSQRLVHSNGNTYLH (SEQ ID NO: 743), a LC CDR2 sequence of RVSNRFP (SEQ ID NO: 744), and a LC CDR3 sequence of SQSTHVPYT (SEQ ID NO: 745).

[0020] In one embodiment, the anti-TRBC1 binding domain (VH) comprises a sequence with at least 80% sequence identity to the sequence EVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWVYKQRPGQGLEWIGFINPYNDDIQ SNERFRGKATLTSDKSSTTAYMELSSLTSEDSAVYYCARGAGYNFDGAYRFEDFWGQGTT LTVSS (SEQ ID NO: 625). In one embodiment, the VL of the anti-TRBC1 binding domain comprises a sequence with at least 80% sequence identity to the sequence DVVMTQSPLSLPYSLGDQASISCRSSQRLVHSNGNTYLHWYLQKPGQSPKLLIYRVSNRFPG VPDRFSGSGSGTDFTLKISRVEAEDLGIYFCSQSTHVPYTFGGGTKLEIKR (SEQ ID NO: 626). In some embodiments, the anti-TRBC1 binding domain comprises a sequence with at least 80% sequence identity to EVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWVYKQRPGQGLEWIGFINPYNDDIQ SNERFRGKATLTSDKSSTTAYMELSSLTSEDSAVYYCARGAGYNFDGAYRFEDFWGQGTT LTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPYSLGDQASISCRSSQRLVHSNGNTYLHWY LQKPGQSPKLLIYRVSNRFPGVPDRFSGSGSGTDFTLKISRVEAEDLGIYFCSQSTHVPYTFG GGTKLEIKR (SEQ ID NO: 624).

[0021] In some embodiments, the anti-TRBC1 binding domain comprises a sequence with at least 80% sequence identity to DVVMTQSPLSLPYSLGDQASISCRSSQRLVHSNGNTYLHWYLQKPGQSPKLLIYRVSNRFPG VPDRFSGSGSGTDFTLKISRVEAEDLGIYFCSQSTHVPYTFGGGTKLEIKRGGGGSGGGGSG GGGSEVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWVYKQRPGQGLEWIGFINPYN DDIQSNERFRGKATLTSDKSSTTAYMELSSLTSEDSAVYYCARGAGYNFDGAYRFEDFWGQ GTTLTVSS (SEQ ID NO: 748).

[0022] In one example, the T cell engager further comprises an anti-CD2 binding domain comprising a heavy chain variable region (VH) that comprises a heavy chain complementarity determining region 3 (HC CDR3) sequence of AAVRDYVGMPYYSGSAYEY (SEQ ID NO: 749) or VRDYVGMPYYSGSAYEY (SEQ ID NO: 750). In some embodiments, the VH of the anti-CD2 binding domain comprises a HC CDR2 sequence of IRPGTIP (SEQ ID NO: 751) or AIRPGTIPYYSESVKG (SEQ ID NO: 752). In some embodiments, the VH of the anti-CD2 binding domain comprises a HC CDR1 sequence of GLTFSSYG (SEQ ID NO: 753) or SYGMA (SEQ ID NO: 754). In some embodiments, the anti-CD2 binding domain is a VHH domain. In some embodiments, the anti-CD2 binding domain comprises: a HC CDR1 sequence of GLTFSSYG (SEQ ID NO: 753), a HC CDR2 sequence of IRPGTIP (SEQ ID NO: 751), and a HC CDR3 sequence of AAVRDYVGMPYYSGSAYEY (SEQ ID NO: 749).

[0050] In some embodiments, the first binding domain of the T cell engager is a first VHH that is fused to the first polypeptide and binds to CD3; and the second binding domain of the T cell engager is a second VHH fused to the second polypeptide and binds to CD2.

[0051] In some embodiments, the first binding domain of the T cell engager is a first VHH that binds to CD2; and the second binding domain of the T cell engager is a second VHH that binds to CD3.

[0052] In some embodiments, the T cell engager is fused with the first or second polypeptide via a peptide linker.

[0053] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain and a TCR alpha constant (TRAC) domain, and (b) a TCR beta variable (TRBV) domain and a TCR beta constant (TRBC) domain; and (ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the one or more binding domains of the T cell engager, the TRBV domain, the TRBC domain, the TRAV domain, and the TRAC domain. In some embodiments, the multispecific molecule further comprises a linker connecting the TRBC domain and the TRAV domain. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the linker comprises 20-38 amino acid sequences. In some embodiments, the linker is a structure guided flexible linker. In some embodiments, the linker comprises Gly, Ser, Ala and/or Glu residues.

[0054] In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 862 without a signal peptide of SEQ ID NO: 668. In some embodiments, the multispecific molecule comprises one or more sequences of SEQ ID NO: 604, SEQ ID NO: 682, SEQ ID NO: 603, (SEQ ID NO: 683) (GGGGS), SEQ ID NO: 620, SEQ ID NO: 863, SEQ ID NO: 864, SEQ ID NO: 619, SEQ ID NO: 865, SEQ ID NO: 866, and SEQ ID NO: 884. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 862. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 862 without a signal peptide of SEQ ID NO: 668.

[0055] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain and a TCR alpha constant (TRAC) domain, and (b) a TCR beta variable (TRBV) domain and a TCR beta constant (TRBC) domain; and (ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the one or more binding domains of the T cell engager, the TRAV domain, the TRAC domain, the TRBV domain, and the TRBC domain. some embodiments, the multispecific molecule

further comprises a linker connecting the TRAC domain and the TRBV domain. In some embodiments, the linker comprises between 20-38 amino acids, comprising Gly, Ser, Ala and/or Glu amino acids. In some embodiments, the one or more binding domains of the T cell engager comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises, from N-terminus to C-terminus, a light chain variable domain (VL) followed by a heavy chain variable domain (VH). In some embodiments, the scFv comprises, from N-terminus to C-terminus, a heavy chain variable domain (VH) followed by a light chain variable domain (VL).

[0056] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain and a TCR alpha constant (TRAC) domain, and (b) a TCR beta variable (TRBV) domain and a TCR beta constant (TRBC) domain; and (ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the TRAV domain, the TRAC domain, the one or more binding domains of the T cell engager, the TRBV domain, and the TRBC domain. . In some embodiments, the multispecific molecule further comprises a linker connecting the TRAC domain and the one or more binding domains of the T cell engager. . In some embodiments, the one or more binding domains of the T cell engager comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises, from N-terminus to C-terminus, a light chain variable domain (VL) followed by a heavy chain variable domain (VH). In some embodiments, the scFv comprises, from N-terminus to C-terminus, a heavy chain variable domain (VH) followed by a light chain variable domain (VL).

[0057] In some embodiments, the multispecific molecule comprises one or more sequences selected from the group consisting of SEQ ID NO: 619, SEQ ID NO: 868, SEQ ID NO: 869, SEQ ID NO: 604, SEQ ID NO: 870, SEQ ID NO: 603, SEQ ID NO:620, SEQ ID NO: 871, SEQ ID NO: 866, and SEQ ID NO: 884. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 867. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 867 without a signal peptide of SEQ ID NO: 667.

[0058] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises:(i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain and a TCR alpha constant (TRAC) domain, and (b) a TCR beta variable (TRBV) domain and a TCR beta constant (TRBC) domain; and (ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the TRBV domain, the TRBC domain, the one or more binding domains of the T cell

engager, the TRAV domain, and the TRAC domain. In some embodiments, the multispecific molecule further comprises a linker connecting the TRBC domain and the one or more binding domains of the T cell engager, wherein the linker comprises 20-37 amino acids and comprises Gly, Ser, Ala and/or Glu amino acids.

[0059] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain, and (b) a TCR beta variable (TRBV) domain; and (ii) a T cell engager comprising a single chain variable fragment (scFv) that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the scFv, the TRBV domain, and the TRAV domain. In some embodiments, the recombinant nucleic acid further comprises a TCR alpha constant (TRAC) domain. In some embodiments, the recombinant nucleic acid further comprises a TCR beta constant (TRBC) domain. In some embodiments, the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the scFv, the TRBV domain, the TRBC domain, the TRAV domain and the TRAC domain. In some embodiments, the recombinant nucleic acid further the T cell engager comprises a VL domain and a VH domain from N-terminus to C-terminus. In some embodiments, the recombinant nucleic acid further the T cell engager comprises a VH domain and a VL domain from N-terminus to C-terminus.

[0060] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain, and (b) a TCR beta variable (TRBV) domain; and (ii) a T cell engager comprising a single chain variable fragment (scFv) that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the scFv, the TRAV domain, and the TRBV domain.

[0061] In some embodiments the recombinant nucleic acid further comprises a TCR alpha constant (TRAC) domain. In some embodiments the recombinant nucleic acid further comprises a TCR beta constant (TRBC) domain. In some embodiments, the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the scFv, the TRBV domain, the TRBC domain, the TRAV domain and the TRAC domain. In some embodiments, the T cell engager comprises a VL domain and a VH domain from N-terminus to C-terminus. In some embodiments, the T cell engager comprises a VH domain and a VL domain from N-terminus to C-terminus.

[0062] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain, and (b) a TCR beta

variable (TRBV) domain; and (ii) a T cell engager comprising a single chain variable fragment (scFv) that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the TRAV domain, the scFv, and the TRBV domain. In some embodiments the recombinant nucleic acid further comprises a TCR alpha constant (TRAC) domain. In some embodiments the recombinant nucleic acid further comprises a TCR beta constant (TRBC) domain.

[0063] In some embodiments, the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the scFv, the TRAV domain, the TRAC domain, the TRBV domain and the TRBC domain. In some embodiments, the T cell engager comprises a VL domain and a VH domain from N-terminus to C-terminus. In some embodiments, the T cell engager comprises a VH domain and a VL domain from N-terminus to C-terminus.

[0064] In one example, provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain, and (b) a TCR beta variable (TRBV) domain; and (ii) a T cell engager comprising a single chain variable fragment (scFv) that bind to an extracellular domain of a receptor expressed by a T cell, wherein the MHC-peptide complex engager and the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the TRBV domain, the scFv, and the TRAV domain. In some embodiments, the recombinant nucleic acid further comprises a TCR alpha constant (TRAC) domain. In some embodiments, the recombinant nucleic acid further comprises a TCR beta constant (TRBC) domain. In some embodiments, the T cell engager form a continuous single polypeptide chain comprising, from N-terminus to C-terminus, the scFv, the TRAV domain, the TRAC domain, the TRBV domain and the TRBC domain. In some embodiments, the T cell engager comprises a VL domain and a VH domain from N-terminus to C-terminus. In some embodiments, the T cell engager comprises a VH domain and a VL domain from N-terminus to C-terminus.

[0065] In some embodiments, the recombinant nucleic acid further encodes a peptide linker, wherein the linker comprises an amino acid sequence having 90% sequence identity with the sequence of SEQ ID NO: 864, or 872 or 873. In some embodiments, the TRAC domain comprises a wildtype murine TRAC sequence or a wildtype human TRAC sequence. In some embodiments, the TRAC domain does not comprise a mutation. In some embodiments, the TRAC domain comprises a mutation compared to a murine TRAC sequence or a wildtype human TRAC sequence. In some embodiments, the mutation is a stability enhancing mutation. In some embodiments, the mutation is selected from the group consisting of S139F, T150I and A190T, the residue positions are numbered based on Kabat numbering scheme.

[0066] In some embodiments, the TRBC domain comprises a wildtype murine TRBC sequence or a wildtype human TRBC sequence. In some embodiments, the TRBC domain does not comprise a

mutation compared to the wildtype sequence. In some embodiments, the TRBC domain comprises a mutation compared to a murine TRBC sequence or a wildtype human TRBC sequence. In some embodiments, the mutation is a stability enhancing mutation. In some embodiments, the mutation is selected from the group consisting of E134K, H139R, D155P and S170D, the residue positions are numbered based on Kabat numbering scheme.

[0067] In one example, provided herein is a composition comprising the multispecific molecule encoded by the sequence of the recombinant nucleic acid of the composition of any one of the embodiments described above.

[0068] In some embodiments, the multispecific molecule is isolated or purified.

[0069] Provided herein is a pharmaceutical composition comprising the composition of any one of the embodiments described above.

[0070] In one example, provided herein is a method of treating cancer in a subject in need thereof comprising administering a therapeutically effective amount of the pharmaceutical described above.

[0071] In one example, provided herein is a method of making a multispecific molecule, wherein the multispecific molecule is an engineered T cell receptor (TCR) construct comprising: (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain, and (b) a TCR beta variable (TRBV) domain; and (ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the multispecific molecule comprises a post-translational modification; wherein the method comprises expressing the multispecific molecule from a recombinant nucleic acid comprising a sequence encoding the multispecific molecule in a mammalian cell, thereby producing a multispecific molecule comprising a mammalian post-translational modification signature. In one embodiment, the method further comprises isolating or purifying the multispecific molecule comprising the mammalian post-translational modification signature. In some embodiments, the cell secretes the first polypeptide and the second polypeptide encoded by the sequence of the recombinant nucleic acid of the composition described above.

[0072] Provided herein is a mammalian cell comprising the recombinant nucleic acid of the composition of any one of the embodiments described above**.**

INCORPORATION BY REFERENCE

[0073] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWING

[0074] The salient features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also "FIG." herein), of which:

[0075] **FIG. 1A** is a schematic that illustrates in word diagram an overview of the work undertaken.

[0076] **FIG. 1B** shows a schematic diagram of a plasmid, expression and tetramer binding assay. SP, signal peptide. Flow cytometry scan is for illustration purpose only and is not data.

[0077] **FIG. 1C** shows expression of TCR library, enrichment and tetramer assay.

[0078] **FIG. 1D** shows a schematic tetramer and monomer assays.

[0079] **FIG. 2** is a schematic that shows a general overview of various experimental bispecific sTCR scaffold designs.

[0080] At center, a schematic diagram shows a p-MHC complex presented by a tumor cell on the top, a multispecific engager (soluble TCR) on the left, comprising TRAV and TRBV domains (an MHC-peptide complex engager) for association with the p-MHC, graphically indicated by the bidirectional arrow. The sTCR TRAC and a TRBC domains form a scaffold, that are interconnected by disulfide bonds at positions 1 and 2 as marked on the schematic diagrams. The T cell engager comprises an anti-CD3 scFv, capable to binding CD3, a surface molecule on a T cell, and is attached to a variable domain via a short peptide linker.

[0081] Top left, a bispecific sTCR as described above, with a CD3 specific scFv as T cell engager attached to TRBV.

[0082] Bottom left, similar to top left, but with the CD3 specific scFv as T cell engager attached to TRAV.

[0083] Top right, a bispecific sTCR as described above, with a CD3 specific scFv as T cell engager attached to TRBC.

[0084] Bottom right, a bispecific sTCR as described above, with a CD3 specific scFv as T cell engager attached to TRAC.

[0085] **FIG. 3** features schematic representations of ten different sTCR scaffold structures constructed and tested. Scaffold 2, TRAV-TRBV make up the p-MHC engager, is the basic structure for evaluating stability, with purification tag 10X HIS (SEQ ID NO: 866) and affinity acceptor molecule Biotin Acceptor Protein (BAP) tag. Positions 1 and 2 indicate inter-chain disulfide linkages. Scaffold 5 illustrates two sets of TCRs being connected to IgG domains. Each sTCR comprises two chains- an alpha chain comprising TRAV-TRAC; and a beta chain comprising a TRBV and TRBC. Scaffold 6 comprises a TRAV and a TRAC that are stabilized by two scaffolding polypeptides having one inter-chain disulfide bond. The two scaffolding polypeptides are immunoglobulin Fv domains arranged in an Fv

clasp. Scaffold 14 comprises the sTCR fused to a immunoglobulin (Ig) Fc structure. The Fc portions are engineered and comprise knob-and-hole configurations for increased stability in bound form. Scaffold 19 comprises a tagged sTCR molecule comprising the TRAV-TRAC-TRBV-TRBC tetrameric structure, fused to an anti-CD3 scFv via a short linker peptide. Scaffold 20 has a basic structural similarity to Scaffold 6, and in addition comprises an scFv attached to TRBV via a short linker peptide. Scaffold 21 comprises the basic structure similar to Scaffold 14, and further comprises an scFv attached to TRBV via a short linker peptide. Scaffold 36 comprises the basic sTCR configuration of Scaffold 2 and is linked to two scFvs in tandem attached to TRBV via peptide linkers. Scaffold 37 comprises a basic tetrameric sTCR configuration, where the TRAC and TRBC are attached tags BAP, Sortase A and 10X HIS (SEQ ID NO: 866). Scaffold 43 comprises the basic tetrameric sTCR structure connected to Ig Fc domains. Arrow points to protease target sites that are used for cleavage of the sTCR from the Ig domains.

[0086] **FIG. 4** upper panel shows a diagrammatic representation of the experimental protocol involving transfection of the constructs and purification. A representative gel filtration result for verification of purity of the product is shown at upper right. Lower panel indicates the workflow and timeline of tumor cell killing by effector T cells in an in vitro assay.

[0087] **FIGs. 5A** and **5B** shows an exemplary representative data from generation and purification of Scaffold 2, showing gel electrophoresis of the purified sTCRs from cell culture media (FIG. 5A), and single protein peak at mid-elusion (FIG. 5B) respectively.

[0088] **FIG. 6** shows a schematic layout of the workflow for target cell cytotoxicity assay, for testing the functional efficacy of the soluble TCR constructs. T cells were obtained from peripheral blood samples of healthy donor. A375 cells transfected with polynucleotides encoding the peptide and the MHC, and expressing the polypeptides were used as target cells. Purified sTCR products were added to culture at the concentrations indicated for assaying T cell activation and cytotoxicity, measured by loss of GFP positive target cells compared to untreated control.

[0089] **FIG. 7A** shows structural design of Scaffold 19 (Ros9a Scaffold 19) construct having TCR alpha and beta variable domains capable of binding mutated RAS epitope bound to MHC encoded by HLA-C:08:02.

[0090] **FIG. 7B** shows cytotoxicity data using the method outlined in the workflow layout of FIG. 6. Cytotoxicity was measured by the area with total number of GFP-positive cells (=total green objects), which gives a measure of live cells. Accordingly higher cytotoxicity correlates with lower green cells. Figure discloses SEQ ID NO: 999.

[0091] **FIG. 8A** shows structural design of a Scaffold 20b (Ros9a Scaffold 20b) construct having TCR alpha and beta variable domains capable of binding mutated RAS epitope bound to MHC encoded by HLA-C:08:02. **FIG. 8B** shows cytotoxicity data. Figure 8B discloses SEQ ID NO: 999.

[0092] **FIG. 9A** shows structural design of a Scaffold 21 (Ros9a Scaffold 21) construct having TCR alpha and beta variable domains capable of binding mutated RAS epitope bound to MHC encoded by HLA-C:08:02. **FIG. 9B** shows cytotoxicity data. Figure 9B discloses SEQ ID NO: 999.

[0093] **FIG. 10A** shows structural design of Scaffold 19 (Ros9d Scaffold 19) construct having TCR alpha and beta variable domains capable of binding mutated RAS epitope bound to MHC encoded by HLA-C:08:02. **FIG. 10B** shows cytotoxicity data. Figure 10B discloses SEQ ID NO: 999.

[0094] **FIG. 11A** shows structural design of Scaffold 21 (Ros9d Scaffold 21) construct having TCR alpha and beta variable domains capable of binding mutated RAS epitope bound to MHC encoded by HLA-C:08:02. **FIG. 11B** shows cytotoxicity data. Figure 11B discloses SEQ ID NO: 999.

[0095] **FIG. 12A** shows structural design of Scaffold 19 (Ros10 Scaffold 19) construct having TCR alpha and beta variable domains capable of binding mutated RAS epitope bound to MHC encoded by HLA-C:08:02. **FIG. 12B** shows cytotoxicity data. Figure 12B discloses SEQ ID NO: 997.

[0096] **FIG. 13** shows a schematic layout of the workflow for target cell cytotoxicity assay, for testing the functional efficacy of the soluble TCR constructs. T cells were obtained from peripheral blood samples of healthy donor. Target cells are tumor cells of HPAC cell line which endogenously express the mutated Ras epitope and the HLA-C:08:02. Purified sTCR products were added to culture at the concentrations indicated for assaying T cell activation and cytotoxicity, measured by apoptosis marker Annexin V and Caspase 3/7 positive target cells compared to untreated control.

[0097] **FIGs. 14A** and **14B** show imaging (FIG. 14A) and quantitative data (FIG. 14B) from the imaging assay of a comparison between Ros9a Scaffold 19 and Ros 10 scaffold 19 treatments showing annexin positive cells (orange) and caspase 3/7 positive cells (green) at different peptide concentrations. **FIG. 14C** shows imaging with only Annexin V positive cells.

[0098] **FIGs. 15A-15B** show cytotoxicity assay with donor's T cells that were rested for shorter period, showing increase in target cell cytotoxicity.

[0099] **FIG. 16** illustrates schematic representations of various multispecific engager scaffolds having an anti-CD3 scFv and an additional T cell engager, comprising a CD8a ectodomain.

[0100] **FIG. 17** illustrates schematic representations of various multispecific engager scaffolds having an anti-CD3 or anti-CD2 VHH domains. The amino acid sequences of Scaffolds 53, 55 and 57 are disclosed elsewhere in the specification, e.g. Table 10.

[0101] **FIG. 18A-18B** illustrates schematic representations of various multispecific engager scaffolds designed for testing. are fused with the basic tetrameric sTCR structure via short peptide linkers. Figure 18B discloses "10XHis" as SEQ ID NO: 866, "GGGGS" as SEQ ID NO: 683, and "$(GGGGS)_3$" as SEQ ID NO: 690.

[0102] Scaffolds 45-48 contain a basic tetrameric structure in which TRAV and TRBV are linked to immunoglobulin constant regions. Heteromeric Igs are included for the two chains. Scaffolds 46 and 48 comprise disulfide bonds to present at position 2, which is omitted in Scaffolds 46 and 48.

[0103] Scaffold 49-51 was designed to include anti -CD3 scFv attached to TRAV or TRBV. In Scaffold 50, TRAV and TRBV are initially linked but comprises a cleavage sequence (F2A). Scaffold 49 comprises two polypeptides, the first polypeptide has the following orientation NH2-aCD3scFv_VH-TRAV-(linker+tag)-COOH, where aCD3scFv stands for antiCD3scFv; the second polypeptide has the following orientation NH2-TRBV-aCD3scFv_VL -(linker+tag)-COOH. Scaffold 50 is generated as a single polypeptide with a cleavable sequence (F2A) in between: NH2-aCD3scFv_VH-TRAV-(F2A)-TRBV--aCD3scFv_VL -(linker+tag)-COOH. Scaffold 51 is a single polypeptide with a linker in connecting the two juxtaposition chains: NH2-aCD3scFv_VH-TRAV-(linker)-TRBV--aCD3scFv_VL -(linker+tag)-COOH. Scaffold 52 is a single polypeptide with a linker in connecting the two juxtaposition chains: NH2- TRAV--aCD3scFv_VH--(linker)-aCD3scFv_VL-TRBV- (linker+tag)-COOH.

[0104] **FIG. 19** illustrates schematic representations of various multispecific engager scaffolds designed for testing. Scaffolds 103, 106 and 137, 108 and 138 comprises the basic tetrameric sTCR structure. Of these, Scaffold 138 has an additional domain that binds TRBC1, in addition to an anti-CD3VHH. Scaffolds 139 and 142 are newer experimental designs that do not contain the basic tetrameric sTCR structure. In Scaffolds 139, each of the TRAV and TRBV are linked via a short peptide linker to an anti-CD3 VHH. Scaffold 142 comprises an anti-CD3 VHH domain attached to one of TRAV and TRBV, and an anti-TRBC1 scFv attached to the other of TRAV and TRBV via short peptide linkers. Figure discloses SEQ ID NO: 690.

[0105] **FIG. 20** illustrates schematic representations of various multispecific engager scaffolds designed for testing. Figure discloses SEQ ID NO: 690.

[0106] **FIG. 21** illustrates schematic representations of a multispecific engager scaffolds having one or more VHH T cell engagers. In these scaffold designs, the linker between the VHH and the TCR is an extended linker.

[0107] **FIG. 22** shows a schematic layout of the workflow for target cell cytotoxicity assay for testing the functional efficacy of the soluble TCR constructs built on Scaffold 22. T cells were obtained from peripheral blood samples of healthy donor. A375 cells transfected with polynucleotides encoding the peptide and the MHC, and expressing the polypeptides were used as target cells. Purified sTCR products were added to culture at the concentrations indicated for assaying T cell activation and cytotoxicity, measured by loss of GFP positive target cells compared to untreated control.

[0108] **FIG. 23** shows the structure and assay results to test the cytotoxic potential of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on TR66-VHVL construct. Figure discloses SEQ ID NOS 999 and 755, respectively, in order of appearance.

[0109] **FIG. 24** shows the structure and assay results to test the cytotoxic potential of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on TR66-VLVH construct. Figure discloses SEQ ID NOS 999 and 763, respectively, in order of appearance.

[0110] **FIG. 25** shows the structure and assay results to test the cytotoxic potential of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on 210112-VHVL construct. Figure discloses SEQ ID NOS 999 and 769, respectively, in order of appearance.

[0111] **FIG. 26** shows the structure and assay results to test the cytotoxic potential of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on 210112-VLVH construct. Figure discloses SEQ ID NOS 999 and 1038, respectively, in order of appearance.

[0112] **FIG. 27** shows the structure and assay results to test the cytotoxic potential of the construct Ros9d Scaffold 22. The anti-CD3 arm is based on TR66-VHVL construct. Figure discloses SEQ ID NOS 999 and 781, respectively, in order of appearance.

[0113] **FIG. 28** shows the structure and assay results to test the cytotoxic potential of the construct Ros9d Scaffold 22. The anti-CD3 arm is based on 210112-VHVL construct. Figure discloses SEQ ID NOS 999 and 786, respectively, in order of appearance.

[0114] **FIG. 29** shows a schematic diagram of the workflow for assaying cytotoxic potential of the sTCR construct against tumor cell lines with endogenous KRAS G12D, an assay similar to as shown in FIG. 13.

[0115] **FIG. 30** shows imaging of target HPAC tumor cells treated with Ros9a Scaffold 22 sTCR construct in presence of T cells, showing annexin positive cells (orange) and caspase 3/7 positive target apoptotic cells (green) at different peptide concentrations.

[0116] **FIG. 31** shows quantitative assessment results from the analysis of cytotoxicity assay of FIG. 30.

[0117] **FIG. 32** shows results from imaging analysis when target cells in the assay were stained from Annexin V alone.

[0118] **FIG. 33** shows an assay similar to that shown in FIGs. 15A and 15B. The donor's T cells that were rested for shorter period than in the protocol for FIGs. 30-32, and this improves target cell cytotoxicity.

[0119] **FIG. 34** shows a graph representing the quantitative assessments of the assay results shown in FIG. 33.

[0120] **FIG. 35A** shows graphical representation of structures of single chain soluble bispecific TCR (BiTE) constructs designed to enable mRNA formatting and delivery, and simplifying the development pipeline. In this design, a TRBC is connected to a TRAV fragment forming a single polypeptide chain comprising both TCR alpha and beta chains with another linker connecting an anti-CD3 scFv. The general framework of the structures are based on primary scaffold structures described herein. In some cases, as shown in the Figure, the linker between the TRBC and TRAV is a GS linker, $(G4S)_7$ (SEQ ID NO: 864). **Top left**: Scaffold Name: Ros9a_SC107βα_antiCD3. Graphical representation of a construct having one or more point mutations in the constant (TRAC and TRBC) regions, that enhance the stability of the polypeptide. Additional mutations are designed to remove glycosylation sites. **Top right**: Scaffold name Ros9a_SC107wβα_antiCD3. Graphical representation of a construct that is devoid of several mutations in the constant domains for stability enhancing purpose as shown in the top left model. AlphaFold 3D modeling of the protein structure depicting spatial orientation of the domains of the polypeptide is shown at the bottom. Nt, N terminus; Ct, C terminus. The designs leave an anti-CD3 scFv N terminal end free. The TCR has an N terminal to C terminal orientation of its alpha and beta chains such that beta chain is N terminal to the alpha chain. Figure discloses "$(G_4S)_7$" as SEQ ID NO: 864 and "10XHis" as SEQ ID NO: 866.

[0121] FIG. 35B shows schematic diagrams for single chain soluble TCR designs, **Top left**, Scaffold name: Ros9a_SC111αβ_antiCD3, with stabilizing mutations in the constant domain; **Top right** Scaffold name: Ros9a_SC111wαβ_antiCD3, and does not contain the stability enhancing constant domain mutations. AlphaFold model image at the bottom, in which a linker (such as $(G_4S)_4$ (SEQ ID NO: 760)) connects the N terminal end of the anti-CD3 scFv with the C terminal end of TRBC, forming the single chain with an internal ant-CD3 scFv, which access the same position as in the construct shown in FIG. 35A, but not constrained by the linker. In this case the alpha chain is N terminal to the beta chain. Figure discloses "10XHis" as SEQ ID NO: 866 and "$(G_4S)_4$" as SEQ ID NO: 760.

[0122] FIG. 36 (top) shows a construct design of soluble BiTE (e.g., an mRNA encoding the polypeptide) having domain layout from N terminus to C terminus, N terminal anti-CD3 scFv comprising variable light chain (VL) and variable heavy chain (VH) domains, followed by a linker connecting the scFv with TCR beta chain comprising TRBV fused to TRBC, which is connected by a second longer linker to TCR alpha chain comprising TRAV fused to TRAC. Exemplary construct, Ros9a_SC107wβα_antiCD3. An exemplary construct Ros9a_SC107wβα_antiCD3 may have a sequence of SEQ ID NO: 862. SEQ ID NO: 862 lacks stability enhancing mutations S>F, T>I and A>T in TCR alpha contact (TRAC) region as shown in Table 12. **FIG. 36** (bottom) shows a schematic of the structure of the polypeptide. Dotted line denotes the linker creating the single chain. Figure discloses "G_4S" as SEQ ID NO: 683, "$(G_4S)_7$" as SEQ ID NO: 864, and "10XHis" as SEQ ID NO: 866.

[0123] FIG. 37 (top) shows a soluble BiTE construct design with layout of domains from N terminus to C terminus, e.g., an mRNA encoding the specific domains: N terminal TCR alpha chain comprising TRAV fused to TRAC, followed by a linker connecting the TCR alpha with anti-CD3 scFv comprising variable light chain (VL) and variable heavy chain (VH) domains, followed by a linker connecting the scFv with TCR beta chain comprising TRBV fused to TRBC. Exemplary Construct Ros9a_SC111wαβ_antiCD3, **FIG. 37** (bottom) shows a schematic structure of the polypeptide. Figure discloses "$(G_4S)_4$" as SEQ ID NO: 760, "G_4S" as SEQ ID NO: 683, and "10XHis" as SEQ ID NO: 866.

[0124] FIG. 38 (left) shows design and exemplary AlphaFold 3D modeling structure of a single polypeptide chain sTCR having a peptide linker connecting the TCR alpha and beta chains. Different linkers, H1 (linker 1), H2 (Linker 2) or GS linker (35GS) were tested, and for polypeptides in which the alpha and beta chain are in an alpha-beta as well as in a beta-alpha orientation from N terminus to C terminus. Cell surface expression and peptide binding assays for each construct were tested. Results shown in the graph at the right side.

[0125] FIG. 39A shows a schematic protocol for assaying the cytotoxic potential of the BiTE sTCRs. Single polypeptide chains having TCR alpha and beta domains and anti-CD3 scFv were cocultured with T cells (effectors) and target cancer cell lines (e.g., A375 cells) and cell death of the target cells were measured. PBMCs from three healthy donors were isolated, rested for one day in non-activating media before assay. In alternate assay set ups, shorter rest (3-5 hrs) were also tested. In alternative sets, T cells were isolated from PBMCs by negative selection ("untouched" purified T cells) and the assay was performed. (E:T, effector cell to target cell ratio).

[0126] **FIG. 39B** shows data depicting comparison of two sTCR BiTE constructs, one with Fc fusion at the TCR constant domains (top figure) , the other without Fc fusion (bottom figure). E, $CD3^+$ (effector) cells expanded and O/N rested; T, (target cancer cell line) A375 transduced to express KRAS G12D + HLA C*08:02. The BiTE is a Ros9a sTCR based construct, added to the culture at a concentration ranging between 100 nM – 0.4 nM. The data shows substantially less cytotoxic potential of the Fc fused construct.

[0127] **FIG. 40** shows data comparing efficacy of the indicated single chain sTCR constructs versus a double chain sTCR construct in promoting T cell mediated target cell cytotoxicity. Upper left, T cell cytotoxicity data using an sTCR construct Ros9aS74_antiCD3, which comprises two separate TCR chains (alpha and beta chains); and which is shown as a control for assessing cytotoxic potential of single chain constructs designed herein. For example, Upper right, cytotoxicity data using single chain sTCR construct Ros9a_SC107_antiCD3, inset showing graphical view of Ros9a_SC107_antiCD3 polypeptide structure. Data shows Ros9a_SC107_antiCD3 has equivalent potency to Ros9aS74_antiCD3. Similar assays were used to assess cytotoxicity potential of single chain sTCR construct Ros9a_SC111_antiCD3 (Lower left), which had slightly lower potential for inducing cytotoxicity than Ros9aS74_antiCD3. However higher doses had considerable improvement on inducing cytotoxicity; and Lower right, data on sTCR Ros9a_SC113_antiCD3 construct. The effects were dose dependent, which is indicated in the index at the right hand column. Target cells express green fluorescence protein, reduction of which as a function of time was a fast and easy measure of the T cell mediated cytotoxicity. Figure discloses "10XHis" as SEQ ID NO: 866, "$(G_4S)_7$" as SEQ ID NO: 864, and "$(G_4S)_4$" as SEQ ID NO: 760.

[0128] **FIG. 41** shows imaging data of target cell depletion in presence of the sTCR BiTE constructs in an assay as shown in FIG. 40. Figure discloses "10XHis" as SEQ ID NO: 866, "$(G_4S)_7$" as SEQ ID NO: 864, and "$(G_4S)_4$" as SEQ ID NO: 760.

DETAILED DESCRIPTION

[0129] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0130] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0131] Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the disclosure can also be implemented in a single embodiment.

[0132] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing"

genus encompassed by the term "vector." A vector typically refers to a nucleic acid sequence containing an origin of replication and other entities necessary for replication and/or maintenance in a host cell. Vectors capable of directing the expression of genes and/or nucleic acid sequence to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility are often in the form of "plasmids" which refer to circular double stranded DNA molecules which, in their vector form are not bound to the chromosome, and typically comprise entities for stable or transient expression or the encoded DNA. Other expression vectors that can be used in the methods as disclosed herein include, but are not limited to plasmids, episomes, bacterial artificial chromosomes, yeast artificial chromosomes, bacteriophages or viral vectors, and such vectors can integrate into the host's genome or replicate autonomously in the cell. A vector can be a DNA or RNA vector. Other forms of expression vectors known by those skilled in the art which serve the equivalent functions can also be used, for example, self-replicating extrachromosomal vectors or vectors capable of integrating into a host genome. Exemplary vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked.

[0161] The terms "spacer" or "linker" as used in reference to a fusion protein may refer to a peptide that joins the proteins comprising a fusion protein. In some embodiments, the constituent amino acids of a spacer can be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity of the molecule. Suitable linkers for use in an embodiment of the present disclosure are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. The linker is used to separate two antigenic peptides by a distance sufficient to ensure that, in some embodiments, each antigenic peptide properly folds. Exemplary peptide linker sequences adopt a flexible extended conformation and do not exhibit a propensity for developing an ordered secondary structure. Typical amino acids in flexible protein regions include Gly, Asn and Ser. Virtually any permutation of amino acid sequences containing Gly, Asn and Ser would be expected to satisfy the above criteria for a linker sequence. Other near neutral amino acids, such as Thr and Ala, also can be used in the linker sequence. In some cases, linkers may be designed with combinations of Gly (G) and Ser (S). For example, G4S (SEQ ID NO: 683) would refer to a linker that may comprise a sequence of GGGGS (SEQ ID NO: 683). For example, in some cases, the linker sequence is GGGGS (SEQ ID NO: 683); or for example, in some cases the linker sequence is a concatemer of GGGGS (SEQ ID NO: 683). Linkers used herein are not limited to those described above. Other near neutral amino acids, such as Thr and Ala, also can be used in the linker sequence. Linkers used herein may include linkers that have been available in the public domain, e.g., published sequences or commercially available. Linkers used herein may include linkers that are designed in-house.

[0162] In some embodiments, the peptide linkers have more than one functional properties, such as the ones described herein. For example, the peptide linker links two or more functional domains,

[0206] Provided herein is a composition comprising a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, e.g., a multispecific engager molecule, wherein the multispecific molecule comprises: (i) an MHC-peptide complex engager comprising: (a) a T cell receptor (TCR) alpha variable (TRAV) domain, and (b) a TCR beta variable (TRBV) domain; wherein the peptide in the MHC-peptide complex comprises a cancer specific peptide, such as a RAS peptide sequence; and (ii) a T cell engager comprising a binding domain that binds to an extracellular domain of a receptor expressed on a T cell. Provided herein is a recombinant nucleic acid comprising a sequence encoding a multispecific molecule that may be used in therapy, for use in vivo in a human subject in need thereof, wherein the recombinant nucleic acid comprising the sequence encoding the multispecific molecules is administered to the human subject, and the recombinant nucleic acid is taken up by a cell in vivo, and the multispecific engager molecule encoded by the recombinant nucleic acid is expressed in vivo. In some embodiments, the multispecific engager molecule is administered to the subject, wherein the multispecific molecule comprises a post-translational modifications of a human cell.

[0207] In some embodiments, the multispecific molecule comprises a first polypeptide and a second polypeptide, wherein the first polypeptide comprises the TRAV domain and the second polypeptide comprises the TRBV domain.

[0208] In some embodiments, the peptide of the MHC-peptide complex is a peptide from a cancer antigen.

[0209] In some embodiments, the peptide of the MHC-peptide complex comprises a mutation.

[0210] In some embodiments, the MHC in the MHC-peptide complex binds a wild-type peptide of the MHC-peptide complex with a lower affinity compared to the peptide comprising the mutation.

[0211] In some embodiments, the peptide of the MHC-peptide complex does not comprise an amino acid sequence YLEPGPVTA (SEQ ID NO: 885).

[0212] In some embodiments, the MHC of the MHC-peptide complex comprises a class I MHC.

[0213] In some embodiments, the MHC of the MHC-peptide complex is a class I MHC polypeptide.

[0214] An MHC of the MHC-peptide complex is encoded by an HLA. In general about six HLA alleles are expressed in every human beings. In general, an allele is described as one of two or more versions of DNA sequence (a single base or a segment of bases) at a given genomic location. An individual inherits two alleles, one from each parent, for any given genomic location where such variation exists. If the two alleles are the same, the individual is homozygous for that allele. If the alleles are different, the individual is heterozygous. The distribution and frequency of HLA antigens vary greatly among different ethnic groups. The genes coding for HLA heterodimers are highly polymorphic, with more than 12,000 class I and 4,000 class II allele variants identified across the human population. It has been postulated that this diversity of HLA polymorphism has evolved under

ROR1, ROR2, SLC44A4, Syndecan-1, TACI, TAG-72, Tenascin, TIM3, TRAILR1, TRAILR2,VEGFR-1, VEGFR-2, or VEGFR-3, among others. In some embodiments, one of the aforementioned targets may be contemplated in generating a target cell specific binder or soluble TCR, having an engager that comprises the TRAV/TRBV domains, that specifically binds to an antigenic peptide of one of the aforementioned exemplary gene products, presented by a suitable MHC protein, and wherein the binder comprises a second engager, that binds to a cell surface component of a cell e.g., a T cell.

[0250] *a. MHC : peptide (p:MHC) complexes*

[0251] In some embodiments, an MHC-peptide complex engager is designed based on the identification of the cancer specific antigen that is to be targeted. In some embodiments, for example, the cancer specific antigen peptide is a KRAS antigen. In some embodiments, the cancer specific KRAS antigen comprises a mutation with respect to KRAS antigens that are present in non-cancerous healthy and normal cells and tissues. In some embodiments, (i) the activating oncogene mutation is a KRAS mutation; (ii) the KRAS mutation is a G12 mutation, optionally wherein the G12 KRAS mutation is selected from a G12D, G12V, G12S, G12C, G12A, and a G12R KRAS mutation; (iii) the KRAS mutation is a G13 mutation, optionally wherein the G13 KRAS mutation is a G13D KRAS mutation; and/or (iv) the activating oncogene mutation is a H-RAS or N-RAS mutation. The KRAS antigens or epitopes, and the corresponding MHC molecules encoded by an HLA allele that can bind and present the epitope to a T cell may be obtained from prior investigations in the field. For example, a mutant RAS peptide sequence may be selected from the group consisting of: DTAGHEEY (SEQ ID NO: 886), TAGHEEYSAM (SEQ ID NO: 887), DILDTAGHE (SEQ ID NO: 888), DILDTAGH (SEQ ID NO: 889), ILDTAGHEE (SEQ ID NO: 890), ILDTAGHE (SEQ ID NO: 891), DILDTAGHEEY (SEQ ID NO: 892), DTAGHEEYS (SEQ ID NO: 893), LLDILDTAGH (SEQ ID NO: 894), DILDTAGRE (SEQ ID NO: 895), DILDTAGR (SEQ ID NO: 896), ILDTAGREE (SEQ ID NO: 897), ILDTAGRE (SEQ ID NO: 898), CLLDILDTAGR (SEQ ID NO: 899), TAGREEYSAM (SEQ ID NO: 900), REEYSAMRD (SEQ ID NO: 901), DTAGKEEYSAM (SEQ ID NO: 902), CLLDILDTAGK (SEQ ID NO: 903), DTAGKEEY (SEQ ID NO: 904), LLDILDTAGK (SEQ ID NO: 905), ILDTAGKE (SEQ ID NO: 906), ILDTAGKEE (SEQ ID NO: 907), DTAGLEEY (SEQ ID NO: 908), ILDTAGLE (SEQ ID NO: 909), DILDTAGL (SEQ ID NO: 910), ILDTAGLEE (SEQ ID NO: 911), GLEEYSAMRDQY (SEQ ID NO: 912), LLDILDTAGLE (SEQ ID NO: 913), LDILDTAGL (SEQ ID NO: 914), DILDTAGLE (SEQ ID NO: 915), DILDTAGLEEY (SEQ ID NO: 916), AGVGKSAL (SEQ ID NO: 917), GAAGVGKSAL (SEQ ID NO: 918), AAGVGKSAL (SEQ ID NO: 919), CGVGKSAL (SEQ ID NO: 920), ACGVGKSAL (SEQ ID NO: 921), DGVGKSAL (SEQ ID NO: 922), ADGVGKSAL (SEQ ID NO: 923), DGVGKSALTI (SEQ ID NO: 924), GARGVGKSA (SEQ ID NO: 925), KLVVVGARGV (SEQ ID NO: 926), VVVGARGV (SEQ ID NO: 927), SGVGKSAL (SEQ ID NO: 928), VVVGASGVGK (SEQ ID NO: 929), GASGVGKSAL

(SEQ ID NO: 930), VGVGKSAL (SEQ ID NO: 931), VVVGAGCVGK (SEQ ID NO: 932), KLVVVGAGC (SEQ ID NO: 933), GDVGKSAL (SEQ ID NO: 934), DVGKSALTI (SEQ ID NO: 935), VVVGAGDVGK (SEQ ID NO: 936), TAGKEEYSAM (SEQ ID NO: 937), DTAGHEEYSAM (SEQ ID NO: 938), TAGHEEYSA (SEQ ID NO: 939), DTAGREEYSAM (SEQ ID NO: 940), TAGKEEYSA (SEQ ID NO: 941), AAGVGKSA (SEQ ID NO: 942), AGCVGKSAL (SEQ ID NO: 943), AGDVGKSAL (SEQ ID NO: 944), AGKEEYSAMR (SEQ ID NO: 945), AGVGKSALTI (SEQ ID NO: 946), ARGVGKSAL (SEQ ID NO: 947), ASGVGKSA (SEQ ID NO: 948), ASGVGKSAL (SEQ ID NO: 949), AVGVGKSA (SEQ ID NO: 950), CVGKSALTI (SEQ ID NO: 951), DILDTAGK (SEQ ID NO: 952), DILDTAGREEY (SEQ ID NO: 953), DTAGHEEYSAMR (SEQ ID NO: 954), DTAGKEEYS (SEQ ID NO: 955), DTAGKEEYSAMR (SEQ ID NO: 956), DTAGLEEYS (SEQ ID NO: 957), DTAGLEEYSA (SEQ ID NO: 958), DTAGLEEYSAMR (SEQ ID NO: 959), DTAGREEYS (SEQ ID NO: 960), DTAGREEYSAMR (SEQ ID NO: 961), GAAGVGKSA (SEQ ID NO: 962), GACGVGKSA (SEQ ID NO: 963), GACGVGKSAL (SEQ ID NO: 964), GADGVGKS (SEQ ID NO: 965), GAGDVGKSA (SEQ ID NO: 966), GAGDVGKSAL (SEQ ID NO: 967), GASGVGKSA (SEQ ID NO: 968), GCVGKSAL (SEQ ID NO: 969), GCVGKSALTI (SEQ ID NO: 970), GHEEYSAM (SEQ ID NO: 971), GKEEYSAM (SEQ ID NO: 972), GLEEYSAMR (SEQ ID NO: 973), GREEYSAM (SEQ ID NO: 974), GREEYSAMR (SEQ ID NO: 975), HEEYSAMRD (SEQ ID NO: 976),

KEEYSAMRD (SEQ ID NO: 977), KLVVVGASG (SEQ ID NO: 978), LDILDTAGR (SEQ ID NO: 979), LEEYSAMRD (SEQ ID NO: 980), LVVVGARGV (SEQ ID NO: 981), LVVVGASGV (SEQ ID NO: 982), REEYSAMRDQY (SEQ ID NO: 983), RGVGKSAL (SEQ ID NO: 984), TAGLEEYSA (SEQ ID NO: 985), TEYKLVVVGAA (SEQ ID NO: 986), VGAAGVGKSA (SEQ ID NO: 987), VGADGVGK (SEQ ID NO: 988), VGASGVGKSA (SEQ ID NO: 989), VGVGKSALTI (SEQ ID NO: 990), VVVGAAGV (SEQ ID NO: 991), VVVGAVGV (SEQ ID NO: 992), YKLVVVGAC (SEQ ID NO: 993), YKLVVVGAD (SEQ ID NO: 994), YKLVVVGAR (SEQ ID NO: 995), and DILDTAGKE (SEQ ID NO: 996); or (b) at least one polynucleotide encoding the at least one polypeptide.

[0252] In some embodiments, a mutant RAS peptide sequence may be selected from the group consisting of : KLVVVGADGV (SEQ ID NO: 219), KLVVVGACGV (SEQ ID NO: 15), KLVVVGAVGV (SEQ ID NO: 221), LVVVGADGV (SEQ ID NO: 220), LVVVGACGV (SEQ ID NO: 30), LVVVGAVGV (SEQ ID NO: 222); GADGVGKSAL (SEQ ID NO: 997), GACGVGKSAL (SEQ ID NO: 964), GAVGVGKSAL (SEQ ID NO: 998), GADGVGKSA (SEQ ID NO: 999), GACGVGKSA (SEQ ID NO: 963), GAVGVGKSA (SEQ ID NO: 1000); and/or VVGADGVGK (SEQ ID NO: 301), VVGACGVGK (SEQ ID NO: 109), VVGAVGVGK (SEQ ID NO: 45), VVVGADGVGK (SEQ ID NO: 302), VVVGACGVGK (SEQ ID NO: 110), VVVGAVGVGK (SEQ ID NO: 46).

[0253] In some embodiments, a mutant RAS peptide sequence may be selected from the group consisting of : LVVVGACGV (SEQ ID NO: 30), KLVVVGACGV (SEQ ID NO: 15), LVVVGADGV (SEQ ID NO: 220), KLVVVGADGV (SEQ ID NO: 219), LVVVGAVGV (SEQ ID NO: 222), KLVVVGAVGV (SEQ ID NO: 221), VVGACGVGK (SEQ ID NO: 109), VVVGACGVGK (SEQ ID NO: 110), VVGADGVGK (SEQ ID NO: 301), VVVGADGVGK (SEQ ID NO: 302), VVGAVGVGK (SEQ ID NO: 45), VVVGAVGVGK (SEQ ID NO: 46), VVGACGVGK (SEQ ID NO: 109), VVGADGVGK (SEQ ID NO: 301), VVVGADGVGK (SEQ ID NO: 302), VVGAVGVGK (SEQ ID NO: 45), and VVVGAVGVGK (SEQ ID NO: 46).

[0254] In some embodiments, a mutant RAS peptide sequence may be selected from the group consisting of : VVGADGVGK (SEQ ID NO: 301), VVGACGVGK (SEQ ID NO: 109), VVGAVGVGK (SEQ ID NO: 45), VVVGADGVGK (SEQ ID NO: 302), VVVGACGVGK (SEQ ID NO: 110), VVVGAVGVGK (SEQ ID NO: 46).

[0255] In some embodiments, a mutant RAS peptide sequence may be selected from the group consisting of : GADGVGKSAL (SEQ ID NO: 997), GACGVGKSAL (SEQ ID NO: 964), GAVGVGKSAL (SEQ ID NO: 998), GADGVGKSA (SEQ ID NO: 999), GACGVGKSA (SEQ ID NO: 963), or GAVGVGKSA (SEQ ID NO: 1000).

[0256] In some embodiments, the mutant RAS peptide sequences comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids.

[0257] In some embodiments, a mutant RAS peptide sequence binds to or is predicted to bind to a protein encoded by an HLA-A02:01 allele, an HLA-A03:01 allele, an HLA-A11:01 allele, and/or an HLA-C08:02 allele.

[0258] In some embodiments, a mutant RAS peptide sequence binds or is predicted to bind to a protein encoded by: an HLA-A02:01 allele and an HLA-A03:01 allele, an HLA-A11:01 allele, an HLA-A03:02 allele, an HLA-A30:01 allele, an HLA-A31:01 allele, an HLA-A33:01 allele, an HLA-A33:03 allele, an HLA-A68:01 allele, or an HLA-A74:01 allele; an HLA-A02:01 allele and an HLA-C08:02 allele; an HLA-A03:01 allele, an HLA-A11:01 allele, an HLA-A03:02 allele, an HLA-A30:01 allele, an HLA-A31:01 allele, an HLA-A33:01 allele, an HLA-A33:03 allele, an HLA-A68:01 allele, or an HLA-A74:01 allele and an HLA-C08:02 allele; or an HLA-A03:01 allele, an HLA-A11:01 allele, an HLA-A03:02 allele, an HLA-A30:01 allele, an HLA-A31:01 allele, an HLA-A33:01 allele, an HLA-A33:03 allele, an HLA-A68:01 allele, or an HLA-A74:01 allele and allele and an HLA-A03:01

allele, an HLA-A11:01 allele, an HLA-A03:02 allele, an HLA-A30:01 allele, an HLA-A31:01 allele, an HLA-A33:01 allele, an HLA-A33:03 allele, an HLA-A68:01 allele, or an HLA-A74:01 allele.

[0259] In some embodiments, the at least one polypeptide comprises at least one mutant RAS peptide sequence that binds to a protein encoded by an HLA allele with an affinity of less than 10 μM, less than 1 μM, less than 500 nM, less than 400 nM, less than 300 nM, less than 250 nM, less than 200 nM, less than 150 nM, less than 100 nM, or less than 50 nM.

[0260] In some embodiments the binding potential of shared antigens such as KRAS mutations is predicted by a MHC-peptide binding prediction analysis software that ranks the peptide:MHC pairs according to predicted binding affinity, rank one having the highest affinity.

[0261] **Table 1.** Peptide Sequences Comprising RAS Q61H Mutation, Corresponding HLA Allele, and Rank of Binding Potential. Ranking of binding potential is a measure of MHC : peptide binding efficiency converted to an arbitrary integer figures, with "1" being the most potent and strongest binding and gradually losing with increasing numerical value. In some embodiments, a peptide sequence provided in Table 1 binds to or is predicted to bind to a protein encoded by an HLA allele, which allele is provided in a corresponding column in Table 1 next to the peptide sequence.

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGHEEY	1001	HLA-A36:01	1
ILDTAGHEEY	1001	HLA-A01:01	2
DTAGHEEYSAM	938	HLA-A26:01	3
DTAGHEEYSAM	938	HLA-A25:01	4
GHEEYSAM	971	HLA-B15:09	4
DTAGHEEY	886	HLA-A26:01	5
ILDTAGHEE	890	HLA-C08:02	5
AGHEEYSAM	1002	HLA-C01:02	6
AGHEEYSAM	1002	HLA-B46:01	6
DTAGHEEY	886	HLA-A25:01	6
DTAGHEEY	886	HLA-A01:01	6
DTAGHEEY	886	HLA-B18:01	7
DTAGHEEY	886	HLA-A36:01	7
ILDTAGHEE	890	HLA-C05:01	7
ILDTAGHEE	890	HLA-A02:07	7
ILDTAGHEEY	1001	HLA-A29:02	7
ILDTAGHEEY	1001	HLA-C08:02	7
HEEYSAMRD	976	HLA-B49:01	8
TAGHEEYSA	939	HLA-B35:03	8

DTAGHEEYS	893	HLA-A68:02	9
DTAGHEEYSAMR	954	HLA-A68:01	9
GHEEYSAM	971	HLA-B39:01	9
ILDTAGHEE	890	HLA-A01:01	9
LDTAGHEEY	1003	HLA-B53:01	9
HEEYSAMRD	976	HLA-B41:01	10
ILDTAGHEE	890	HLA-A36:01	10
DTAGHEEY	886	HLA-B58:01	11
LLDILDTAGH	894	HLA-A01:01	12
TAGHEEYSAM	887	HLA-B35:03	12
LDTAGHEEY	1003	HLA-B35:01	13
DILDTAGHE	888	HLA-A26:01	14
DTAGHEEY	886	HLA-C12:03	14
ILDTAGHEEY	1001	HLA-C05:01	14
AGHEEYSAM	1002	HLA-A30:02	15
DILDTAGHEEY	892	HLA-A25:01	15
DTAGHEEY	886	HLA-C02:02	15
ILDTAGHEE	890	HLA-C04:01	15
DILDTAGH	889	HLA-A26:01	16
ILDTAGHEE	890	HLA-A02:01	16
LDTAGHEEY	1003	HLA-A29:02	16
ILDTAGHE	891	HLA-A01:01	17
LDTAGHEEY	1003	HLA-B18:01	17
AGHEEYSAM	1002	HLA-C14:03	18
DILDTAGHEEY	892	HLA-A29:02	18
DTAGHEEYS	893	HLA-A26:01	18
ILDTAGHEEY	1001	HLA-B15:01	18
DTAGHEEYSA	1004	HLA-A68:02	19
ILDTAGHE	891	HLA-C05:01	19
ILDTAGHEEY	1001	HLA-A02:07	19
ILDTAGHEEY	1001	HLA-A30:02	19
LDTAGHEEY	1003	HLA-A36:01	19
AGHEEYSAM	1002	HLA-C14:02	20
AGHEEYSAM	1002	HLA-B15:03	20
LLDILDTAGH	894	HLA-A02:07	20

[0262] In some embodiments, a peptide comprising a RAS Q61R mutation comprises a sequence of TCLLDILDTAGREEYSAMRDQYM (SEQ ID NO: 1005). In some embodiments, a peptide comprising a RAS Q61R mutation comprises a sequence provided in Table 2.

[0263] Table 2. Peptide Sequences Comprising RAS Q61R Mutation, Corresponding HLA Allele, and Rank of Binding Potential obtained from Neon MHC program.

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGREEY	1006	HLA-A36:01	1
ILDTAGREEY	1006	HLA-A01:01	2
DTAGREEYSAM	940	HLA-A26:01	3
DILDTAGR	896	HLA-A33:03	4
DILDTAGR	896	HLA-A68:01	5
DTAGREEY	1007	HLA-A26:01	6
DTAGREEYSAM	940	HLA-A25:01	6
CLLDILDTAGR	899	HLA-A74:01	7
DTAGREEY	1007	HLA-A01:01	7
REEYSAMRD	901	HLA-B41:01	7
GREEYSAMR	975	HLA-B27:05	8
ILDTAGREE	897	HLA-C08:02	8
ILDTAGREEY	1006	HLA-A29:02	8
REEYSAMRD	901	HLA-B49:01	8
AGREEYSAM	1008	HLA-B46:01	9
DTAGREEY	1007	HLA-B18:01	9
DTAGREEY	1007	HLA-A25:01	9
DTAGREEY	1007	HLA-A36:01	9
DILDTAGR	896	HLA-A74:01	10
DILDTAGRE	895	HLA-A26:01	10
ILDTAGREE	897	HLA-C05:01	10
DILDTAGR	896	HLA-A26:01	11
GREEYSAM	974	HLA-B39:01	11
AGREEYSAM	1008	HLA-B15:03	12
GREEYSAM	974	HLA-C07:02	12
ILDTAGREE	897	HLA-A01:01	12
TAGREEYSA	1009	HLA-B35:03	12
ILDTAGREEY	1006	HLA-A30:02	13
DTAGREEYS	960	HLA-A68:02	14

ILDTAGRE	898	HLA-A01:01	14
CLLDILDTAGR	899	HLA-A31:01	15
DTAGREEYSAMR	961	HLA-A68:01	15
LLDILDTAGR	1010	HLA-A01:01	15
DTAGREEY	1007	HLA-B58:01	16
ILDTAGREEY	1006	HLA-C08:02	16
DILDTAGR	896	HLA-A31:01	17
ILDTAGREE	897	HLA-C04:01	17
ILDTAGREEY	1006	HLA-A32:01	17
LLDILDTAGR	1010	HLA-A74:01	17
TAGREEYSAM	900	HLA-B35:03	17
DILDTAGREEY	953	HLA-A32:01	18
ILDTAGRE	898	HLA-C05:01	18
ILDTAGREE	897	HLA-A02:07	18
REEYSAMRD	901	HLA-B40:01	18
AGREEYSAM	1008	HLA-B15:01	19
AGREEYSAMR	1011	HLA-A31:01	19
ILDTAGRE	898	HLA-A36:01	19
LDILDTAGR	979	HLA-A68:01	19
LDTAGREEY	1012	HLA-A29:02	19
LDTAGREEY	1012	HLA-B35:01	19
REEYSAMRD	901	HLA-B45:01	19
REEYSAMRDQY	983	HLA-A36:01	19
DTAGREEY	1007	HLA-C02:02	20

[0264] In some embodiments, a peptide comprising a RAS Q61K mutation comprises a sequence of TCLLDILDTAGKEEYSAMRDQYM (SEQ ID NO: 1013). In some embodiments, a peptide comprising a RAS Q61K mutation comprises a sequence provided in Table 3. In some embodiments, a peptide sequence provided in Table 3 binds to or is predicted to bind to a protein encoded by an HLA allele, which allele is provided in a corresponding column in Table 3 next to the peptide sequence.

[0265] Table 3. Peptide Sequences Comprising RAS Q61K Mutation, Corresponding HLA Allele, and Rank of Binding Potential

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGKEEY	1014	HLA-A36:01	1
ILDTAGKEEY	1014	HLA-A01:01	2

DTAGKEEYSAM	902	HLA-A26:01	3
CLLDILDTAGK	903	HLA-A03:01	4
DTAGKEEY	904	HLA-A01:01	5
DTAGKEEY	904	HLA-A26:01	5
DTAGKEEYSAM	902	HLA-A25:01	5
AGKEEYSAM	1015	HLA-B46:01	6
DILDTAGKE	996	HLA-A26:01	7
KEEYSAMRD	977	HLA-B41:01	7
DTAGKEEY	904	HLA-B18:01	8
GKEEYSAM	972	HLA-B15:03	8
ILDTAGKEE	907	HLA-C08:02	8
ILDTAGKEEY	1014	HLA-A29:02	8
DTAGKEEYS	955	HLA-A68:02	9
LDTAGKEEY	1016	HLA-B53:01	9
TAGKEEYSA	941	HLA-B35:03	9
DILDTAGK	952	HLA-A68:01	10
DTAGKEEY	904	HLA-A36:01	10
KEEYSAMRD	977	HLA-B49:01	10
LDTAGKEEY	1016	HLA-C07:01	10
DTAGKEEYSAMR	956	HLA-A68:01	11
ILDTAGKEE	907	HLA-C05:01	11
ILDTAGKEEY	1014	HLA-C08:02	11
LLDILDTAGK	905	HLA-A01:01	12
AGKEEYSAM	1015	HLA-A30:02	13
DTAGKEEY	904	HLA-A25:01	13
DTAGKEEYS	955	HLA-A26:01	13
ILDTAGKE	906	HLA-C05:01	13
LDTAGKEEY	1016	HLA-B35:01	13
AGKEEYSAMR	945	HLA-A31:01	14
DILDTAGK	952	HLA-A33:03	14
ILDTAGKE	906	HLA-A01:01	14
ILDTAGKEE	907	HLA-A01:01	14
ILDTAGKEE	907	HLA-A02:07	14
TAGKEEYSAM	937	HLA-B35:03	14
AGKEEYSAM	1015	HLA-B15:01	15

ILDTAGKEEY	1014	HLA-A30:02	15
LDTAGKEEY	1016	HLA-B46:01	15
DTAGKEEY	904	HLA-B58:01	16
ILDTAGKEEY	1014	HLA-C05:01	17
AGKEEYSAM	1015	HLA-A30:01	18
AGKEEYSAM	1015	HLA-B15:03	18
DTAGKEEY	904	HLA-C02:02	18
LDTAGKEEY	1016	HLA-A29:02	18

[0266] In some embodiments, a peptide comprising a RAS Q61L mutation comprises a sequence of TCLLDILDTAGLEEYSAMRDQYM (SEQ ID NO: 1017). In some embodiments, a peptide comprising a RAS Q61L mutation comprises a sequence provided in Table 4. In some embodiments, a peptide sequence provided in Table 4 binds to or is predicted to bind to a protein encoded by an HLA allele, which allele is provided in a corresponding column in Table 4 next to the peptide sequence.

[0267] Table 4. Peptide Sequences Comprising RAS Q61L Mutation, Corresponding HLA Allele, and Rank of Binding Potential

Peptide	SEQ ID NO:	Allele	Rank of Binding Potential
ILDTAGLEEY	1018	HLA-A36:01	1
ILDTAGLEEY	1018	HLA-A01:01	2
LLDILDTAGL	1019	HLA-A02:07	3
GLEEYSAMRDQY	912	HLA-A36:01	4
DTAGLEEY	908	HLA-A25:01	5
DTAGLEEY	908	HLA-A26:01	5
DTAGLEEYSAM	1020	HLA-A26:01	5
DTAGLEEY	908	HLA-A01:01	6
ILDTAGLEE	911	HLA-C08:02	6
ILDTAGLEE	911	HLA-A01:01	6
CLLDILDTAGL	1021	HLA-A02:04	7
ILDTAGLEE	911	HLA-A36:01	7
LLDILDTAGL	1019	HLA-A01:01	7
DILDTAGL	910	HLA-B14:02	8
DILDTAGLEEY	916	HLA-A25:01	8
DTAGLEEYS	957	HLA-A68:02	8
DTAGLEEYSAM	1020	HLA-A25:01	8
GLEEYSAMR	973	HLA-A74:01	8

ILDTAGLE	909	HLA-A01:01	8
DILDTAGLEEY	916	HLA-A26:01	9
DTAGLEEY	908	HLA-A36:01	9
ILDTAGLEEY	1018	HLA-A29:02	9
DILDTAGL	910	HLA-B08:01	10
DTAGLEEY	908	HLA-B18:01	10
ILDTAGLEE	911	HLA-A02:07	10
LDTAGLEEY	1022	HLA-B35:01	10
CLLDILDTAGL	1021	HLA-A02:01	11
DTAGLEEY	908	HLA-C02:02	11
ILDTAGLEE	911	HLA-C05:01	11
ILDTAGLEEY	1018	HLA-C08:02	11
ILDTAGLEEY	1018	HLA-A02:07	11
LLDILDTAGL	1019	HLA-C08:02	11
DILDTAGL	910	HLA-A26:01	12
LDTAGLEEY	1022	HLA-B53:01	12
DTAGLEEY	908	HLA-C03:02	13
DTAGLEEY	908	HLA-B58:01	13
ILDTAGLEEY	1018	HLA-A30:02	13
LLDILDTAGL	1019	HLA-C05:01	13
LLDILDTAGL	1019	HLA-C04:01	13
DTAGLEEYSAMR	959	HLA-A68:01	14
ILDTAGLE	909	HLA-A36:01	15
LLDILDTAGL	1019	HLA-A02:01	15
AGLEEYSAM	1023	HLA-B15:03	16
DTAGLEEYSA	958	HLA-A68:02	16
GLEEYSAMRDQY	912	HLA-A01:01	16
ILDTAGLE	909	HLA-C04:01	16
ILDTAGLEEY	1018	HLA-B15:01	16
LDILDTAGL	914	HLA-B37:01	16
AGLEEYSAM	1023	HLA-A30:02	17
AGLEEYSAM	1023	HLA-B48:01	17
AGLEEYSAMR	1024	HLA-A31:01	17
ILDTAGLEE	911	HLA-C04:01	17
LDTAGLEEY	1022	HLA-C03:02	17

AGLEEYSAM	1023	HLA-C14:02	18
GLEEYSAMR	973	HLA-A31:01	18
LEEYSAMRD	980	HLA-B41:01	18
LLDILDTAGLE	913	HLA-A01:01	18
AGLEEYSAM	1023	HLA-C14:03	19
LDILDTAGL	914	HLA-B40:02	19
LDTAGLEEY	1022	HLA-A29:02	19
DILDTAGLE	915	HLA-A26:01	20
DTAGLEEY	908	HLA-B15:01	20
ILDTAGLEEY	1018	HLA-A02:01	20
LDTAGLEEY	1022	HLA-A36:01	20
LDTAGLEEY	1022	HLA-B46:01	20
DTAGLEEY	908	HLA-A68:02	21
DTAGLEEY	908	HLA-C12:03	21
ILDTAGLE	909	HLA-C05:01	21
LDTAGLEEY	1022	HLA-B18:01	21
LEEYSAMRD	980	HLA-B49:01	21
TAGLEEYSA	985	HLA-B54:01	21
DILDTAGLEEY	916	HLA-A29:02	22
GLEEYSAM	1025	HLA-C05:01	22

[0268] In some embodiments, the variable domain sequences may be derived from TCR sequences isolated and characterized in earlier investigations. A TCR contains variable complementarity determining regions (CDRs), as well as framework regions (FRs) and a constant region. The amino acid sequence of the third complementarity-determining region (CDR3) loops of the α and β chain variable domains largely determines the sequence diversity of αβ T cells arising from recombination between variable (Vβ), diversity (Dβ), and joining (Jβ) gene segments in the β chain locus, and between analogous Vα and Jα gene segments in the α chain locus, respectively. The existence of multiple such gene segments in the TCR α and β chain loci allows for a large number of distinct CDR3 sequences to be encoded. Independent addition and deletion of nucleotides at the Vβ-Dβ, Dβ-Jβ, and Vα-Jα junctions during the process of TCR gene rearrangement further increases CDR3 sequence diversity. In this respect, immunocompetence is reflected in the diversity of TCRs.

[0269] Table 5. Some exemplary RAS-TCR sequences that were previously identified are provided below. An exemplary sTCR design for MHC-RAS complex may be designed using the relevant variable regions from the following TCRs

Name	SEQ ID NO	Sequence
RAS TCR-28 beta chain	569	MGTSLLCWMALCLLGADHADTGVSQNPRHKITKRGQNVTFRCDPIS EHNRLYWYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERPKGS FSTLEIQRTEQGDSAMYLCASSEYTMGTQYFGPGTRLLVLKDLRNV TPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHVELSWWVNGKEV HSGVSTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHG LSEEDKWPEGSPKPVTQNISAEAWGRADCGITSASYHQGVLSATILY EILLGKATLYAVLVSGLVLMAMVKKKNS
RAS TCR-28 peptide	600	VVGAVGVGK

[0270] In some embodiments, the antigen is an ovarian cancer antigen or a T lymphoma antigen.

[0271] In some embodiments, the TRAV:TRBV comprises sequences for binding a G12D mutated RAS antigen (GADGVGKSA (SEQ ID NO: 999)) presented on an MHC protein encoded by an HLA:C*08:02. The TRAV comprises a sequence: LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFI PADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSD (SEQ ID NO: 619). The respective CDR sequences are:

CDR1: NIATNDY (SEQ ID NO: 874)

CDR2: GYKTK (SEQ ID NO: 875)

CDR3: LVGDMDQAGTALI (SEQ ID NO: 876): (CDR sequences according to IMGT)

[0272] The TRBV comprises a sequence: AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPD RFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLTVV (SEQ ID NO: 620). The respective CDR sequences are:

CDR1: SGHDT (SEQ ID NO: 877)

CDR2: YYEEEE (SEQ ID NO: 878)

CDR3: ASSLGEGRVDGYT (SEQ ID NO: 879); (CDR sequences according to IMGT)

[0273] In some embodiments, the TRAV: TRBV may comprise a sequence for binding specificity for any other non-RAS epitope, as might be necessary. For example, a TRAV:TRBV for the multispecific recombinant soluble polypeptide may comprise a binding specificity for MART1 epitope, presented on an MHC protein encoded by an HLA:A*02:01. The antigen binding affinity (KD) of pHLA in this case is 16nM. For example, a TCR may comprise a TRAV domain having a sequence of:

such as, for example, a CD3 receptor, and can activate a receptor mediated cell signaling pathway in the T cell. In some embodiments, the linker is designed such as to be able to bind and activate at least an inflammatory pathway in the T cell or potentiate T cell mediated killing of a target cell. In some embodiments, the linker peptide may have a function of blocking or inhibiting a target cell mediated downregulation of a T cell function. In some embodiments, the nucleic acid constructs for expressing a bispecific scFv engager comprises an N terminal signal peptide sequence for secretion of the bispecific scFv engager.

[0284] In one embodiment, an exemplary CD3 binding domain is an scFv. In one embodiment, the anti-CD3 binder is an scFV, the particular domains and sub-domains, respective sequences and/or variations are listed in Table 6A. In one embodiment the anti-CD3 scFv comprises a sequence that is at least 80% identical to SEQ ID NO: 601. In some embodiments, an exemplary CD3 binding domain is an scFv comprising a sequence that is at least 85%, at least 90%, at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 601. In one embodiment the anti-CD3 scFv comprises a sequence that is at least 80% identical to SEQ ID NO: 602. In some embodiments, an exemplary CD3 binding domain is an scFv comprising a sequence that is at least 85%, at least 90%, at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 602. In one embodiment the anti-CD3 scFv comprises a sequence that is at least 80% identical to SEQ ID NO: 612, 613 or 614. In some embodiments, an exemplary CD3 binding domain is an scFv comprising a sequence that is at least 85%, at least 90%, at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 612. In some embodiments, an exemplary CD3 binding domain is an scFv comprising a sequence that is at least 85%, at least 90%, at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 613. In some embodiments, an exemplary CD3 binding domain is an scFv comprising a sequence that is at least 85%, at least 90%, at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 614. In some embodiments, an exemplary CD3 binding domain is an scFv comprising a sequence of SEQ ID NO: 601, 602, 612, 613, or 614. In some embodiments, an exemplary CD3 binding domain comprises a heavy variable domain that is at least 80% identical to SEQ ID NO: 603 and SEQ ID NO: 884. In some embodiments, an exemplary CD3 binding domain comprises a heavy variable domain that is at least 85% identical to SEQ ID NO: 603 and SEQ ID NO: 884. In some embodiments, an exemplary CD3 binding domain comprises a heavy variable domain that is at least 90% identical to SEQ ID NO: 603 and SEQ ID NO: 884. In some embodiments, an exemplary CD3 binding domain comprises a heavy variable domain that is at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 603 and SEQ ID NO: 884. In some embodiments, an exemplary CD3 binding domain comprises a heavy variable domain having a sequence of SEQ ID NO: 603 and SEQ ID NO: 884. In some embodiments, an exemplary CD3 binding domain comprises a light variable domain that is at least 80% identical to SEQ ID NO: 604. In some embodiments, an exemplary CD3 binding domain comprises a light variable domain that is at least 90% identical to SEQ ID NO: 604. In some embodiments, an exemplary CD3 binding

QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRF SGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK (SEQ ID NO: 672).

[0299] In some embodiments, the CD3 binder scFv can have the configuration: VH-linker-VL, having a sequence that is at least 80% identical to the sequence: QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNY NQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSG GGGSGGGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGT SPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKL ELK (SEQ ID NO: 727).

[0300] In some embodiments, the CD3 binder scFv can have the configuration: VL-linker-VH, having a sequence that is at least 80% identical to the sequence: QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRF SGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSGGGGSGGGGSG GGGSGGGGSQVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGY INPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWG QGTTLTVSS SEQ ID NO: 670).

[0301] In some embodiments, the CD3 binding domains comprise a HCDR1, 2, and 3 and an LCDR1, 2, and 3 as follows according to Kabat and IMGT nomenclature as shown below:

[0302] Table 6D. CDR sequences and variations of TR66 antibody.

Name	Numbering	CDR1	CDR2	CDR3
VH(TR66)	IMGT	GYTFTRYT (SEQ ID NO: 718)	INPSRGYT (SEQ ID NO: 719)	ARYYDDHYCLDY (SEQ ID NO: 720)
VH(TR66(C114S))	IMGT	GYTFTRYT (SEQ ID NO: 718)	INPSRGYT (SEQ ID NO: 719)	ARYYDDHYSLDY (SEQ ID NO: 721)
VL(TR66)	IMGT	SSVSY (SEQ ID NO: 722)	DTS (SEQ ID NO: 881)	QQWSSNPLT (SEQ ID NO: 724)
VL(TR66(C114S))	IMGT	SSVSY (SEQ ID NO: 722)	DTS (SEQ ID NO: 881)	QQWSSNPLT (SEQ ID NO: 724)
VH(TR66)	Kabat	RYTMH (SEQ ID NO:673)	YINPSRGYTNYNQKFKD (SEQ ID NO: 674)	YYDDHYCLDY (SEQ ID NO: 675)
VH(TR66(C114S))	Kabat	RYTMH (SEQ ID NO:673)	YINPSRGYTNYNQKFKD (SEQ ID NO: 674)	YYDDHYSLDY (SEQ ID NO: 717)
VL(TR66)	Kabat	RASSSVSYMN (SEQ ID NO: 676)	DTSKVAS (SEQ ID NO: 677)	QQWSSNPLT (SEQ ID NO: 678)
VL(TR66(C114S))	Kabat	RASSSVSYMN (SEQ ID NO: 676)	DTSKVAS (SEQ ID NO: 677)	QQWSSNPLT (SEQ ID NO: 678)

[0303] In some embodiments, the anti-CD3 binding domain (scFv) comprises a sequence QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNY

	LRAEDTAVYYCARGIYSDSSDFIGNLWGQGTLVTVSS (SEQ ID NO: 703)
210112 VL	AIQMTQSPSSLSASVGDRVTITCQASQTIYNYLAWYQQKPGKVPELLIYKASTLASGVPSRFMGTGFGTDFTLTIDSLQPEDAATYYCQQYNTIINVDRTFGGGTKVEIK (SEQ ID NO: 704)
210112 VH-CDR1 (Kabat)	NNYYMC (SEQ ID NO: 705)
210112 VH-CDR2 (Kabat)	CIYTNNIGSTWYASWAKG (SEQ ID NO: 706)
210112 VH-CDR3 (Kabat)	GIYSDSSDFIGNL (SEQ ID NO: 707)
210112 VL-CDR1 (Kabat)	QASQTIYNYLA (SEQ ID NO: 708)
210112 VL-CDR2 (Kabat)	KASTLAS (SEQ ID NO: 709)
210112 VL-CDR3 (Kabat)	QQYNTIINVDRT (SEQ ID NO: 710)
210112 VH-CDR1 (IMGT)	GFSFSNNYY (SEQ ID NO: 711)
210112 VH-CDR2 (IMGT)	IYTNNIGST (SEQ ID NO: 712)
210112 VH-CDR3 (IMGT)	ARGIYSDSSDFIGNL (SEQ ID NO: 713)
210112 VL-CDR1 (IMGT)	QTIYNY (SEQ ID NO: 714)
210112 VL-CDR2 (IMGT)	KAS (SEQ ID NO: 715)
210112 VL-CDR3 (IMGT)	QQYNTIINVDRT (SEQ ID NO: 716)

[0308] In one embodiment, the anti-CD3 binding domain comprises a heavy chain CDR3 sequence GIYSDSSDFIGNL (SEQ ID NO: 707). In some embodiments, the heavy chain CDR1 sequence of SEQ ID NO: 705. In some embodiments, the heavy chain CDR2 sequence of SEQ ID NO: 706. In one embodiment, the anti-CD3 binding domain comprises a light chain CDR3 sequence QQYNTIINVDRT (SEQ ID NO:710). In one embodiment, the anti-CD3 binding domain comprises a light chain CDR1 sequence (SEQ ID NO: 708). In some embodiments, the light chain CDR2 sequence of (SEQ ID NO:709).

including, for example, swapping domains that are N terminal to a linker sequence to C terminal, with respect to the linker.

[0328] As an example Arndt et al. (Biochemistry 37:12918-12926, 1998) describe the so called domain swapping as a possible explanation for the appearance of non-covalently linked oligomers of scFv fragments. Under this model the protein state is subjected to a possible thermodynamic equilibrium between a monomeric and a dimeric/oligomeric form due to a constantly occurring intra- and intermolecular exchange of the VL/VH interface contacts. These oligomers could be present already in the cell culture supernatant and should be eliminated during the purification process. However these molecular species could be also formed during the storage of purified monomeric species. The preferred energetic status of the protein is strongly influenced by its overall design (primary sequence, linker length, VL/VH orientation etc.). Worn and Pltickthun (JMB 305:989-1010, 1999) mentioned that forms with higher content of monomeric species could be obtained by using a linker of 20 or more residues. Desplancq et al., (Protein Eng. 7:1027-1033, 1994) indicated that the variable domain orientation could also have an impact on the formation of dimers and high molecular forms. In the same publication Desplancq showed that a linker of 25 or 30 amino acids (aa) gave the best ratio of monomer over dimer for their particular antibody. The distance between the C-terminus of VL and the N- terminus of VH is around 39-43 A, and the distance between the C-terminus of VH and N-terminus of VL is 32-34 A (Pltickthun et al., From PCR to fermentation. (J. McCafferty, H. R.Hoogenboom, & D. J. Chriswell, Eds.). In: (IRL Press., pp. 203-252, 1996). To obtain similar molecular properties, a linker for the orientation VL-VH has to be longer than a VH-VL linker. Pltickthun et al., (From PCR to fermentation. (J. McCafferty, H. R. Hoogenboom, & D. J. Chriswell, Eds.). In: (IRL Press., pp. 203-252, 1996) recommended using linkers with a length of 15 or 20 amino acids in the orientation VH/VL and linkers with a length of 20 or 25 amino acids in the orientation VL/VH. Another possibility to force the formation of monomers and to stabilize the VH/VL domain interaction is to engineer an interface disulfide bond into the contact surface between the two domains. The introduction of a disulfide bridge at the position H44-L100 (Kabat numbering) has been the most frequently used in scFvs with satisfactory results (Brinkmann et al., PNAS. 90:7538-7542, 1993; Worn and Plockthun, Biochemistry 38: 8739-8750, 1999; Weatherill et al., PEDS. 25:321-329, 2012). This strategy has been used successfully to stabilize IgG-like bispecific antibodies combining scFv fused to full length IgG (Michaelson et al., mAbs 1: 128- 141, 2009; Schanzer et al., Antimicrob. Agents. Chemother. 55:2369-2378, 2011). Weatherill et al., (PEDS 25:321-239, 2012) stabilized human scFvs (VH-(G4S)4-VL and VL-(G4S)4-VH ("(G4S)4" is disclosed as SEQ ID NO: 760)) with a disulfide bond between the position VH44 and VL-100. Moreover this publication address the problem of possible domain swapping with scFv containing no interface disulfide bond by performing different SE-HPLC experiments at different load volume and concentration. The assays gave different results depending on the sample loading conditions for the non-stabilized scFv, but independent of the conditions used the disulfide stabilized molecules eluted

like a monomer. Zhao et al., (Int. J. Mol. Sci. 12:1-11, 2011) introduced the same mutation in a scFv and observed higher stability of the stabilized molecule after storage for 20 h at 37 °C. For the bispecific format using scFv fused to full length IgG, Schanzer et al., (Antimicrob. Agents Chemother. 55:2369-2378 2011) compared the effect of the linker length and interface disulfide bond. They fused the parental scFv or scdFv (VH-(G4S)3-VL ("(G4S)3" is disclosed as SEQ ID NO: 690)) either at the C- or N terminal part of the heavy chain or light chains. For the different linker length (20, 25 and 30 amino acids) they fused the parental scFv to the C-terminal part either of the heavy or light chains. The results obtained with the different linker length identified the 30 aa peptide as the more preferable linker for the production of stable monomers. The level of aggregates after 7 days storage at 40°C was 50% for scFv15, 18% for scFv20, 8% for scFv25 and 6% for scFv30. But the disulfide scFv15 stabilized with the interface disulfide bond was slightly superior to the scFv30. The same approach was used by Michaelson et al., (mAbs, 1:128-141 2009), and they improved their parental IgG-like bispecific antibody containing scFv with 15 aa linker in VH/VL orientation (generating 40% aggregates), by increasing the linker length to 20 aa of the scFv and introducing the interface disulfide bond between the position VH44 and VL-100. The resulting molecule yielded more than 98% monomers that were stable after three months at 4°C. The authors took the decision to work on the improvement of the scFv molecule before going to the bispecific format.

[0329] In the case of anti-RAS specific bispecific proteins as described in the disclosure, it is not known if the formation of dimers and high molecular forms could occur and what is the implication on the anti-RAS and/or the anti-CD3 scFv molecules. In order to assess an optimal overall molecule for the anti RAS specific bi-scFv protein for each separate scFv the following modifications are evaluated: domain orientation, linker length, introduction of an interface disulfide bond and a combination of the three modifications.

[0330] In another example, addition of cleavage sequences, such as autocleavable sequences for example, T2A, P2A F2A sequences between adjoining domains, within linkers, or between two amino acid chains to generate mature proteins is easily conceivable by one of skill in the art. An exemplary autocleavable sequence is T2A: TNFSLLKQAGDVEENPGP (SEQ ID NO: 627) Conjugating or inserting tag sequences, such as HIS tag at any region of the construct can be easily conceivable in the art. Functionalization of any of the linker sequences as known in the art can be achieved by one of ordinary skill in the art given the sequences and descriptions provided.

[0331] In some embodiments, described herein is a recombinant nucleic acid sequence encoding a first polypeptide, and a second polypeptide for a bispecific molecule, which comprises: (a) a nucleic acid sequence encoding a (a) TRAV/TRBV domains fused to a nucleic acid sequence encoding a TRAC/TRBC respectively; that binds to a cancer specific peptide in the MHC complex on the cancer cell, (b) a VHH domain that binds to a cell surface component of an effector cell, e.g. the T cell, (c) a short linker operably linking (a) and (b).

amino acids or less, 9 amino acids or less, 8 amino acids or less, 7 amino acids or less, 6 amino acids or less, or 5 amino acids or less.

[0337] In some embodiments, the linker may comprise one or more amino acids, wherein each amino acid is linked to the adjacent amino acid via natural peptide bonds, e.g., bond between amino group of one amino acid to the carboxyl group of a neighboring amino acid. In some embodiments, the antigen binding domains, scFvs or binding domains are linked with each other by a linker. In some embodiments, where there are more than one scFvs, the more than scFvs are linked with each other by linkers.

[0338] In some embodiments the linkers are flexible. In some embodiments the linkers comprise a hinge region. Linkers are usually short peptide sequences. In some embodiments the linkers are stretches of Glycine and one or more Serine residues. Other amino acids preferred for a peptide linker include but are not limited to threonine (Thr), serine (Ser), proline (Pro), glycine (Gly), aspartic acid (Asp), lysine (Lys), glutamine (Gln), asparagine (Asn), and alanine (Ala) arginine (Arg), phenylalanine (Phe), glutamic acid (Glu). Of these Pro, Thr, and Gln are frequently used amino acids for natural linkers. Pro is a unique amino acid with a cyclic side chain which causes a very restricted conformation. Pro-rich sequences are used as interdomain linkers, including the linker between the lipoyl and E3 binding domain in pyruvate dehydrogenase ($GA_2PA_3PAKQEA_3PAPA_2KAEAPA_3PA_2KA$) (SEQ ID NO: 628). Empirical linkers may be flexible linkers, rigid linkers, and cleavable linkers. Sequences such as (G4S)x (SEQ ID NO: 683) (where x is multiple copies of the moiety, designated as 1, 2, 3, 4, and so on) comprise a flexible linker sequence. Other flexible sequences used herein include several repeats of glycine, e.g., (Gly)6 (SEQ ID NO: 1026) or (Gly)8 (SEQ ID NO: 1027). On the other hand, a rigid linker may be used, for example, a linker (EAAAK)x (SEQ ID NO: 1028), where x is an integer, 1, 2, 3, 4 etc. gives rise to a rigid linker. In some embodiments, the linker or spacer may be made of nonreactive amino acid moieties, for example, a series of glycine or serine or alanine residues. An exemplary linker may comprise an amino acid sequence GSGS (SEQ ID NO: 1029), or SGGG (SEQ ID NO: 1030), or SGGGGSG (SEQ ID NO: 629). An exemplary linker may comprise an amino acid sequence SSGGGGSGGGGSGGGGS. (SEQ ID NO: 630).

[0339] The length of a linker peptide can be crucial in the design of a multispecific engager. For example, limiting the linker peptide length to less than 10 amino acids restricts the association between two adjacent domains. In some embodiments, the linker may comprise an anchoring or clasping function and may comprise a crosslinking moiety. The crosslinking moiety may be a peptide or a chemical crosslinking moiety.

[0340] In some embodiments, a peptide linker may further function as a conditionally cleavable linker. By conditionally cleavable it may be understood that the peptide is cleaved when the agent that cleaves it is available. By conditionally cleavable it may be understood that the peptide is cleaved when the agent that cleaves it is activated, for example if the agent that cleaves is an enzyme, is activated. By conditionally cleavable it may be understood that the peptide is cleaved when the agent

TRBC stabilizing domain; which may be connected at the C terminal with an optional marker or tag peptide for example a hexa-HIS peptide (SEQ ID NO: 1031) or another functional domain. In some embodiments, a linker sequence comprising approximately 1-10 amino acids may be present between any two proximal domains. Upon post-translational cleavage, the mature protein may comprise self-assembled TRAV: TRBV domains on separate polypeptides, wherein the TRAV is operably linked with a TRAC, and the TRBV is operably linked with a TRBC, wherein the TRAC:TRBC form the stabilization domains. In some embodiments, the TRAC and the TRBC domains comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more point mutations conferring increased stabilization of the domains. In some embodiments the human TRAC comprises a disulfide lock position between T48C and T166C on the human TRAC one polypeptide domain. In some embodiments, the TRBC comprises disulfide lock positions S57C or S173C. In some embodiments, such a scaffold structure may be about 312 amino acids in length.

[0364] In one embodiment, an exemplary recombinant nucleic acid encoding an sTCR polypeptide uses a basic scaffold design as exemplified above but comprises leucine zipper stabilization domains for increased TRAV: TRBV stability. Accordingly, the recombinant nucleic acid comprises a sequence encoding a polypeptide comprising, from N- to C- terminus: an N terminal native signal sequence; a first insert sequence comprising a TRAV that specifically binds to the desired antigen in association with a TRBV; a TRAC sequence, optionally linked to a tag sequence (e.g., a FLAG tag); a sequence for a leucine zipper chain; a P2A sequence; a native signal sequence; a second insert sequence comprising the TRBV; a TRBC domain; a tag sequence, a leucine zipper sequence that oligomerizes with the other leucine zipper chain in the polypeptide, optionally connected at the C terminal with marker or tag peptide, for example a hexa-HIS tag (SEQ ID NO: 1031), or another functional domain. The basic scaffold design of this type may comprise a 442 amino acid polypeptide.

[0365] In some embodiments, the scaffold comprises a human TRAC engineered to comprise one or more of stability enhancing mutations: S139F, T150I, A190T and the TRBC comprises E134K, H139R, D155P, S170D.

[0366] In some embodiments, the scaffold may comprise Fc domains in addition to the TCR constant regions for additional stabilization. In some embodiments the Fc domains comprise a knob-in-hole configuration. In some embodiments, the FcA domain comprises a T366W “knob” mutation. In some embodiments, the FcB domain comprises a T336S “hole” mutation. In some embodiments, the FcB domain comprises a L368A “hole” mutation. In some embodiments, the FcB domain comprises a Y407V “hole” mutation. In some embodiments, the scaffold comprises an Fc region from human IgG1. In some embodiments, the FcB comprises one or more amino acid substitutions, selected from M252E, I253A, and H435A substitutions.

[0367] In some embodiments, the scaffold comprises the basic configuration described in the previous paragraph, comprising an FcA-FcB domain in knob-and-hole configuration as described above, but lacks the TRAC and TRBC domains.

[0368] In some embodiments, the human TRAC may be linked via a short linker to a 10X His tag (SEQ ID NO: 866) followed by a furin cleavage site. In some embodiments, the self-cleavable sequence may be F2A sequence; and the human TRBC sequence may be linked to a BAP tag.

[0369] The stabilization domains may comprise a disulfide bond between the human TRAC contributed by engineered cysteine on T166 (T166C) and human TRBC contributed by engineered cysteine at S173 (S173C). A natural disulfide bond exists between TRAC (C213) and TRBC (C247). One or more glycosylation sites may be removed in TRAC and TRBC.

[0370] In some embodiments, the scaffold comprises a human TRAC engineered to comprise one or more of stability enhancing mutations: S139F, T150I, A190T and the TRBC comprises S170D, C191A or N205D. In some embodiments, both TRAC and TRBC sequences may comprise one or more N>Q substitutions.

[0371] The numbering of the amino acid residues in the TRAC and TRBC domains used herein follows the Kabat numbering system (Kabat, et al. Sequences of Immunological Interest Vol. 1 Fifth Edition 1991 US Department of Health and Human Services, Public Health Service, NIH).

[0372] In one embodiment, human TRAC may be fused to human IgG1 or a portion thereof, comprising the upper hinge of IgG1, the core hinge of IgG1 and the lower hinge of IgG1 followed by the Fc of IgG1. Accordingly, in one embodiment, the recombinant nucleic acid encoding this exemplary sTCR comprises a heavy chain (364 aa long) and a light chain (154 aa long) polypeptide; the heavy chain comprising, from N- to C- terminus: an N terminal native signal sequence; a first insert sequence comprising a TRAV that specifically binds to the desired antigen in association with a TRBV; a TRAC sequence that is fused at the C terminus to the upper hinge domain of human IgG1. A 10X-HIS tag (SEQ ID NO: 866) may be attached to the C terminal end of the human IgG1. In some embodiments, the light chain comprises a signal sequence, a second insert sequence comprising the TRBV, operably linked to a human TRBC. In one embodiment, the human TRAC comprises one, two or three N>Q substitutions. In some embodiments one N>Q substitution is upstream of disulfide lock position 1 (T48C or T 166C), and two N>Q substitutions are downstream. The human IgG1 sequence has a disulfide site with TRBC C-terminal cysteine. In some embodiments the light chain comprises a disulfide lock position contributed by a S57C or S173C on the human TRBC sequence. In some embodiments, there are engineered disulfide bonds between IgG1 hinge region and human TRBC (C247).

[0373] In some embodiments, the dimerization or stabilization domains comprise SARAH domain A and SARAH domain B. In some embodiments, a recombinant nucleic acid encoding an engineered T cell receptor (TCR) construct, wherein the engineered TCR construct comprises (a) a first polypeptide comprising a TCR alpha variable (TRAV) domain comprising CDR1a, a CDR2a and a CDR3a; one or more TRAV framework region (TRAV-FR) sequences; (b) a second polypeptide comprising a TCR beta variable (TRBV) domain comprising CDR1b, CDR2b and a CDR3b; one or

comprises the TCR-alpha and TCR beta chains and one or more tags (HIS tag and BAP tag), to which one or more T cell engagers may be conjugated to obtain a bi- or multispecific engager. In some embodiments, the scaffold comprises an engager molecule that is at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, identical to the sequence: METDTLLLWVLLLWVPGSTGD**LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQ FPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAG TALIFGKGTTLSVSSD*YIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITD KCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC***GSGHHHHHHHHH HRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMETDTLLLWVLLLWVPGSTGDDAGVTQSPT HLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFP NYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLTVVEDLKNVFPPEVAVFEPS KAEISRTQKATLVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALS SRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGSGGL NDIFEAQKIEWHE (SEQ ID NO: 633);

wherein the TRAV sequence is: LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFI PADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSD (SEQ ID NO: 619); the TRAC sequence is YIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNS AVAWSQKSDFTCANAFQNSIIPEDTFFPSPESS (SEQ ID NO: 1032);

the TRBV sequence is: AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPD RFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLTVV (SEQ ID NO: 620); and the TRBC sequence is EDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQ PLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVS AEAWGRADCGSGGLNDIFEAQKIEWHE (SEQ ID NO: 1033). The TRAV and TRAC form the first polypeptide and the TRBV and the TRBC form the second polypeptide.

[0392] In some embodiments, the TRBV is N terminal to the TRAV. In some embodiments, the TRAV is N terminal with respect to TRBV. In some embodiments, the TRAV is associated with a tag, e.g., a HIS tag. In some embodiments the TRBV is associated with a tag, for example, a HIS tag or a BAP tag. In some embodiments, the TRAC is associated with a HIS tag or a BAP tag. In some embodiments, the TRAC is associated with a HIS tag or a BAP tag. In some embodiments, the TRAC is associated with a HIS tag or a BAP tag. In some embodiments, the TRBC is associated with a HIS-tag or a BAP tag.

[0393] In some embodiments, the alpha chain comprises one or more of a tag, a signal peptide a cleavage domain. In some embodiments, the beta chain comprises one or more of a tag, a signal peptide a cleavage domain.

[0394] In some embodiments, there is a cleavable sequence between the alpha chain and the beta chain. In some embodiments, the cleavable sequence is a P2A, F2A or T2A sequence.

[0395] In some embodiments, the scaffold comprises an engager molecule comprising a TRAV and a TRBV domain connected with a TRAC domain and a TRBC domain respectively; further comprising immunoglobulin chains conjugated to the TCR alpha and beta chains. An exemplary representative diagram is shown as **Scaffold 5** in **FIG. 3**. In some embodiments, such a scaffold can comprise a sequence that is at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, identical to the sequence:

METLLGVSLVILWLQLARVNS**QQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYR QYSGKSPELIMSIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAGGGGAD GLTFGKGTHLIIQP*YIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKC VLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESS***VEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGSGHHHHHHHHH (**Scaffold 5,** *199.16 TCR as example having binding affinity for MART1/A*02:01*) (SEQ ID NO: 634); and a corresponding beta chain can comprise a sequence that is at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, identical to the sequence:

MGTRLLCWAALCLLGAELTEAGITQSPKYLFRKEGQNVTLSCEQNLNHDAMYWYRQDPG QGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSQGLAGAGE LFFGEGSRLTVLEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGK EVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWT QDRAKPVTQIVSAEAWGRADC (SEQ ID NO:635). The TRAV sequence is marked as bold, and the TRBV sequence is marked by underline. The 10X Histidine tag, HHHHHHHHHH (SEQ ID NO: 866) is conjugated at a terminus.

[0396] In some embodiments, the scaffold comprises an engager molecule comprising a TRAV and a TRBV domain; further comprising molecular clasp or linker between the two chains. In some embodiments, the molecular clasp is a Fv clasp (**FIG. 3, scaffold 6**). In some embodiments, the scaffold comprises an engager molecule comprising a TRAV, a TRBV and two stabilizer polypeptide segments connected via Fv clasp. In some embodiments, such a scaffold comprises a sequence:

LVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQ DPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGGGSGLNDIFEAQKIE WHEGGGGSGGGGSLPETGG (**Ros10 Scaffold 19, without signal peptide**) (SEQ ID NO: 652).

[0410] In some embodiments, the scaffold comprises an engager molecule that is at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence: In some embodiments, the scaffold comprises an engager molecule that is at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence: MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQG PRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKG TTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRS MDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GSGHHHHHHHHHHHRIRRSGSGV KQTLNFDLLKLAGDVESNPGPMMGVKVLFALICIAVAEAQIVLTQSPAIMSASPGEKVTMTCRA SSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQ WSSNPLTFGAGTKLELKGGGGSGGGGSGGGGSGGGGSGGGGSQVQLQQSGAELARPGASVKM SCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQL SSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSGGGGSAGVTQSPTHLIKTRGQQVTLRC SPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSA LYLCASSLGEGRVDGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYP PHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQF YGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLP ETGG* (SEQ ID NO: 763) (**Ros9a Scaffold 22**).

[0411] In some embodiments, the scaffold comprises an engager molecule that is at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence: In some embodiments, the scaffold comprises an engager molecule that is at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93 at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence: **LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEV ASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNPD PAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVA WSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GSGHHHHHHHHHHHRIRRSGSGVKQTLNFDL LKLAGDVESNPGPMMGVKVLFALICIAVAEAQIVLTQSPAIMSASPGEKVTMTCRASSSVSY MNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWS SNPLTFGAGTKLELKGGGGSGGGGSGGGGSGGGGSGGGGSQVQLQQSGAELARPGASVK

MSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYM
QLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSGGGGSAGVTQSPTHLIKTRGQQVTL
RCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGD
SALYLCASSLGEGRVDGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGF
YPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQV
QFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGGGSGLNDIFEAQKIEWHEGGGGSGGGG
SLPETGG* (SEQ ID NO: 1034) (**Ros9a Scaffold 22, without signal peptide**).

[0412] In some embodiments, provided herein is a polypeptide chain comprising a third binding domain that specifically interacts with an extracellular protein of the T cell. In some embodiments, the extracellular protein to which the third domain binding specifically interacts is different from the extracellular protein to which the second binding domain specifically interacts. In some embodiments, the extracellular protein to which the third domain binding specifically interacts are present on the same T cell. In some embodiments, the third binding domain is a CD8 molecule or a fragment thereof.

[0413] In one embodiment, the recombinant polynucleic acid is an mRNA.

[0414] In one aspect, provided herein is a cell comprising a polynucleic acid of any one of embodiments disclosed and described herein.

[0415] In one aspect, provided herein is pharmaceutical composition for use in treating cancer, comprising any one of the recombinant polynucleic acids, recombinant polypeptides or the cell of any one of embodiments disclosed and described herein.

[0416] In some embodiments, the antigen of the target cell is a cancer antigen peptide, a pathogenic antigen peptide or an autoimmune antigen peptide.

[0417] In one embodiment, the first binding domain, the second binding domain, the third binding domain, the additional binding domain of the first polypeptide chain, the additional binding domain of the second polypeptide chain and/or the additional binding domain of the third polypeptide chain comprises a TRAV/TRBV domain, a VH domain, a VL domain, a VNAR domain, a VHH domain, a single chain variable fragment (scFv), an Fab, a single-domain antibody (sdAb), a nanobody, a bispecific antibody or a diabody.

[0418] In any of the above embodiments, the BiTE or the TRiTE comprises any one or more of (i) an immunoglobulin Fc region connecting between two binding domains, (ii) a modified immunoglobulin Fc region connecting between two binding domains (e.g., with a knob and hole configuration), connects to a binding domain that binds to a different heterologous protein, e.g., a T cell expressing protein.

Methods for Preparing Multispecific T cell Engagers

[0419] The engagers described herein are produced as recombinant proteins. Generally, a polynucleotide sequence is constructed that encodes the recombinant protein is prepared and inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for

GPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVD GYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWV NGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSEN DEWTQDRAKPVTQIVSAEAWGRADCGGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPE TGG (**Ros9a Scaffold 19**) (SEQ ID NO: 641).

Domain/fragment	**SEQUENCE**	
N terminal signal peptide	MMGVKVLFALICIAVAEAD (SEQ ID NO: 667)	
TCR alpha chain, TRAV:TRAC fusion	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQ GYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQ AGTALIFGKGTTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDS QIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANA FQNSIIPEDTFFPSPESSC (SEQ ID NO: 680)	
	TRAV	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQ QFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLP RVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSS D (SEQ ID NO: 619) CDR1: NIATNDY (SEQ ID NO: 874) CDR2: GYKTK (SEQ ID NO: 875) CDR3: LVGDMDQAGTALI (SEQ ID NO: 876) (According to IMGT numbering scheme)
	TRAC	YIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQS KDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFT CANAFQNSIIPEDTFFPSPESSC (SEQ ID NO: 681)
Linker, HIS-tag, cleavable linker	GSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 696)	
Signal peptide	MMGVKVLFALICIAVAEA (SEQ ID NO: 668)	
anti-CD3 VL	AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLL IYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLP WTFGQGTKVEIK (SEQ ID NO: 604) LC CDR1 QDIRNY (SEQ ID NO: 732), LC CDR2 YTS (SEQ ID NO: 733), LC CDR3 QQGNTLPWT (SEQ ID NO: 610). (IMGT numbering scheme)	

linker between anti-CD3 VL and VH	GGGGSGGGGSGGGGSGGGGSGGGS (SEQ ID NO: 682)	
anti-CD3 VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGL EWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDT AVYYCARSGYYGDSDWYFDVWGQGTLVTVSS (SEQ ID NO: 603) HC CDR1 GYSFTGYT (SEQ ID NO: 729), HC CDR2 INPYKGVS (SEQ ID NO: 730), HC CDR3 ARSGYYGDSDWYFDV (SEQ ID NO: 731); (IMGT numbering scheme)	
short linker	GGGGS (SEQ ID NO: 683)	
TCR beta chain-TRBV:TRBC fusion	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQ YYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSL GEGRVDGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKAT LVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSR YALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVT QIVSAEAWGRADC (SEQ ID NO: 684)	
	TRBV	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQ ALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSS ELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTR LTVV (SEQ ID NO: 620) CDR1: SGHDT (SEQ ID NO: 877) CDR2: YYEEEE (SEQ ID NO: 878) CDR3: ASSLGEGRVDGYT (SEQ ID NO: 879) (Underlined, CDR sequences according to IMGT)
	TRBC	EDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFY PPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDS RYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADC (SEQ ID NO: 685)
linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG (SEQ ID NO: 686)	

[0485] Ros9a Scaffold 19, can also be without signal peptide (SEQ ID NO: 642) in its mature form.

[0486] **Ros9a Scaffold 20b (SEQ ID NO: 643).** The diagrammatic representation of the general design and sequence is depicted in FIG. 8A.

[0487] The above sequence (SEQ ID NO: 643) consists of the following segments from N terminal to C terminal as shown in **Table 8B**:

MMGVKVLFALICIAVAEAAIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGK APKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTK VEIKGGGGSGGGGSGGGGSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTG YTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAE DTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSGGGGSAGVTQSPTHLIKTRGQQVTL RCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNAL LLGDSALYLCASSLGEGRVDGYTFGSGTRLTVVGSDYEFLKSWTVEDLQKRLLALDPMM EQEIEEIRQKYQCKRQPILDAIEAKGSGHHHHHHHHHHHRIRRSGSGATNFSLLKQAGDVE ENPGPMMGVKVLFALICIAVAEADLAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWY QQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAG TALIFGKGTTLSVCPDGSDYEFLKSWTVEDLQKRLLALDPMMEQEIEEIRQKYQSKRQPIL DAIEAKGSGSAWSHPQFEK (**Ros9a Scaffold 20b**) (SEQ ID NO: 643).	
Domain/fragment	SEQUENCE
N terminal signal peptide	MMGVKVLFALICIAVAEAD (SEQ ID NO: 667)
Anti-CD3-VL	AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLL IYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLP WTFGQGTKVEIK (SEQ ID NO: 604) LC CDR1 QDIRNY (SEQ ID NO: 732), LC CDR2 YTS (SEQ ID NO: 733), LC CDR3 QQGNTLPWT (SEQ ID NO: 610). (IMGT numbering scheme)
Linker sequence	GGGGSGGGGSGGGGSGGGGSGGGS (SEQ ID NO: 682)
Anti-CD3 VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGL EWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDT AVYYCARSGYYGDSDWYFDVWGQGTLVTVSS (SEQ ID NO: 603) HC CDR1 : GYSFTGYT (SEQ ID NO: 729), HC CDR2 INPYKGVS (SEQ ID NO: 730), HC CDR3 ARSGYYGDSDWYFDV (SEQ ID NO: 731); (IMGT numbering scheme)

Short linker	GGGGS (SEQ ID NO: 683)
TCR beta chain, TRBV	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQ YYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSL GEGRVDGYTFGSGTRLTVV (SEQ ID NO: 620) CDR1: SGHDT (SEQ ID NO: 877) CDR2: YYEEEE (SEQ ID NO: 878) CDR3: ASSLGEGRVDGYT (SEQ ID NO: 879) (IMGT numbering scheme)
Fv clasp domain	GSDYEFLKSWTVEDLQKRLLALDPMMEQEIEEIRQKYQCKRQPILD AIEAK (SEQ ID NO: 687)
short linker-HIS tag, cleavable linker	GSGHHHHHHHHHHRIRRSGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 688)
Signal peptide sequence	MMGVKVLFALICIAVAEAD (SEQ ID NO: 667)
TRAV sequence	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQ GYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQ AGTALIFGKGTTLSV (SEQ ID NO:883)
TRAV sequence (alternate)	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQ GYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQ AGTALIFGKGTTLSVSSD (SEQ ID NO: 619)
Fv clasp domain	CPDGSDYEFLKSWTVEDLQKRLLALDPMMEQEIEEIRQKYQSKRQPI LDAIEAKGSGSAWSHPQFEK (SEQ ID NO: 689).

[0488] The polypeptide Ros9a Scaffold 20b can also be without signal peptide and having the SEQ ID NO: 644.

[0489] **Ros9a Scaffold 21**. The diagrammatic representation of the general design and sequence is depicted in **FIG. 9A**. The first polypeptide chain (Chain A, SEQ ID NO: 645) and the second polypeptide chain (Chain B, SEQ ID NO: 646) were generated. These polypeptides comprise sequences set forth below:

[0490] **Chain A:**

MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPS QGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTAL IFGKGTTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDK CVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GGGGSGGGGSG

GGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSLP
ETGGHHHHHHHHHH* (**Ros9a Scaffold 21)** (SEQ ID NO: 645).

[0491] **Chain B:**

MMGVKVLFALICIAVAEAAIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKA
PKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEI
KGGGGSGGGGSGGGGSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTM
NWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAV
YYCARSGYYGDSDWYFDVWGQGTLVTVSSGGGGSDAGVTQSPTHLIKTRGQQVTLRCSPK
SGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSA
LYLCASSLGEGRVDGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATG
FYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFR
CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGGGSGGGGSGGGGSDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLEASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV
DKSRWQQGNVFSCSVMHEALHNAYTQKSLSLSPGKGGGGSGLNDIFEAQKIEWHE (**Ros9a Scaffold 21)** (SEQ ID NO: 646).

[0492] SEQ ID NOs: 645-646 consists of the following segments from N terminal to C terminal as shown in **Table 8C**:

Chain A		
Signal Peptide	MMGVKVLFALICIAVAEAD (SEQ ID NO: 667)	
TCR-alpha chain	**LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRF IIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGD MDQAGTALIFGKGTTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCL FTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSQKS DFTCANAFQNSIIPEDTFFPSPESSC** (SEQ ID NO: 680)	
	TRAV	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPS QGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDT AVYYCLVGDMDQAGTALIFGKGTTLSVSSD (SEQ ID NO: 619) CDR1: NIATNDY (SEQ ID NO: 874)

		CDR2: GYKTK (SEQ ID NO: 875) CDR3: LVGDMDQAGTALI (SEQ ID NO: 876) (IMGT)
	TRAC	YIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDS DVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQ NSIIPEDTFFPSPESSC (SEQ ID NO: 681)
Linker	GGGGSGGGGSGGGGS (SEQ ID NO: 690)	
Immunoglobulin Fc domain chain 1	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 691)	
Linker with SLPETG (SEQ ID NO: 1036) and HIS-tag	GGGGSGGGGSLPETGGHHHHHHHHHH (SEQ ID NO: 695)	
Chain B		
Signal Peptide	MMGVKVLFALICIAVAEAD (SEQ ID NO: 667)	
Anti-CD3 VL domain	AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLI YYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPW TFGQGTKVEIK (SEQ ID NO: 604) LC CDR1 QDIRNY (SEQ ID NO: 732), LC CDR2 YTS (SEQ ID NO: 733), LC CDR3 QQGNTLPWT (SEQ ID NO: 610). (IMGT numbering scheme)	
linker	GGGGSGGGGSGGGGSGGGGSGGGS (SEQ ID NO: 682)	
Anti-CD3 VH domain	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLE WVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTA VYYCARSGYYGDSDWYFDVWGQGTLVTVSS (SEQ ID NO: 603) HC CDR1 : GYSFTGYT (SEQ ID NO: 729), HC CDR2 INPYKGVS (SEQ ID NO: 730), HC CDR3 ARSGYYGDSDWYFDV (SEQ ID NO: 731); (IMGT numbering scheme)	

linker	GGGGSD (SEQ ID NO: 693)	
TCR-beta chain (TRBV:TRBC fusion)	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADC (SEQ ID NO: 684)	
	TRBV	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLTVV (SEQ ID NO: 620) CDR1: SGHDT (SEQ ID NO: 877); CDR2: YYEEEE (SEQ ID NO: 878); CDR3: ASSLGEGRVDGYT (SEQ ID NO: 879).
	TRBC	EDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADC (SEQ ID NO: 685)
linker	GGGGSGGGGSGGGGS (SEQ ID NO: 690)	
Immunoglobulin Fc domain chain 2	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLEASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNAYTQKSLSLSPGK (SEQ ID NO: 692)	
linker and BAP tag	GGGGSGLNDIFEAQKIEWHE (SEQ ID NO: 694)	

[0493] **Ros9d Scaffold 19.** The diagrammatic representation of the general design and sequence is depicted in FIG. 10A.

Table 8D:

MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVY**

ITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSCGSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMMGVKVLFALICIAVAEAAIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSGGGGSAGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGQTNYGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG* (**Ros9d Scaffold 19**) (SEQ ID NO: 647).

Domain	Sequence
Signal Peptide	MMGVKVLFALICIAVAEAD (SEQ ID NO: 667)
TCR alpha chain (TRAV:TRAC fusion)	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC (SEQ ID NO: 680)
Linker, HIS-tag, cleavable linker	GSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 696)
Signa Peptide	MMGVKVLFALICIAVAEA (SEQ ID NO: 668)
Anti-CD3 -VL	AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIK (SEQ ID NO: 604) LC CDR1 QDIRNY (SEQ ID NO: 732), LC CDR2 YTS (SEQ ID NO: 733), LC CDR3 QQGNTLPWT (SEQ ID NO: 610). (IMGT numbering scheme)
linker	GGGGSGGGGSGGGGSGGGGSGGGS (SEQ ID NO: 682)
Anti-CD-VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSS (SEQ ID NO: 603) HC CDR1 : GYSFTGYT (SEQ ID NO: 729),

	HC CDR2 INPYKGVS (SEQ ID NO: 730), HC CDR3 ARSGYYGDSDWYFDV (SEQ ID NO: 731); (IMGT numbering scheme)
linker	GGGGS (SEQ ID NO: 683)
TCR beta chain (TRBV:TRBC fusion)	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQY YEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGQ TNYGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCL ATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSR LRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEA WGRADC (SEQ ID NO: 785)
linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG (SEQ ID NO: 686)

[0494] The polypeptide Ros9d Scaffold 19 can also be without signal peptide and having the SEQ ID NO: 648.

[0495] **Ros9d Scaffold 21 :** The diagrammatic representation of the general design and sequence is depicted in FIG. 11A. This scaffold has 2 polypeptide chains, the first polypeptide chain (Chain A) has the following sequence

[0496] **Chain A:**

MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQG**
PRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKG
TTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRS
MDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSCGGGGSGGGGSGGGGSDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSLPETGGHHHHHHHHHH* (**Ros9d**
Scaffold 21) (SEQ ID NO: 649).

[0497] The second polypeptide (Chain B) has the sequence:

[0498] **Chain B:**

MMGVKVLFALICIAVAEAAIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKL
LIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGS
GGGGSGGGGSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGK
GLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAV

YYCARSGYYGDSDWYFDVWGQGTLVTVSSGGGGSDAGVTQSPTHLIKTRGQQVTLRCSPKSG
HDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCA
SSLGQTNYGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELS
WWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSEN
DEWTQDRAKPVTQIVSAEAWGRADCGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLEASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH
NAYTQKSLSLSPGKGGGGSGLNDIFEAQKIEWHE* (**Ros9d Scaffold 21**) (SEQ ID NO: 650).

[0499] The above scaffold has the following parts as shown in **Table 8E**:

Chain A	
Signal Peptide	MMGVKVLFALICIAVAEAD (SEQ ID NO: 667)
TRAV: TRAC fusion	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKT KVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKG TTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYIT DKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC (SEQ ID NO: 680)
linker	GGGGSGGGGSGGGGS (SEQ ID NO: 690)
Immunoglobulin Fc	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 691).
linker, LPETG-HIS Tag ("LPETG" disclosed as SEQ ID NO: 1035)	GGGGSGGGGSLPETGGHHHHHHHHHH (SEQ ID NO: 695)
Chain B	
Signal Peptide	MMGVKVLFALICIAVAEA (SEQ ID NO: 668)

Anti-CD3 VL	AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTS RLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKV EIK (SEQ ID NO: 604) LC CDR1 QDIRNY (SEQ ID NO: 732), LC CDR2 YTS (SEQ ID NO: 733), LC CDR3 QQGNTLPWT (SEQ ID NO: 610). (IMGT numbering scheme)
Linker	GGGGSGGGGSGGGGSGGGGSGGGS (SEQ ID NO: 682)
Anti-CD3 -VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVAL INPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGY YGDSDWYFDVWGQGTLVTVSSGGGGS (SEQ ID NO: 884) HC CDR1 : GYSFTGYT (SEQ ID NO: 729), HC CDR2 INPYKGVS (SEQ ID NO: 730), HC CDR3 ARSGYYGDSDWYFDV (SEQ ID NO: 731); (IMGT numbering scheme)
TRBV:TR BC fusion	DAGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYE EEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGQTNYGYT FGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVEL SWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNH FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADC (SEQ ID NO: 697)
Linker	GGGGSGGGGSGGGGS (SEQ ID NO: 690)
Immunogl obulin Fc region	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLEASRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCS VMHEALHNAYTQKSLSLSPGK (SEQ ID NO: 692)
Linker and tag	GGGGSGLNDIFEAQKIEWHE (SEQ ID NO: 694)

[0500] **Ros10 Scaffold 19.** The diagrammatic representation of the general design and sequence is depicted in **FIG. 12A**.

[0501] The above scaffold has the following parts as shown in **Table 8F**:

MMGVKVLFALICIAVAEA**QKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYS GKSPELIMFIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAAAMDSSYK LIFGSGTRLLVRPDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYIT DKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GSGHHHHH HHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMMGVKVLFALICIAVAEAAIQMTQ SPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGS GTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGSGGGG SGGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINP YKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDSDWYFDV WGQGTLVTVSSGGGGSAGITQSPRYKITETGRQVTLMCHQTWSHSYMFWYRQDLGHGL RLIYYSAAADITDKGEVPDGYVVSRSKTENFPLTLESATRSQTSVYFCASSDPGTEAFFGQ GTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGKEV HDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQ DRAKPVTQIVSAEAWGRADCGGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG

(**Ros10 Scaffold 19**) (SEQ ID NO: 651).

Domain	Sequence
Signal peptide	MMGVKVLFALICIAVAEA (SEQ ID NO: 668)
TRAV:TRAC fusion	QKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAAAMDSSYKLIFGSGTRLLVRPDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC (SEQ ID NO: 698)
Linker with cleavable sequence	GSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:699)
Signal peptide	MMGVKVLFALICIAVAEA (SEQ ID NO: 668)
Anti-CD3 - VL	AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIK (SEQ ID NO: 604) LC CDR1 QDIRNY (SEQ ID NO: 732), LC CDR2 YTS (SEQ ID NO: 733),

	LC CDR3 QQGNTLPWT (SEQ ID NO: 610); (IMGT numbering scheme)
linker	GGGGSGGGGSGGGGSGGGGSGGGS (SEQ ID NO: 682)
Anti-CD3 VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALIN PYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYGD SDWYFDVWGQGTLVTVSS (SEQ ID NO: 603) HC CDR1 : GYSFTGYT (SEQ ID NO: 729), HC CDR2 INPYKGVS (SEQ ID NO: 730), HC CDR3 ARSGYYGDSDWYFDV (SEQ ID NO: 731); (IMGT numbering scheme)
Linker	GGGGS (SEQ ID NO: 683)
TRBV:TRB C fusion	AGITQSPRYKITETGRQVTLMCHQTWSHSYMFWYRQDLGHGLRLIYYSAAADI TDKGEVPDGYVVSRSKTENFPLTLESATRSQTSVYFCASSDPGTEAFFGQGTRL TVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGK EVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYG LSENDEWTQDRAKPVTQIVSAEAWGRADC (SEQ ID NO: 700)
linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG (SEQ ID NO: 686)

[0502] **Example 3. Generation and purification of sTCR scaffolds.**

[0503] In this example, sTCRs are isolated in small scale productions for testing the functionality. Human epithelial cells Expi293 were transfected with mRNA constructs (or alternatively with plasmid vectors consisting of the sequence encoding an sTCR, Cells were grown in culture for a week and the supernatants collected. The secreted sTCR products were passed through IMAC nickel NTA columns and size exclusion gel filtration columns and collected. Typically, the process yields an estimated 5-30 mg/L of culture supernatant. Shown in FIG. 4 (top), a cartoon of a cell culture from left collection of the purified product. **FIG. 4** bottom shows representative purification products run on gel electrophoresis. **FIGs. 5A** and **5B** additionally show purification products of scaffold 2 eluents collected at different times, run on a native gel: left early collection, right, mid-elution. Right-most, protein size marker. **FIG. 5B** shows single elution peak.

[0504] **Example 4. Functional Assay of sTCR Scaffolds on Cells Transfected with Peptide and HLA.**

[0505] In this example, functional assays of the bispecific TCRs were performed. **FIG. 6** shows a schematic diagram of the process. The effector cells are T cells from a human donor. Target cells are A375 cells expressing the HLA and the epitope specific for each sTCR. A375 cells were electroporated

with vector or mRNA expressing the HLA, and the KRAS mutated peptide and rested overnight. Effector T cells were purified from peripheral blood sample of a healthy donor and rested for 1-2 days in non-activating media before assay. sTCRs were added to the media and the Effector to target cell rations were between 0.04:1 – 10:1. Survival of the A375 cells were evaluated. FIGs 7A-12B illustrate the results for four representative bispecific sTCR scaffolds consisting of Ros9 and Ros 10 TCR backbones having TRAV and TRBV sequences against RAS G12D peptide GADGVGKSA (SEQ ID NO: 999) (KRAS G12D 9mer) on HLA-C*08:02; and an anti CD3 scFv. The amino acid sequences and representative diagrammatic view of the structures are provided for each set of experiment with a different bispecific molecule on **FIGs. 7A, 8A, 9A, 10A, 11A**, and **12A**. The amino acid sequences of the are color coded in accordance to the fill color scheme of the domains in the corresponding diagram of the BiTE scaffold structure to the left. The corresponding results for cytotoxic assay is represented in the graphs on the FIGs 7B, 8B, 9B, 10B, 11B and 12B respectively. The higher the cytotoxicity, in this case, the lower the values on the Y axis, determining the total green object area, since the target cells express green fluorescence protein (GFP). **FIG. 7B** shows, Scaffold 19 shows higher cytotoxicity of the target cells with increasing sTCR concentration. This indicated that the sTCR Scaffold 19 potentiated T cell activation and cytotoxicity. Likewise, **FIG. 9B** showed similar effect with Scaffold 21; **FIG. 10B** with Scaffold 19, and **FIG. 11B** with Scaffold 21. However, data shown in **FIG. 8B** and **FIG. 12B** indicate that the scaffolds shown in these figures did not result in successful potentiation of the T cell in inducing cytotoxicity to target cells.

[0506] **Example 5. Functional Assay of sTCR Scaffolds on Tumor Cells Expressing Endogenous Peptide and HLA**

[0507] In this example, effectiveness of the representative sTCR scaffolds are evaluated with tumor cells as target cells that express the peptide and HLA for the TCR. HPAC cells represent tumor cell line that are not engineered and known to express the KRAS G12D mutation, and HLA-C*08:02. One goal of this experiment is to determine if tumor cell lines with native levels of HLA/neoantigen have sufficient antigen to be targeted by BiTE, and also to compare BiTE efficacy across scaffolds (CD3 binders; affinity) against physiologically relevant tumor cells. Cells were stained for presence of Annexin V and Caspase 3/7 (nuc-light rapid in future tests) as apoptotic cell markers after the treatment – as depicted in a representative schematic diagram in **FIG. 13**.

[0508] The Ros 9 Scaffold 19 and Ros 10 Scaffold 19 structures were compared in this assay and results presented in data on **FIGs. 14A-14C**. FIG. 14A shows fluorescent images of target cells positive for Annexin V (orange) and caspase 3/7 apoptotic signals (green), with the indicated doses of sTCRs in culture and T isolated cells from a human donor. As in indicated, presence of T cells and the sTCR at the highest concentration of 100 microM as well as at 50 microM showed high expression of Annexin V at least in case of incubation with Scaffold 19 (top panel). It was noted from this assay that the caspase signal was detectable throughout and including in the control sets, particularly, one without

scFv from BNT New Scaffolds group (NSG23)–TR66-VHVL **Ros9a Scaffold 22**	MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNI ATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTL SLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNP DPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLD MRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GS GHHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMMGV KVLFALICIAVAEAQVQLQQSGAELARPGASVKMSCKTSGYTFTRYT MHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTA YMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSGGGGSG GGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCRASSSVSYMN WYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSMEAE DAATYYCQQWSSNPLTFGAGTKLELKGGGGSAGVTQSPTHLIKTRG QQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDR FSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTR LTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELS WWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQD PRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGG GSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	755
	Signal Peptide	MMGVKVLFALICIAVAEAD	756
	Human TCR-alpha chain	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQ QFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLP RVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSS DYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQS KDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTC ANAFQNSIIPEDTFFPSPESSC	757
	Linker -10X HIS (SEQ ID NO: 866) (underlined) and cleavage domain (*italicized*)	GSGHHHHHHHHHH*RIRR*SGSGVKQTLNFDLLKLAGD VESNPGP	758
	Second signal peptide	MMGVKVLFALICIAVAEA	668

	CD3-VH domain	QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMH WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLT TDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLD YWGQGTTLTVSS (Underlined CDR sequences, Kabat) According to IMGT numbering scheme: HCDR1: GYTFTRYT (SEQ ID NO: 718) HCDR2: INPSRGYT (SEQ ID NO: 719) HCDR3: ARYYDDHYSLDY (SEQ ID NO: 721)	759
	linker	GGGGSGGGGSGGGGSGGGGS	760
	CD3-VL domain	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQ QKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTI SSMEAEDAATYYCQQWSSNPLTFGAGTKLELK (Underlined CDR sequences, Kabat) According to IMGT numbering scheme: HCDR1: SSVSY (SEQ ID NO: 722) HCDR2: INPSRGYT (SEQ ID NO: 723) HCDR3: ARYYDDHYSLDY (SEQ ID NO: 721)	761
	linker	GGGGS	683
	Human TCR-beta TRBV:TRBC	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQ ALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSE LNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLT VVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATG FYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQD SRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADC	762
	Linker with LPETG (SEQ ID NO: 1035) sequence	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	686
scFv from BNT New Scaffolds group – TR66-VLVH	MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNI ATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTL SLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNP DPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLD MRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GS GHHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPM	763

Ros9a Scaffold 22	MGVKVLFALICIAVAEAQIVLTQSPAIMSASPGEKVTMTCRASSSVSY MNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSME AEDAATYYCQQWSSNPLTFGAGTKLELKGGGGSGGGGSGGGGSGG GGSGGGGSQVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWV KQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLS SLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSGGGGSAGVTQS PTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEE RQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVD GYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATG FYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLR VSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWG RADCGGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG*		
	Signal peptide	MMGVKVLFALICIAVAEAD	756
	Human TCR-alpha chain TRAV:TRAC	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQ QFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLP RVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSS DYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQS KDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTC ANAFQNSIIPEDTFFPSPESSC	764
	Linker -10X HIS (SEQ ID NO: 866) and cleavage domain	GSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGD VESNPGP	765
	Second signal peptide	MMGVKVLFALICIAVAEA	668
	Anti-CD3-VH domain	QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMH WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLT TDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLD YWGQGTTLTVSS	767
	linker	GGGGSGGGGSGGGGSGGGGSGGGGS	611

	Anti-CD3-VL domain	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQ QKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTI SSMEAEDAATYYCQQWSSNPLTFGAGTKLELK	766
	Linker	GGGGS	683
	Human TCR-beta chain: TRBV:TRBC	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQ ALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSE LNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLT VVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATG FYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQD SRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADC	768
	linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	686
scFv from BNT New Scaffolds group – NSG23-210112-VHVL **Ros9a Scaffold 22**	MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNI ATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTL SLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNP DPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLD MRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GS GHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMMGV KVLFALICIAVAEAQVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYY MCWVRQAPGKGLEWVSCIYTNNIGSTWYASWAKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARGIYSDSSDFIGNLWGQGTLVTVSSGGG GSGGGGSGGGGSGGGGSAIQMTQSPSSLSASVGDRVTITCQASQTIYN YLAWYQQKPGKVPELLIYKASTLASGVPSRFMGTGFGTDFTLTIDSLQ PEDAATYYCQQYNTIINVDRTFGGGTKVEIKGGGGSAGVTQSPTHLIK TRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNF PDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGS GTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHV ELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFW QDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCG GGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG*		769
	Signal peptide	MMGVKVLFALICIAVAEAD	756

	linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	686
scFv from New Scaffolds group – NSG23-210112-VLVH **Ros9a Scaffold 22**	**YIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**EDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCMMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GSGHHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMMGVKVLFALICIAVAEAAIQMTQSPSSLSASVGDRVTITCQASQTIYNYLAWYQQKPGKVPELLIYKASTLASGVPSRFMGTGFGTDFTLTIDSLQPEDAATYYCQQYNTIINVDRTFGGGTKVEIKGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYYMCWVRQAPGKGLEWVSCIYTNNIGSTWYASWAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIYSDSSDFIGNLWGQGTLVTVSSGGGGSAGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	775
	Signal peptide sequence	MMGVKVLFALICIAVAEAD	756
	Human TCR-alpha chain	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC	776

	His tag +Linker	GSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDV ESNPGP	777
	Second signal peptide	MMGVKVLFALICIAVAEA	668
	Anti-CD3-VL domain [210112-VL]	AIQMTQSPSSLSASVGDRVTITCQASQTIYNYLAWYQQ KPGKVPELLIYKASTLASGVPSRFMGTGFGTDFTLTIDS LQPEDAATYYCQQYNTIINVDRTFGGGTKVEIK (CDRs underlined according to IMGT)	778
	Linker	GGGGSGGGGSGGGGSGGGGSGGGGS	611
	Anti-CD3-VH domain [210112-VH]	QVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYYMCW VRQAPGKGLEWVSCIYTNNIGSTWYASWAKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCARGIYSDSSDFIGNL WGQGTLVTVSS (CDRs underlined according to IMGT)	779
	Linker	GGGGS	683
	Human TCR-beta chain	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQA LGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSELN VNALLLGDSALYLCASSLGEGRVDGYTFGSGTRLTVVE DLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPH VELSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALS SRLRVSATFWQDPRNHFRCQVQFYGLSENDEWTQDRA KPVTQIVSAEAWGRADC	780
	linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	686
scFv from BNT New Scaffolds group – NSG23-TR66-VHVL **Ros9d Scaffold 22**	MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNIAT NDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPR VSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNPDPAVYQ LRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLDMRSMDFK SNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**CGSGHHHHHHHH HHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMMGVKVLFALICIAVAE AQVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLE WIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYY CARYYDDHYSLDYWGQGTTLTVSSGGGGSGGGGSGGGGSGGGGSQIVL TQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKV ASGVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKL ELKGGGGSAGVTQSPT	781

	HLIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRG NFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGQTNYGYTFGSGT RLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVELSW WVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQDPRNH FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGGGGSGLNDI FEAQKIEWHEGGGGSGGGGSLPETGG		
	Signal peptide	MMGVKVLFALICIAVAEAD	756
	Human TCR-alpha chain	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQ QFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLP RVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSS DYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQS KDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTC ANAFQNSIIPEDTFFPSPESSC	782
	Linker peptide	GSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGD VESNPGP	696
	Second signal peptide sequence	MMGVKVLFALICIAVAEA	668
	Anti-CD3-VH domain	QVQLQQSGAELARPGASVKMSCKTSGYTFTRYTMH WVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLT TDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYSLD YWGQGTTLTVSS	783
	Linker	GGGGSGGGGSGGGGSGGGGS	760
	Anti-CD3-VL domain	QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQ QKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTI SSMEAEDAATYYCQQWSSNPLTFGAGTKLELK	784
	Linker	GGGGS	683
	TCR beta chain	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQ ALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSE LNVNALLLGDSALYLCASSLGQTNYGYTFGSGTRLTV VEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGF YPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDS RYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADC	785

	linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	686
scFv from BNT New Scaffolds group – NSG23-210112-VHVL **Ros9d Scaffold 22**	MMGVKVLFALICIAVAEAD**LAKTTQPISVDSYEGQEVNITCSHNNI ATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTL SLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSSDYIQNP DPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQSKDSDVYITDKCVLD MRSMDFKSNSAVAWSQKSDFTCANAFQNSIIPEDTFFPSPESSC**GS GHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGDVESNPGPMMGV KVLFALICIAVAEAQVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYY MCWVRQAPGKGLEWVSCIYTNNIGSTWYASWAKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARGIYSDSSDFIGNLWGQGTLVTVSSGGG GSGGGGSGGGGSGGGGSAIQMTQSPSSLSASVGDRVTITCQASQTIYN YLAWYQQKPGKVPELLIYKASTLASGVPSRFMGTGFGTDFTLTIDSLQ PEDAATYYCQQYNTIINVDRTFGGGTKVEIKGGGGSAGVTQSPTHLIK TRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRGNF PDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGQTNYGYTFGSG TRLTVVEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGFYPPHVE LSWWVNGKEVHDGVCTDPQPLKEQPALQDSRYALSSRLRVSATFWQ DPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGG GGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	786
		MMGVKVLFALICIAVAEAD	756
	Human TCR-alpha chain	LAKTTQPISVDSYEGQEVNITCSHNNIATNDYITWYQ QFPSQGPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLP RVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVSS DYIQNPDPAVYQLRDSKSSDKFVCLFTDFDSQIQVSQS KDSDVYITDKCVLDMRSMDFKSNSAVAWSQKSDFTC ANAFQNSIIPEDTFFPSPESSC	787
	HIS tag+ cleavable peptide Linker	GSGHHHHHHHHHHRIRRSGSGVKQTLNFDLLKLAGD VESNPGP	788
	Second signal peptide	MMGVKVLFALICIAVAEA	668

	Anti-CD3 VH domain	QVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYYMC WVRQAPGKGLEWVSCIYTNNIGSTWYASWAKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARGIYSDSSDF IGNLWGQGTLVTVSS	779
	Linker	GGGGSGGGGSGGGGSGGGGS	760
	Anti-CD3 VL domain	AIQMTQSPSSLSASVGDRVTITCQASQTIYNYLAWYQ QKPGKVPELLIYKASTLASGVPSRFMGTGFGTDFTLTI DSLQPEDAATYYCQQYNTIINVDRTFGGGTKVEIK	789; alternati vely 778
	Linker	GGGGS	683
	TCR beta chain	AGVTQSPTHLIKTRGQQVTLRCSPKSGHDTVSWYQQ ALGQGPQFIFQYYEEEERQRGNFPDRFSGHQFPNYSSE LNVNALLLGDSALYLCASSLGQTNYGYTFGSGTRLTV VEDLKNVFPPEVAVFEPSKAEISRTQKATLVCLATGF YPPHVELSWWVNGKEVHDGVCTDPQPLKEQPALQDS RYALSSRLRVSATFWQDPRNHFRCQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADC	790
	linker- BAP-LPETG tag (SEQ ID NO: 1035)	GGGGSGLNDIFEAQKIEWHEGGGGSGGGGSLPETGG	686

[0514] **Example 7. Functional assay of the constructs in Table 9.**

[0515] **FIG. 23** shows a diagrammatic view of the structure (left) and amino acid sequence (bottom) of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on TR66-VHVL construct. **FIG. 24** shows a diagrammatic view of the structure (left) and amino acid sequence (bottom) of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on TR66-VLVH construct. **FIG. 25** shows a diagrammatic view of the structure (left) and amino acid sequence (bottom) of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on 210112-VHVL construct. **FIG. 26** shows the a diagrammatic view of the structure (left) and amino acid sequence (bottom) of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on 210112-VLVH construct. **FIG. 27** shows the a diagrammatic view of the structure (left) and amino acid sequence (bottom) of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on 210112-VLVH construct. The anti-CD3 arm is based on TR66-VHVL construct. **FIG. 28** shows a diagrammatic view of the structure (left) and amino acid sequence (bottom) of the construct Ros9a Scaffold 22. The anti-CD3 arm is based on 210112-VLVH construct. The anti-CD3 arm is based on 210112-VHVL construct. The functional assays are described below.

additionally incorporated one or more cleavable sequence elements and linkers, where the long polypeptide may be cleaved upon translation to effectively engage without experiencing steric hindrance. Various design modifications were explored and tested as detailed herein.

[0520] In this Example, protein structure guided soluble TCR constructs were designed to incorporate a linker between anti-CD3 binder and TRBV to orient the anti-CD3 scFv. In one exemplary design, suitable constructs were designed using scaffolds based on platform scaffolds 74, 107, 109, 111 or 113 described earlier. Exemplary soluble TCR alpha-beta chains were designed, having an N terminal to C-terminal orientation where beta chains appear N-terminal to an alpha chain. Conversely, exemplary soluble TCR alpha beta chains were designed where the alpha chain appears N terminal to the beta chain. (**Table 12**). Additionally, BiTEs were designed with these variations, including a variation where T cell engagers (e.g., anti CD3 engagers, e.g., an anti-CD3 scFV as described above) were designed as an N-terminal subunit connected to the TCR chains with linkers, or as an internal moiety connected to individual TCR chains, whereas spatially occupying the same three-dimensional structure with respect to TCR chains, where it is connected to one or the other chain vial linkers, as well as whether it is terminally placed on the polypeptide or internally placed.

[0521] Use of protein structure-guided design to engineer flexible glycine-serine linkers to physically link the extracellular domains of TCRα and TCRβ with an anti-CD3 single-chain variable fragment (scFv) to generate soluble TCR (sTCR) BiTEs encoded by a single mRNA or DNA construct. The TCRα and TCRβ extracellular domains (including the variable and constant domains) are liberated from the cell membrane via truncation precisely 5' of the conserved cysteine residues at the end of the TCRα and TCRβ constant domains. The TCRα, TCRβ, and anti-CD3 scFv components were linked in two different orientations as explained earlier – TCRαβ or TCRβα. To determine approximate, compatible glycine-serine linker lengths, experimental TCR crystal structures solved in-house were used to visualize and measure distances in angstroms between the N- and C-termini of the extracellular portions of the TCRα and TCRβ chains. Glycine-serine linkers in increasing increments of (GGGGS)n (SEQ ID NO: 683) were built between the chains and the resulting fusion proteins with anti-CD3 scFvs were modeled via AlphaFold2 to determine the linker lengths that minimally bridged the distances between desired termini without disrupting the three-dimensional conformation of the TCRαβ heterodimer. For the TCRαβ orientation, a minimal linker of 20 amino acids – (GGGGS)4 (SEQ ID NO: 760) –was constructed to join the C-terminus of the TCRα chain to the N-terminus of the anti-CD3 scFv, which was in turn joined via a 5 amino acid linker – (GGGGS) (SEQ ID NO: 683)–from the C-terminus of the scFv to the N-terminus of the TCRβ chain. For the TCRβα orientation, the anti-CD3 scFv is at the N-terminus of the construct and is joined at the C-terminus via a 5 amino acid linker – (GGGGS) (SEQ ID NO: 683) –to the N-terminus of the TCRβ chain followed by a minimal linker of 35 amino acids – (GGGGS)7 (SEQ ID NO: 864) –to join the C-terminus of the TCRβ chain to the N-terminus of the TCRα chain. The sequences below also contain secretion signal sequences, Ni-NTA

Ros9a_αβ_35GS Linker	αβ	35 residues	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS $(G_4S)_7$ (SEQ ID NO: 864)
Ros9a_βα_Linker 1	βα	37 residues	GGSSGSG-X_{25}-GSGSG, where X is an amino acid selected from A, G, S and T. (SEQ ID NO: 872)
Ros9a_αβ_Linker 1	αβ	37 residues	GGSSGSG-X_{25}-GSGSG, where X is an amino acid selected from A, G, S and T (SEQ ID NO: 872)
Ros9a_βα_Linker 2	βα	36 residues	SGGGSGGGSEGGGSEGGGSEGGGSEGGGSGGGSG (SEQ ID NO: 873)
Ros9a_αβ_Linker 2	αβ	36 residues	SGGGSGGGSEGGGSEGGGSEGGGSEGGGSGGGSG(SEQ ID NO: 873)

[0527] The respective sequences and individual domains of the single chain sTCR constructs are detailed in Table 12. In some constructs certain amino acid positions are mutated for increasing stability of the soluble TCR polypeptide. For example, S139F, T150I and A190T mutations can be incorporated in TRAC fragment in various constructs, the positions are indicated by *sign right after the amino acid residue that can be mutated and shown in its non-mutated versions in the Table 12 below. Similarly for TRBC, several mutations have been reported to enhance stability, which were prepared for testing, including E134K, H139R, D155P, or S170D. Additionally, one or more N-glycosylation sites may be mutated from TRAC/TRBC (e.g., the Asparagine residue underlined and bolded in some sequences shown in Table 12). However, single chain soluble TCR structures may lack several TRAC modifications, for example S139F, T150I and A190T mutations; and are still found to be stable and efficient in activity at least as compared to the corresponding recombinant TCRs with the stability enhancing modifications described above. Similarly, single chain soluble TCR structures may lack TRBC modifications, for example E134K, H139R, D155P, or S170D. (See, for example **FIG. 40**). AlphaFold 2 modeling informs linker length decisions. In some embodiments, the multispecific molecule comprises one or more sequences of SEQ ID NO: 604, SEQ ID NO: 682, SEQ ID NO: 603, (SEQ ID NO: 683) (GGGGS), SEQ ID NO: 620, SEQ ID NO: 863, SEQ ID NO: 864, SEQ ID NO: 619, SEQ ID NO: 865, SEQ ID NO: 866, and SEQ ID NO: 884. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 862. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 862 without a signal peptide of SEQ ID NO: 668. In some

embodiments, wherein the multispecific molecule comprises one or more sequences selected from the group consisting of SEQ ID NO: 619, SEQ ID NO: 868, SEQ ID NO: 869, SEQ ID NO: 604, SEQ ID NO: 870, SEQ ID NO: 603, SEQ ID NO:620, SEQ ID NO: 871, SEQ ID NO: 866, and SEQ ID NO: 884. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 867. In some embodiments, the multispecific molecule comprises a sequence of SEQ ID NO: 867, but without the signal peptide sequence of SEQ ID NO: 667.

[0528] Table 12. Single Chain Bispecific Structure Sequences and Domains

	Amino Acid Sequence	
TCRβα single-chain sTCR BiTE Full Sequence of sTCR Scaffold name: Ros9a_SC107wβα_antiCD3	MMGVKVLFALICIAVAEAAIQMTQSPSSLSASVGDRVTITCRASQDIRN YLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQP EDFATYYCQQGNTLPWTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGS GGGSEVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGK GLEWVALINPYKGVSTYNQKFKDRFTISVDKSKNTAYLQMNSLRAED TAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSGGGGSAGVTQSPTH LIKTRGQQVTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQRG NFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASSLGEGRVDGYTFG SGTRLTVVEDLKNVFPPEVAVFEPSE*AEISH*TQKATLVCLATGFYPD* HVELSWWVNGKEVHS*GVCTDPQPLKEQPALQDSRYALSSRLRVSAT FWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADG GGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSLAKTTQPISVDSY EGQEVNITCSHNNIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASL FIPADRKSSTLSLPRVSLSDTAVYYCLVGDMDQAGTALIFGKGTTLSVS SDYIQNPDPAVYQLRDSKSSDKS*VCLFTDFDSQT*QVSQSKDSDVYIT DKCVLDMRSMDFKSNSAVAWSQKSDFA*CANAFQNSIIPEDTFFPSPES SGSGLPETGGGSGHHHHHHHHHH (SEQ ID NO: 862)	
	Signal Peptide	MMGVKVLFALICIAVAEA (SEQ ID NO: 668)
	Anti-CD3-(scFv) VL	AIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGK APKLLIYYTSRLESGVPSRFSGSGSGTDYTLTISSLQPEDFATY YCQQGNTLPWTFGQGTKVEIK (SEQ ID NO: 604) LC CDR1 QDIRNY (SEQ ID NO: 732), LC CDR2 YTS (SEQ ID NO: 733), LC CDR3 QQGNTLPWT (SEQ ID NO: 610). (IMGT numbering scheme)
	linker between	GGGGSGGGGSGGGGSGGGGSGGGS (SEQ ID NO: 682)

CLAIMS

What is claimed is:

1. A composition comprising a recombinant nucleic acid for expression in a mammalian cell, the recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule is an engineered T cell receptor (TCR) construct, comprising:

(i) an MHC-peptide complex engager comprising:

(a) a T cell receptor (TCR) alpha variable (TRAV) domain, and

(b) a TCR beta variable (TRBV) domain; and

(ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the T cell engager comprises an anti-CD3 binding domain comprising a heavy chain variable region (VH) that comprises a heavy chain complementarity determining region 3 (HC CDR3) sequence of ARGIYSDSSDFIGNL (SEQ ID NO: 713) or GIYSDSSDFIGNL (SEQ ID NO: 707).

2. A composition comprising a recombinant nucleic acid comprising a sequence encoding a multispecific molecule, wherein the multispecific molecule comprises:

(i) an MHC-peptide complex engager comprising:

(a) a TCR alpha variable (TRAV) domain, and

(b) a TCR beta variable (TRBV) domain;

wherein the peptide in the MHC-peptide complex comprises a RAS peptide sequence; and

(ii) a T cell engager comprising one or more binding domains that bind to an extracellular domain of a receptor expressed by a T cell, wherein the T cell engager comprises an anti-CD3 binding domain comprising a heavy chain variable region (VH) that comprises a heavy chain complementarity determining region 3 (HC CDR3) sequence of ARGIYSDSSDFIGNL (SEQ ID NO: 713) or GIYSDSSDFIGNL (SEQ ID NO: 707).

3. The composition of claim 1 or 2, wherein the multispecific molecule comprises a post-translational modification; or wherein the multispecific molecule comprises a mammalian glycosylation signature.

4. The composition of any one of claims 1-3, wherein the multispecific molecule is soluble and lacks a trans-membrane domain.

5. The composition of any one of claims 1-4, wherein the multispecific molecule consists of two polypeptides, a first polypeptide and a second polypeptide, wherein the first polypeptide and the second polypeptide comprise one or more disulfide bonds between the two polypeptides, and wherein the first polypeptide comprises the TRAV domain and the second polypeptide comprises the TRBV domain.

6. The composition of claim 5, wherein the first polypeptide further comprises a dimerizing domain fused to the TRAV domain, and the second polypeptide comprises a dimerizing domain fused to the TRBV domain.

7. The composition of claim 6, wherein the dimerizing domain of the first polypeptide comprises a TCR alpha constant (TRAC) domain or portion thereof; and the dimerizing domain of the second polypeptide comprises a TCR beta constant (TRBC) domain or portion thereof.

8. The composition of any one of claims 1-4, wherein the MHC-peptide complex engager comprises a TCR alpha constant (TRAC) domain or portion thereof and a TCR beta constant (TRBC) domain or portion thereof, and wherein the multispecific molecule further comprises a linker connecting the TRAC domain and the TRBV domain.

9. The composition of any of claims 1-8, wherein the peptide of the MHC-peptide complex is a mutant peptide, and wherein the MHC of the MHC-peptide complex binds the mutant peptide with a higher affinity compared to a corresponding wild-type peptide.

10. The composition of any one of claims 1-9, wherein the one or more binding domains of the T cell engager binds to CD3 delta, CD3 gamma or CD3 epsilon.

11. The composition of any one of claims 1-10, wherein the one or more binding domains of the T cell engager comprises an antibody domain or antigen binding fragment thereof, and wherein the antigen binding fragment is an ScFv, an SdAb or a VHH.

12. The composition of any one of claims 1-11, wherein the VH of the anti-CD3 binding domain further comprises a HC CDR2 sequence of IYTNNIGST (SEQ ID NO: 712) or CIYTNNIGSTWYASWAKG (SEQ ID NO: 706) and a HC CDR1 sequence of GFSFSNNYY (SEQ ID NO: 711) or NNYYMC (SEQ ID NO: 705).

13. The composition of any one of claims 1-12, wherein the anti-CD3 binding domain comprises a light chain variable region (VL) that comprises a light chain complementarity determining region 3 (LC CDR3) sequence of QQYNTIINVDRT (SEQ ID NO: 713), and further comprises an LC CDR2 sequence of KAS (SEQ ID NO: 715) or KASTLAS (SEQ ID NO: 709) and an LC CDR1 sequence of QTIYNY (SEQ ID NO: 714) or QASQTIYNYLA (SEQ ID NO: 708).

14. The composition of any one of claims 1-13, wherein the anti-CD3 binding domain comprises:

(a) a HC CDR1 sequence of GFSFSNNYY (SEQ ID NO: 711),

(b) a HC CDR2 sequence of IYTNNIGST (SEQ ID NO: 712),

(c) a HC CDR3 sequence of ARGIYSDSSDFIGNL (SEQ ID NO: 713),

(d) a LC CDR1 sequence of QTIYNY (SEQ ID NO: 714),

(e) a LC CDR2 sequence of KAS (SEQ ID NO: 715), and

(f) a LC CDR3 sequence of QQYNTIINVDRT (SEQ ID NO: 716), wherein the residues are numbered according to IMGT nomenclature.

15. The composition of any one of claims 1-14, wherein the VH of the anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to the sequence QVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYYMCWVRQAPGKGLEWVSCIYTNNIGST WYASWAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIYSDSSDFIGNLWGQGTLV TVSS (SEQ ID NO: 703), and/or the VL of the anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to the sequence AIQMTQSPSSLSASVGDRVTITCQASQTIYNYLAWYQQKPGKVPELLIYKASTLASGVPSRF MGTGFGTDFTLTIDSLQPEDAATYYCQQYNTIINVDRTFGGGTKVEIK (SEQ ID NO: 704).

16. The composition of any one of claims 1-15, wherein the anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to QVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYYMCWVRQAPGKGLEWVSCIYTNNIGST WYASWAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIYSDSSDFIGNLWGQGTLV TVSSGGGGSGGGGSGGGGSGGGGSAIQMTQSPSSLSASVGDRVTITCQASQTIYNYLAWYQ QKPGKVPELLIYKASTLASGVPSRFMGTGFGTDFTLTIDSLQPEDAATYYCQQYNTIINVDRT FGGGTKVEIK (SEQ ID NO: 701), or a sequence that is identical to SEQ ID NO: 701.

17. The composition of any one of claims 1-15, wherein the anti-CD3 binding domain comprises a sequence with at least 80% sequence identity to AIQMTQSPSSLSASVGDRVTITCQASQTIYNYLAWYQQKPGKVPELLIYKASTLASGVPSRF MGTGFGTDFTLTIDSLQPEDAATYYCQQYNTIINVDRTFGGGTKVEIKGGGGSGGGGSGGG GSGGGGSGGGGSQVQLVQSGGGLVQPGGPLRLSCTASGFSFSNNYYMCWVRQAPGKGLE WVSCIYTNNIGSTWYASWAKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGIYSDSSD FIGNLWGQGTLVTVSS (SEQ ID NO: 702), or a sequence that is identical to SEQ ID NO: 702.