PROTEINS COMPRISING KALLIKREIN RELATED PEPTIDASE 2 ANTIGEN BINDING DOMAINS AND THEIR USES

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 23, 2020, is named JBI6125USNP1_SL.txt and is 764,265 bytes in size.

TECHNICAL FIELD

The invention provides antigen binding domains that bind kallikrein related peptidase 2 (hK2) protein comprising the antigen binding domains that bind hK2, polynucleotides encoding them, vectors, host cells, methods of making and using them.

BACKGROUND

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males worldwide. The course of prostate cancer from diagnosis to death is best categorized as a series of clinical stages based on the extent of disease, hormonal status, and absence or presence of detectable metastases: localized disease, rising levels of prostate-specific antigen (PSA) after radiation therapy or surgery with no detectable metastases, and clinical metastases in the non-castrate or castrate stage. Although surgery, radiation, or a combination of both can be curative for patients with localized disease, a significant proportion of these patients have recurrent disease as evidenced by a rising level of PSA, which can lead to the development of metastases, especially in the high-risk group—a transition to the lethal stage of the disease.

Androgen depletion therapy (ADT) is the standard treatment with a generally predictable outcome: decline in PSA, a period of stability in which the tumor does not proliferate, followed by rising PSA and regrowth as castration-resistant disease. Historically, ADT has been the standard of care for patients with metastatic prostate cancer.

Kallikrein related peptidase 2 (hK2, HK2) is a trypsin-like enzyme with androgen receptor (AR)-driven expression specific to prostate tissue and prostate cancer. hK2 is activated by Transmembrane Protease, Serine 2 (TMPRSS2) and secreted into the ducts of the prostate, where it initiates a cascade that cleaves semenogelin, the extracellular matrix in ejaculate, to enhance sperm motility. hK2 expression is restricted to the prostate and prostate cancer tissue, however it has recently been demonstrated that hK2 was detectable in breast cancer lines and primary patient samples after appropriate activation of the AR-pathway by steroid hormones (U.S. Pat. Publ. No. 2018/0326102). Similar to PSA, retrograde release of catalytically inactive hK2 into the blood occurs when the highly structured organization of the prostate is compromised upon hypertrophy or malignant transformation.

There is a need for next generation hK2 binding domains for therapeutic and diagnostic purposes.

SUMMARY

The disclosure provides an isolated protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises:

a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205.

The disclosure also provides an isolated antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain variable region (VH) of SEQ ID NO: 75 and a light chain variable region (VL) of SEQ ID NO: 74.

The disclosure also provides isolated antigen binding domains that bind hK2 comprising certain VH and VL amino acid sequences.

The disclosure also provides an isolated multispecific protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205. In a particular embodiment, the disclosure provides an isolated multispecific protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 162 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 163.

The disclosure also provides an isolated multispecific protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain variable region (VH) of SEQ ID NO: 75 and a light chain variable region (VL) of SEQ ID NO: 74.

The disclosure also provides isolated multispecific protein comprising an antigen binding domain that bind hK2 comprising certain VH and VL amino acid sequences.

The disclosure also provides an isolated chimeric antigen receptor (CAR) comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2).

The disclosure also provides an isolated chimeric antigen receptor (CAR) comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a

HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205. In a particular embodiment, the disclosure provides an isolated chimeric antigen receptor (CAR) comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 162 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 163.

The disclosure also provides an isolated chimeric antigen receptor (CAR) comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain variable region (VH) of SEQ ID NO: 75 and a light chain variable region (VL) of SEQ ID NO: 74.

The disclosure also provides an isolated multispecific protein comprising a first antigen binding domain that binds hK2 and a second antigen binding domain that binds a lymphocyte antigen (such as CD3).

In a particular embodiment, the present the disclosure provides an isolated multispecific protein comprising a first antigen binding domain that binds hK2 and a second antigen binding domain that binds a lymphocyte antigen, wherein the isolated multispecific protein is an isolated anti hK2/anti CD3 protein.

The disclosure also provides an immunoconjugate comprising the isolated antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides an immunoconjugate comprising the isolated protein comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides an immunoconjugate comprising the isolated multispecific protein comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides an immunoconjugate comprising the isolated CAR comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides a pharmaceutical composition comprising the isolated antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides a pharmaceutical composition comprising the isolated protein comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides a pharmaceutical composition comprising the isolated multispecific protein comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides a pharmaceutical composition comprising the isolated multispecific protein of the disclosure.

The disclosure also provides a pharmaceutical composition comprising the isolated CAR comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides an isolated polynucleotide encoding the isolated antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides an isolated polynucleotide encoding the isolated protein comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides an isolated polynucleotide encoding the isolated multispecific protein comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides an isolated polynucleotide encoding the isolated CAR comprising the antigen binding domain that binds hK2 of the disclosure.

The disclosure also provides a vector comprising the polynucleotide of the disclosure.

The disclosure also provides a host cell comprising the polynucleotide or the vector of the disclosure.

The disclosure also provides a method of treating a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the antigen binding domain that binds hK2, the protein comprising the antigen binding domain that binds hK2, the multispecific protein comprising the antigen binding domain that binds hK2, the CAR-T comprising the antigen binding domain that binds hK2, the immunoconjugate of the disclosure or the pharmaceutical composition of the disclosure to the subject in need thereof for a time sufficient to treat the hK2 expressing cancer.

The disclosure also provides a method of reducing the amount of hK2 expressing tumor cells in a subject, comprising administering to the subject the antigen binding domain that binds hK2, the protein comprising the antigen binding domain that binds hK2, the multispecific protein comprising the antigen binding domain that binds hK2, the CAR-T comprising the antigen binding domain that binds hK2, the immunoconjugate of the disclosure or the pharmaceutical composition of the disclosure for a time sufficient to reduce the amount of hK2 expressing tumor cells.

The disclosure also provides a method of preventing establishment of a hK2 expressing cancer in a subject, comprising administering the antigen binding domain that binds hK2, the protein comprising the antigen binding domain that binds hK2, the multispecific protein comprising the antigen binding domain that binds hK2, the CAR-T comprising the antigen binding domain that binds hK2, the immunoconjugate of the disclosure or the pharmaceutical composition of the disclosure to the subject in need thereof to prevent establishment of the hK2 expressing cancer in the subject.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a hK2 expressing cancerous condition, comprising administering the antigen binding domain that binds hK2, the protein comprising the antigen binding domain that binds hK2, the multispecific protein comprising the antigen binding domain that binds hK2, the CAR-T comprising the antigen binding domain that binds hK2, the immunoconjugate of the disclosure or the pharmaceutical composition of the disclosure to the subject in need thereof to treat the noncancerous condition.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering a therapeutically effective amount of the antigen binding domain that binds hK2, the protein comprising the antigen binding domain that binds hK2, the multispecific protein comprising the antigen binding domain that binds hK2, the CAR-T comprising the antigen binding domain that binds hK2, the immunoconjugate of the disclosure or the pharmaceutical composition of the disclosure to the subject in need thereof for a time sufficient to treat the prostate cancer.

The disclosure also provides a method of treating breast cancer in a subject, comprising administering a therapeutically effective amount of the antigen binding domain that binds hK2, the protein comprising the antigen binding domain that binds hK2, the multispecific protein comprising the antigen binding domain that binds hK2, the CAR-T comprising the antigen binding domain that binds hK2, the immunoconjugate of the disclosure or the pharmaceutical composition of the disclosure to the subject in need thereof for a time sufficient to treat the breast cancer.

The disclosure also provides a method of detecting prostate cancer or breast cancer in a subject, comprising administering to the subject the immunoconjugate of the disclosure, and detecting binding of the immunoconjugate to hK2, thereby detecting prostate cancer or breast cancer.

The disclosure also provides a kit comprising the antigen binding domain that binds hK2, the protein comprising the antigen binding domain that binds hK2, the multispecific protein comprising the antigen binding domain that binds hK2, the CAR-T comprising the antigen binding domain that binds hK2, the immunoconjugate of the disclosure or the pharmaceutical composition of the disclosure.

The disclosure also provides an anti-idiotypic antibody binding to the antigen binding domain that binds hK2 of the disclosure

The disclosure also provides a chimeric antigen receptor (CAR) comprising: an extracellular domain comprising an antigen binding domain that binds hK2; a transmembrane domain; and an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing will be apparent from the following more particular description of example embodiments, as illustrated in the accompanying drawings. TM: transmembrane.

FIG. 1 shows the sequence alignment of the VH domains of mu11B6 (SEQ ID NO: 125), hu11B6 (SEQ ID NO: 5), KL2B357 (SEQ ID NO: 159), KL2B358 (SEQ ID NO: 161), KL2B359 (SEQ ID NO: 139), KL2B360 (SEQ ID NO: 159), HCF3 (SEQ ID NO: 6) and HCG5 (SEQ ID NO: 4).

FIG. 2 shows the sequence alignment of the VL domains of mu11B6 (SEQ ID NO: 124), hu11B6 (SEQ ID NO: 2), KL2B357 (SEQ ID NO: 160), KL2B358 (SEQ ID NO: 140), KL2B359 (SEQ ID NO: 140), KL2B360 (SEQ ID NO: 140), LDC6 (SEQ ID NO: 1) and LCB7 (SEQ ID NO: 3).

FIG. 3 shows the binding epitopes of selected hK2 antibodies mapped onto the sequence of hK2 antigen. The figure discloses each sequence as SEQ ID NO: 467.

FIG. 4A and 4B show binding of hybridoma supernatants to primary human T cells. Clone UCHT1 was used as a positive control (Fig. 1B); mouse IgG1 isotype (mIgG1) was used as a negative control.

FIG. 5 shows binding of anti-CD3 scFv variants, expressed in E. coli, to CD3.

FIG. 6 shows the alignment of the VL regions of CD3B815 (SEQ ID NO: 249), CD3W244 (SEQ ID NO: 250), CD3W245 (SEQ ID NO: 251), CD3W246 (SEQ ID NO: 252), CD3W247 (SEQ ID NO: 253), and CD3W248 (SEQ ID NO: 254).

FIG. 7 shows hydrogen-deuterium exchange mass spectrometry (HDX-MS) of CD3W245 bound to human CD3ε (CD3ε:CD3W245) and OKT3 bound to CD3ε (CD3ε:OKT). The amino acid sequence shown represent residues 2-93 of the 105 residues of the ECD domain of CD3ε and corresponds to residue 1-91 of CD3ε SEQ ID NO: 340 Single underline indicates segments with 10% - 30% decrease in deuteration levels and double underline indicates segments with >30% decrease in deuteration levels in the presence of the antibody, as compared to CD3ε alone.

FIG. 8A shows in vitro target cytotoxicity of KL2BxCD3 bi-specific molecules measured by incuCyte imaging system in real-time for quantifying target cell death.

FIG. 8B shows in vitro target cytotoxicity of KL2BxCD3 bi-specific molecules measured by fluorescent caspase 3/7 reagent to measure apoptosis signal from target cell death.

FIG. 9A shows in vitro T cell activation and proliferation by KLK2xCD3 bi-specific antibodies by showing the frequency of CD25 positive cells at different doses.

FIG. 9B shows in vitro T cell activation and proliferation by KLK2xCD3 bi-specific antibodies by showing the frequency of cells entering into proliferation gate.

FIG. 10A shows in vitro T cell INF-γ release by KLK2xCD3 bi-specific antibodies.

FIG. 10B shows in vitro T cell TNF-α release by KLK2xCD3 bi-specific antibodies.

FIG. 11 show the cartoon of the design of the hK2 binding chimeric artificial receptors (CAR). The hK2 binding scFv was cloned in either VH-VL or VL-VH orientation in the various CARs.

FIG. 12A and FIG. 12B show hK2 CAR expression on T cells-surface. Primary human T cells were electroporated with no mRNA (MOCK) or 10μg of mRNA expressing either an hK2 scFv CAR or irrelevant control CAR. 24 hours post-electroporation CAR surface expression was measured by flow cytometry following staining with 2 μg/ml biotinylated L-protein and streptavidin-conjugated PE (top panel) or 2 μg/ml biotinylated L-protein and streptavidin-conjugated PE (bottom panel) or biotinylated hK2 (1 μg/ml) and streptavidin-conjugated PE.

FIG. 13 shows cytotoxicity of hK2 positive (VCaP, top panel) and negative (DU145, bottom panel) tumor cells by hK2 CAR-T cells in 20-hour flow-based assay at the indicated effector-to-target cell (E/T) ratio. 24 hours after transient transfection, target cells were labeled with Cell Trace Violet (CTV)

fluorescent dye and then co-cultured with hK2 CAR-T cells. Mock T cells served as negative effector controls. Percent killing was measured as the ratio of the absolute number of live (viability dye negative) target (CTV positive) cells remaining in the co-culture relative to the number of live targets cultured without CAR-T cells.

FIG. 14 shows real-time hK2 CAR-T cell-mediated cytotoxicity. Normalized cell index (CI) plot for VCaP target cells (5E4) incubated with Mock, 10μg mRNA electroporated (24 hours post transfection) hK2 11B6 CAR LH or control CAR-T cells at different E:T ratios for approximately 72 hours. When seeded alone, target cells adhered to the plate and proliferated, increasing the CI readout. When T cells were added to target cells, hK2 CAR and control CAR-T cells mediated hK2 positive VCaP cell cytolysis and subsequent progressive decrease in CI at an E/T ratio from 5:1 to 0.156:1. The reduction in CI value after addition of effector cells reflected the loss of viability of target cells. The Y-axis shows the normalized CI generated by the RTCA software and displayed in real time. X-axis is the time of cell culture and treatment time in hour. Mean values of the CI were plotted ± standard deviation.

FIG. 15 shows lack of real-time hK2 CAR-T cell-mediated cytotoxicity of target cells not expressing hK2. Normalized cell index (CI) plot for DU145 target cells (5E3) incubated with Mock, 10μg mRNA electroporated (24 hours post transfection) hK2 11B6 CAR LH or control CAR-T cells at different E:T ratios for approximately 72 hours. When seeded alone, target cells adhered to the plate and proliferated, increasing the CI readout. When T cells were added to target cells, hK2 CAR-T and control CAR-T cells did not reduce CI after addition which displayed no cytolytic activity. The Y-axis shows the normalized CI generated by the RTCA software and displayed in real time. X-axis is the time of cell culture and treatment time in hour. Mean values of the CI were plotted ± standard deviation.

FIG. 16 shows interferon-γ (IFN-γ) production by antigen-stimulated hK2 CAR-T cells. Supernatant was collected from xCELLigence based killing assay approximately 70 hours co-culture (VCap#5E4, DU145#5E3). hK2 CAR_LH and Control CAR modified T cells secreted IFN-γ during co-culture with hK2-expressing VCaP cells, but not with hK2-negative DU145 cells. Mean IFN-γ concentration ± standard deviation (pg/ml) from duplicate cultures is shown.

FIG. 17 shows expression of hK2 CAR constructs (constructs 1-10) on Jurkat cells containing luciferase gene driven by the signaling-responsive NFAT promoter (JNL cells) transduced with the various hK2 CAR constructs as shown in the Figure. Expression was determined by biotinylated hK2 followed by streptavidin-conjugated PE.

FIG. 18 shows binding between the CAR1-10 constructs and its cognate cellular antigen (hK2 on target cells) detected by luciferase expression in the JNL cells upon binding. JNL cells transduced with the indicated CAR constructs (CAR1-10) and un-transduced JNL cells (UTD) were co-cultured with target cells lines (VCaP or DU145 cells) and luciferase activity was measured as luminescence intensity.

Constructs were considered active when the luminescence intensity exceeded 1.5-fold the level of UTD cells in the presence of antigen-expressing cells.

FIG. 19 shows hK2 CAR1-10 or parental 11B6_HL and 11B6_LH expression on T cell surface. Primary human T cells were transduced with 11B6 thermally stabilized and parental scFv CAR lentivirus (multiplicity of infection (MOI): 3) and CAR expression was determined by biotinylated hK2 (1μg/ml) followed by streptavidin-conjugated PE 14 days post transduction. UTD: untransduced control.

FIG. 20 shows percent tumor cell growth inhibition of hK2 positive VCaP cells at effector:target ratio of 1:1 or 0.5:1 by T cells transduced with CAR1-10 or the parental 11B6_HL or 11B6_LH. in the real-time IncuCyte killing assay assessing antigen-dependent cytotoxicity. Tumor cell growth inhibition (%) =(Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%).

FIG. 21 shows percent tumor cell growth inhibition of PC3 cells at effector:target ratio of 1:1 by T cells transduced with CAR1-10 or the parental 11B6_HL or 11B6_LH. in the real-time IncuCyte killing assay assessing antigen-dependent cytotoxicity. Tumor cell growth inhibition (%) =(Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%).

FIG. 22 shows cytokine release by hK2 CAR-T cells. Supernatant collected from overnight (approximately 20 hours) co-culture of hK2 CAR-T cells with VCaP cells at 1:1 of E/T ratio was analyzed using 13-plex Milliplex Human High Sensitivity T cell kit (HSTCMAG28SPMX13). hK2 CAR-T cells secreted cytokines during co-culture with hK2-expressing VCaP cells, but minimally during co-culture with un-transduced T cells (UTD). Mean cytokine concentration ± standard deviation (pg/ml) from duplicate cultures is shown.

FIG. 23 shows IFN-γ release by hK2 CAR-T cells. Supernatant collected from overnight (approximately 20 hours) co-culture of hK2 CAR-T cells with VCap, DU145 (5E4 cells) cells at 1:1 of E/T ratio. hK2 CAR modified T cells secrete IFN-γ during co-culture with hK2-expressing VCaP cells, but not hK2-negative DU145 cells. Mean IFN-γ concentration ± standard deviation (pg/ml) from duplicate cultures is shown. CD3/28 beads stimulated T cells and T cells only were used as positive and negative controls, respectively.

FIG. 24A and **FIG. 24B** shows flow cytometry histograms of CellTrace Violet (CTV) labeled untransduced T cells (T cells only in the Figure) or CAR1-10-transduced CAR-T after 5-day co-culture with VCaP or DU145 cells, or after stimulation with CD3/28 beads.

FIG. 25A and **25B** shows flow cytometry histograms of CD25 positive CellTrace Violet (CTV) labeled untransduced T cells (T cells only in the Figure) or CAR1-10-transduced CAR-T after 5-day co-culture with VCaP or DU145 cells, or after stimulation with CD3/28 beads.

FIG. 26 shows that hK2 CAR-T cells proliferated more robustly than CD3/28 beads positive control after 5 days of coculture with VCaP cells. Different CAR constructs engineered T cells had different proliferation activity and displayed different CAR+ T cells counts. The CAR+ T cells counts were based on mean absolute cell count +/- SEM from three technical replicates.

FIG. 27 shows the surface expression of CAR17 (KL2B413_HL), CAR18 (KL2B413_LH), CAR19 (KL2B359_HL) and CAR20 (HK2B359_LH on the surface of primary T cells. The numbers inside each histogram indicate the percentage of cells expressing the indicated CARs.

FIG. 28 shows the surface expression of CAR17 (KL2B413_HL), CAR18 (KL2B413_LH), CAR19 (KL2B359_HL) and CAR20 (KL2B359_LH) on JNL cells.

FIG. 29 shows the relative light units (RLU) resulting from luciferase expression in JNL cells mediated by binding of the test CAR-T to its cognate receptor on various target cells as indicated in the Figure. JNL cells containing the indicated CAR clones and untransduced JNL cells (UTD) were co-cultured with target cells lines (VCaP, LNCaP/hK2, LNCaP, C4-2B, 22Rv1 or DU145 cells) and luciferase activity was measured as luminescence intensity (RLU, relative light units). KL2B413_HL: CAR17, KL2B413_LH: CAR18, HL2B359_HL: CAR19, KL2B359_LH: CAR20.

FIG. 30 shows percent tumor cell growth inhibition of hK2 positive VCaP cells by CAR-T cells transduced with CAR17 (B413HL in the Figure), CAR18 (B413LH in the Figure), CAR19 (B359HL in the Figure) and CAR20 (B359LH in the Figure) in the real-time IncuCyte killing assay assessing antigen-dependent cytotoxicity. Tumor cell growth inhibition (%) =(Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%).

FIG. 31 shows percent tumor cell growth inhibition of hK2 negative DU145 cells by CAR-T cells transduced with CAR17 (B413HL in the Figure), CAR18 (B413LH in the Figure), CAR19 (B359HL in the Figure) and CAR20 (B359LH in the Figure) in the real-time IncuCyte killing assay assessing antigen-dependent cytotoxicity. Tumor cell growth inhibition (%) =(Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%).

FIG. 32 shows IFN-γ production by CAR-T cells transduced with CAR17 (KLK2B413HL in the Figure), CAR18 (KLK2B413LH in the Figure), CAR19 (KLK2B359HL in the Figure) and CAR20 (KLK2B359LH in the Figure) or untransduced T cells (UTD) in co-cultures with cells indicated in the Figure.

FIG. 33A and **FIG. 33B** show that co-culture of CAR-T cells transduced with CAR17 (B413HL in the Figure), CAR18 (B413LH in the Figure), CAR19 (B359HL in the Figure) and CAR20 (B359LH in the Figure) and VCap cells result in increase in CD107a+hK2-CAR-T+ cells, indicative of immune cell activation and cytotoxic degranulation, whereas co-culture with hK2 negative DU145 cells had no effect.

FIG. 34A and **FIG. 34B** show flow cytometry histograms of CellTrace Violet (CTV) labeled untransduced T cells (T cells only in the Figure) or CAR-T cells transduced with CAR17 (KLB413HL in the Figure), CAR18 (KLB413LH in the Figure), CAR19 (KLB359HL in the Figure) and CAR20 (KLB359LH in the Figure) after 5-day co-culture with VCAp or DU145 cells. UTD: untransduced.

FIG. 35 shows the percentage of proliferating cells in co-cultures of CellTrace Violet (CTV) labeled untransduced T cells (T cells only in the Figure) or CAR-T cells transduced with CAR17 (KLB413HL in the Figure), CAR18 (KLB413LH in the Figure), CAR19 (KLB359HL in the Figure) and CAR20 (KLB359LH in the Figure) after 5-day co-culture with VCAP or DU145 cells. UTD: untransduced.

FIG. 36A and FIG. 36B show flow cytometry histograms of CTV+CD25+ CAR-T cells transduced with CAR17 (KLB413HL in the Figure), CAR18 (KLB413LH in the Figure), CAR19 (KLB359HL in the Figure) and CAR20 (KLB359LH in the Figure) after 5-day co-culture with VCaP or DU145 cells.

FIG. 37 shows the percentage of CTV expressing un-transduced T cells (UTD) or CAR-T cells transduced with CAR17 (KLB413HL in the Figure), CAR18 (KLB413LH in the Figure), CAR19 (KLB359HL in the Figure) and CAR20 (KLB359LH in the Figure) after 5-day co-culture with VCaPs or DU145 cells, alone (T cells only) or after CD2/28 bead stimulation.

FIG. 38A-38F shows the binding paratope of selected anti-hK2 antibodies and selected anti-hK2/CD3 bispecific antibodies. Underlined sequences indicate CDR regions and highlighted sequences indicate paratope regions. FIG. 38A discloses SEQ ID NOS 219 and 220, respectively, in order of appearance. FIG. 38B discloses SEQ ID NOS 213 and 224, respectively, in order of appearance. FIG. 38C discloses SEQ ID NOS 203 and 215, respectively, in order of appearance. FIG. 38D discloses SEQ ID NOS 468 and 469, respectively, in order of appearance. FIG. 38E discloses SEQ ID NOS 354 and 221, respectively, in order of appearance. FIG. 38F discloses SEQ ID NOS 356 and 222, respectively, in order of appearance.

FIG. 39A shows in vivo efficacy of KLK2xCD3 bi-specific antibody in VCaP xenograft mouse model and cytokine profile. Three KLK2xCD3 bispecific antibodies were tested at 3 dose levels: 5mg/kg, 1mg/kg, and 0.2mg/kg. Tumor growth inhibition was plotted based on tumor volume measurement.

FIG. 39B shows in vivo efficacy of KLK2xCD3 bi-specific antibody in VCaP xenograft mouse model and cytokine profile. 100µl blood samples were collected from animals via retro-orbital bleed 6 hours post first dose. Plasma was separated from blood sample by high speed centrifugation. A Luminex assay was carried out to quantify IFN-γ and TNF-α concentrations at different KLK2 bi-specific doses.

DETAILED DESCRIPTION

The disclosed methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed methods are not limited to the specific methods described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed methods.

All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

When a list is presented, unless stated otherwise, it is to be understood that each individual element of that list, and every combination of that list, is a separate embodiment. For example, a list of embodiments presented as "**A, B, or C**" is to be interpreted as including the embodiments, "A," "B," "C," "A or B," "A or C," "B or C," or "A, B, or C."

As used in this specification and the appended claims, the singular forms "**a**," "**an**," and "**the**" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

The transitional terms "**comprising**," "**consisting essentially of**," and "**consisting of**" are intended to connote their generally accepted meanings in the patent vernacular; that is, (i) "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps; (ii) "consisting of" excludes any element, step, or ingredient not specified in the claim; and (iii) "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. Embodiments described in terms of the phrase "comprising" (or its equivalents) also provide as embodiments those independently described in terms of "consisting of" and "consisting essentially of."

"**About**" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. Unless explicitly stated otherwise within the Examples or elsewhere in the Specification in the context of a particular assay, result or embodiment, "about" means within one standard deviation per the practice in the art, or a range of up to 5%, whichever is larger.

"**Activation**" or "**stimulation**" or "**activated**" or "**stimulated**" refers to induction of a change in the biologic state of a cell resulting in expression of activation markers, cytokine production, proliferation or mediating cytotoxicity of target cells. Cells may be activated by primary stimulatory signals. Co-stimulatory signals can amplify the magnitude of the primary signals and suppress cell death following initial stimulation resulting in a more durable activation state and thus a higher cytotoxic capacity. A "**co-stimulatory signal**" refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell and/or NK cell proliferation and/or upregulation or downregulation of key molecules.

"**Alternative scaffold**" refers to a single chain protein framework that contains a structured core associated with variable domains of high conformational tolerance. The variable domains tolerate variation to be introduced without compromising scaffold integrity, and hence the variable domains can be engineered and selected for binding to a specific antigen.

"**Antibody-dependent cellular cytotoxicity**", "**antibody-dependent cell-mediated cytotoxicity**" or "**ADCC**" refers to the mechanism of inducing cell death that depends upon the interaction of antibody-coated target cells with effector cells possessing lytic activity, such as natural killer cells (NK), monocytes, macrophages and neutrophils via Fc gamma receptors (FcγR) expressed on effector cells.

"**Antibody-dependent cellular phagocytosis**" or "**ADCP**" refers to the mechanism of elimination of antibody-coated target cells by internalization by phagocytic cells, such as macrophages or dendritic cells.

"**Antigen**" refers to any molecule (e.g., protein, peptide, polysaccharide, glycoprotein, glycolipid, nucleic acid, portions thereof, or combinations thereof) capable of being bound by an antigen binding domain or a T-cell receptor capable of mediating an immune response. Exemplary immune responses include antibody production and activation of immune cells, such as T cells, B cells or NK cells. Antigens may be expressed by genes, synthetized, or purified from biological samples such as a tissue sample, a tumor sample, a cell or a fluid with other biological components, organisms, subunits of proteins/antigens, killed or inactivated whole cells or lysates.

"**Antigen binding fragment**" or "**antigen binding domain**" refers to a portion of the protein that binds an antigen. Antigen binding fragments may be synthetic, enzymatically obtainable or genetically engineered polypeptides and include portions of an immunoglobulin that bind an antigen, such as the VH, the VL, the VH and the VL, Fab, Fab', $F(ab')_2$, Fd and Fv fragments, domain antibodies (dAb) consisting of one VH domain or one VL domain, shark variable IgNAR domains, camelized VH domains, VHH

domains, minimal recognition units consisting of the amino acid residues that mimic the CDRs of an antibody, such as FR3-CDR3-FR4 portions, the HCDR1, the HCDR2 and/or the HCDR3 and the LCDR1, the LCDR2 and/or the LCDR3, alternative scaffolds that bind an antigen, and multispecific proteins comprising the antigen binding fragments. Antigen binding fragments (such as VH and VL) may be linked together via a synthetic linker to form various types of single antibody designs where the VH/VL domains may pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate single chains, to form a monovalent antigen binding domain, such as single chain Fv (scFv) or diabody. Antigen binding fragments may also be conjugated to other antibodies, proteins, antigen binding fragments or alternative scaffolds which may be monospecific or multispecific to engineer bispecific and multispecific proteins.

"**Antibodies**" is meant in a broad sense and includes immunoglobulin molecules including monoclonal antibodies including murine, human, humanized and chimeric monoclonal antibodies, antigen binding fragments, multispecific antibodies, such as bispecific, trispecific, tetraspecific , dimeric, tetrameric or multimeric antibodies, single chain antibodies, domain antibodies and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding site of the required specificity. "Full length antibodies" are comprised of two heavy chains (HC) and two light chains (LC) inter-connected by disulfide bonds as well as multimers thereof (e.g. IgM). Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (comprised of domains CH1, hinge, CH2 and CH3). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The VH and the VL regions may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged from amino-to-carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. Immunoglobulins may be assigned to five major classes, IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Antibody light chains of any vertebrate species may be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"**Bispecific**" refers to a molecule (such as an antibody) that specifically binds two distinct antigens or two distinct epitopes within the same antigen. The bispecific molecule may have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca cynomolgus* (cynomolgus, cyno) or *Pan troglodytes*, or may bind an epitope that is shared between two or more distinct antigens.

"**Bispecific anti-hK2/anti-CD3 antibody**", "**hk2/CD3 antibody**", "**hk2xCD3 antibody,**" "**anti-hK2/anti-CD3 protein,**" and the like refer to an antibody that binds hk2 and CD3 and that comprises at least one binding domain specifically binding hK2 and at least one binding domain specifically binding CD3. The domains specifically binding hK2 and CD3 are typically V_H/V_L pairs. The bispecific anti-hk2×CD3 antibody may be monovalent in terms of its binding to either hk2 or CD3.

"**Cancer**" refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

"**Chimeric antigen receptor**" (CAR) as used herein is defined as a cell-surface receptor comprising an extracellular target-binding domain, a transmembrane domain and an intracellular signaling domain, all in a combination that is not naturally found together on a single protein. This includes receptors wherein the extracellular domain and the intracellular signaling domain are not naturally found together on a single receptor protein. CARs are intended primarily for use with lymphocyte such as T cells and natural killer (NK) cells.

"**Complement-dependent cytotoxicity**" or "**CDC**", refers to the mechanism of inducing cell death in which the Fc effector domain of a target-bound protein binds and activates complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate CDC by binding complement receptors (e.g., CR3) on leukocytes.

"**Complementarity determining regions**" (CDR) are antibody regions that bind an antigen. There are three CDRs in the VH (HCDR1, HCDR2, HCDR3) and three CDRs in the VL (LCDR1, LCDR2, LCDR3). CDRs may be defined using various delineations such as Kabat (Wu et al. (1970) J Exp Med 132: 211-50; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991), Chothia (Chothia et al. (1987) J Mol Biol 196: 901-17), IMGT (Lefranc et al. (2003) Dev Comp Immunol 27: 55-77) and AbM (Martin and Thornton J Bmol Biol 263: 800-15, 1996). The correspondence between the various delineations and variable region numbering is described (see e.g. Lefranc et al. (2003) Dev Comp Immunol 27: 55-77; Honegger and Pluckthun, J Mol Biol (2001) 309:657-70; International ImMunoGeneTics (IMGT) database; Web resources, http://www_imgt_org). Available programs such as abYsis by UCL Business PLC may be used to delineate CDRs. The term "CDR", "HCDR1", "HCDR2", "HCDR3", "LCDR1", "LCDR2" and "LCDR3" as used herein includes CDRs defined by any of the methods described supra, Kabat, Chothia, IMGT or AbM, unless otherwise explicitly stated in the specification.

"**CD3**" refers to an antigen which is expressed on T cells as part of the multimolecular T cell receptor (TCR) complex and which consists of a homodimer or heterodimer formed from the association of two or four receptor chains: CD3 epsilon, CD3 delta, CD3 zeta and CD3 gamma. Human CD3 epsilon comprises the amino acid sequence of **SEQ ID NO: 442.** All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, "CD3" means human CD3 unless specified as being from a non-human species, e.g., "mouse CD3" "monkey CD3," etc.

Throughout the specification, "**CD3-specific**" or "**specifically binds CD3**" or "**anti-CD3 antibody**" refers to antibodies that bind specifically to the CD3-epsilon polypeptide (**SEQ ID NO:442**), including antibodies that bind specifically to the CD3-epsilon extracellular domain (ECD) (**SEQ ID NO:443**). CD3-epsilon, together with CD3-gamma, -delta and -zeta, and the T-cell receptor alpha/beta and gamma/delta heterodimers, forms the T-cell receptor-CD3 complex. This complex plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways. The CD3 complex mediates signal transduction, resulting in T cell activation and proliferation. CD3 is required for the immune response.

SEQ ID NO: 442 (Human CD3 epsilon)

MQSGTHWRVLGLCLLSVGVWGQDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDK
NIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVCENCMEMDV
MSVATIVIVDICITGGLLLLVYYWSKNRKAKAKPVTRGAGAGGRQRGQNKERPPPVPNPDYEPIR
KGQRDLYSGLNQRRI

SEQ ID NO: 443 (Human CD3 epsilon extracellular domain)

DGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFS
ELEQSGYYVCYPRGSKPEDANFYLYLRARVCENCMEMD

"**Decrease,**" "**lower,**" "**lessen,**" "**reduce,**" or "**abate**" refers generally to the ability of a test molecule to mediate a reduced response (*i.e.*, downstream effect) when compared to the response mediated by a control or a vehicle. Exemplary responses are T cell expansion, T cell activation or T-cell mediated tumor cell killing or binding of a protein to its antigen or receptor, enhanced binding to a Fcγ or enhanced Fc effector functions such as enhanced ADCC, CDC and/or ADCP. Decrease may be a

statistically significant difference in the measured response between the test molecule and the control (or the vehicle), or a decrease in the measured response, such as a decrease of about 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 30 fold or more, such as 500, 600, 700, 800, 900 or 1000 fold or more (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.).

"**Differentiation**" refers to a method of decreasing the potency or proliferation of a cell or moving the cell to a more developmentally restricted state.

"**Encode**" or "**encoding**" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

"**Enhance,**" "**promote,**" "**increase,**" "**expand**" or "**improve**" refers generally to the ability of a test molecule to mediate a greater response (*i.e.*, downstream effect) when compared to the response mediated by a control or a vehicle. Exemplary responses are T cell expansion, T cell activation or T-cell mediated tumor cell killing or binding of a protein to its antigen or receptor, enhanced binding to a Fcγ or enhanced Fc effector functions such as enhanced ADCC, CDC and/or ADCP. Enhance may be a statistically significant difference in the measured response between the test molecule and control (or vehicle), or an increase in the measured response, such as an increase of about 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 30 fold or more, such as 500, 600, 700, 800, 900 or 1000 fold or more (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.).

"**Epitope**" refers to a portion of an antigen to which an antibody specifically binds. Epitopes typically consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be composed of contiguous and/or discontinuous amino acids that form a conformational spatial unit. For a discontinuous epitope, amino acids from differing portions of the linear sequence of the antigen come in close proximity in 3-dimensional space through the folding of the protein molecule. Antibody "epitope" depends on the methodology used to identify the epitope.

"**Expansion**" refers to the outcome of cell division and cell death.

"**Express**" and "**expression**" refers the to the well-known transcription and translation occurring in cells or *in vitro*. The expression product, *e.g.*, the protein, is thus expressed by the cell or *in vitro* and may be an intracellular, extracellular or a transmembrane protein.

"**Expression vector**" refers to a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

"**dAb**" or "**dAb fragment**" refers to an antibody fragment composed of a VH domain (Ward et al., Nature 341:544 546 (1989)).

"**Fab**" or "**Fab fragment**" refers to an antibody fragment composed of VH, CH1, VL and CL domains.

"**F(ab')$_2$**" or "**F(ab')$_2$ fragment**" refers to an antibody fragment containing two Fab fragments connected by a disulfide bridge in the hinge region.

"**Fd**" or "**Fd fragment**" refers to an antibody fragment composed of VH and CH1 domains.

"**Fv**" or "**Fv fragment**" refers to an antibody fragment composed of the VH and the VL domains from a single arm of the antibody. Fv fragments lack the constant regions of Fab (CH1 and CL) regions. The VH and VL in Fv fragments are held together by non-covalent interactions.

"**Fc" polypeptide**" of a dimeric Fc refers to one of the two polypeptide forming the dimeric Fc domain. For example, an Fc polypeptide of a dimeric IgG FC comprises an IgG CH2 and an IgG CH3 constant domain sequence).

"**Full length antibody**" is comprised of two heavy chains (HC) and two light chains (LC) inter-connected by disulfide bonds as well as multimers thereof (e.g. IgM). Each heavy chain is comprised of a heavy chain variable domain (VH) and a heavy chain constant domain, the heavy chain constant domain comprised of subdomains CH1, hinge, CH2 and CH3. Each light chain is comprised of a light chain variable domain (VL) and a light chain constant domain (CL). The VH and the VL may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged from amino-to-carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

"**Genetic modification**" refers to the introduction of a "foreign" (*i.e.*, extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a "cloned" or "foreign" gene or sequence, may include regulatory or control sequences operably linked to polynucleotide encoding the chimeric antigen receptor, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's

genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been "genetically engineered." The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or from a different genus or species.

"**Heterologous**" refers to two or more polynucleotides or two or more polypeptides that are not found in the same relationship to each other in nature.

"**Heterologous polynucleotide**" refers to a non-naturally occurring polynucleotide that encodes two or more neoantigens as described herein.

"**Heterologous polypeptide**" refers to a non-naturally occurring polypeptide comprising two or more neoantigen polypeptides as described herein.

"**Host cell**" refers to any cell that contains a heterologous nucleic acid. An exemplary heterologous nucleic acid is a vector (*e.g.*, an expression vector).

"**Human antibody**" refers to an antibody that is optimized to have minimal immune response when administered to a human subject. Variable regions of human antibody are derived from human immunoglobulin sequences. If human antibody contains a constant region or a portion of the constant region, the constant region is also derived from human immunoglobulin sequences. Human antibody comprises heavy and light chain variable regions that are "derived from" sequences of human origin if the variable regions of the human antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such exemplary systems are human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice or rats carrying human immunoglobulin loci. "Human antibody" typically contains amino acid differences when compared to the immunoglobulins expressed in humans due to differences between the systems used to obtain the human antibody and human immunoglobulin loci, introduction of somatic mutations or intentional introduction of substitutions into the frameworks or CDRs, or both. Typically, "human antibody" is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical in amino acid sequence to an amino acid sequence encoded by human germline immunoglobulin or rearranged immunoglobulin genes. In some cases, "human antibody" may contain consensus framework sequences derived from human framework sequence analyses, for example as described in Knappik et al., (2000) J Mol Biol 296:57-86, or a synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in Shi et al., (2010) J Mol Biol 397:385-96, and in Int. Patent Publ. No. WO2009/085462. Antibodies in which at least one CDR is derived from a non-human species are not included in the definition of "human antibody".

"**Humanized antibody**" refers to an antibody in which at least one CDR is derived from non-human species and at least one framework is derived from human immunoglobulin sequences. Humanized antibody may include substitutions in the frameworks so that the frameworks may not be exact copies of expressed human immunoglobulin or human immunoglobulin germline gene sequences.

"**In combination with**" means that two or more therapeutic agents are be administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

"**Intracellular signaling domain**" or "**cytoplasmic signaling domain**" refers to an intracellular portion of a molecule. It is the functional portion of the protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CAR-T cell.

"**Isolated**" refers to a homogenous population of molecules (such as synthetic polynucleotides or polypeptides) which have been substantially separated and/or purified away from other components of the system the molecules are produced in, such as a recombinant cell, as well as a protein that has been subjected to at least one purification or isolation step. "Isolated" refers to a molecule that is substantially free of other cellular material and/or chemicals and encompasses molecules that are isolated to a higher purity, such as to 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% purity.

"**Kallikrein related peptidase 2**" or "**hK2**" refers to a known protein which is also called kallikrein-2, grandular kallikrein 2, or HK2. hK2 is produced as a preproprotein and cleaved during proteolysis to generate active protease. All hK2 isoforms and variants are encompassed in "hK2". The amino acid sequences of the various isoforms are retrievable from GenBank accession numbers NP_005542.1, NP_001002231.1 and NP_001243009. The amino acid sequence of a full length hK2 is shown in SEQ ID NO: 62. The sequence includes the signal peptide (residues 1-18) and the pro-peptide region (residues 19-24).

SEQ ID NO: 62

MWDLVLSIALSVGCTGAVPLIQSRIVGGWECEKHSQPWQVAVYSHGWAHCGGVLVHPQWV

LTAAHCLKKNSQVWLGRHNLFEPEDTGQRVPVSHSFPHPLYNMSLLKHQSLRPDEDSSHD

LMLLRLSEPAKITDVVKVLGLPTQEPALGTTCYASGWGSIEPEEFLRPRSLQCVSLHLLS

NDMCARAYSEKVTEFMLCAGLWTGGKDTCGGDSGGPLVCNGVLQGITSWGPEPCALPEKP

AVYTKVVHYRKWIKDTIAANP

"**Modulate**" refers to either enhanced or decreased ability of a test molecule to mediate an enhanced or a reduced response (*i.e.*, downstream effect) when compared to the response mediated by a control or a vehicle.

"**Monoclonal antibody**" refers to an antibody obtained from a substantially homogenous population of antibody molecules, i.e., the individual antibodies comprising the population are identical except for possible well-known alterations such as removal of C-terminal lysine from the antibody heavy chain or post-translational modifications such as amino acid isomerization or deamidation, methionine oxidation or asparagine or glutamine deamidation. Monoclonal antibodies typically bind one antigenic epitope. A bispecific monoclonal antibody binds two distinct antigenic epitopes. Monoclonal antibodies may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may be monospecific or multispecific such as bispecific, monovalent, bivalent or multivalent.

"**Multispecific**" refers to a molecule, such as an antibody that specifically binds two or more distinct antigens or two or more distinct epitopes within the same antigen. Multispecific molecule may have cross-reactivity to other related antigens, for example to the same antigen from other species (homologs), such as human or monkey, for example *Macaca fascicularis* (cynomolgus, cyno) or *Pan troglodytes*, or may bind an epitope that is shared between two or more distinct antigens.

"**Natural killer cell**" and "**NK cell**" are used interchangeably and synonymously herein. NK cell refers to a differentiated lymphocyte with a $CD16^+$ $CD56^+$ and/or $CD57^+$ TCR^- phenotype. NK cells are characterized by their ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

"**Operatively linked**" and similar phrases, when used in reference to nucleic acids or amino acids, refers to the operational linkage of nucleic acid sequences or amino acid sequence, respectively, placed in functional relationships with each other. For example, an operatively linked promoter, enhancer elements, open reading frame, 5' and 3' UTR, and terminator sequences result in the accurate production of a nucleic acid molecule (e.g., RNA) and in some instances to the production of a polypeptide (i.e., expression of the open reading frame). Operatively linked peptide refers to a peptide in which the functional domains of the peptide are placed with appropriate distance from each other to impart the intended function of each domain.

The term "**paratope**" refers to the area or region of an antibody molecule which is involved in binding of an antigen and comprise residues that interact with an antigen. A paratope may composed of continuous and/or discontinuous amino acids that form a conformational spatial unit. The paratope for a

given antibody can be defined and characterized at different levels of details using a variety of experimental and computational methods. The experimental methods include hydrogen/deuterium exchange mass spectrometry (HX-MS). The paratope will be defined differently depending on the mapping method employed.

"**Pharmaceutical combination**" refers to a combination of two or more active ingredients administered either together or separately.

"**Pharmaceutical composition**" refers to a composition that results from combining an active ingredient and a pharmaceutically acceptable carrier.

"**Pharmaceutically acceptable carrier**" or "excipient" refers to an ingredient in a pharmaceutical composition, other than the active ingredient, which is nontoxic to a subject. Exemplary pharmaceutically acceptable carriers are a buffer, stabilizer or preservative.

"**Polynucleotide**" or "**nucleic acid**" refers to a synthetic molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. cDNA is a typical example of a polynucleotide. Polynucleotide may be a DNA or a RNA molecule.

"**Prevent,**" "**preventing,**" "**prevention,**" or "**prophylaxis**" of a disease or disorder means preventing that a disorder occurs in a subject.

"**Proliferation**" refers to an increase in cell division, either symmetric or asymmetric division of cells.

"**Promoter**" refers to the minimal sequences required to initiate transcription. Promoter may also include enhancers or repressor elements which enhance or suppress transcription, respectively.

"**Protein**" or "**polypeptide**" are used interchangeably herein are refers to a molecule that comprises one or more polypeptides each comprised of at least two amino acid residues linked by a peptide bond. Protein may be a monomer, or may be protein complex of two or more subunits, the subunits being identical or distinct. Small polypeptides of less than 50 amino acids may be referred to as "peptides". Protein may be a heterologous fusion protein, a glycoprotein, or a protein modified by post-translational modifications such as phosphorylation, acetylation, myristoylation, palmitoylation, glycosylation, oxidation, formylation, amidation, citrullination, polyglutamylation, ADP-ribosylation, pegylation or biotinylation. Protein may be recombinantly expressed.

"**Recombinant**" refers to polynucleotides, polypeptides, vectors, viruses and other macromolecules that are prepared, expressed, created or isolated by recombinant means.

"**Regulatory element**" refers to any cis-or trans acting genetic element that controls some aspect of the expression of nucleic acid sequences.

"**Relapsed**" refers to the return of a disease or the signs and symptoms of a disease after a period of improvement after prior treatment with a therapeutic.

"**Refractory**" refers to a disease that does not respond to a treatment. A refractory disease can be resistant to a treatment before or at the beginning of the treatment, or a refractory disease can become resistant during a treatment.

"**Single chain Fv**" or "**scFv**" refers to a fusion protein comprising at least one antibody fragment comprising a light chain variable region (VL) and at least one antibody fragment comprising a heavy chain variable region (VH), wherein the VL and the VH are contiguously linked via a polypeptide linker, and capable of being expressed as a single chain polypeptide. Unless specified, as used herein, a scFv may have the VL and VH variable regions in either order, e.g., with respect to the N- terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

"**(scFv)$_2$**" or "**tandem scFv**" or "**bis-scFv**" fragments refers to a fusion protein comprising two light chain variable region (VL) and two heavy chain variable region (VH), wherein the two VL and the two VH regions are contiguously linked via polypeptide linkers, and capable of being expressed as a single chain polypeptide. The two VL and two VH regions fused by peptide linkers form a bivalent molecule VL_A-linker-VH_A-linker-VL_B-linker-VH_B to form two binding sites, capable of binding two different antigens or epitopes concurrently.

"**Specifically binds,**" "**specific binding,**" "**specifically binding**" or "**binds**" refer to a proteinaceous molecule binding to an antigen or an epitope within the antigen with greater affinity than for other antigens. Typically, the proteinaceous molecule binds to the antigen or the epitope within the antigen with an equilibrium dissociation constant (K_D) of about $1x10^{-7}$ M or less, for example about $5x10^{-8}$ M or less, about $1x10^{-8}$ M or less, about $1x10^{-9}$ M or less, about $1x10^{-10}$ M or less, about $1x10^{-11}$ M or less, or about $1x10^{-12}$ M or less, typically with the K_D that is at least one hundred fold less than its K_D for binding to a non-specific antigen (e.g., BSA, casein). In the context of the prostate neoantigens described here, "specific binding" refers to binding of the proteinaceous molecule to the prostate neoantigen without detectable binding to a wild-type protein the neoantigen is a variant of.

"**Subject**" includes any human or nonhuman animal. "Nonhuman animal" includes all vertebrates, *e.g.*, mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. The terms "subject" and "patient" can be used interchangeably herein.

"**T cell**" and "**T lymphocyte**" are interchangeable and used synonymously herein. T cell includes thymocytes, naïve T lymphocytes, memory T cells, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Th1) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; $CD4^+$ T cell) $CD4^+$ T cell, a cytotoxic T cell (CTL; $CD8^+$ T cell), a tumor infiltrating cytotoxic T cell (TIL; $CD8^+$ T cell), $CD4^+CD8^+$ T cell, or any other subset of T cells. Also included are "NKT cells", which refer to a

specialized population of T cells that express a semi-invariant αβ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells include $NK1.1^+$ and $NK1.1^-$, as well as $CD4^+$, $CD4^-$, $CD8^+$ and $CD8^-$ cells. The TCR on NKT cells is unique in that it recognizes glycolipid antigens presented by the MHC I-like molecule CD Id. NKT cells can have either protective or deleterious effects due to their abilities to produce cytokines that promote either inflammation or immune tolerance. Also included are "gamma-delta T cells (γδ T cells)," which refer to a specialized population that to a small subset of T cells possessing a distinct TCR on their surface, and unlike the majority of T cells in which the TCR is composed of two glycoprotein chains designated α- and β-TCR chains, the TCR in γδ T cells is made up of a γ-chain and a δ-chain . γδ T cells can play a role in immunosurveillance and immunoregulation and were found to be an important source of IL-17 and to induce robust $CD8^+$ cytotoxic T cell response. Also included are "regulatory T cells" or "Tregs" which refer to T cells that suppress an abnormal or excessive immune response and play a role in immune tolerance. Tregs are typically transcription factor Foxp3-positive $CD4^+$T cells and can also include transcription factor Foxp3-negative regulatory T cells that are IL-10-producing $CD4^+$T cells.

"**Therapeutically effective amount**" or "**effective amount**" used interchangeably herein, refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of a therapeutic or a combination of therapeutics to elicit a desired response in the individual. Example indicators of an effective therapeutic or combination of therapeutics that include, for example, improved wellbeing of the patient, reduction of a tumor burden, arrested or slowed growth of a tumor, and/or absence of metastasis of cancer cells to other locations in the body.

"**Transduction**" refers to the introduction of a foreign nucleic acid into a cell using a viral vector.

"**Treat,**" "**treating**" or "**treatment**" of a disease or disorder such as cancer refers to accomplishing one or more of the following: reducing the severity and/or duration of the disorder, inhibiting worsening of symptoms characteristic of the disorder being treated, limiting or preventing recurrence of the disorder in subjects that have previously had the disorder, or limiting or preventing recurrence of symptoms in subjects that were previously symptomatic for the disorder.

"**Tumor cell**" or a "cancer cell" refers to a cancerous, pre-cancerous or transformed cell, either *in vivo*, *ex vivo*, or in tissue culture, that has spontaneous or induced phenotypic changes. These changes do not necessarily involve the uptake of new genetic material. Although transformation may arise from infection with a transforming virus and incorporation of new genomic nucleic acid, uptake of exogenous nucleic acid or it can also arise spontaneously or following exposure to a carcinogen, thereby mutating an endogenous gene. Transformation/cancer is exemplified by morphological changes, immortalization of

cells, aberrant growth control, foci formation, proliferation, malignancy, modulation of tumor specific marker levels, invasiveness, tumor growth in suitable animal hosts such as nude mice, and the like, *in vitro*, *in vivo*, and *ex vivo*.

"**Variant,**" "**mutant**" or "**altered**" refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications, for example one or more substitutions, insertions or deletions.

The numbering of amino acid residues in the antibody constant region throughout the specification is according to the EU index as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991), unless otherwise explicitly stated.

Mutations in the Ig constant regions are referred to as follows: L351Y_F405A_Y407V refers to L351Y, F405A and Y407V mutations in one immunoglobulin constant region. L351Y_F405A_Y407V/T394W refers to L351Y, F405A and Y407V mutations in the first Ig constant region and T394W mutation in the second Ig constant region, which are present in one multimeric protein.

"VHH" refers to a single-domain antibody or nanobody, exclusively composed of the antigen binding domain of a heavy chain. A VHH single domain antibody lacks the light chain and the CH1 domain of the heavy chain of conventional Fab region.

COMPOSITIONS OF MATTER

Antigen binding domains that bind hK2

The disclosure provides antigen binding domains that bind hK2, monospecific and multispecific proteins (particularly bispecific proteins) comprising the antigen binding domains that bind hK2, chimeric antigen receptors (CAR) comprising the antigen binding domains that bind hK2, polynucleotides encoding the foregoing, vectors, host cells and methods of making and using the foregoing. The antigen binding domains that bind hK2 identified herein demonstrated improved properties in terms of improved thermostability. The multispecific proteins disclosed herein may be particularly effective at mediating T cell mediated cytotoxicity, promoting T cell activation and proliferation, increasing T cell cytokine release and/or displaying increased anti-tumor efficacy.

The disclosure provides an isolated protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 binds to an epitope on hK2 set forth in SEQ ID NO: 111, 112, 113, 114, or 115.

In a particular embodiment, the antigen binding domain that binds hK2 binds to an epitope on hK2 set forth in SEQ ID NOs: 111 and 112.

In some embodiments, the antigen binding domain that binds hK2 binds to an epitope on hK2 set forth in SEQ ID NOs: 113, 114 and 115.

The invention also provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antibody or the antigen binding fragment thereof binds within residues KVTEF (SEQ ID NO: 111) or HYRKW (SEQ ID NO: 112) or SHGWAH (SEQ ID NO: 113) or RHNLFEPEDTGQRVP (SEQ ID NO: 114) or GWGSIEPEE (SEQ ID NO: 115) of hK2. In a particular embodiment, the invention also provides an isolated protein comprising an antigen binding domain that binds hK2, wherein said antigen binding domain binds to hK2 within epitopes having sequences of KVTEF (SEQ ID NO: 111) and HYRKW (SEQ ID NO: 112).

An H/D exchange assay may be used to determine the residues within hK2 to which an antibody binds. In an H/D exchange assay, recombinantly expressed soluble hK2 is incubated in the presence or absence of the antibody in deuterated water for predetermined times resulting in deuterium incorporation at exchangeable hydrogen atoms which are unprotected by the antibody, followed by protease digestion of the protein and analyses of the peptide fragments using LC-MS. H/D exchange assay can be performed using known protocols. An exemplary protocol is described in Example 3. In some embodiments, the H/D exchange mixture is quenched by the addition of a quenching buffer (*e.g.* 8 M urea, 1M TCEP, pH 3.0) before being passed over an equilibrated immobilized pepsin/FPXIII column at room temperature (*e.g.* 600 µL/min). The peptic fragments are then loaded onto a reverse phase trap column (*e.g.* at 600 µL/min) and desalted (*e.g.* for 1 min at 600 µL), separated (*e.g.* on a C18 column) and analyzed by mass spectrometry (*e.g.* using an LTQ™ Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific) with the capillary temperature at 275 °C, resolution 150,000, and mass range (m/z) 300-1,800).

The invention also provides an isolated protein comprising an antigen binding domain, wherein the antigen binding domain of said reference antibody binds to an epitope on hK2 having a sequence selected from the group consisting of SEQ ID NO: 111, 112, 113, 114, and 115 and wherein the antigen binding domain that binds hK2 competes for binding to hK2 with a reference antibody disclosed herein. In some embodiments, the reference antibody comprises:

a. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 138; or

b. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163; or
c. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165; or
d. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167; or
e. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or
f. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205; or
g. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 444.

In certain such embodiments, the reference antibody comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 162 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 163.

Competition for binding of a test antibody that binds to SEQ ID NO: 111, 112, 113, 114, or 115 of soluble hK2 with the reference antibodies of the invention may be assayed *in vitro* using well known methods. For example, binding of labeled antibody to hK2 the membrane proximal region of hK2 in the presence of an unlabeled reference antibody may be assessed by ELISA, or Bioacore analyses or flow cytometry may be used to demonstrate competition. The test antibody competes for binding to hk2 with the reference antibody when the test antibody inhibits binding of the reference antibody to soluble hK2 by 85% or more, for example 90% or more, or 95% or more.

The disclosure provides an isolated protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167; or the HCDR1, the HCDR2 and the

HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205. In a particular embodiment, the isolated protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163.

The disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

SEQ ID NOs: 63, 65, 66, 67, 69, 71, respectively;

SEQ ID NOs: 63, 65, 66, 68, 70, 71, respectively;

SEQ ID NOs: 72, 73, 66, 67, 69, 71, respectively;

SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively;

SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively;

SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively.

SEQ ID NOs: 170,183, 184, 185, 186, and 187, respectively;

SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively;

SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively;

SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively;

SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively;

SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively;

SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively;

SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively; or

SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In a particular embodiment, the disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively.

In another particular embodiment, the disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

The disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the VH of SEQ ID NOs: 137, 162, 164, 166, 168 or 204 and the VL of SEQ ID NOs: 138, 163, 165, 167 or 169.

The disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises
the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169; or
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In a particular embodiment, the disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of of SEQ ID NO: 163.

The disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NOs: 133, 134, 308, 316, 324,325, 404, 405, 406, 407, 408, or 409.In a particular embodiment, the disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 404.

In another embodiment, the disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 405.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises an amino acid sequence at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 404.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises an amino acid sequence at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 405.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein the antigen binding domain that binds hK2 comprises a heavy chain variable region (VH) of SEQ ID NO: 75 and a light chain variable region (VL) of SEQ ID NO: 74. SEQ ID NO: 75 and SEQ ID NO: 74 represent genus VH and VL amino acid sequences encompassing variants demonstrating improved thermostability when compared to the parent antibody hu11B6. The positions engineered which conferred improved thermostability were residues P41, I49, M70, and A88 in the VH (residue numbering according to the hu11B6_VH of SEQ ID NO: 5) and S80, L82, A88 and Y91 in the VL (residue numbering according to the hu11B6_VL of SEQ ID NO: 2).

SEQ ID NO: 75 (VH consensus sequence)

QVQLQESGPGLVKPSX$_1$TLSLTCX$_2$VSGNSITSDYAWNWIRQX$_3$PGKX$_4$LEWX$_5$GYISYSGSTT
YNPSLKSRVTX$_6$SRDTSKNQFSLKLSSVTX$_7$X$_8$DTAVYYCATGYYYGSGFWGQGTLVTVSS

SEQ ID NO: 74 (VL consensus sequence)

X_1IVLTQSPX_2X_3LX_4X_5SX_6GERATX$_7X_8$CX_9ASESVEYFGTSLMHWYQQKPGQPPX_{10}LLIYAASNX_{11}ESGX$_{12}$PX_{13}RFSGSGSGTDFTLTIX_{14}S$X_{15}X_{16}$QX_{17}EDX$_{18}X_{19}$VYX$_{20}$CQQTRKVPYTFGX$_{21}$GTKX$_{22}$EIK

The disclosure provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the VH of SEQ ID NOs: 4, 5, 6, 139, 159 or 161 and the VL of SEQ ID NOs: 1, 2, 3, 140 or 160, with the proviso that the antigen binding domain that binds hK2 does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises

the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1;

the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140; or
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

The disclosure also provides an isolated protein comprising an antigen binding domain that binds hK2, wherein the antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 135, 136, 318, 319, 320, 321, 322 or 323.

In some embodiments, the antigen binding domain that binds hK2 is a scFv.

In some embodiments, the antigen binding domain that binds hK2 is a $(scFv)_2$.

In some embodiments, the antigen binding domain that binds hK2 is a Fv.

In some embodiments, the antigen binding domain that binds hK2 is a Fab.

In some embodiments, the antigen binding domain that binds hK2 is a $F(ab')_2$.

In some embodiments, the antigen binding domain that binds hK2 is a Fd.

In some embodiments, the hK2 antigen binding domain is a dAb.

In some embodiments, the hK2 antigen binding domain is a VHH.

In a particular embodiment, the antigen binding domain that binds hK2 is a Fab.

hK2 binding scFvs

Any of the VH and the VL domains identified herein that bind hK2 may be engineered into scFv format in either VH-linker-VL or VL-linker-VH orientation. Any of the VH and the VL domains identified herein may also be used to generate $sc(Fv)_2$ structures, such as VH-linker-VL-linker-VL-linker-VH, VH-linker-VL-linker-VH-linker-VL, VH-linker-VH-linker-VL-linker-VL, VL-linker-VH-linker-VH-linker-VL, VL-linker-VH-linker-VL-linker-VH or VL-linker-VL-linker-VH-linker-VH.

The VH and the VL domains identified herein may be incorporated into a scFv format and the binding and thermostability of the resulting scFv to hK2 may be assessed using known methods. Binding may be assessed using ProteOn XPR36, BIAcore 3000 or KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art. Binding may be evaluated using purified scFvs or Ecoli supernatants or lysed cells containing the expressed scFv. The measured affinity of a test scFv to hK2 may vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity

and other binding parameters (e.g., K_D, K_{on}, K_{off}) are typically made with standardized conditions and standardized buffers. Thermostability may be evaluated by heating the test scFv at elevated temperatures, such as at 50°C, 55°C or 60°C for a period of time, such as 5 minutes (min), 10 min, 15 min, 20 min, 25 min or 30 min and measuring binding of the test scFv to hK2. The scFvs retaining comparable binding to hK2 when compared to a non-heated scFv sample are referred to as being thermostable.

In recombinant expression systems, the linker is a peptide linker and may include any naturally occurring amino acid. Exemplary amino acids that may be included into the linker are Gly, Ser Pro, Thr, Glu, Lys, Arg, Ile, Leu, His and The. The linker should have a length that is adequate to link the VH and the VL in such a way that they form the correct conformation relative to one another so that they retain the desired activity, such as binding to hK2.

The linker may be about 5-50 amino acids long. In some embodiments, the linker is about 10-40 amino acids long. In some embodiments, the linker is about 10-35 amino acids long. In some embodiments, the linker is about 10-30 amino acids long. In some embodiments, the linker is about 10-25 amino acids long. In some embodiments, the linker is about 10-20 amino acids long. In some embodiments, the linker is about 15-20 amino acids long. In some embodiments, the linker is 6 amino acids long. In some embodiments, the linker is 7 amino acids long. In some embodiments, the linker is 8 amino acids long. In some embodiments, the linker is 9 amino acids long. In some embodiments, the linker is 10 amino acids long. In some embodiments, the linker is 11 amino acids long. In some embodiments, the linker is 12 amino acids long. In some embodiments, the linker is 13 amino acids long. In some embodiments, the linker is 14 amino acids long. In some embodiments, the linker is 15 amino acids long. In some embodiments, the linker is 16 amino acids long. In some embodiments, the linker is 17 amino acids long. In some embodiments, the linker is 18 amino acids long. In some embodiments, the linker is 19 amino acids long. In some embodiments, the linker is 20 amino acids long. In some embodiments, the linker is 21 amino acids long. In some embodiments, the linker is 22 amino acids long. In some embodiments, the linker is 23 amino acids long. In some embodiments, the linker is 24 amino acids long. In some embodiments, the linker is 25 amino acids long. In some embodiments, the linker is 26 amino acids long. In some embodiments, the linker is 27 amino acids long. In some embodiments, the linker is 28 amino acids long. In some embodiments, the linker is 29 amino acids long. In some embodiments, the linker is 30 amino acids long. In some embodiments, the linker is 31 amino acids long. In some embodiments, the linker is 32 amino acids long. In some embodiments, the linker is 33 amino acids long. In some embodiments, the linker is 34 amino acids long. In some embodiments, the linker is 35 amino acids long. In some embodiments, the linker is 36 amino acids long. In some embodiments, the linker is 37 amino acids long. In some embodiments, the linker is 38 amino acids long. In some embodiments, the linker is 39 amino acids long. In some embodiments, the linker is 40 amino acids long.

Exemplary linkers that may be used are Gly rich linkers, Gly and Ser containing linkers, Gly and Ala containing linkers, Ala and Ser containing linkers, and other flexible linkers.

Other linker sequences may include portions of immunoglobulin hinge area, CL or CH1 derived from any immunoglobulin heavy or light chain isotype. Alternatively, a variety of non-proteinaceous polymers, including polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers. Exemplary linkers that may be used are shown in **Table 1**. Additional linkers are described for example in Int. Pat. Publ. No. WO2019/060695.

In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL).

In some embodiments, the scFv comprises, from the N-to C-terminus, the VL, the L1 and the VH (VL-L1-VH).

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 7.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 76.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 77.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 78.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 79.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 80.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 81.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 82.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 83.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 84.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 85.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 86.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 87.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 88.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 89.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 90.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 91.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 92.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 93.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 94.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 95.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 96.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 97.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 98.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 99.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 100.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 101.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 102.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 103.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 104.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 105.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 106.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 107

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

Table 1.

Linker name	Amino acid sequence	SEQ ID NO:
Linker 1	GGSEGKSSGSGSESKSTGGS	7
Linker 2	GGGSGGGS	76
Linker 3	GGGSGGGSGGGS	77
Linker 4	GGGSGGGSGGGSGGGS	78
Linker 5	GGGSGGGSGGGSGGGSGGGS	79
Linker 6	GGGGSGGGGSGGGGS	80
Linker 7	GGGGSGGGGSGGGGSGGGGS	81
Linker 8	GGGGSGGGGSGGGGSGGGGSGGGGS	82
Linker 9	GSTSGSGKPGSGEGSTKG	83
Linker 10	IRPRAIGGSKPRVA	84
Linker 11	GKGGSGKGGSGKGGS	85
Linker 12	GGKGSGGKGSGGKGS	86
Linker 13	GGGKSGGGKSGGGKS	87
Linker 14	GKGKSGKGKSGKGKS	88
Linker 15	GGGKSGGKGSGKGGS	89
Linker 16	GKPGSGKPGSGKPGS	90

Linker 17	GKPGSGKPGSGKPGSGKPGS	91
Linker 18	GKGKSGKGKSGKGKSGKGKS	92
Linker 19	STAGDTHLGGEDFD	93
Linker 20	GEGGSGEGGSGEGGS	94
Linker 21	GGEGSGGEGSGGEGS	95
Linker 22	GEGESGEGESGEGES	96
Linker 23	GGGESGGEGSGEGGS	97
Linker 24	GEGESGEGESGEGESGEGES	98
Linker 25	GSTSGSGKPGSGEGSTKG	99
Linker 26	PRGASKSGSASQTGSAPGS	100
Linker 27	GTAAAGAGAAGGAAAGAAG	101
Linker 28	GTSGSSGSGSGGSGSGGGG	102
Linker 29	GKPGSGKPGSGKPGSGKPGS	103
Linker 30	GSGS	104
Linker 31	APAPAPAPAP	105
Linker 32	APAPAPAPAPAPAPAPAPAP	106
Linker 33	AEAAAKEAAAKEAAAAKEAAAAKEAAAAKAAA	107
Linker 34	GTEGKSSGSGSESKST	108

In a particular embodiment, the L1 comprises or consists of the amino acid sequence of SEQ ID NO: 7.

In some embodiments, the scFv comprises

a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ

ID NO: 205; or the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 139 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

SEQ ID NOs: 63, 65, 66, 67, 69, 71, respectively;

SEQ ID NOs: 63, 65, 66, 68, 70, 71, respectively;

SEQ ID NOs: 72, 73, 66, 67, 69, 71, respectively;

SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively;

SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively;

SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively.

SEQ ID NOs: 170,183, 184, 185, 186, and 187, respectively;

SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively;

SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively;

SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively;

SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively;

SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively;

SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively;

SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively; or

SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186, and 187, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 188, 189, 190, 182, 470, and 209, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 63, 65, 66, 68, 70 and 71, respectively.

In some embodiments, the scFv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 72, 73, 66, 67, 69 and 71, respectively.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 137 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 138.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 164 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 165.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 166 and a VL which is at least

80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 167.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 168 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 169.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 204 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 205.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 159 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 161 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 139 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 159 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises a VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of SEQ ID NO: 163.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NOs: 133, 134, 308, 316, 324,325, 404, 405, 406, 407, 408, or 409.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 133, 134, 308, 316, 324,325, 404, 405, 406, 407, 408, or 409.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 404.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 405.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 75 and the VL of SEQ ID NO: 74.

In some embodiments, the scFv comprises the VH of SEQ ID NOs: 4, 5 or 6 and the VL of SEQ ID NOs: 1, 2 or 3.

In some embodiments, the scFv comprises the VH of SEQ ID NOs: 4, 5, 6, 139, 159 or 161 and the VL of SEQ ID NOs: 1, 2, 3, 140 or 160, with the proviso that the antigen binding domain that binds hK2 does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140.

In some embodiments, the scFv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 8.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 9.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 10.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 13.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 14.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 15.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 16.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 17.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 19.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 20.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 21.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 22.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 23.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 135.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 136.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 318.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 319.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 320.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 321.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 322.

In some embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 323.

Other antigen binding domains that bind hK2

Any of the VH and the VL domains identified herein that bind hK2 may also be engineered into Fab, $F(ab`)_2$, Fd or Fv format and their binding to hK2 and thermostability may be assessed using the assays described herein.

In some embodiments, the Fab comprises
a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or

the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205.
In a particular embodiment, the Fab comprises the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
SEQ ID NOs: 63, 65, 66, 67, 69, 71, respectively;
SEQ ID NOs: 63, 65, 66, 68, 70, 71, respectively;
SEQ ID NOs: 72, 73, 66, 67, 69, 71, respectively;
SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively;
SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively;
SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively;
SEQ ID NOs: 170,183, 184, 185, 186, and 187, respectively;
SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively;
SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively;
SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively;
SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively;
SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively;
SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively;
SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively; or
SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170,183, 184, 185, 186, and 187, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively.

In some embodiments, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In a particular embodiment, the Fab comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively or of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 75 and the VL of SEQ ID NO: 74.

In a particular embodiment, the Fab comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163.

In some embodiments, the Fab comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 137 and a VL which is at least

80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 138.

In some embodiments, the Fab comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 164 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 165.

In some embodiments, the Fab comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 166 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 167.

In some embodiments, the Fab comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 168 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 169.

In some embodiments, the Fab comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 204 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 205.

In some embodiments, the Fab comprises the VH of SEQ ID NOs: 4, 5, 6, 139, 159 or 161 and the VL of SEQ ID NOs: 1, 2, 3, 140 or 160, with the proviso that the Fab does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140.

In some embodiments, the Fab comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

The VH and VL of the Fab comprising the antigen binding domain that binds hK2 may be engineered into Fab-Fc HC (VH-CH1-hinge-CH2-CH3) and Fab-Fc LC (VL-CL) formats respectively. In certain such embodiments, the Fab-Fc HC comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 354. In a particular embodiment, the Fab-Fc HC comprises an amino acid sequence which is identical to SEQ ID NO: 354

In some embodiments, the Fab-Fc HC comprises a C-terminal lysine residue (e.g. K477). In some embodiments, the Fab-Fc HC comprises an amino acid sequence having a C-terminal lysine residue and a sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 361. In a particular embodiment, the Fab-Fc HC comprises an amino acid sequence of SEQ ID NO:361.

In some embodiments, the Fab-Fc LC comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 221. In a particular embodiment, the Fab-Fc LC comprises an amino acid sequence which is identical to SEQ ID NO: 221

As shown in the examples, a particularly suitable antigen binding domain that binds hK2 for incorporating into a multispecific construct comprises a Fab-Fc HC having the amino acid sequence of SEQ ID NO: 354 and a Fab-Fc LC having the amino acid sequence of SEQ ID NO: 221.

In some embodiments, the $F(ab')_2$ comprises
a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively;
SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively;
SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively;
SEQ ID NOs: 170,183, 184, 185, 186, and 187, respectively;
SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively;
SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively;
SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively;
SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively;
SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively;
SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively;

SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively; or
SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170,183, 184, 185, 186, and 187, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively.

In some embodiments, the $F(ab')_2$ comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 75 and the VL of SEQ ID NO: 74.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NOs: 4, 5, 6, 139, 159 or 161 and the VL of SEQ ID NOs: 1, 2, 3, 140 or 160, with the proviso that the Fab does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3.

In some embodiments, the F(ab')$_2$ comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140.

In some embodiments, the $F(ab')_2$ comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

In some embodiments, the Fv comprises

a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 137 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 138; or
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167;
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169; or
the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively;
SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively;
SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively;
SEQ ID NOs: 170, 183, 184, 185, 186, and 187, respectively;
SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively;
SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively;
SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively;
SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively;
SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively;
SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively;
SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively; or
SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186, and 187, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 196, 197, 178, 179, 180, 181, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 200, 201, 190, 191, 192, and 193, respectively.

In some embodiments, the Fv comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 75 and the VL of SEQ ID NO: 74.

In some embodiments, the Fv comprises the VH of SEQ ID NOs: 4, 5 or 6 and the VL of SEQ ID NOs: 1, 2 or 3.

In some embodiments, the Fv comprises the VH of SEQ ID NOs: 4, 5, 6, 139, 159 or 161 and the VL of SEQ ID NOs: 1, 2, 3, 140 or 160, with the proviso that the Fab does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140.

In some embodiments, the Fv comprises the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

In some embodiments, the Fd comprises the VH of SEQ ID NO: 75.

In some embodiments, the Fd comprises the VH of SEQ ID NO: 4.

In some embodiments, the Fd comprises the VH of SEQ ID NO: 5.

In some embodiments, the Fd comprises the VH of SEQ ID NO: 6.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC of SEQ ID NO: 210 and a LC of SEQ ID NO: 221.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC of SEQ ID NO: 211 and a LC of SEQ ID NO: 222.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC of SEQ ID NO: 212 and a LC of SEQ ID NO: 223.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC of SEQ ID NO: 213 and a LC of SEQ ID NO: 224.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC of SEQ ID NO: 219 and a LC of SEQ ID NO: 220.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 354 and a LC which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 221.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 354 and a LC of SEQ ID NO: 221.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC of SEQ ID NO: 354 and a LC which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 221.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC which is at least 95% identical to SEQ ID NO: 354 and a LC which is at least 95% identical to SEQ ID NO: 221.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC which is at least 99% identical to SEQ ID NO: 354 and a LC which is at least 95% identical to SEQ ID NO: 221.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC which is at least 99% identical to SEQ ID NO: 354 and a LC which is at least 99% identical to SEQ ID NO: 221.

In some embodiments, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC which is at least 95% identical to SEQ ID NO: 354 and a LC which is at least 99% identical to SEQ ID NO: 221.

In a particular embodiment, the isolated anti-hK2 antibody or the antigen binding fragment thereof comprises a HC of SEQ ID NO: 354 and a LC of SEQ ID NO: 221

Homologous antigen binding domains and antigen binding domains with conservative substitutions

Variants of the antigen binding domains that bind hK2 are within the scope of the disclosure. For example, variants may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acid substitutions in the antigen binding domain that bind hK2 as long as they retain or have improved functional properties when compared to the parent antigen binding domains. In some embodiments, the sequence identity may be about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% to the antigen binding domains that bind hK2 of the disclosure. In some embodiments, the variation is in the framework regions. In some embodiments, variants are generated by conservative substitutions.

For example, the antigen binding domains that bind hK2 may comprise substitutions at residue positions D16, A23, P41, G45, I49, M70, and A88, and V89 in the VH (residue numbering according to the hu11B6_VH of SEQ ID NO: 5) and D1, D9, S10, A12, V13, L15, I21, N22, K24, K49, R58, V62, D64, S80, L82, Q83, A84, V87, A88, Y91, Q104, and L108 in the VL (residue numbering according to the hu11B6_VL of SEQ ID NO: 2). Conservative substitutions may be made at any indicated positions and the resulting variant antigen binding domains that bind hK2 are tested for their desired characteristics in the assays described herein.

In some embodiments, an isolated protein comprising an antigen binding domain that binds hK2 comprises a VH and a VL which are at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH and VL, respectively, of an antigen binding domain that binds hK2 disclosed herein.

Also provided are antigen binding domains that bind hK2 comprising the VH and the VL which are at least 80% identical to

the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;

the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169; or
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In some embodiments, the identity is 85%. In some embodiments, the identity is 90%. In some embodiments, the identity is 91%. In some embodiments, the identity is 91%. In some embodiments, the identity is 92%. In some embodiments, the identity is 93%. In some embodiments, the identity is 94%. In some embodiments, the identity is 94%. In some embodiments, the identity is 95%. In some embodiments, the identity is 96%. In some embodiments, the identity is 97%. In some embodiments, the identity is 98%. In some embodiments, the identity is 99%.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 137 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 138.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 164 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 165.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID

NO: 166 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 167.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 166 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 444.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 168 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 169.

In some embodiments, the antigen binding domains that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 204 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 205.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 85% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 90% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 91% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 92% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 93% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 94% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 96% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 97% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 98% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 85% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 90% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 91% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 92% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 93% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 94% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 96% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 97% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 98% identical to the VL of SEQ ID NO: 163

In some embodiments, the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of SEQ ID NO: 163

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions ×100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The percent identity between two amino acid sequences may be determined using the algorithm of E. Meyers and W. Miller (*Comput Appl Biosci* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid or nucleic acid sequences may be determined using the Needleman and Wunsch (*J Mol Biol* 48:444-453 (1970)) algorithm which has

been incorporated into the GAP program in the GCG software package (available at http // www gcg com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

In some embodiments, variant antigen binding domains that bind hK2 comprise one or two conservative substitutions in any of the CDR regions, while retaining desired functional properties of the parent antigen binding fragments that bind hK2.

"**Conservative modifications**" refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid modifications. Conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are those in which the amino acid is replaced with an amino acid residue having a similar side chain. The families of amino acid residues having similar side chains are well defined and include amino acids with acidic side chains (e.g., aspartic acid, glutamic acid), basic side chains (e.g., lysine, arginine, histidine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), uncharged polar side chains (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, tryptophan), aromatic side chains (e.g., phenylalanine, tryptophan, histidine, tyrosine), aliphatic side chains (e.g., glycine, alanine, valine, leucine, isoleucine, serine, threonine), amide (e.g., asparagine, glutamine), beta-branched side chains (e.g., threonine, valine, isoleucine) and sulfur-containing side chains (cysteine, methionine). Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan *et al.*, (1988) *Acta Physiol Scand Suppl* 643:55-67; Sasaki *et al.*, (1988) *Adv Biophys* 35:1-24). Amino acid substitutions to the antibodies of the invention may be made by known methods for example by PCR mutagenesis (US Pat. No. 4,683,195). Alternatively, libraries of variants may be generated for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp). The resulting variants may be tested for their characteristics using assays described herein.

Methods of generating antigen binding fragment that bind hK2

Antigen binding domains that bind hK2 provided in the disclosure may be generated using various technologies. For example, the hybridoma method of Kohler and Milstein may be used to identify VH/VL pairs that bind hK2. In the hybridoma method, a mouse or other host animal, such as a

hamster, rat or chicken is immunized with human and/or cyno hK2, followed by fusion of spleen cells from immunized animals with myeloma cells using standard methods to form hybridoma cells. Colonies arising from single immortalized hybridoma cells may be screened for production of the antibodies containing the antigen binding domains that bind hK2 with desired properties, such as specificity of binding, cross-reactivity or lack thereof, affinity for the antigen, and any desired functionality.

Antigen binding domains that bind hK2 generated by immunizing non-human animals may be humanized. Exemplary humanization techniques including selection of human acceptor frameworks include CDR grafting (U.S. Patent No. 5,225,539), SDR grafting (U.S. Patent No. 6,818,749), Resurfacing (Padlan, (1991) *Mol Immunol* 28:489-499), Specificity Determining Residues Resurfacing (U.S. Patent Publ. No. 2010/0261620), human framework adaptation (U.S. Patent No. 8,748,356) or superhumanization (U.S. Patent No. 7,709, 226). In these methods, CDRs or a subset of CDR residues of parental antibodies are transferred onto human frameworks that may be selected based on their overall homology to the parental frameworks, based on similarity in CDR length, or canonical structure identity, or a combination thereof.

Humanized antigen binding domains may be further optimized to improve their selectivity or affinity to a desired antigen by incorporating altered framework support residues to preserve binding affinity (backmutations) by techniques such as those described in Int. Patent Publ. Nos. WO1090/007861 and WO1992/22653, or by introducing variation at any of the CDRs for example to improve affinity of the antigen binding domain.

Transgenic animals, such as mice, rat or chicken carrying human immunoglobulin (Ig) loci in their genome may be used to generate antigen binding fragments that bind hK2, and are described in for example U.S. Patent No. 6,150,584, Int. Patent Publ. No. WO1999/45962, Int. Patent Publ. Nos. WO2002/066630, WO2002/43478, WO2002/043478 and WO1990/04036. The endogenous immunoglobulin loci in such animal may be disrupted or deleted, and at least one complete or partial human immunoglobulin locus may be inserted into the genome of the animal using homologous or non-homologous recombination, using transchromosomes, or using minigenes. Companies such as Regeneron (http://_www_regeneron_com), Harbour Antibodies (http://_www_harbourantibodies_com), Open Monoclonal Technology, Inc. (OMT) (http://_www_omtinc_net), KyMab (http://_www_kymab_com), Trianni (http://_www.trianni_com) and Ablexis (http://_www_ablexis_com) may be engaged to provide human antibodies directed against a selected antigen using technologies as described above. In some embodiments, Ablexis mice and OmniRats rats were immunized with soluble full length KLK2 protein (human Kallikrein-2 6-His protein) (SEQ ID NO: 454).

Antigen binding domains that bind hK2 may be selected from a phage display library, where the phage is engineered to express human immunoglobulins or portions thereof such as Fabs, single chain antibodies (scFv), or unpaired or paired antibody variable regions. The antigen binding domains that bind hK2 may be isolated for example from phage display library expressing antibody heavy and light chain variable regions as fusion proteins with bacteriophage pIX coat protein as described in Shi *et al.*, (2010) *J Mol Biol* 397:385-96, and Int. Patent Publ. No. WO09/085462). The libraries may be screened for phage binding to human and/or cyno hK2 and the obtained positive clones may be further characterized, the Fabs isolated from the clone lysates, and converted to scFvs or other configurations of antigen binding fragments.

Preparation of immunogenic antigens and expression and production of antigen binding domains of the disclosure may be performed using any suitable technique, such as recombinant protein production. The immunogenic antigens may be administered to an animal in the form of purified protein, or protein mixtures including whole cells or cell or tissue extracts, or the antigen may be formed *de novo* in the animal's body from nucleic acids encoding said antigen or a portion thereof.

Conjugation to half-life extending moieties

The antigen binding domains that bind hK2 of the disclosure may be conjugated to a half-life extending moiety. Exemplary half-life extending moieties are albumin, albumin variants, albumin-binding proteins and/or domains, transferrin and fragments and analogues thereof, immunoglobulins (Ig) or fragments thereof, such as Fc regions. Amino acid sequences of the aforementioned half-life extending moieties are known. Ig or fragments thereof include all isotypes, *i.e.*, IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE.

Additional half-life extending moieties that may be conjugated to the antigen binding domains that bind hK2 of the disclosure include polyethylene glycol (PEG) molecules, such as PEG5000 or PEG20,000, fatty acids and fatty acid esters of different chain lengths, for example laurate, myristate, stearate, arachidate, behenate, oleate, arachidonate, octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like, polylysine, octane, carbohydrates (dextran, cellulose, oligo- or polysaccharides) for desired properties. These moieties may be direct fusions with the antigen binding domains that bind hK2 of the disclosure and may be generated by standard cloning and

expression techniques. Alternatively, well known chemical coupling methods may be used to attach the moieties to recombinantly produced antigen binding domains that bind hK2 of the disclosure.

A pegyl moiety may for example be conjugated to the antigen binding domain that bind hK2 of the disclosure by incorporating a cysteine residue to the C-terminus of the antigen binding domain that bind hK2 of the disclosure, or engineering cysteines into residue positions that face away from the hK2 binding site and attaching a pegyl group to the cysteine using well known methods.

In some embodiments, the antigen binding fragment that binds hK2 is conjugated to a half-life extending moiety.

In some embodiments, the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, a fragment of the Ig constant region, a Fc region, transferrin, albumin, an albumin binding domain or polyethylene glycol. In some embodiments, the half-life extending moiety is an Ig constant region.

In some embodiments, the half-life extending moiety is the Ig.

In some embodiments, the half-life extending moiety is the fragment of the Ig.

In some embodiments, the half-life extending moiety is the Ig constant region.

In some embodiments, the half-life extending moiety is the fragment of the Ig constant region.

In some embodiments, the half-life extending moiety is the Fc region.

In some embodiments, the half-life extending moiety is albumin.

In some embodiments, the half-life extending moiety is the albumin binding domain.

In some embodiments, the half-life extending moiety is transferrin.

In some embodiments, the half-life extending moiety is polyethylene glycol.

The antigen binding domains that bind hK2 conjugated to a half-life extending moiety may be evaluated for their pharmacokinetic properties utilizing known *in vivo* models.

Conjugation to immunoglobulin (Ig) constant regions or fragments of the Ig constant regions

The antigen binding domains that bind hK2 of the disclosure may be conjugated to an Ig constant region or a fragment of the Ig constant region to impart antibody-like properties, including Fc effector functions C1q binding, complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis or down regulation of cell surface receptors (e.g., B cell receptor; BCR). The Ig constant region or the fragment of the Ig constant region functions also as a half-life extending moiety as discussed herein. The antigen binding domains that bind hK2 of the disclosure may be engineered into conventional full-length antibodies using standard methods. The full-length antibodies comprising the antigen binding domain that binds hK2 may further be engineered as described herein.

Immunoglobulin heavy chain constant region comprised of subdomains CH1, hinge, CH2 and CH3. The CH1 domain spans residues A118-V215, the CH2 domain residues A231-K340 and the CH3 domain residues G341-K447 on the heavy chain, residue numbering according to the EU Index. In some instances, G341 is referred as a CH2 domain residue. Hinge is generally defined as including E216 and terminating at P230 of human IgG1. Ig Fc region comprises at least the CH2 and the CH3 domains of the Ig constant region, and therefore comprises at least a region from about A231 to K447 of Ig heavy chain constant region.

The invention also provides an antigen binding domain that binds hK2 conjugated to an immunoglobulin (Ig) constant region or a fragment of the Ig constant region.

In some embodiments, the Ig constant region is a heavy chain constant region

In some embodiments, the Ig constant region is a light chain constant region.

In some embodiments, the fragment of the Ig constant region comprises a Fc region.

In some embodiments, the fragment of the Ig constant region comprises a CH2 domain.

In some embodiments, the fragment of the Ig constant region comprises a CH3 domain.

In some embodiments, the fragment of the Ig constant region comprises the CH2 domain and the CH3 domain.

In some embodiments, the fragment of the Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain. Portion of the hinge refers to one or more amino acid residues of the Ig hinge.

In some embodiments, the fragment of the Ig constant region comprises the hinge, the CH2 domain and the CH3 domain.

In some embodiments, the antigen binding domain that binds hK2 is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region.

In some embodiments, the antigen binding domain that binds hK2 is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region.

In some embodiments, the antigen binding domain that binds hK2 is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2).

In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NOs: 7, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, or 108.

In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 7.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 76.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 77.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 78.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 79.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 80.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 81.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 82.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 83.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 84.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 85.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 86.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 87.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 88.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 89.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 90.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 91.

In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 92.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 93.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 94.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 95.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 96.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 97.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 98.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 99.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 100.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 101.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 102.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 103.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 104.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 105.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 106.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 107.
In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NO: 108.

The antigen binding domains that binds hK2 of the disclosure conjugated to Ig constant region or the fragment of the Ig constant region may be assessed for their functionality using several known assays. Binding to hK2 may be assessed using methods described herein. Altered properties imparted by the Ig constant domain or the fragment of the Ig constant region such as Fc region may be assayed in Fc receptor binding assays using soluble forms of the receptors, such as the FcγRI, FcγRII, FcγRIII or FcRn receptors, or using cell-based assays measuring for example ADCC, CDC or ADCP.

ADCC may be assessed using an *in vitro* assay using hK2 expressing cells as target cells and NK cells as effector cells. Cytolysis may be detected by the release of label (e.g. radioactive substrates, fluorescent dyes or natural intracellular proteins) from the lysed cells. In an exemplary assay, target cells are used with a ratio of 1 target cell to 4 effector cells. Target cells are pre-labeled with BATDA and combined with effector cells and the test antibody. The samples are incubated for 2 hours and cell lysis measured by measuring released BATDA into the supernatant. Data is normalized to maximal cytotoxicity with 0.67% Triton X-100 (Sigma Aldrich) and minimal control determined by spontaneous release of BATDA from target cells in the absence of any antibody.

ADCP may be evaluated by using monocyte-derived macrophages as effector cells and any hK2 expressing cells as target cells which are engineered to express GFP or other labeled molecule. In an exemplary assay, effector:target cell ratio may be for example 4:1. Effector cells may be incubated with target cells for 4 hours with or without the antibody of the invention. After incubation, cells may be detached using accutase. Macrophages may be identified with anti-CD11b and anti-CD14 antibodies coupled to a fluorescent label, and percent phagocytosis may be determined based on % GFP fluorescence in the $CD11^{+}CD14^{+}$ macrophages using standard methods.

CDC of cells may be measured for example by plating Daudi cells at 1×10^{5} cells/well (50 µL/well) in RPMI-B (RPMI supplemented with 1% BSA), adding 50 µL of test protein to the wells at final concentration between 0-100 µg/mL, incubating the reaction for 15 min at room temperature, adding 11 µL of pooled human serum to the wells, and incubation the reaction for 45 min at 37° C. Percentage (%) lysed cells may be detected as % propidium iodide stained cells in FACS assay using standard methods.

In some embodiments, the antigen binding domain that binds hK2 is conjugated to an IgG1 heavy chain constant region or a fragment of the IgG1 heavy chain constant region. In some embodiments, the antigen binding domain that binds hK2 is conjugated to an IgG1 heavy chain constant region or a fragment of the IgG1 heavy chain constant region (*e.g.* comprising the hinge-CH2-CH3) comprising an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 110. In some embodiments, the IgG1 heavy chain constant region comprises the Fc silencing mutation (L234A_L235A_D265S) and the T350V_T366L_K392L_T394W mutations designed to promote selective heterodimerization. In some embodiments, the antigen binding domain that binds hK2 is conjugated to an IgG1 heavy chain constant region or a fragment of the IgG1 heavy chain constant region having the amino acid sequence of SEQ ID NO: 110.

In some embodiments, the antigen binding domain that binds hK2 is conjugated to an IgG1 heavy chain constant region (*e.g.* CH1-hinge-CH2-CH3) comprising an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 378. In a particular embodiment, the antigen binding domain that binds hK2 is conjugated to an IgG1 heavy chain constant region or a fragment of the IgG1 heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 378.

In some embodiments, the antigen binding domain that binds hK2 is conjugated to an IgG1 light chain constant region comprising an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least

90%, at least 95% or at least 99%) identical to SEQ ID NO: 309. In some embodiments, the antigen binding domain that binds hK2 is conjugated to an IgG1 light chain constant region comprising an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 447. In some embodiments, the antigen binding domain that binds hK2 is conjugated to an IgG1 light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309 or 447. In a particular embodiment, the antigen binding domain that binds hK2 is conjugated to an IgG1 light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises an antigen binding domain that binds hK2 which is conjugated to an IgG1 heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 378 and an antigen binding domain that binds hK2 which is conjugated to an IgG1 light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3) and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first

Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309, and wherein the isolated protein further comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3) and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173,

174 and 175, respectively, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309, and wherein the isolated protein further comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the amino acid sequence of SEQ ID NO: 331, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309, and wherein the isolated protein further comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the amino acid sequence of SEQ ID NO: 331, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

Proteins comprising the antigen binding domains that bind hK2 of the disclosure

The antigen binding domains that bind hK2 of the disclosure may be engineered into monospecific or multispecific proteins of various designs using standard methods.

The disclosure also provides a monospecific protein comprising the antigen binding domain that binds hK2 of the disclosure.

In some embodiments, the monospecific protein is an antibody.

The disclosure also provides a multispecific protein comprising the antigen binding domain that binds hK2 of the disclosure.

In some embodiments, the multispecific protein is bispecific.

In some embodiments, the multispecific protein is trispecific.

In some embodiments, the multispecific protein is tetraspecific.

In some embodiments, the multispecific protein is monovalent for binding to hK2.

In some embodiments, the multispecific protein is bivalent for binding to hK2.

The disclosure also provides an isolated multispecific protein comprising a first antigen binding domain that binds hK2 and a second antigen binding domain that binds a lymphocyte antigen (such as CD3).

In some embodiments, the lymphocyte antigen is a T cell antigen.

In some embodiments, the T cell antigen is a $CD8^+$ T cell antigen.

In some embodiments, the lymphocyte antigen is a NK cell antigen.

In some embodiments, the lymphocyte antigen is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C.

In some embodiments, the lymphocyte antigen is CD3ε.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

In some embodiments the anti-hK2/anti-CD3 protein is bispecific.

In some embodiments, the first antigen binding domain that binds hK2 and/or the second antigen binding domain that binds the lymphocyte antigen comprise a scFv, a $(scFv)_2$, a Fv, a Fab, a $F(ab')_2$, a Fd, a dAb or a VHH.

In some embodiments, the first antigen binding domain that binds hK2 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fab.

In particular embodiments, the first antigen binding domain that binds hK2 comprises the Fab.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises the Fab.

In some embodiments, the first antigen binding domain that binds hK2 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the $F(ab')_2$.

In some embodiments, the first antigen binding domain that binds hK2 comprises the $F(ab')_2$.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprise the $F(ab')_2$.

In some embodiments, the first antigen binding domain that binds hK2 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the VHH.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VHH.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises the VHH.

In some embodiments, the first antigen binding domain that binds hK2 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fv.

In some embodiments, the first antigen binding domain that binds hK2 comprises the Fv.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises the Fv.

In some embodiments, the first antigen binding domain that binds hK2 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the Fd.

In some embodiments, the first antigen binding domain that binds hK2 comprises the Fd.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises the Fd.

In some embodiments, the first antigen binding domain that binds hK2 and/or the second antigen binding domain that binds the lymphocyte antigen comprise the scFv.

In a particular embodiment, the multispecific protein is bispecific, wherein the first antigen binding domain that binds hK2 comprises a Fab and the second antigen binding domain that binds the lymphocyte antigen (*e.g.* CD3) comprises a scFv.

In some embodiments, the first antigen binding domain that binds comprises the scFv.

In some embodiments, the second antigen binding domain that binds the lymphocyte antigen comprises the scFv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a scFv and the second antigen binding domain that binds the lymphocyte antigen comprises a Fab.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises a Fab and the second antigen binding domain that binds the lymphocyte antigen comprises a scFv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a scFv and the second antigen binding domain that binds the lymphocyte antigen comprises a Fab'.

In some embodiments, the first antigen binding domain that binds hK2 comprises a Fab' and the second antigen binding domain that binds the lymphocyte antigen comprises a scFv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a scFv and the second antigen binding domain that binds the lymphocyte antigen comprises a Fv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a dAb and the second antigen binding domain that binds the lymphocyte antigen comprises a scFv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a scFv and the second antigen binding domain that binds the lymphocyte antigen comprises a dAb.

In some embodiments, the first antigen binding domain that binds hK2 comprises a Fd and the second antigen binding domain that binds the lymphocyte antigen comprises a scFv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a scFv and the second antigen binding domain that binds the lymphocyte antigen comprises a VHH.

In some embodiments, the first antigen binding domain that binds hK2 comprises a VHH and the second antigen binding domain that binds the lymphocyte antigen comprises a scFv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a Fv and the second antigen binding domain that binds the lymphocyte antigen comprises a scFv.

In some embodiments, the first antigen binding domain that binds hK2 comprises a scFv and the second antigen binding domain that binds the lymphocyte antigen comprises a Fd.

In some embodiments, the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).

In some embodiments, the L1 comprises about 5-50 amino acids.

In some embodiments, the L1 comprises about 5-40 amino acids.

In some embodiments, the L1 comprises about 10-30 amino acids.

In some embodiments, the L1 comprises about 10-20 amino acids.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NOs: 7, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, or 108.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 7.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 76.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 77.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 78.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 79.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 80.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 81.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 82.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 83.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 84.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 85.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 86.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 87.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 88.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 89.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 90.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 91.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 92.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 93.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 94.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 95.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 96.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 97.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 98.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 99.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 100.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 101.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 102.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 103.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 104.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 105.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 106.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 107.

In some embodiments, the L1 comprises the amino acid sequence of SEQ ID NO: 108.

In a particular embodiment, the L1 comprises the amino acid sequence of SEQ ID NO: 7.

In some embodiments, the first antigen binding domain that binds hK2 comprises the HCDR1 of SEQ ID NOs: 63, 72, 141, 147, 170, 176, 188, 194, 196, 198, 200, 206, or 216, the HCDR2 of SEQ ID NOs: 64, 65, 73, 142, 148, 171, 177, 188, 189, 195, 197, 199, 201, 207, or 217, the HCDR3 of SEQ ID NOs: 66, 143, 172, 178, 184, 190, 208, or 218, the LCDR1 of SEQ ID NOs: 67, 68, 144, 173, 179, 182, 185 or 191, the LCDR2 of SEQ ID NOs: 69, 70, 145, 174, 180, 186, 192, or 470 and the LCDR3 of SEQ ID NOs: 71, 146, 175, 181, 187, 193, or 209.

In some embodiments, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of

SEQ ID NOs: 63, 65, 66, 68, 70 and 71, respectively;

SEQ ID NOs: 63, 64, 66, 67, 69 and 71, respectively;

SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively;

SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively;

SEQ ID Nos: 170, 171, 172, 173, 174 and 175, respectively;

SEQ ID NO: 176, 177, 178, 179, 180 and 181, respectively;

SEQ ID NO: 170, 183, 184, 185, 186 and 187, respectively;

SEQ ID NO: 188, 189, 190, 191, 192 and 193, respectively;

SEQ ID NO: 206, 207, 208, 182, 470 and 209, respectively;

SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively;

SEQ ID NOs: 72, 73, 66, 68, 70 and 71, respectively;

SEQ ID NOs: 72, 73, 66, 67, 69 and 71, respectively;

SEQ ID NO: 194, 195, 172, 173, 174 and 175, respectively;

SEQ ID NO: 196, 197, 178, 179, 190 and 181 respectively;

SEQ ID NO: 198, 199, 184, 185, 186 and 187, respectively;

SEQ ID NO: 200, 201, 190, 191, 192 and 193 respectively; or

SEQ ID NO: 216, 217, 218, 182, 470, and 209 respectively.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively or of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second antigen binding domain that binds a lymphocyte antigen, optionally which is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C, such as CD3.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively, and the second antigen binding domain that binds a lymphocyte antigen, optionally which is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C, such as CD3.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 75 and the VL of SEQ ID NO: 74.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 75 and the VL of SEQ ID NO: 74.

In some embodiments, the first antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID

NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the first antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In some embodiments, the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In some embodiments, the first antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163, and the second antigen binding domain that binds a lymphocyte antigen, optionally which is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C, such as CD3.

In some embodiments, the first antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second antigen binding domain that binds a lymphocyte antigen, optionally which is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C, such as CD3.

In some embodiments, the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163, and the second antigen binding domain that binds a lymphocyte antigen, optionally which is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C, such as CD3.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second antigen binding domain that binds a lymphocyte antigen, optionally which is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C, such as CD3.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NOs: 4, 5, 6, 139, 159 or 161 and the VL of SEQ ID NOs: 1, 2, 3, 140 or 160, with the proviso that the Fab does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.

In some embodiments, the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NOs: 4, 5 or 6 and the VL of SEQ ID NOs: 1, 2 or 3, with the proviso that the first antigen binding domain that binds hK2 does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.

In some embodiments, the first antigen binding domain that binds hK2 comprises:

the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;

the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140; or
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NOs: : 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 133, 134, 135, 136, 308, 316, 318, 319, 320, 321, 322, 323, 324, 325, 404, 405, 406, 407, 408, or 409.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 8.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 9.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 10.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 13.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 14.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 15.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 16.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 17.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 19.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 20.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 21.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 22.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 23.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 133.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 134.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 135.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 136.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 308.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 316.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 318.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 319.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 320.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 321.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 322.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 323.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 324.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 325.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 404.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 405.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 406.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 407.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 408.

In some embodiments, the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 409.

In some embodiments, the first antigen binding domain that binds hK2 comprises an amino acid sequence at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 404,

In some embodiments, the first antigen binding domain that binds hK2 comprises an amino acid sequence at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 405.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises an amino acid sequence of SEQ ID NO: 404 or 405.The disclosure also provides a second binding domain that binds lymphocyte antigen, wherein the antigen binding domain that binds lymphocyte comprises the heavy chain variable region (VH) of SEQ ID NO: 248 and the light chain variable region (VL) of SEQ ID NO: 157. SEQ ID NO: 248 and SEQ ID NO: 157 represent genus VH and VL amino acid sequences of the parent antibody CD3B815 antibody.

VL consensus (SQ ID NO: 157)

$DIQX_1TQSPX_2X_3LSX_4SX_5GX_6RVX_7X_8X_9CRARQSIGTAIHWYQQKX_{10}X_{11}X_{12}X_{13}PX_{14}LLIX_{15}YASESI$
$SGX_{16}PSRFSGSGSGTDFTLTIX_{17}SX_{18}QX_{19}EDX_{20}AX_{21}YYCQQSX_{22}SWPYTFGX_{23}GTKLEIK$

In some embodiments, the second antigen binding domain that binds a lymphocyte antigen comprises:

the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and a LCDR3 of SEQ ID NO: 260; or

the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

In some embodiments, the second antigen binding domain that binds a lymphocyte antigen comprises:

the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249; or

the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 250; or

the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251; or

the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 252; or

the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 253; or

the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 254.

In some embodiments, the second binding domain that binds a lymphocyte antigen comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 248 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 251.

In some embodiments, the second binding domain that binds a lymphocyte antigen comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 251.

In some embodiments, the second binding domain that binds a lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 251.

In a particular embodiment, the second binding domain that binds a lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 251.

In some embodiments, the second antigen binding domain that binds a lymphocyte antigen comprises

the HCDR1 of SEQ ID NO: 116, the HCDR2 of SEQ ID NO: 117, the HCDR3 of SEQ ID NO: 118, the LCDR1 of SEQ ID NO: 119, the LCDR2 of SEQ ID NO: 120 and the LCDR3 of SEQ ID NO: 121; or

the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123.

In a particular embodiment, the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively and the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively and the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively and the second antigen binding domain that binds a lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 251.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively and the second antigen binding domain that binds a lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 251.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163 and the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

In a particular embodiment, the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163 and the second antigen binding domain that binds a lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 251.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein

the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 275, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second

domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186 and 187, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259, and 261, respectively; or
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 275, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186 and 187, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259, and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, , wherein

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:139 and the VL of SEQ ID NO:140, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:139 and the VL of SEQ ID NO:140, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123;

wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:137 and the VL of SEQ ID NO:138, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:137 and the VL of SEQ ID NO:138, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:168 and the VL of SEQ ID NO:169, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:162 and the VL of SEQ ID NO:163, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:166 and the VL of SEQ ID NO:444, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:164 and the VL of SEQ ID NO:165, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:168 and the VL of SEQ ID NO:169, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:204 and the VL of SEQ ID NO:205, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123;

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:204 and the VL of SEQ ID NO:205, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251; or

the first binding domain that binds hK2 comprises the VH of SEQ ID NO:162 and the VL of SEQ ID NO:163, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:139 and the VL of SEQ ID NO:140, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:139 and the VL of SEQ ID NO:140, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:137 and the VL of SEQ ID NO:138, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:137 and the VL of SEQ ID NO:138, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:168 and the VL of SEQ ID NO:169, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:162 and the VL of SEQ ID NO:163, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:166 and the VL of SEQ ID NO:444, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:164 and the VL of SEQ ID NO:165, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:168 and the VL of SEQ ID NO:169, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:204 and the VL of SEQ ID NO:205, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO:123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:204 and the VL of SEQ ID NO:205, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a lymphocyte antigen, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO:162 and the VL of SEQ ID NO:163, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO:251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.In some embodiments, the first antigen binding domain that binds hK2 is conjugated to a first immunoglobulin (Ig) constant region or a fragment of the first Ig constant region and/or the second antigen binding domain that binds the lymphocyte antigen is conjugated to a second immunoglobulin (Ig) constant region or a fragment of the second Ig constant region.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises a Fc region.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises a CH2 domain.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises a CH3 domain.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises the CH2 domain and the CH3 domain.

In some embodiments, the fragment of the first Ig constant region and/or the fragment of the second Ig constant region comprises at least portion of a hinge, the CH2 domain and the CH3 domain.

In some embodiments, the fragment of the Ig constant region comprises the hinge, the CH2 domain and the CH3 domain.

In some embodiments, the multispecific protein further comprises a second linker (L2) between the first antigen binding domain that binds hK2 and the first Ig constant region or the fragment of the first Ig constant region and the second antigen binding domain that binds the lymphocyte antigen and the second Ig constant region or the fragment of the second Ig constant region.

In some embodiments, the L2 comprises the amino acid sequence of SEQ ID NOs: 7, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, or 108.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1, an IgG2, and IgG3 or an IgG4 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG2 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG3 isotype.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG4 isotype.

In a particular embodiment, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region is an IgG1 isotype.

The first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region can further be engineered as described herein.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in reduced binding of the multispecific protein to a FcγR.

In some embodiments, the at least one mutation that results in reduced binding of the multispecific protein to the FcγR is selected from the group consisting of F234A/L235A, L234A/L235A, L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, wherein residue numbering is according to the EU index. In a particular embodiment, the first Ig constant region or the fragment of the first Ig constant region and/or the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations: L234A_L235A_D265S.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that results in enhanced binding of the multispecific protein to a Fcγ receptor (FcγR).

In some embodiments, the at least one mutation that results in enhanced binding of the multispecific protein to the FcγR is selected from the group consisting of S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E, wherein residue numbering is according to the EU index.

In some embodiments, the FcγR is FcγRI, FcγRIIA, FcγRIIB or FcγRIII, or any combination thereof.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprises at least one mutation that modulates a half-life of the multispecific protein.

In some embodiments, the at least one mutation that modulates the half-life of the multispecific protein is selected from the group consisting of H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R, wherein residue numbering is according to the EU index.

In some embodiments, the multispecific protein comprises at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region.

In some embodiments, the at least one mutation in a CH3 domain of the first Ig constant region or in a CH3 domain of the fragment of the first Ig constant region and/or at least one mutation in a CH3 domain of the second Ig constant region or in a CH3 domain of the fragment of the second Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, F405W, T394W, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, L351Y/Y407A, T366A/K409F, L351Y/Y407A, T366V/K409F, T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index. In a particular embodiment, the first Ig constant region or the fragment of the first Ig constant region comprises the following mutations T350V_T366L_K392L_T394W and/or the second Ig constant region or the fragment of the second Ig constant region comprises the following mutations T350V_L351Y_F405A_Y407V.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations

L234A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and
L235A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or
L235A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and
L235A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

In some embodiments, the first Ig constant region or the fragment of the first Ig constant region and the second Ig constant region or the fragment of the second Ig constant region comprise the following mutations

L234A_L235A_D265S_T350V_L351Y_F405A_Y407V in the first Ig constant region and
L234A_L235A_D265S_T350V_T366L_K392L_T394W in the second Ig constant region; or

L234A_L235A_D265S_T350V_T366L_K392L_T394W in the first Ig constant region and L234A_L235A_D265S_T350V_L351Y_F405A_Y407V in the second Ig constant region.

In some embodiments, the first Ig heavy chain constant region or the fragment of the first Ig heavy chain constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 378.

In a particular embodiment, the first Ig heavy chain constant region or the fragment of the first Ig heavy chain constant region comprises an amino acid sequence which is identical to SEQ ID NO: 378.

In some embodiments, the first Ig light chain constant region or the fragment of the first Ig light chain constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 309.

In a particular embodiment, the first Ig light chain constant region or the fragment of the first Ig light chain constant region comprises an amino acid sequence which is identical to SEQ ID NO: 309.

In some embodiments, the second Ig constant region or the fragment of the second Ig constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to SEQ ID NO: 109.

In a particular embodiment, the second Ig constant region or the fragment of the second Ig constant region comprises an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the first Ig heavy chain constant region comprises an amino acid sequence which is identical to SEQ ID NO: 378, the first Ig light chain constant region comprises an amino acid sequence which is identical to SEQ ID NO: 309 and the second Ig constant region comprises an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3) and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2 and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309, and wherein the isolated protein further comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3) and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii)

a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309, and wherein the isolated protein further comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309.

In a particular embodiment, the isolated protein disclosed herein comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the amino acid sequence of SEQ ID NO: 331, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

In a particular embodiment, the isolated protein disclosed herein comprises a first antigen binding domain that binds hK2, wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and (i) a first Ig heavy chain constant region or fragment of a first Ig heavy chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 378; and (ii) a first Ig light chain constant region or fragment of a first Ig light chain constant region comprising an amino acid sequence which is identical to SEQ ID NO: 309, and wherein the isolated protein further comprises a second antigen binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the second antigen binding domain that binds a lymphocyte antigen comprises the amino acid sequence of SEQ ID NO: 331, and a second Ig constant region or a fragment of a second Ig constant region comprising an amino acid sequence which is identical to SEQ ID NO: 109.

Generation of multispecific proteins that comprise antigen binding fragments that bind hK2

The antigen binding fragments that bind hK2 of the disclosure may be engineered into multispecific antibodies which are also encompassed within the scope of the invention.

The antigen binding fragments that bind hK2 may be engineered into full length multispecific antibodies which are generated using Fab arm exchange, in which substitutions are introduced into two monospecific bivalent antibodies within the Ig constant region CH3 domain which promote Fab arm exchange *in vitro*. In the methods, two monospecific bivalent antibodies are engineered to have certain substitutions at the CH3 domain that promote heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond

isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2- mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably a reducing agent selected from the group consisting of: 2- mercaptoethylamine, dithiothreitol and tris(2-carboxyethyl)phosphine. For example, incubation for at least 90 min at a temperature of at least 20°C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

CH3 mutations that may be used include technologies such as Knob-in-Hole mutations (Genentech), electrostatically-matched mutations (Chugai, Amgen, NovoNordisk, Oncomed), the Strand Exchange Engineered Domain body (SEEDbody) (EMD Serono), Duobody® mutations (Genmab), and other asymmetric mutations (e.g. Zymeworks).

Knob-in-hole mutations are disclosed for example in WO1996/027011 and include mutations on the interface of CH3 region in which an amino acid with a small side chain (hole) is introduced into the first CH3 region and an amino acid with a large side chain (knob) is introduced into the second CH3 region, resulting in preferential interaction between the first CH3 region and the second CH3 region. Exemplary CH3 region mutations forming a knob and a hole are T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S_L368A_Y407V.

Heavy chain heterodimer formation may be promoted by using electrostatic interactions by substituting positively charged residues on the first CH3 region and negatively charged residues on the second CH3 region as described in US2010/0015133, US2009/0182127, US2010/028637 or US2011/0123532.

Other asymmetric mutations that can be used to promote heavy chain heterodimerization are L351Y_F405A_Y407V/T394W, T366I_K392M_T394W/F405A_Y407V, T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, Y407A/T366A_K409F, or T350V_L351Y_F405A_Y407V/T350V_T366L_K392L_T394W as described in US2012/0149876 or US2013/0195849 (Zymeworks).

SEEDbody mutations involve substituting select IgG residues with IgA residues to promote heavy chai heterodimerization as described in US20070287170.

Other exemplary mutations that may be used are R409D_K370E/D399K_E357K, S354C_T366W/Y349C_T366S_L368A_Y407V, Y349C_T366W/S354C_T366S_L368A_Y407V, T366K/L351D, L351K/Y349E, L351K/Y349D, L351K/L368E, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, K392D/D399K, K392D/ E356K, K253E_D282K_K322D/D239K_E240K_K292D, K392D_K409D/D356K_D399K as described in WO2007/147901, WO 2011/143545, WO2013157954, WO2013096291 and US2018/0118849.

Duobody® mutations (Genmab) are disclosed for example in US9150663 and US2014/0303356 and include mutations F405L/K409R, wild-type/F405L_R409K, T350I_K370T_F405L/K409R, K370W/K409R, D399AFGHILMNRSTVWY/K409R, T366ADEFGHILMQVY/K409R, L368ADEGHNRSTVQ/K409AGRH, D399FHKRQ/K409AGRH, F405IKLSTVW/K409AGRH and Y407LWQ/K409AGRH.

Additional bispecific or multispecific structures into which the antigen binding domains that bind hK2 can be incorporated include Dual Variable Domain Immunoglobulins (DVD) (Int. Pat. Publ. No. WO2009/134776; DVDs are full length antibodies comprising the heavy chain having a structure VH1-linker-VH2-CH and the light chain having the structure VL1-linker-VL2-CL; linker being optional), structures that include various dimerization domains to connect the two antibody arms with different specificity, such as leucine zipper or collagen dimerization domains (Int. Pat. Publ. No. WO2012/022811, U.S. Pat. No. 5,932,448; U.S. Pat. No. 6,833,441), two or more domain antibodies (dAbs) conjugated together, diabodies, heavy chain only antibodies such as camelid antibodies and engineered camelid antibodies, Dual Targeting (DT)-Ig (GSK/Domantis), Two-in-one Antibody (Genentech), Cross-linked Mabs (Karmanos Cancer Center), mAb2 (F-Star) and CovX-body (CovX/Pfizer), IgG-like Bispecific (InnClone/Eli Lilly), Ts2Ab (MedImmune/AZ) and BsAb (Zymogenetics), HERCULES (Biogen Idec) and TvAb (Roche), ScFv/Fc Fusions (Academic Institution), SCORPION (Emergent BioSolutions/Trubion, Zymogenetics/BMS), Dual Affinity Retargeting Technology (Fc-DART) (MacroGenics) and Dual(ScFv)$_2$-Fab (National Research Center for Antibody Medicine--China), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock (DNL) (ImmunoMedics), Bivalent Bispecific (Biotecnol) and Fab-Fv (UCB-Celltech). ScFv-, diabody-based, and domain antibodies, include but are not limited to, Bispecific T Cell Engager (BiTE) (Micromet), Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DART) (MacroGenics), Single-chain Diabody (Academic), TCR-like Antibodies (AIT, ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack) and COMBODY (Epigen Biotech), dual targeting nanobodies (Ablynx), dual targeting heavy chain only domain antibodies.

The antigen binding domains that bind hK2 of the disclosure may also be engineered into multispecific proteins which comprise three polypeptide chains. In such designs, at least one antigen

binding domain is in the form of a scFv. Exemplary designs include (in which "1" indicates the first antigen binding domain, "2" indicates the second antigen binding domain and "3" indicates the third antigen binding domain:

Design 1: Chain A) scFv1- CH2-CH3; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

Design 2: Chain A) scFv1- hinge- CH2-CH3; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

Design 3: Chain A) scFv1- CH1-hinge- CH2-CH3; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

Design 4: Chain A) CH2-CH3-scFv1; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3

CH3 engineering may be incorporated to the Designs 1-4, such as mutations L351Y_F405A_Y407V/T394W, T366I_K392M_T394W/F405A_Y407V, T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, Y407A/T366A_K409F, or T350V_L351Y_F405A_Y407V/T350V_T366L_K392L_T394W as described in US2012/0149876 or US2013/0195849 (Zymeworks).

In a particular embodiment, the design is: Chain A) scFv-hinge-CH2-CH3; Chain B) VL2-CL; Chain C) VH2-CH1-hinge-CH2-CH3.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (such as CD3), wherein the first antigen binding domain that binds hK2 comprises a HCDR1 of SEQ ID NOs: 63, 72, 141, 147, 170, 176, 188, 194, 196, 198, 200, 206, or 216, a HCDR2 of SEQ ID NOs: 64, 65, 73, 142, 148, 171, 177, 188, 189, 195, 197, 199, 201, 207, or 217, a HCDR3 of SEQ ID NOs: 66, 143, 172, 178, 184, 190, 208, or 218, a LCDR1 of SEQ ID NOs: 67, 68, 144, 173, 179, 182, 185 or 191 a LCDR2 of SEQ ID NOs: 69, 70, 145, 174, 180, 186, 192 or 470, and a LCDR3 of SEQ ID NOs: 71, 146, 175, 181, 187, 193, or 209.

In some embodiment the lymphocyte antigen is CD3.In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (such as CD3), wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively;
SEQ ID Nos: 170, 171, 172, 173, 174 and 175, respectively;
SEQ ID NO: 176, 177, 178, 179, 180 and 181, respectively;
SEQ ID NO: 170, 183, 184, 185, 186 and 187, respectively;
SEQ ID NO: 188, 189, 190, 191, 192 and 193, respectively;
SEQ ID NO: 206, 207, 208, 182, 470 and 209, respectively;

SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively;
SEQ ID NO: 194, 195, 172, 173, 174 and 175, respectively;
SEQ ID NO: 196, 197, 178, 179, 190 and 181 respectively;
SEQ ID NO: 198, 199, 184, 185, 186 and 187, respectively;
SEQ ID NO: 200, 201, 190, 191, 192 and 193 respectively; or
SEQ ID NO: 216, 217, 218, 182, 470, and 209 respectively.

In some embodiment the lymphocyte antigen is CD3.In a particular embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively or of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (such as CD3), wherein the first antigen binding domain that binds hK2 comprises
the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169; or
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.

In some embodiment the lymphocyte antigen is CD3.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (such as CD3), wherein the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NO: 75 and the VL of SEQ ID NO: 74.

In some embodiment the lymphocyte antigen is CD3.In some embodiments, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In a particular embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first

antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In a particular embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In a particular embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 163.

In a particular embodiment the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (such as CD3), wherein the first antigen binding domain that binds hK2 comprises the VH of SEQ ID NOs: 4, 5, 6, 137, 139, 159, or 161 and the VL of SEQ ID NOs: 1, 2, 3, 140, or 160 with the proviso that the antigen binding domain that binds hK2 does not comprise the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 2.
In some embodiment the lymphocyte antigen is CD3.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (such as CD3), wherein wherein the first antigen binding domain that binds hK2 comprises:
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140;

the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140; or
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

In some embodiment the lymphocyte antigen is CD3.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (such as CD3), wherein the first antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 133, 134, 135, 136, 308, 316, 318, 319, 320, 321, 322, 323, 324, 325, 404, 405, 406, 407, 408, or 409.

In some embodiment the lymphocyte antigen is CD3.

In some embodiments, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first antigen binding domain that binds hK2 comprises an amino acid sequence at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 404 or 405.

In a particular embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen (*e.g.* CD3), wherein the first antigen binding domain that binds hK2 comprises an amino acid sequence of SEQ ID NO: 404 or 405.

In some embodiments, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises a HCDR1 of SEQ ID NOs: 255 or 116, a HCDR2 of SEQ ID NOs: 256, or 117, a HCDR3 of SEQ ID NOs: 257, or 118, a LCDR1 of SEQ ID NOs: 258 or 119 a LCDR2 of SEQ ID NOs: 259, or 120, and a LCDR3 of SEQ ID NOs: 260, 261, or 121.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 157.

In some embodiments, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises:
the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and a LCDR3 of SEQ ID NO: 260; or
the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

In some embodiments, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises:
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249;
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 250;
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251;
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 252;
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 253; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 254.

In some embodiments, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VH SEQ ID NO: 248 and a VL which is

at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the VL of SEQ ID NO: 251.

In a particular embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 251.

In some embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises
the HCDR1 of SEQ ID NO: 116, the HCDR2 of SEQ ID NO: 117, the HCDR3 of SEQ ID NO: 118, the LCDR1 of SEQ ID NO: 119, the LCDR2 of SEQ ID NO: 120 and the LCDR3 of SEQ ID NO: 121; or the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123.

In a particular embodiment, the isolated multispecific protein comprises a first binding domain that binds hK2 and a second binding domain that binds a lymphocyte antigen, wherein the second antigen binding domain that binds the lymphocyte antigen comprises the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

In some embodiments, the second binding domain of the isolated multispecific protein, that binds the lymphocyte antigen, comprises the amino acid sequence of SEQ ID NOs: 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336 or 337.

In a particular embodiment, the second binding domain of the isolated multispecific protein, that binds the lymphocyte antigen, comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 331.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 275, 258, 259 and 260, respectively;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251; and/or

the isolated multispecific protein comprises a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 260, respectively;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that

binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 259 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 260, respectively;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 260, respectively;
the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186 and 187, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 260, respectively;
the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 444, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 355 a LC1 of SEQ ID NO: 445 and a HC2 of SEQ ID NO: 360.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 260, respectively;
the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 356, a LC1 of SEQ ID NO: 222 and a HC2 of SEQ ID NO: 360.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259, and 260, respectively;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 151; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein

the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 260, respectively;

the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331; and/or

the isolated multispecific protein comprises a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein

the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 275, 258, 259 and 261, respectively;

wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;

wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;

wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;

wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second

domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186 and 187, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively;
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259, and 261, respectively; or
wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 275, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186 and 187, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively, and the second domain that binds the lymphocyte antigen

comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds a CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259, and 261, respectively.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170,

171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251;

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123;

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251;

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123;

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251;

the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331;

the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 444, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331;

the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331;

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123;

the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123;
the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 151; or
the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2

comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 444, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2

comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 151.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated protein comprises:

a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267;
a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272;
a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267;
a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272;
a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267;
a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360;
a HC1 of SEQ ID NO: 355 a LC1 of SEQ ID NO: 223 and a HC2 of SEQ ID NO: 360;

a HC1 of SEQ ID NO: 356, a LC1 of SEQ ID NO: 222 and a HC2 of SEQ ID NO: 360;
a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272;
a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272;
a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267; or
a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated protein comprises a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein, the isolated multispecific protein comprises a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 355 a LC1 of SEQ ID NO: 223 and a HC2 of SEQ ID NO: 360.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 356, a LC1 of SEQ ID NO: 222 and a HC2 of SEQ ID NO: 360.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

The disclosure also provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

In some embodiments the isolated multispecific protein is an anti-hK2/anti-CD3 protein.

In a particular embodiment, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds CD3 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv of SEQ ID NO: 331; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360.

In some embodiments, the isolated multispecific protein comprises a lysine (e.g. K477) at the C-terminus of both of the Fc domains (*i.e.* the HC1 and HC2 domains). An additional lysine may enhance expression of the construct. Accordingly, in a particular embodiment, the isolated multispecific protein comprises a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds CD3 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv of SEQ ID NO: 331; and/or
the isolated multispecific protein comprises a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 80% identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 80% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 85% identical to the VH of SEQ ID NO: 162 and a VL which is at least 85% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 85% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 90% identical to the VH of SEQ ID NO: 162 and a VL which is at least 90% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 90% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 95% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 99% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 95% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of SEQ

ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 99% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 95% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv which is at least 99% identical to the scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 95% identical to the VH of SEQ ID NO: 162 and a VL which is at least 95% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH which is at least 99% identical to the VH of SEQ ID NO: 162 and a VL which is at least 99% identical to the VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv of SEQ ID NO: 331.

In a particular embodiment, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second binding domain that binds CD3 comprises a scFv of SEQ ID NO: 331.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 which is at least 80% identical to the HC1 of SEQ ID NO: 354, a LC1 which is at least 80% identical to the LC1 of SEQ ID NO: 221 and a HC2 which is at least 80% identical to the HC2 of SEQ ID NO: 360.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 which is at least 85% identical to the HC1 of SEQ ID NO: 354, a LC1 which is at least

85% identical to the LC1 of SEQ ID NO: 221 and a HC2 which is at least 85% identical to the HC2 of SEQ ID NO: 360.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 which is at least 90% identical to the HC1 of SEQ ID NO: 354, a LC1 which is at least 90% identical to the LC1 of SEQ ID NO: 221 and a HC2 which is at least 90% identical to the HC2 of SEQ ID NO: 360.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 which is at least 95% identical to the HC1 of SEQ ID NO: 354, a LC1 which is at least 95% identical to the LC1 of SEQ ID NO: 221 and a HC2 which is at least 95% identical to the HC2 of SEQ ID NO: 360.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the isolated multispecific protein comprises a HC1 which is at least 99% identical to the HC1 of SEQ ID NO: 354, a LC1 which is at least 99% identical to the LC1 of SEQ ID NO: 221 and a HC2 which is at least 99% identical to the HC2 of SEQ ID NO: 360.

In some embodiments, the isolated multispecific protein comprises a lysine (e.g. K477) at the C-terminus of both of the Fc domains (*i.e.* the HC1 and HC2 domains). Accordingly, in some embodiments, the isolated multispecific protein comprises a HC1 which comprises a C-terminal lysine and is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the HC1 of SEQ ID NO: 361 and a HC2 which comprises a C-terminal lysine and is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%) identical to the HC2 of SEQ ID NO: 362.

In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the antigen binding domain binding to hK2 binds to hK2 at three or more residues within epitopes having sequences of SEQ ID NO: 111 and SEQ ID NO: 112. In some embodiments, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the antigen binding domain binding to CD3 binds to CD3 at three or more residues within epitopes having sequences of SEQ ID NO: 341, SEQ ID NO: 448 and SEQ ID NO: 449.

In a particular embodiment, the disclosure provides an isolated multispecific protein, comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the antigen binding domain

binding to hK2 binds to hK2 within epitopes having sequences of SEQ ID NO: 111 and SEQ ID NO: 112 and wherein the antigen binding domain binding to CD3 binds to CD3 within epitopes having sequences of SEQ ID NO: 341, SEQ ID NO: 448 and SEQ ID NO: 449.

Isotypes, allotypes and Fc engineering

The Ig constant region or the fragment of the Ig constant region, such as the Fc region present in the proteins of the disclosure may be of any allotype or isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG1 isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG2 isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG3 isotype.

In some embodiments, the Ig constant region or the fragment of the Ig constant region is an IgG4 isotype.

The Ig constant region or the fragment of the Ig constant region may be of any allotype. It is expected that allotype has no influence on properties of the Ig constant region, such as binding or Fc-mediated effector functions. Immunogenicity of therapeutic proteins comprising Ig constant regions of fragments thereof is associated with increased risk of infusion reactions and decreased duration of therapeutic response (Baert *et al.*, (2003) *N Engl J Med* 348:602-08). The extent to which therapeutic proteins comprising Ig constant regions of fragments thereof induce an immune response in the host may be determined in part by the allotype of the Ig constant region (Stickler *et al.*, (2011) *Genes and Immunity* 12:213-21). Ig constant region allotype is related to amino acid sequence variations at specific locations in the constant region sequences of the antibody. **Table 2** shows select IgG1, IgG2 and IgG4 allotypes.

Table 2.

Allotype	Amino acid residue at position of diversity (residue numbering: EU Index)

	IgG2		IgG4		IgG1			
	189	282	309	422	214	356	358	431
G2m(n)	T	M						
G2m(n-)	P	V						
G2m(n)/(n-)	T	V						
nG4m(a)			L	R				
G1m(17)					K	E	M	A
G1m(17,1)					K	D	L	A
G1m(3)					R	E	M	A

In a particular embodiment, the Ig constant region allotype is huIgG1_G1m(17).

C-terminal lysine (CTL) may be removed from the Ig constant region by endogenous circulating carboxypeptidases in the blood stream (Cai *et al.*, (2011) *Biotechnol Bioeng* 108:404-412). During manufacturing, CTL removal may be controlled to less than the maximum level by control of concentration of extracellular Zn^{2+}, EDTA or EDTA – Fe^{3+} as described in U.S. Patent Publ. No. US20140273092. CTL content of proteins may be measured using known methods.

In some embodiments, the antigen binding fragment that binds hK2 conjugated to the Ig constant region has a C-terminal lysine content from about 10% to about 90%. In some embodiments, the C-terminal lysine content is from about 20% to about 80%. In some embodiments, the C-terminal lysine content is from about 40% to about 70%. In some embodiments, the C-terminal lysine content is from about 55% to about 70%. In some embodiments, the C-terminal lysine content is about 60%.

Fc region mutations may be made to the antigen binding domains that bind hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region to modulate their effector functions such as ADCC, ADCP and/or ADCP and/or pharmacokinetic properties. This may be achieved by introducing mutation(s) into the Fc that modulate binding of the mutated Fc to activating FcγRs (FcγRI, FcγRIIa, FcγRIII), inhibitory FcγRIIb and/or to FcRn.

In some embodiments, the antigen binding domain that binds hK2s conjugated to the Ig constant region or the fragment of the Ig constant region comprises at least one mutation in the Ig constant region or in the fragment of the Ig constant region.

In some embodiments, the at least one mutation is in the Fc region.

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen mutations in the Fc region.

In some embodiments, the antigen binding domain that binds hK2s conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation in the Fc region that modulates binding of the antibody to FcRn.

Fc positions that may be mutated to modulate half-life (e.g. binding to FcRn) include positions 250, 252, 253, 254, 256, 257, 307, 376, 380, 428, 434 and 435. Exemplary mutations that may be made singularly or in combination are mutations T250Q, M252Y, I253A, S254T, T256E, P257I, T307A, D376V, E380A, M428L, H433K, N434S, N434A, N434H, N434F, H435A and H435R. Exemplary singular or combination mutations that may be made to increase the half-life are mutations M428L/N434S, M252Y/S254T/T256E, T250Q/M428L, N434A and T307A/E380A/N434A. Exemplary singular or combination mutations that may be made to reduce the half-life are mutations H435A, P257I/N434H, D376V/N434H, M252Y/S254T/T256E/H433K/N434F, T308P/N434A and H435R.

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises M252Y/S254T/T256E mutation.

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation in the Fc region that reduces binding of the protein to an activating Fcγ receptor (FcγR) and/or reduces Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis (ADCP).

Fc positions that may be mutated to reduce binding of the protein to the activating FcγR and subsequently to reduce effector function include positions 214, 233, 234, 235, 236, 237, 238, 265, 267, 268, 270, 295, 297, 309, 327, 328, 329, 330, 331 and 365. Exemplary mutations that may be made singularly or in combination are mutations K214T, E233P, L234V, L234A, deletion of G236, V234A,

F234A, L235A, G237A, P238A, P238S, D265A, S267E, H268A, H268Q, Q268A, N297A, A327Q, P329A, D270A, Q295A, V309L, A327S, L328F, A330S and P331S in IgG1, IgG2, IgG3 or IgG4. Exemplary combination mutations that result in proteins with reduced ADCC are mutations L234A/L235A on IgG1, L234A/L235A/D265S on IgG1, V234A/G237A/ P238S/H268A/V309L/A330S/P331S on IgG2, F234A/L235A on IgG4, S228P/F234A/ L235A on IgG4, N297A on all Ig isotypes, V234A/G237A on IgG2, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M on IgG1, H268Q/V309L/A330S/P331S on IgG2, S267E/L328F on IgG1, L234F/L235E/D265A on IgG1, L234A/L235A/G237A/P238S/H268A/A330S/P331S on IgG1, S228P/F234A/L235A/G237A/P238S on IgG4, and S228P/F234A/L235A/G236-deleted/G237A/P238S on IgG4. Hybrid IgG2/4 Fc domains may also be used, such as Fc with residues 117-260 from IgG2 and residues 261-447 from IgG4.

Exemplary mutation that result in proteins with reduced CDC is a K322A mutation.

Well-known S228P mutation may be made in IgG4 to enhance IgG4 stability.

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation selected from the group consisting of K214T, E233P, L234V, L234A, deletion of G236, V234A, F234A, L235A, G237A, P238A, P238S, D265A, S267E, H268A, H268Q, Q268A, N297A, A327Q, P329A, D270A, Q295A, V309L, A327S, L328F, K322, A330S and P331S.

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises L234A/L235A/D265S mutation. In a particular embodiment, the antigen binding domain that binds hK2 is conjugated to an IgG1 constant region or to the fragment of an IgG1 constant region comprising L234A_L235A_D265S mutations.

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises L234A/L235A mutation.

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region comprises at least one mutation in the Fc region that enhances binding of the protein to an Fcγ receptor (FcγR) and/or enhances Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and/or phagocytosis (ADCP).

Fc positions that may be mutated to increase binding of the protein to the activating FcγR and/or enhance Fc effector functions include positions 236, 239, 243, 256,290,292, 298, 300, 305, 312, 326, 330, 332, 333, 334, 345, 360, 339, 378, 396 or 430 (residue numbering according to the EU index). Exemplary mutations that may be made singularly or in combination are G236A, S239D, F243L, T256A, K290A, R292P, S298A, Y300L, V305L, K326A, A330K, I332E, E333A, K334A, A339T and P396L. Exemplary combination mutations that result in proteins with increased ADCC or ADCP are a S239D/I332E, S298A/E333A/K334A, F243L/R292P/Y300L, F243L/R292P/Y300L/P396L, F243L/R292P/Y300L/V305I/P396L and G236A/S239D/I332E.

Fc positions that may be mutated to enhance CDC include positions 267, 268, 324, 326, 333, 345 and 430. Exemplary mutations that may be made singularly or in combination are S267E, F1268F, S324T, K326A, K326W, E333A, E345K, E345Q, E345R, E345Y, E430S, E430F and E430T. Exemplary combination mutations that result in proteins with increased CDC are K326A/E333A, K326W/E333A, H268F/S324T, S267E/H268F, S267E/S324T and S267E/H268F/S324T.

The specific mutations described herein are mutations when compared to the IgG1, IgG2 and IgG4 wild-type amino acid sequences of SEQ ID NOs: 130, 131 and 132, respectively.

SEQ ID NO: 130, wild-type IgG1

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS

GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG

PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE

LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW

QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 131; wild-type IgG2

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS

GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVF

LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFR
VVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 132; wild-type IgG4

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG
NVFSCSVMHEALHNHYTQKSLSLSLGK

Binding of the antibody to FcγR or FcRn may be assessed on cells engineered to express each receptor using flow cytometry. In an exemplary binding assay, $2x10^5$ cells per well are seeded in 96-well plate and blocked in BSA Stain Buffer (BD Biosciences, San Jose, USA) for 30 min at 4°C. Cells are incubated with a test antibody on ice for 1.5 hour at 4°C. After being washed twice with BSA stain buffer, the cells are incubated with R-PE labeled anti-human IgG secondary antibody (Jackson Immunoresearch Laboratories) for 45 min at 4°C. The cells are washed twice in stain buffer and then resuspended in 150 µL of Stain Buffer containing 1:200 diluted DRAQ7 live/dead stain (Cell Signaling Technology, Danvers, USA). PE and DRAQ7 signals of the stained cells are detected by Miltenyi MACSQuant flow cytometer (Miltenyi Biotec, Auburn, USA) using B2 and B4 channel respectively. Live cells are gated on DRAQ7 exclusion and the geometric mean fluorescence signals are determined for at least 10,000 live events collected. FlowJo software (Tree Star) is used for analysis. Data is plotted as the logarithm of antibody concentration versus mean fluorescence signals. Nonlinear regression analysis is performed.

Glycoengineering

The ability of the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region to mediate ADCC can be enhanced by engineering the Ig constant region or the fragment of the Ig constant region oligosaccharide component. Human IgG1 or IgG3 are N-glycosylated at Asn297 with the majority of the glycans in the well-known biantennary G0, G0F, G1, G1F, G2 or G2F forms. Ig constant region containing proteins may be produced by non-engineered CHO cells typically have a glycan fucose content of about at least 85%. The removal of the core fucose from the biantennary complex-type oligosaccharides attached to the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region enhances the ADCC of the protein via improved FcγRIIIa binding without altering antigen binding or CDC activity. Such proteins can be achieved using different methods reported to lead to the successful expression of relatively high defucosylated immunoglobulins bearing the biantennary complex-type of Fc oligosaccharides such as control of culture osmolality (Konno *et al.*, Cytotechnology 64(:249-65, 2012), application of a variant CHO line Lec13 as the host cell line (Shields *et al.*, *J Biol Chem* 277:26733-26740, 2002), application of a variant CHO line EB66 as the host cell line (Olivier *et al.*, *MAbs*;2(4): 405-415, 2010; PMID:20562582), application of a rat hybridoma cell line YB2/0 as the host cell line (Shinkawa *et al.*, *J Biol Chem* 278:3466-3473, 2003), introduction of small interfering RNA specifically against the a 1,6-fucosyltrasferase (*FUT8)* gene (Mori *et al.*, *Biotechnol Bioeng* 88:901-908, 2004), or coexpression of β-1,4-*N*-acetylglucosaminyltransferase III and Golgi α-mannosidase II or a potent alpha-mannosidase I inhibitor, kifunensine (Ferrara *et al.*, *J Biol Chem* 281:5032-5036, 2006, Ferrara *et al.*, *Biotechnol Bioeng* 93:851-861, 2006; Xhou *et al.*, *Biotechnol Bioeng* 99:652-65, 2008).

In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region of the disclosure has a biantennary glycan structure with fucose content of about between 1% to about 15%, for example about 15%, 14%, 13%, 12%, 11% 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%. In some embodiments, the antigen binding domain that binds hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region has a glycan structure with fucose content of about 50%, 40%, 45%, 40%, 35%, 30%, 25%, or 20%.

"Fucose content" means the amount of the fucose monosaccharide within the sugar chain at Asn297. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures. These may be characterized and quantified by multiple methods, for example: 1) using MALDI-TOF of N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures) as described in Int Pat. Publ. No. WO2008/077546 2); 2) by enzymatic release of the Asn297

glycans with subsequent derivatization and detection/ quantitation by HPLC (UPLC) with fluorescence detection and/or HPLC-MS (UPLC-MS); 3) intact protein analysis of the native or reduced mAb, with or without treatment of the Asn297 glycans with Endo S or other enzyme that cleaves between the first and the second GlcNAc monosaccharides, leaving the fucose attached to the first GlcNAc; 4) digestion of the mAb to constituent peptides by enzymatic digestion (e.g., trypsin or endopeptidase Lys-C), and subsequent separation, detection and quantitation by HPLC-MS (UPLC-MS); 5) Separation of the mAb oligosaccharides from the mAb protein by specific enzymatic deglycosylation with PNGase F at Asn 297. The oligosaccharides thus released can be labeled with a fluorophore, separated and identified by various complementary techniques which allow: fine characterization of the glycan structures by matrix-assisted laser desorption ionization (MALDI) mass spectrometry by comparison of the experimental masses with the theoretical masses, determination of the degree of sialylation by ion exchange HPLC (GlycoSep C), separation and quantification of the oligosaccharide forms according to hydrophilicity criteria by normal-phase HPLC (GlycoSep N), and separation and quantification of the oligosaccharides by high performance capillary electrophoresis-laser induced fluorescence (HPCE-LIF).

"Low fucose" or "low fucose content" as used herein refers to the antigen binding domain that bind hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region with fucose content of about between 1%-15%.

"Normal fucose" or 'normal fucose content" as used herein refers to the antigen binding domain that bind hK2 conjugated to the Ig constant region or to the fragment of the Ig constant region with fucose content of about over 50%, typically about over 80% or over 85%.

Anti-idiotypic antibodies

Anti-idiotypic antibodies are antibodies that specifically bind to the antigen binding domain that binds hK2 of the disclosure.

The invention also provides an anti-idiotypic antibody that specifically binds to the antigen binding domain that binds hK2 of the disclosure.

The invention also provides an anti-idiotypic antibody that specifically binds to the antigen binding domain that binds hK2 comprising
the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;

the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169;
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140; or
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

An anti-idiotypic (Id) antibody is an antibody which recognizes the antigenic determinants (*e.g.* the paratope or CDRs) of the antibody. The Id antibody may be antigen-blocking or non-blocking. The antigen-blocking Id may be used to detect the free antigen binding domain in a sample (*e.g.* the antigen binding domain that binds hK2 of the disclosure). The non-blocking Id may be used to detect the total antibody (free, partially bond to antigen, or fully bound to antigen) in a sample. An Id antibody may be prepared by immunizing an animal with the antibody to which an anti-Id is being prepared.

An anti-Id antibody may also be used as an immunogen to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. An anti-anti-Id may be epitopically identical to the original antigen binding domain which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of the antigen binding domain, it is possible to identify other clones expressing antigen binding domains of identical specificity. Anti-Id antibodies may be varied (thereby producing anti-Id antibody variants) and/or derivatized by any suitable technique, such as those described elsewhere herein.

Immunoconjugates

The antigen binding domains that bind hK2 of the disclosure, the proteins comprising the antigen binding domains that bind hK2 or the multispecific proteins that comprise the antigen binding domains that bind hK2 (collectively referred herein as to hK2 binding proteins) may be conjugated to a heterologous molecule. hK2 binding protein also includes chimeric antigen receptors (CAR) which comprises the antigen biding domains that bind hK2 of the disclosure.

In some embodiments, the heterologous molecule is a detectable label or a cytotoxic agent.

The invention also provides an antigen binding domain that binds hK2 conjugated to a detectable label.

The invention also provides a protein comprising an antigen binding domain that binds hK2 conjugated to a detectable label.

The invention also provides a multispecific protein comprising an antigen binding domain that binds hK2 conjugated to a detectable label.

The invention also provides a CAR comprising an antigen binding domain that binds hK2 conjugated to a detectable label.

The invention also provides an antigen binding domain that binds hK2 conjugated to a cytotoxic agent.

The invention also provides a protein comprising an antigen binding domain that binds hK2 conjugated to a cytotoxic agent.

The invention also provides a multispecific protein comprising an antigen binding domain that binds hK2 conjugated to a cytotoxic agent.

The invention also provides a CAR comprising an antigen binding domain that binds hK2 conjugated to a cytotoxic agent.

hK2 binding proteins of the disclosure may be used to direct therapeutics to hK2 expressing cells. such as prostate or breast cancer cells. Alternatively, hK2 expressing cells may be targeted with a hK2 binding protein of the disclosure coupled to a therapeutic intended to modify cell function once internalized.

In some embodiments, the detectable label is also a cytotoxic agent.

The hK2 binding proteins of the disclosure conjugated to a detectable label may be used to evaluate expression of hK2 on a variety of samples.

Detectable label includes compositions that when conjugated to the hK2 binding proteins of the disclosure renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

Exemplary detectable labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, haptens, luminescent molecules, chemiluminescent molecules, fluorochromes, fluorophores, fluorescent quenching agents, colored molecules, radioactive isotopes, scintillates, avidin, streptavidin, protein A, protein G, antibodies or fragments thereof, polyhistidine, Ni^{2+}, Flag tags, myc tags, heavy metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron donors/acceptors, acridinium esters, and colorimetric substrates.

A detectable label may emit a signal spontaneously, such as when the detectable label is a radioactive isotope. In other cases, the detectable label emits a signal as a result of being stimulated by an external field.

Exemplary radioactive isotopes may be γ-emitting, Auger-emitting, β-emitting, an alpha-emitting or positron-emitting radioactive isotope. Exemplary radioactive isotopes include ^{3}H, ^{11}C, ^{13}C, ^{15}N, ^{18}F, ^{19}F, ^{55}Co, ^{57}Co, ^{60}Co, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{68}Ga, ^{72}As, ^{75}Br, ^{86}Y, ^{89}Zr, ^{90}Sr, ^{94m}Tc, ^{99m}Tc, ^{115}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{211}At, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{226}Ra, ^{225}Ac and ^{227}Ac.

Exemplary metal atoms are metals with an atomic number greater than 20, such as calcium atoms, scandium atoms, titanium atoms, vanadium atoms, chromium atoms, manganese atoms, iron atoms, cobalt atoms, nickel atoms, copper atoms, zinc atoms, gallium atoms, germanium atoms, arsenic atoms, selenium atoms, bromine atoms, krypton atoms, rubidium atoms, strontium atoms, yttrium atoms, zirconium atoms, niobium atoms, molybdenum atoms, technetium atoms, ruthenium atoms, rhodium atoms, palladium atoms, silver atoms, cadmium atoms, indium atoms, tin atoms, antimony atoms, tellurium atoms, iodine atoms, xenon atoms, cesium atoms, barium atoms, lanthanum atoms, hafnium atoms, tantalum atoms, tungsten atoms, rhenium atoms, osmium atoms, iridium atoms, platinum atoms, gold atoms, mercury atoms, thallium atoms, lead atoms, bismuth atoms, francium atoms, radium atoms, actinium atoms, cerium atoms, praseodymium atoms, neodymium atoms, promethium atoms, samarium atoms, europium atoms, gadolinium atoms, terbium atoms, dysprosium atoms, holmium atoms, erbium atoms, thulium atoms, ytterbium atoms, lutetium atoms, thorium atoms, protactinium atoms, uranium atoms, neptunium atoms, plutonium atoms, americium atoms, curium atoms, berkelium atoms, californium atoms, einsteinium atoms, fermium atoms, mendelevium atoms, nobelium atoms, or lawrencium atoms.

In some embodiments, the metal atoms may be alkaline earth metals with an atomic number greater than twenty.

In some embodiments, the metal atoms may be lanthanides.

In some embodiments, the metal atoms may be actinides.

In some embodiments, the metal atoms may be transition metals.

In some embodiments, the metal atoms may be poor metals.

In some embodiments, the metal atoms may be gold atoms, bismuth atoms, tantalum atoms, and gadolinium atoms.

In some embodiments, the metal atoms may be metals with an atomic number of 53 (i.e. iodine) to 83 (i.e. bismuth).

In some embodiments, the metal atoms may be atoms suitable for magnetic resonance imaging.

The metal atoms may be metal ions in the form of +1, +2, or +3 oxidation states, such as Ba^{2+}, Bi^{3+}, Cs^{+}, Ca^{2+}, Cr^{2+}, Cr^{3+}, Cr^{6+}, Co^{2+}, Co^{3+}, Cu^{+}, Cu^{2+}, Cu^{3+}, Ga^{3+}, Gd^{3+}, Au^{+}, Au^{3+}, Fe^{2+}, Fe^{3+}, F^{3+}, Pb^{2+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{7+}, Hg^{2+}, Ni^{2+}, Ni^{3+}, Ag^{+}, Sr^{2+}, Sn^{2+}, Sn^{4+}, and Zn^{2+}. The metal atoms may comprise a metal oxide, such as iron oxide, manganese oxide, or gadolinium oxide.

Suitable dyes include any commercially available dyes such as, for example, 5(6)-carboxyfluorescein, IRDye 680RD maleimide or IRDye 800CW, ruthenium polypyridyl dyes, and the like.

Suitable fluorophores are fluorescein isothiocyanate (FITC), fluorescein thiosemicarbazide, rhodamine, Texas Red, CyDyes (e.g., Cy3, Cy5, Cy5.5), Alexa Fluors (e.g., Alexa488, Alexa555, Alexa594; Alexa647), near infrared (NIR) (700-900 nm) fluorescent dyes, and carbocyanine and aminostyryl dyes.

The antigen binding domain that binds hK2 conjugated to a detectable label may be used as an imaging agent.

The protein comprising an antigen binding domain that binds hK2 conjugated to a detectable label may be used as an imaging agent.

The multispecific protein comprising an antigen binding domain that binds hK2 conjugated to a detectable label may be used as an imaging agent.

The CAR comprising an antigen binding domain that binds hK2 conjugated to a detectable label may be used as an imaging agent.

In some embodiments, the cytotoxic agent is a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

In some embodiments, the cytotoxic agent is daunomycin, doxorubicin, methotrexate, vindesine, bacterial toxins such as diphtheria toxin, ricin, geldanamycin, maytansinoids or calicheamicin. The cytotoxic agent may elicit their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

In some embodiments, the cytotoxic agent is an enzymatically active toxin such as diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins,

dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In some embodiments, the cytotoxic agent is a radionuclide, such as ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

In some embodiments, the cytotoxic agent is dolastatins or dolostatin peptidic analogs and derivatives, auristatin or monomethyl auristatin phenylalanine. Exemplary molecules are disclosed in U.S. Pat No. 5,635,483 and 5,780,588. Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob Agents and Chemother. 45(12):3580-3584) and have anticancer and antifungal activity. The dolastatin or auristatin drug moiety may be attached to the antibody of the invention through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO02/088172), or via any cysteine engineered into the antibody.

The hK2 binding proteins of the disclosure may be conjugated to a detectable label using known methods.

In some embodiments, the detectable label is complexed with a chelating agent.

In some embodiments, the detectable label is conjugated to the hK2 binding proteins of the disclosure via a linker.

The detectable label or the cytotoxic moiety may be linked directly, or indirectly, to the hK2 binding proteins of the disclosure using known methods. Suitable linkers are known in the art and include, for example, prosthetic groups, non-phenolic linkers (derivatives of N-succimidyl-benzoates; dodecaborate), chelating moieties of both macrocyclics and acyclic chelators, such as derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10,tetraacetic acid (DOTA), derivatives of diethylenetriaminepentaacetic avid (DTPA), derivatives of S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and derivatives of 1,4,8,11-tetraazacyclodocedan-1,4,8,11-tetraacetic acid (TETA), N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene) and other chelating moieties. Suitable peptide linkers are well known.

In some embodiments, the hK2 binding proteins of the disclosure is removed from the blood via renal clearance.

Kits

The invention also provides a kit comprising the antigen binding domain that binds hK2.

The invention also provides a kit comprising the protein comprising an antigen binding domain that binds hK2.

The invention also provides a kit comprising the multispecific protein comprising an antigen binding domain that binds hK2.

The invention also provides a kit comprising the CAR comprising an antigen binding domain that binds hK2.

The kit may be used for therapeutic uses and as diagnostic kits.

The kit may be used to detect the presence of hK2 in a sample.

In some embodiments, the kit comprises the hK2 binding protein of the disclosure and reagents for detecting the hK2 binding protein. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

In some embodiments, the kit comprises the antigen binding domain that binds hK2 in a container and instructions for use of the kit.

In some embodiments, the kit comprises the protein comprising an antigen binding domain that binds hK2 in a container and instructions for use of the kit.

In some embodiments, the kit comprises the multispecific protein comprising an antigen binding domain that binds hK2 in a container and instructions for use of the kit.

In some embodiments, the antigen binding domain that binds hK2 in the kit is labeled.

In some embodiments, the protein comprising an antigen binding domain that binds hK2 in the kit is labeled.

In some embodiments, the multispecific protein comprising an antigen binding domain that binds hK2 in the kit is labeled.

In some embodiments, the kit comprises the antigen binding domain that binds hK2 comprising
the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169;
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205.
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1;

the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140; or
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160.

In a particular embodiment, the kit comprises the antigen binding domain that binds hK2 comprising a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163.

In some embodiments, the kit comprises the antigen binding domain that binds hK2 comprising SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 133, 134, 135, 136, 308, 316, 318, 319, 320, 321, 322, 323, 324, 325, 404, 405, 406, 407, 408, or 409.

Methods of detecting hK2

The invention also provides a method of detecting hK2 in a sample, comprising obtaining the sample, contacting the sample with the antigen binding domain that binds hK2 of the disclosure and detecting the bound hK2 in the sample.

In some embodiments, the sample may be derived from urine, blood, serum, plasma, saliva, ascites, circulating cells, synovial fluid, circulating cells, cells that are not tissue associated (*i.e.*, free cells), tissues (*e.g.*, surgically resected tissue, biopsies, including fine needle aspiration), histological preparations, and the like.

The antigen binding domain that binds hK2 of the disclosure may be detected using known methods. Exemplary methods include direct labeling of the antibodies using fluorescent or chemiluminescent labels, or radiolabels, or attaching to the antibodies of the invention a moiety which is readily detectable, such as biotin, enzymes or epitope tags. Exemplary labels and moieties are ruthenium, ^{111}In-DOTA, ^{111}In- diethylenetriaminepentaacetic acid (DTPA), horseradish peroxidase, alkaline phosphatase and beta-galactosidase, poly-histidine (HIS tag), acridine dyes, cyanine dyes, fluorone dyes, oxazin dyes, phenanthridine dyes, rhodamine dyes and Alexafluor® dyes.

The antigen binding domain that binds hK2 of the disclosure may be used in a variety of assays to detect hK2 in the sample. Exemplary assays are western blot analysis, radioimmunoassay, surface plasmon resonance, immunoprecipitation, equilibrium dialysis, immunodiffusion, electrochemiluminescence (ECL) immunoassay, immunohistochemistry, fluorescence-activated cell sorting (FACS) or ELISA assay.

Chimeric antigen receptors (CAR) comprising the antigen binding domains that bind hK2 of the disclosure

Any of the antigen binding domains that bind hK2 identified herein may be engineered into a chimeric antigen receptor (CAR) to provide CARs that bind hK2. The disclosure provides for CARs that target hK2, cells comprising such CARs, and methods of treating hK2 producing cancer, such as prostate cancer, using the CARs described herein.

The CARs of the invention have antigen specificity for hK2. The phrases "have antigen specificity" and "elicit antigen-specific response" as used herein mean that the CAR can specifically bind to and immunologically recognize an antigen, such that binding of the CAR to the hK2 antigen elicits an immune response. Methods of testing the CARs for antigen specificity and for the ability to recognize target cells are known in the art.

The disclosure relates to the use of T cells which have been genetically modified to stably express a desired chimeric antigen receptor. A chimeric antigen receptor (CAR) is an artificially constructed heterologous protein or polypeptide containing an antigen binding domain of an antibody (such as scFv) linked to a T-cell signaling domain. Characteristics of CARs include their ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize antigens independent of antigen processing, thus bypassing a major mechanism of tumor evasion. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

The CARs described herein provide recombinant polypeptide constructs comprising at least an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain (also referred to herein as a cytoplasmic signaling domain) comprising a functional signaling domain derived from a stimulatory molecule as described herein. T cells expressing a CAR are referred to herein

as CAR T cells, CAR-T cells or CAR modified T cells, and these terms are used interchangeably herein. The cell can be genetically modified to stably express an antibody binding domain on its surface, conferring novel antigen specificity that is MHC independent.

In some instances, the T cell is genetically modified to stably express a CAR that combines an antigen recognition domain of an antibody with an intracellular domain of the CD3-zeta chain or FcγRI protein into a single chimeric protein. In one embodiment, the stimulatory molecule is the zeta chain associated with the T cell receptor complex.

An "intracellular signaling domain," refers to an intracellular portion of a molecule. It is the functional portion of the protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CAR-T cell. Examples of immune effector function, e.g., in a CAR-T cell, include cytolytic activity and helper activity, including the secretion of cytokines.

In one embodiment, the intracellular signaling domain comprises a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent simulation. In one embodiment, the intracellular signaling domain comprises a co-stimulatory intracellular domain. Exemplary co-stimulatory intracellular signaling domains include those derived from molecules responsible for co-stimulatory signals, or antigen independent stimulation. F or example, in the case of a CAR-T, a primary intracellular signaling domain comprises a cytoplasmic sequence of a T cell receptor, and a co-stimulatory intracellular signaling domain comprises a cytoplasmic sequence from co-receptor or co-stimulatory molecule.

A primary intracellular signaling domain comprises a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Exemplary ITAM containing primary cytoplasmic signaling sequences include those derived from CD3-zeta, FcRγ, FcR beta, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, CD66d, DAP10 and DAP12.

The term "zeta" or alternatively "zeta chain", "CD3-zeta" or "TCR-zeta" is defined as the protein provided as GenBank Acc. No. BAG36664.1, or the equivalent residues from a nonhuman species, e.g., murine, rabbit, primate, mouse, rodent, monkey, ape and the like, and a "zeta stimulatory domain" or alternatively a "CD3-zeta stimulatory domain" or a "TCR-zeta stimulatory domain" is defined as the

amino acid residues from the cytoplasmic domain of the zeta chain that are sufficient to functionally transmit an initial signal necessary for T cell activation. In one aspect, the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, that are functional orthologs thereof. In one aspect, the "zeta stimulatory domain" or a "CD3-zeta stimulatory domain" is the sequence provided as SEQ ID NO: 28, or a sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99%, sequence identity with SEQ ID NO: 28.

SEQ ID NO: 28 CD3-zeta stimulatory domain

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

The term "co-stimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the T cell, such as, but not limited to, proliferation. Co-stimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. Co-stimulatory molecules include, but are not limited to an MHC class 1 molecule, BTLA and a Toll ligand receptor, as well as OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18) and 4-1BB (CD137).

A co-stimulatory intracellular signaling domain can be the intracellular portion of a co-stimulatory molecule. A co-stimulatory molecule can be represented in the following protein families: TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), and activating NK cell receptors. Exemplary such molecules include CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, MyD88, CD40, ICOS, BAFFR, HVEM, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, and a ligand that specifically binds with CD83, and the like.

The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment thereof.

The term "4-1BB" or alternatively "CD137" refers to a member of the TNFR superfamily with an amino acid sequence provided as GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like; and a "4-1BB co-stimulatory domain" is

defined as amino acid residues 214-255 of GenBank accession no. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In one aspect, the "4-1BB co-stimulatory domain" or "CD137 co-stimulatory domain" is the sequence provided as SEQ ID NO: 27 (KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL; SEQ ID NO: 27) or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, or a sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with SEQ ID NO: 27.

In one embodiment, a transmembrane domain that is naturally associated with one of the other domains in the CAR is used. In another embodiment, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. In one embodiment, the transmembrane domain comprises the CD8α hinge domain.

In some embodiments, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one co-stimulatory molecule as defined herein. In one embodiment, the co-stimulatory molecule is chosen from 4-1BB (*i.e.*, CD137), CD27, CD3-zeta and/or CD28. CD28 is a T cell marker important in T cell co-stimulation. CD27 is a member of the tumor necrosis factor receptor superfamily and acts as a co-stimulatory immune checkpoint molecule. 4-1BB transmits a potent co-stimulatory signal to T cells, promoting differentiation and enhancing long-term survival of T lymphocytes. CD3-zeta associates with TCRs to produce a signal and contains immunoreceptor tyrosine-based activation motifs (ITAMs). In another embodiment, the co-stimulatory molecule is MyD88 or CD40.

In one embodiment, the CAR comprises an intracellular hinge domain comprising CD8 and an intracellular T cell receptor signaling domain comprising CD28, 4-1BB, and CD3-zeta. In another embodiment, the CAR comprises an intracellular hinge domain and an intracellular T cell receptor signaling domain comprising CD28, 4-1BB, and CD3-zeta, wherein the hinge domain comprises all or part of the extracellular region of CD8, CD4 or CD28; all or part of an antibody constant region; all or part of the FcγRIIIA receptor, an IgG hinge, an IgM hinge, an IgA hinge, an IgD hinge, an IgE hinge, or an Ig hinge. The IgG hinge may be from IgG1, IgG2, IgG3, IgG4, IgM1, IgM2, IgA1, IgA2, IgD, IgE, or a chimera thereof.

CARs described herein provide recombinant polypeptide constructs comprising at least an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain (also referred to herein as "a cytoplasmic signaling domain") comprising, *e.g.*, a functional signaling domain derived from a stimulatory molecule as defined below

In one embodiment, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one embodiment, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a co-stimulatory molecule and a functional signaling domain derived from a stimulatory molecule . In one embodiment, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule.

The CARs of the disclosure can be designed to comprise the CD28 and/or 4-1BB signaling domain by itself or be combined with any other desired cytoplasmic domain(s) useful in the context of the CARs of the invention. In one embodiment, the cytoplasmic domain of the CAR can further comprise the signaling domain of CD3-zeta. For example, the cytoplasmic domain of the CAR can include but is not limited to CD3-zeta, 4-1BB and CD28 signaling modules and combinations thereof. Accordingly, the invention provides CAR T cells and methods of their use for adoptive therapy.

The disclosure further provides variants, e.g., functional variants, of the CARs. The term "functional variant" as used herein refers to a CAR having substantial or significant sequence identity or similarity to a parent CAR, which functional variant retains the biological activity of the CAR for which it is a variant. Functional variants encompass, e.g., those variants of the CAR that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional variant can, for example, be at least about 30%, about 40%, about 50%, about 60%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent CAR.

A functional variant can, for example, comprise the amino acid sequence of the parent CAR with at least one conservative amino acid substitution. In one embodiment, the functional variant comprises the amino

acid sequence of the parent CAR with at least one non-conservative amino acid substitution. In this case, the non-conservative amino acid substitution may not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant such that the biological activity of the functional variant is increased as compared to the parent CAR.

Conservative amino acid substitutions are known in the art and described herein.

The CAR of the disclosure can consist essentially of the specified amino acid sequence or sequences described herein, such that other components e.g., other amino acids, do not materially change the biological activity of the functional variant.

The CARs of the disclosure (including functional portions and functional variants) can be of any length, *i.e.*, can comprise any number of amino acids, provided that the CARs (or functional portions or functional variants thereof) retain their biological activity, e.g., the ability to specifically bind to an antigen, detect diseased cells (e.g., cancer cells) in a host, or treat or prevent disease in a host, etc. For example, the CARs can be about 50 to about 5000 amino acids long, such as about 50, about 70, about 75, about 100, about 125, about 150, about 175, about 200, about 225, about 250, about 275, about 300, about 325, about 350, about 375, about 400, about 425, about 450, about 475, about 500, about 525, about 550, about 575, about 600, about 625, about 650, about 675, about 700, about 725, about 750, about 775, about 800, about 825, about 850, about 875, about 900, about 925, about 950, about 975, about 1000 or more amino acids in length.

The CARs of the disclosure (including functional portions and functional variants of the invention) may comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, N′-benzyl-N′-methyl-lysine, N′,N′-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, and α-tert-butylglycine.

The CARs of the disclosure (including functional portions and functional variants) can be subject to post-translational modifications. They can be glycosylated, esterified, N-acylated, amidated, carboxylated, phosphorylated, esterified, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt. In some embodiments, they are dimerized or polymerized, or conjugated.

The CARs of the disclosure (including functional portions and functional variants thereof) can be obtained by methods known in the art. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan et al., *Fmoc Solid Phase Peptide Synthesis*, Oxford University Press, Oxford, United Kingdom, 2000; *Peptide and Protein Drug Analysis*, ed. Reid, R., Marcel Dekker, Inc.,2000; and *Epitope Mapping*, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2001. Also, the CARs can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. Further, some of the CARs (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, etc. Methods of isolation and purification are known in the art. Alternatively, the CARs, of the disclosure (including functional portions and functional variants thereof) can be commercially synthesized. In this respect, the CARs, polypeptides, and proteins can be synthetic, recombinant, isolated, and/or purified.

The disclosure also provides an antibody or an antigen binding fragment thereof that binds to an epitope of the CAR of the disclosure. The antibody or the antigen binding fragment thereof may have any level of affinity or avidity for the functional portion of the CAR. In some embodiments, the antibody or the antigen binding fragment may bind hK2 with a range of affinities (K_D). In one embodiment the antibody binds to hK2 with the K_D equal to or less than about 10^{-7} M, such as but not limited to, 1-9.9 (or any range or value therein, such as 1, 2, 3, 4, 5, 6, 7, 8, or 9)$\times 10^{-8}$ M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, 10^{-14} M, 10^{-15} M or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art. One exemplary affinity is equal to or less than 1×10^{-8} M. Another exemplary affinity is equal to or less than 1×10^{-9} M.

Methods of testing antibodies for their ability to bind to any functional portion of the CARs of the disclosure are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), Western blot, enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, and competitive inhibition assays.

The portion of the CAR comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen binding domain is expressed as part of a contiguous polypeptide chain

including, for example, a single domain antibody fragment (sdAb), a scFv and a human chimeric or humanized antibody (Harlow et al., 1999, *In: Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, N.Y.; Harlow et al., 1989, *In: Antibodies: A Laboratory Manual*, Cold Spring Harbor, N.Y.; Houston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; Bird et al., 1988, *Science* 242:423-426). In one aspect, the antigen binding domain of a CAR of the disclosure comprises an antibody fragment. In one embodiment, the CAR of the disclosure comprises an antibody fragment that comprises a scFv.

The disclosure also provides a CAR comprising an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds hK2.

The disclosure also provides a CAR comprising

an extracellular domain comprising an antigen binding domain that binds hK2;

a transmembrane domain; and

an intracellular signaling domain optionally comprising at least one co-stimulatory domain.

In some embodiments, the CAR further comprises a CD8a-hinge region.

In some embodiments, the CAR comprises a CD8a transmembrane region (CD8a-TM) polypeptide and the intracellular signaling domain comprising a co-stimulatory domain comprising a TNF receptor superfamily member 9 (CD137) component and a primary signaling domain comprising a T-cell surface glycoprotein CD3 zeta chain (CD3z) component.

In some embodiments, the CAR comprises

the CD8a-hinge region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 25;

the transmembrane domain comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 26; and/or

the intracellular signaling domain comprising a co-stimulatory domain having an amino acid sequence that is at least 90% identical to SEQ ID NO: 27, and a primary signaling domain having an amino acid sequence that is at least 90% identical to SEQ ID NO: 28.

The disclosure also provides a CAR comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds hK2 and comprises:

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 138 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 161;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 138 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 165 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 164;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 167 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 169 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 168;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 205 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 204;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 444 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 166;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 160 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 159;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 140 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 161;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 140 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 139;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 140 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 159;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 1 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 4;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 2 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 4;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 3 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 4;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 1 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 5;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 2 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 5;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 3 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 5;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 1 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 6;

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 2 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 6; or

the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 3 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 6.

In a particular embodiment, the disclosure provides a CAR comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds hK2 and comprises the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 163 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 162.

In another particular embodiment, the disclosure provides a CAR comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds hK2 and comprises the LCDR1, the LCDR2 and the LCDR3

of the VL of SEQ ID NO: 138 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 137; or the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 140 and the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 139.

In some embodiments, the CDRs are defined according to Kabat.

In some embodiments, the CDRs are defined according to Chothia.

In some embodiments, the CDRs are defined according to IMGT.

In some embodiments, the CDRs are defined according to AbM.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1 of SEQ ID NOs: 63, 72, 141, 147, 170, 176, 188, 194, 196, 198, 200, 206 or 216, the HCDR2 of SEQ ID NOs: 64, 65, 73, 142, 148, 171, 177, 183, 189, 195, 197, 199, 201, 207, or 217, the HCDR3 of SEQ ID NOs: 66, 143, 172, 178, 184, 190, 208, or 218, the LCDR1 of SEQ ID NOs: 67, 68, 144, 173, 179, 182,185 or 191, the LCDR2 of SEQ ID NOs: 69, 70, 145, 174, 180, 186, 192 or 470 and the LCDR3 of SEQ ID NOs: 71, 146, 175, 181, 187, 193, or 209.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1 of SEQ ID NOs: 63, 72, 141, 147, 170, 176, 188, 194, 196, 198, 200, 206 or 216, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR2 of SEQ ID NOs: 64, 65, 73, 142, 148, 171, 177, 183, 189, 195, 197, 199, 201, 207, or 217, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR3 of SEQ ID NOs: 66, 143, 172, 178, 184, 190, 208, or 218, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular

antigen binding domain that binds hK2 comprises the LCDR1 of SEQ ID NOs: 67, 68, 144, 173, 179, 185 or 191,or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the LCDR2 of SEQ ID NOs: 69, 70, 145, 174, 180, 186, 192 or 470, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the LCDR3 of SEQ ID NOs: 71, 146, 175, 181, 187, 193, or 209, or conservative substitutions thereof.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174, and 175, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186, and 187, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192, and 193, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, and 209, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 147, 148, 143, 144, 145 and 146, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 194, 195, 172, 173, 174, and 175, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 196, 197, 178, 179, 180, and 181, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 198, 199, 184, 185, 186, and 187, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 200, 201, 190, 191, 192, 193, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 216, 217, 218, 182, 470, and 209, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular

antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 72, 73, 66, 67, 69 and 71, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 63, 64, 66, 67, 69 and 71, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 63, 65, 66, 68, 70 and 71, respectively.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 72, 73, 66, 68, 70 and 71, respectively.

In some embodiments, the extracellular antigen binding domain that binds hK2 comprises the VL of SEQ ID NOs: 1, 2, 3, 138, 140, 160, 163, 165, 167, 169, or 205.

In some embodiments, the extracellular antigen binding domain that binds hK2 comprises the VH of SEQ ID NOs: 4, 5, 6, 137, 139, 159, 161, 162, 164, 166, 168, or 204.

In some embodiments, the extracellular antigen binding domain that binds hK2 comprises the VL of SEQ ID NOs: 1, 2, 3, 138, 140, 160, 163, 165, 167, 169, 205, or 444 and the VH of SEQ ID NOs: 4, 5, 6, 137, 139, 159, 161, 162, 164, 166, 168, or 204.

In some embodiments, the extracellular antigen binding domain that binds hK2 comprises

the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;

the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;

the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;

the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 140;

the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 160;

the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140; or
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 160

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 138 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 137.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 163 and the VH comprising the amino acid sequence that is

at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 162.

In some embodiments, the extracellular antigen-binding domain comprises the VL of SEQ ID NO: 163 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 162.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 163 and the VH of SEQ ID NO: 162.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 165 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 164.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 167 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 166.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 168 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 169.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 204 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 205.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99%

identical with the polypeptide of SEQ ID NO: 160 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 159.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 140 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 161.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 140 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 139.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 140 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 159.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 1 and the VH comprising the amino acid sequence that is at at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 4.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 1 and the VH comprising the amino acid sequence that is at least 50%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 5.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99%

identical with the polypeptide of SEQ ID NO: 1 and the VH comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 6.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 2 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 4.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 2 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 5.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 2 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 6.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 3 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 4.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 3 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 5.

In some embodiments, the extracellular antigen-binding domain comprises the VL comprising the amino acid sequence that is at 85%, at least 90%, at least 95%, at least 98% or at least 99% identical

with the polypeptide of SEQ ID NO: 3 and the VH comprising the amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the polypeptide of SEQ ID NO: 6.

In a particular embodiment, the CAR comprises an extracellular antigen binding domain comprising a VL comprising the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO: 140 and a VH comprising the amino acid sequence of SEQ ID NO: 137 or SEQ ID NO: 139.

In some embodiments, the extracellular antigen binding domain that binds hK2 comprises a scFv. In some embodiments, the scFv comprises a linker polypeptide between the VL and the VH.

In some embodiments, the linker polypeptide comprises the amino acid sequence of SEQ ID NO: 7. In some embodiments, the linker polypeptide comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with SEQ ID NO: 7.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 149, 150, 151, 152, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422. 423, 424, or 425.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 29.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 30.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 31.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 32.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 33.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 34.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 34.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 36.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 37.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 38.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 39.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 40.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 41.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 42.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 43.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 44.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 149.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 150.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 151.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 152.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 410.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 411.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 412.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 413.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 414.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 415.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 416.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 417.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 418.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 419.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 420.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 421.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 422.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 423.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 424.

In some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence SEQ ID NO: 425.

In a some embodiments, the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence SEQ ID NO: 416 or SEQ ID NO: 417.

In a particular embodiment the extracellular antigen binding domain that binds hK2 is a scFv which comprises an amino acid sequence which is identical to the amino acid sequence SEQ ID NO: 416 or SEQ ID NO: 417

In some embodiments, the CAR comprises an extracellular antigen binding domain that binds hK2 comprising a scFv which comprises an amino acid sequence which is at least 80% (*e.g.* at least

90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151 or SEQ ID NO: 152

In a particular embodiment, the CAR comprises an extracellular antigen binding domain that binds hK2 comprising a scFv which comprises an amino acid sequence which is identical to the amino acid sequence SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151 or SEQ ID NO: 152 .

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 8.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 9.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 10.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 13.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 14.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 15.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 16.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 17.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 19.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 20.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 21.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 22.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 23.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 133.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 134.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 135.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 136.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 318.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 319.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 320.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 321.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 322.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 323.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 404.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 405.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 322.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 323.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 404.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 405.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 406.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 407.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 408.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 409.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 324.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 325.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 308.

In some embodiments, the scFv comprises the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 316.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 404.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 405.

In some embodiments, the scFv comprises an amino acid sequence which is identical to the amino acid sequence of SEQ ID NO: 404 or SEQ ID NO: 405.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135 or SEQ ID NO: 136.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 134.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 135.

In some embodiments, the scFv comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 136.

In a particular embodiment, the scFv comprises an amino acid sequence which is identical to the amino acid sequence of SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135 or SEQ ID NO: 136.

In some embodiment, the extracellular antigen binding domain that binds hK2 comprises a signal polypeptide. In some embodiments, the signal sequence comprises an amino acid sequence of SEQ ID NO: 24.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 149, 150, 151, 152, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422. 423, 424, or 425.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 33.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at

least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 33.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 34.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 34.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 35.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 35.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 36.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 36.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 37.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 37.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 38.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 38.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 39.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 39.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 40.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 40.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 41.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 41.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 42.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 42.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 43.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 43.

The disclosure also provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NO: 44.

In one embodiment, the extracellular antigen binding domain comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical with the amino acid sequence of SEQ ID NO: 44.

In some embodiments, the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member (such as, e.g., CD28 or inducible T-cell co-stimulator (ICOS) component, and a combination thereof.

In some embodiments, the CD137 component comprises an amino acid sequence of SEQ ID NO: 27. In some embodiments, the CD137 component comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98 or at least 99% identical to the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the CD3z component comprises an amino acid sequence of SEQ ID NO: 28. In some embodiments, the CD3z component comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98 or at least 99% identical to the amino acid sequence of SEQ ID NO: 28.

In some embodiments, the intracellular signaling domain comprises an amino acid sequence of SEQ ID NO: 45. In some embodiments, the intracellular signaling domain comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98 or at least 99% identical to the amino acid sequence of SEQ ID NO: 45.

SEQ ID NO: 45

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR

In some embodiments, the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide. In some embodiments, the CD8a-TM polypeptide comprises an amino acid sequence of **SEQ ID NO: 26**. (IYIWAPLAGTCGVLLLSLVITLYC; **SEQ ID NO: 26**).

In some embodiments, the CD8a-TM polypeptide comprises an amino acid sequence that is least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 26.

In some embodiments, the transmembrane domain comprises at least the transmembrane region(s) of) the α, β or ζ chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD8α, CD9, CD16, CD22, CD33, CD37, CD40, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, the transmembrane domain comprises at least the transmembrane domain of ζ, η or FcεR1γ and -β, MB1 (Igα.), B29 or CD3- γ, ζ, or η. In some embodiments, the transmembrane domain is synthetic, e.g., comprising predominantly hydrophobic residues such as leucine and valine, a triplet of phenylalanine, or tryptophan.

In some embodiments, the CAR further comprises a hinge region linking the transmembrane domain to the extracellular antigen binding domain that binds hK2. In some embodiments, the hinge region is a CD8a-hinge region. In some embodiments, CD8a-hinge region comprises an amino acid sequence of **SEQ ID NO: 25** (TSTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD; SEQ ID NO: 25).

In some embodiments, the CD8a-hinge region comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of **SEQ ID NO: 25**. In some embodiments, the hinge region comprises the amino acid sequence EPKSCDKTHTCPPCP (**SEQ ID NO: 126**), or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% amino acid sequence identity with EPKSCDKTHTCPPCP (SEQ ID NO: 126). In some embodiments, the hinge region comprises the sequence ERKCCVECPPCP (**SEQ ID NO: 127**) or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with ERKCCVECPPCP (SEQ ID

NO: 127). In some embodiments, the hinge region comprises the sequence ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$, (SEQ ID NO: 128) or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with ELKTPLGDTTHTCPRCP(EPKSCDTPPPCPRCP)$_3$ (SEQ ID NO: 128). In some embodiments, the hinge region comprises the sequence ESKYGPPCPSCP (SEQ ID NO: 129), or comprises an amino acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with ESKYGPPCPSCP (SEQ ID NO: 129).

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NOs: 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 153, 154, 155, 156, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 435, 436, 437, 438, 439, 440, or 441.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 46.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 46.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 47.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 47.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 48.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 48.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 49.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 49.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 50.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 51.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 52.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 52.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 53.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 53.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 54.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 54.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 55.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 55.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 56.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 56.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 57.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 57.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 58.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 58.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 59.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 59.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 60.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 60.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 61.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 61.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 153.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 153.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 154.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 154.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 155.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 155.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 156.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 156.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 426.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 426.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 427.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 427.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 428.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO:428.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 429.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 429.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 430.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 430.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 431.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 431.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 432.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 432.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 433.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 433.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 434.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 434.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 435.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 435.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 436.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 436.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 437.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 437.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 438.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 438.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 439.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 439.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 440.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 440.

The disclosure also provides a CAR comprising the amino acid sequence of SEQ ID NO: 441.

The disclosure also provides a CAR comprising the amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of SEQ ID NO: 441.

The disclosure also provides a CAR comprising an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 432.

The disclosure also provides a CAR comprising an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 433.

In some embodiments, the CAR comprises an amino acid sequence which is identical to the amino acid sequence of SEQ ID NO: 432 or SEQ ID NO: 433.

In some embodiments, the CAR comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155 or SEQ ID NO: 156.

In some embodiments, the CAR comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 154.

In some embodiments, the CAR comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 155.

In some embodiments, the CAR comprises an amino acid sequence which is at least 80% (*e.g.* at least 90%, at least 95%, at least 98% or at least 99%) identical to the amino acid sequence of SEQ ID NO: 156.

In a particular embodiment, the CAR comprises an amino acid sequence which is identical to the amino acid sequence of SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155 or SEQ ID NO: 156

In a particular embodiment, the disclosure provides a CAR comprising an extracellular antigen binding domain that binds hK2, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen binding domain binds to hK2 at three or more residues within epitopes having sequences of SEQ ID NO: 111 and SEQ ID NO: 112.

CAR constructs and immunoresponsive cells expressing CARs

The disclosure also provides isolated polynucleotides encoding the CARs described herein.

Unless otherwise specified, a "polynucleotide encoding an amino acid sequence" includes all polynucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase polynucleotide that encodes a protein or a RNA may also include introns to the extent that the polynucleotide encoding the protein may in some variants contain an intron(s) that is spliced into the expressed protein.

The disclosure also provides an expression vector comprising the nucleic acid molecule encoding the CAR of the disclosure.

The term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression

can be supplied by the host cell or in an *in vitro* expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The disclosure also provides isolated immunoresponsive cells comprising the CAR of the disclosure. In some embodiments, the isolated immunoresponsive cell is transduced with the CAR, for example, the CAR is constitutively expressed on the surface of the immunoresponsive cell. In certain embodiments, the isolated immunoresponsive cell is further transduced with at least one co-stimulatory ligand such that the immunoresponsive cell expresses the at least one co-stimulatory ligand. In certain embodiments, the at least one co-stimulatory ligand is selected from the group consisting of 4-1BBL, CD48, CD70, CD80, CD86, OX40L, TNFRSF14, and combinations thereof. In certain embodiments, the isolated immunoresponsive cell is further transduced with at least one cytokine such that the immunoresponsive cell secretes the at least one cytokine. In certain embodiments, the at least cytokine is selected from the group consisting of IL-2, IL-3, IL-6, IL-7, IL-11, IL-12, IL-15, IL-17, IL-21, and combinations thereof. In some embodiments, the isolated immunoresponsive cell is selected from the group consisting of a T lymphocyte (T cell), a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell (Treg), a human embryonic stem cell, a lymphoid progenitor cell, a T cell-precursor cell, and a pluripotent stem cell from which lymphoid cells may be differentiated.

In some embodiments, the isolated immunoresponsive cell is the T cell.

For purposes herein, the T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to bone marrow, blood, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, $CD4^+/CD8^+$ double positive T cells, $CD8^+$ T cells (e.g., cytotoxic T cells), $CD4^+$ helper T cells, e.g., Th1 and Th2 cells, peripheral blood mononuclear cells (PBMCs), peripheral blood leukocytes (PBLs), tumor infiltrating cells, memory T cells, naïve T cells, and the like. The T cell may be a $CD8^+$ T cell or a $CD4^+$ T cell.

In some embodiments, the isolated immunoresponsive cell is the NK cell.

In some embodiments, the isolated immunoresponsive cell is the CTL.

In some embodiments, the isolated immunoresponsive cell is the Treg.

In some embodiments, the isolated immunoresponsive cell is the human embryonic stem cell.

In some embodiments, the isolated immunoresponsive cell is the lymphoid progenitor cell.

In some embodiments, the isolated immunoresponsive cell is the pluripotent stem cell.

In one embodiment, the CAR T cells of the disclosure can be generated by introducing a lentiviral vector comprising a desired CAR, for example, a CAR comprising an extracellular domain comprising an antigen binding domain that binds hK2, CD8α hinge and transmembrane domain, and human 4-1BB and CD3-zeta signaling domains, into the cells. The CAR T cells of the disclosure are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control.

Embodiments of the invention further provide host cells comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, or algae, fungi, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5α *E.coli* cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell may be a prokaryotic cell, e.g., a DH5α cell. For purposes of producing a recombinant CAR, polypeptide, or protein, the host cell may be a mammalian cell. The host cell may be a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell may be a peripheral blood lymphocyte (PBL). The host cell may be a T cell.

Also provided are a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, an erythrocyte, a neutrophil, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

Polynucleotides, host cells and vectors

The disclosure also provides an isolated polynucleotide encoding any of the hK2 binding proteins of the disclosure. The hK2 binding protein includes the antigen binding domains that bind hK2, the proteins comprising the antigen binding domains that bind hK2, the multispecific proteins that comprise the antigen binding domains that bind hK2 and the chimeric antigen receptors (CAR) comprising the antigen binding domains that bind hK2 of the disclosure.

The invention also provides an isolated polynucleotide encoding any of hK2 biding proteins or fragments thereof.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 137.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 162.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 164.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 166.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 168.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 204.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 159.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 161.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 139.

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NOs: 4, 5 or 6 .

The invention also provides an isolated polynucleotide encoding the VH of SEQ ID NO: 75.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 138.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 163.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 165.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 167.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 169.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 205.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 140.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 160.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NO: 74.

The invention also provides an isolated polynucleotide encoding the VL of SEQ ID NOs: 1, 2 or 3.

The invention also provides for an isolated polynucleotide encoding
the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169;
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2; or
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

In a particular embodiment, the invention provides an isolated polynucleotide encoding the VH of SEQ ID NO: 162 and/or an isolated polynucleotide encoding the VL of SEQ ID NO: 163.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NOs: SEQ ID NOs: : 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 133, 134, 135, 136, 308, 316, 318, 319, 320, 321, 322, 323, 324, 325, 404, 405, 406, 407, 408, or 409.

The invention also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NOs: 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 149, 150, 151, 152, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422. 423, 424, or 425.

In some embodiments, the isolated polynucleotide sequence encoding an hK2 binding protein of the disclosure is at least 85% identical with the polynucleotide of SEQ ID NOs: 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 263, 271, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 292, 293, 294, 295, 296, 297, 303, 304, 305, 306, 312, 316, 317, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 446, 450 or 451.

In some embodiments, the isolated polynucleotide sequence encoding an hK2 binding protein of the disclosure is at least 90% identical with the polynucleotide of SEQ ID NOs: 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 263, 271, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 292, 293, 294, 295, 296, 297, 303, 304, 305, 306, 312, 316, 317, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 446, 450 or 451.

In some embodiments, the isolated polynucleotide sequence encoding an hK2 binding protein of the disclosure is at least 95% identical with the polynucleotide of SEQ ID NOs: 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 263, 271, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 292, 293, 294, 295, 296, 297, 303, 304, 305, 306, 312, 316, 317, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 446, 450 or 451.

In some embodiments, the isolated polynucleotide sequence encoding an hK2 binding protein of the disclosure is at least 98% identical with the polynucleotide of SEQ ID NOs: 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 263, 271, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 292, 293, 294, 295, 296, 297, 303, 304, 305, 306, 312, 316, 317, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 446, 450 or 451.

In some embodiments, the isolated polynucleotide sequence encoding an hK2 binding protein of the disclosure is at least 99% identical with the polynucleotide of SEQ ID NOs: 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 263, 271, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 292, 293, 294, 295, 296, 297, 303, 304, 305, 306, 312, 316, 317, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 446, 450 or 451.

The invention also provides an isolated polynucleotide of SEQ ID NOs: 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 263, 267, 271, 267, 274, 282, 292, 293, 294, 295, 303, 304, 305, 306,312, 316, 363, 364, 365, 367, 368, 369, 370, 371, 372, 373, or 374.

The invention also provides an isolated polynucleotide of SEQ ID NOs: 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 263, 271, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 292, 293, 294, 295, 296, 297, 303, 304, 305, 306, 312, 316, 317, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 446, 450 or 451.In a particular embodiment, the disclosure provides an isolated polynucleotide sequence encoding a polypeptide sequence of SEQ ID NO: 354.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence encoding a polypeptide sequence of SEQ ID NO: 221.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence encoding a polypeptide sequence of SEQ ID NO: 360.

In a particular embodiment, the disclosure provides isolated polynucleotide sequences encoding polypeptide sequences of SEQ ID NOs: 354, 221 and 360.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99%, or 100%) identical to the polynucleotide of SEQ ID NO: 367.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99% or 100%) identical to the polynucleotide of SEQ ID NO: 303.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99% or 100%) identical to the polynucleotide of SEQ ID NO: 373.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence encoding a polypeptide sequence of SEQ ID NO: 361.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence encoding a polypeptide sequence of SEQ ID NO: 221.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence encoding a polypeptide sequence of SEQ ID NO: 362. In a particular embodiment, the disclosure provides isolated polynucleotide sequences encoding polypeptide sequences of SEQ ID NOs: 361, 221 and 362.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99% or 100%) identical to the polynucleotide of SEQ ID NO: 370.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99% or 100%) identical to the polynucleotide of SEQ ID NO: 303.

In a particular embodiment, the disclosure provides an isolated polynucleotide sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95% or at least 99% or 100%) identical to the polynucleotide of SEQ ID NO: 374.

Some embodiments of the disclosure also provide an isolated or purified nucleic acid comprising a polynucleotide which is complementary to the polynucleotides encoding the hK2 binding proteins of the disclosure or polynucleotides which hybridize under stringent conditions to the polynucleotides encoding the hK2 binding proteins of the disclosure.

The polynucleotides which hybridize under stringent conditions may hybridize under high stringency conditions. By "high stringency conditions" is meant that the polynucleotide specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-12 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70° C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the CARs described herein. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

The polynucleotide sequences of the disclosure may be operably linked to one or more regulatory elements, such as a promoter or enhancer, that allow expression of the nucleotide sequence in the intended host cell. The polynucleotide may be a cDNA. The promoter bay be a strong, weak, tissue-specific, inducible or developmental-specific promoter. Exemplary promoters that may be used are hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin, human myosin, human hemoglobin, human muscle creatine, and others. In addition, many viral promoters function constitutively in eukaryotic cells and are suitable for use with the described embodiments. Such viral promoters include Cytomegalovirus (CMV) immediate early promoter, the early and late promoters of SV40, the Mouse Mammary Tumor Virus (MMTV) promoter, the long terminal repeats (LTRs) of Maloney leukemia virus, Human Immunodeficiency Virus (HIV), Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV), and other retroviruses, and the thymidine kinase promoter of Herpes Simplex Virus. Inducible promoters such as the metallothionein promoter, tetracycline-inducible promoter, doxycycline-inducible promoter, promoters that contain one or more interferon-stimulated response elements (ISRE) such as protein kinase R 2',5'-oligoadenylate synthetases, Mx genes, ADAR1, and the like may also be sued.

The invention also provides a vector comprising the polynucleotide of the invention. The disclosure also provides an expression vector comprising the polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon-based vectors or any other vector suitable for introduction of the synthetic polynucleotide of the invention into a given organism or genetic background by any means. Polynucleotides encoding the hK2 binding proteins of the disclosure may be operably linked to control sequences in the expression vector(s) that ensure the expression of the hK2 binding proteins. Such regulatory elements may include a transcriptional promoter, sequences encoding suitable mRNA ribosomal binding sites, and sequences that control the termination of transcription and translation. Expression vectors may also include one or more nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, other 5' or 3' flanking nontranscribed sequences, 5' or 3' nontranslated sequences (such as necessary ribosome binding sites), a polyadenylation site, splice donor and acceptor sites, or transcriptional termination sequences. An origin of replication that confers the ability to replicate in a host may also be incorporated.

The expression vectors can comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. The non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the hK2 binding proteins of the disclosure encoded by the incorporated polynucleotides. The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. Exemplary vectors may be constructed as described by Okayama and Berg, 3 *Mol. Cell. Biol.* 280 (1983).

Vectors of the disclosure may also contain one or more Internal Ribosome Entry Site(s) (IRES). Inclusion of an IRES sequence into fusion vectors may be beneficial for enhancing expression of some proteins. In some embodiments, the vector system will include one or more polyadenylation sites (e.g., SV40), which may be upstream or downstream of any of the aforementioned nucleic acid sequences. Vector components may be contiguously linked or arranged in a manner that provides optimal spacing for expressing the gene products (i.e., by the introduction of "spacer" nucleotides between the ORFs) or positioned in another way. Regulatory elements, such as the IRES motif, may also be arranged to provide optimal spacing for expression.

Vectors of the disclosure may be circular or linear. They may be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColE1, SV40, 2μ plasmid, λ, bovine papilloma virus, and the like.

The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

The vectors may also comprise selection markers, which are well known in the art. Selection markers include positive and negative selection marker. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Exemplary marker genes include antibiotic resistance genes (e.g., neomycin resistance gene, a hygromycin resistance gene, a kanamycin resistance gene, a tetracycline resistance gene, a penicillin resistance gene, histidinol resistance gene, histidinol x resistance gene), glutamine

synthase genes, HSV-TK, HSV-TK derivatives for ganciclovir selection, or bacterial purine nucleoside phosphorylase gene for 6-methylpurine selection (Gadi et al., 7 *Gene Ther.* 1738-1743 (2000)). A nucleic acid sequence encoding a selection marker or the cloning site may be upstream or downstream of a nucleic acid sequence encoding a polypeptide of interest or cloning site.

Exemplary vectors that may be used are Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia), pEE6.4 (Lonza) and pEE12.4 (Lonza). Additional vectors include the pUC series (Fermentas Life Sciences, Glen Burnie, Md.), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as λGT10, λGT11, λEMBL4, and λNM1149, λZapII (Stratagene) can be used. Exemplary plant expression vectors include pBI01, pBI01.2, pBI121, pBI101.3, and pBIN19 (Clontech). Exemplary animal expression vectors include pEUK-Cl, pMAM, and pMAMneo (Clontech). The expression vector may be a viral vector, e.g., a retroviral vector, e.g., a gamma retroviral vector.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 137.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 162.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 164.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 166.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 168.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 204.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 159.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 161.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 139.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NOs: 4, 5 or 6 .

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 75.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 138.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 163.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 165.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 167.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 169.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 205.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 140.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 160.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NO: 74.

In some embodiments, the vector comprises the polynucleotide encoding the VH of SEQ ID NOs: 1, 2 or 3.

In some embodiments, the vector comprises the polynucleotide encoding
the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169;
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2; or
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

In some embodiments, the vector comprises the polynucleotide encoding polypeptide of SEQ ID NOs: SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 133, 134, 135, 136, 308, 316, 318, 319, 320, 321, 322, 323, 324, 325, 404, 405, 406, 407, 408, or 409.

In some embodiments, the vector comprises the polynucleotide encoding the polypeptide of SEQ ID NOs: 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 149, 150, 151, 152, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422. 423, 424, or 425.

In a particular embodiment, the vector comprises the polynucleotide encoding the polypeptide sequence of SEQ ID NO: 354. In a particular embodiment, the vector comprises the polynucleotide encoding the polypeptide sequence of SEQ ID NO: 221. In a particular embodiment, the vector comprises the polynucleotide encoding the polypeptide sequence of SEQ ID NO: 360. In some embodiments, the disclosure provides a vector the polypeptide sequences of SEQ ID NOs: 354, 221 and 360.

In a particular embodiment, the vector comprises the polynucleotide of SEQ ID NO: 367. In a particular embodiment, the vector comprises the polynucleotide of SEQ ID NO: 303. In a particular embodiment, the vector comprises the polynucleotide of SEQ ID NO: 373. In some embodiments, the vector comprises the polynucleotide sequences of SEQ ID NOs: 367, 303 and 373.

In a particular embodiment, the vector comprises the polynucleotide encoding the polypeptide sequence of SEQ ID NO: 361. In a particular embodiment, the vector comprises the polynucleotide encoding the polypeptide sequence of SEQ ID NO: 221. In a particular embodiment, the vector comprises the polynucleotide encoding the polypeptide sequence of SEQ ID NO: 362.

In a particular embodiment, the vector comprises the polynucleotide of SEQ ID NO: 370. In a particular embodiment, the vector comprises the polynucleotide of SEQ ID NO: 303. In a particular embodiment, the vector comprises the polynucleotide of SEQ ID NO: 374.

The invention also provides for a host cell comprising one or more vectors of the invention. "Host cell" refers to a cell into which a vector has been introduced. It is understood that the term host cell is intended to refer not only to the particular subject cell but to the progeny of such a cell, and also to a stable cell line generated from the particular subject cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Such host cells may be eukaryotic cells, prokaryotic cells, plant cells or archeal cells. *Escherichia coli*, bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species are examples of prokaryotic host cells. Other microbes, such as yeast, are also useful for expression. Saccharomyces (e.g., S. cerevisiae) and Pichia are examples of suitable yeast host cells. Exemplary eukaryotic cells may be of mammalian, insect, avian or other animal origins. Mammalian eukaryotic cells include immortalized cell lines such as hybridomas or myeloma cell lines such as SP2/0 (American Type Culture Collection (ATCC), Manassas, VA, CRL-1581), NS0 (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATTC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary (CHO) cells such as CHO-K1SV (Lonza Biologics, Walkersville, MD), CHO-K1 (ATCC CRL-61) or DG44.

The disclosure also provides a method of producing the hK2 binding protein of the disclosure comprising culturing the host cell of the disclosure in conditions that the hK2 binding protein is expressed, and recovering the hK2 binding protein produced by the host cell. Methods of making

proteins and purifying them are known. Once synthesized (either chemically or recombinantly), the hK2 binding proteins may be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, high performance liquid chromatography (HPLC) purification, gel electrophoresis, and the like (see generally Scopes, Protein Purification (Springer- Verlag, N.Y., (1982)). A subject protein may be substantially pure, e.g., at least about 80% to 85% pure, at least about 85% to 90% pure, at least about 90% to 95% pure, or at least about 98% to 99%, or more, pure, e.g., free from contaminants such as cell debris, macromolecules, etc. other than the subject protein

The polynucleotides encoding the hK2 binding proteins of the disclosure may be incorporated into vectors using standard molecular biology methods. Host cell transformation, culture, antibody expression and purification are done using well known methods.

Modified nucleotides may be used to generate the polynucleotides of the disclosure. Exemplary modified nucleotides are 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5″-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queuosine, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine.

Pharmaceutical Compositions/Administration

The disclosure also provides a pharmaceutical composition comprising the hK2 binding protein of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the antigen binding domain that binds hK2 of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the protein comprising the

antigen binding domain that binds hK2 of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the multispecific protein comprising the antigen binding domain that binds hK2 of the disclosure and a pharmaceutically acceptable carrier.

The disclosure also provides a pharmaceutical composition comprising the CAR comprising the antigen binding domain that binds hK2 of the disclosure and a pharmaceutically acceptable carrier.

For therapeutic use, the hK2 binding protein of the disclosure may be prepared as pharmaceutical compositions containing an effective amount of the antibody as an active ingredient in a pharmaceutically acceptable carrier. "Carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the antibody of the invention is administered. Such vehicles may be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine may be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the antibodies of the invention in such pharmaceutical formulation may vary, from less than about 0.5%, usually to at least about 1% to as much as 15 or 20% by weight and may be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, See especially pp. 958-989.

The mode of administration of the hK2 binding protein of the disclosure may be any suitable route such as parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, transmucosal (oral, intranasal, intravaginal, rectal) or other means appreciated by the skilled artisan, as known in the art.

In embodiments of the present disclosure, the CAR-expressing cells may be provided in compositions, e.g., suitable pharmaceutical composition(s) comprising the CAR-expressing cells and a pharmaceutically acceptable carrier. In one aspect, the present disclosure provides pharmaceutical compositions comprising an effective amount of a lymphocyte expressing one or more of the CARs

described and a pharmaceutically acceptable excipient. Pharmaceutical compositions of the present disclosure may comprise a CAR-expressing cell, e.g., a plurality of CAR-expressing cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, excipients or diluents. A pharmaceutically acceptable carrier can be an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to the subject.

A pharmaceutically acceptable carrier can include a buffer, excipient, stabilizer, or preservative. Examples of pharmaceutically acceptable carriers are solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible, such as salts, buffers, antioxidants, saccharides, aqueous or non-aqueous carriers, preservatives, wetting agents, surfactants or emulsifying agents, or combinations thereof. The amounts of pharmaceutically acceptable carrier(s) in the pharmaceutical compositions may be determined experimentally based on the activities of the carrier(s) and the desired characteristics of the formulation, such as stability and/or minimal oxidation.The term "pharmaceutically acceptable," as used herein with regard to pharmaceutical compositions, means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals and/or in humans.

Method of treatment and uses

The disclosure also provides a method of treating a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the antigen biding domain that binds hK2 of the disclosure to the subject in need thereof for a time sufficient to treat the hK2 expressing cancer.

The disclosure also provides a method of treating a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the protein comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to treat the hK2 expressing cancer

The disclosure also provides a method of treating a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to treat the hK2 expressing cancer.

The disclosure also provides a method of treating a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the CAR comprising the antigen biding

domain that binds hK2 of the disclosure to the subject for a time sufficient to treat the hK2 expressing cancer.

The disclosure also provides a method of treating a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the immunoconjugate comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to treat the hK2 expressing cancer.

The disclosure also provides a method of treating a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the pharmaceutical composition comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to treat the hK2 expressing cancer.

The disclosure also provides a method of administering a genetically modified T cell expressing a CAR for the treatment of a subject having cancer or at risk of having cancer using lymphocyte infusion.

In at least one embodiment, autologous lymphocyte infusion is used in the treatment. Autologous PBMCs are collected from a subject in need of treatment and T cells are activated and expanded using the methods described herein and known in the art and then infused back into the subject.

In one aspect, the disclosure relates generally to the treatment of a subject at risk of developing cancer. The invention also includes treating a malignancy or an autoimmune disease in which chemotherapy and/or immunotherapy results in significant immunosuppression in a subject, thereby increasing the risk of the subject developing cancer.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the antigen binding domain that bind hK2 of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the protein comprising the antigen binding domain that bind hK2 of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the multispecific protein comprising the antigen binding domain that bind hK2 of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the immunoconjugate of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the pharmaceutical composition of the disclosure to the subject to treat the noncancerous condition.

The disclosure also provides a method of treating a noncancerous condition in a subject at risk of developing a cancerous condition, comprising administering the CAR of the disclosure to the subject to treat the noncancerous condition.

In some embodiments, the subject at risk of developing the cancerous condition has an enlarged prostate.

In some embodiments, the subject at risk of developing the cancerous condition has a benign prostate hyperplasia (BPH).

In some embodiments, the subject at risk of developing the cancerous condition has a and high PSA levels in absence of diagnosed prostate cancer.

The disclosure also provides a method of preventing hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to prevent the hK2 expressing cancer.

The disclosure also provides a method of preventing a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the protein comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to prevent the hK2 expressing cancer

The disclosure also provides a method of preventing a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to prevent the hK2 expressing cancer.

The disclosure also provides a method of preventing a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the CAR comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to prevent the hK2 expressing cancer.

The disclosure also provides a method of preventing a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the immunoconjugate comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to prevent the hK2 expressing cancer.

The disclosure also provides a method of preventing a hK2 expressing cancer in a subject, comprising administering a therapeutically effective amount of the pharmaceutical composition comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to prevent the hK2 expressing cancer.

The disclosure also provides a method of reducing the amount of hK2 expressing tumor cells in a subject, comprising administering the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to reduce the amount of hK2 expressing tumor cells.

The disclosure also provides a method of reducing the amount of hK2 expressing tumor cells in a subject, comprising administering the protein comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to reduce the amount of hK2 expressing tumor cells.

The disclosure also provides a method of reducing the amount of hK2 expressing tumor cells in a subject, comprising administering the multispecific protein comprising the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to reduce the amount of hK2 expressing tumor cells.

The disclosure also provides a method of reducing the amount of hK2 expressing tumor cells in a subject, comprising administering the CAR that comprises the antigen biding domain that binds hK2 of the disclosure to the subject for a time sufficient to reduce the amount of hK2 expressing tumor cells.

The disclosure also provides a method of reducing the amount of hK2 expressing tumor cells in a subject, comprising administering the immunoconjugate of the disclosure to the subject for a time sufficient to reduce the amount of hK2 expressing tumor cells.

The disclosure also provides a method of reducing the amount of hK2 expressing tumor cells in a subject, comprising administering the pharmaceutical composition of the disclosure to the subject for a time sufficient to reduce the amount of hK2 expressing tumor cells.

In some embodiments, the hK2 expressing cancer is prostate cancer.

In some embodiments, the hK2 expressing cancer is prostate derived cancer.

In some embodiments, the hK2 expressing cancer has metastasized to bone.

In some embodiments, the hK2 expressing cancer is a breast cancer.

In some embodiments, the hK2 expressing cancer is an androgen receptor (AR) expressing breast cancer.

In some embodiments, the prostate cancer is relapsed, refractory, malignant or castration resistant prostate cancer, or any combination thereof.

In some embodiments, the breast cancer is relapsed, refractory or malignant breast cancer, or any combination thereof.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds hK2 to the subject for a time sufficient to treat the prostate cancer, wherein the antigen binding domain that bind hK2 comprises
the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169;
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2; or
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds hK2 to the subject for a time sufficient to treat the prostate cancer, wherein the

antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 133, 134, 135, 136, 308, 316, 318, 319, 320, 321, 322, 323, 324, 325, 404, 405, 406, 407, 408, or 409.

The disclosure also provides a method of treating breast cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds hK2 to the subject for a time sufficient to treat the breast cancer, wherein the antigen binding domain that bind hK2 comprises

the VH of SEQ ID NO: 137 and the VL of SEQ ID NO: 138;
the VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163;
the VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165;
the VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 167;
the VH of SEQ ID NO: 168 and the VL of SEQ ID NO: 169;
the VH of SEQ ID NO: 204 and the VL of SEQ ID NO: 205;
the VH of SEQ ID NO: 159 and the VL of SEQ ID NO: 160;
the VH of SEQ ID NO: 161 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 139 and the VL of SEQ ID NO: 140;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 2;
the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 5 and the VL of SEQ ID NO: 3;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 1;
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 2; or
the VH of SEQ ID NO: 6 and the VL of SEQ ID NO: 3.

The disclosure also provides a method of treating breast cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds hK2 to the subject for a time sufficient to treat the breast cancer, wherein the antigen binding domain that binds hK2 comprises the amino acid sequence of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 133, 134, 135, 136, 308, 316, 318, 319, 320, 321, 322, 323, 324, 325, 404, 405, 406, 407, 408, or 409.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen

binding domain that binds a lymphocyte antigen to the subject for a time sufficient to treat the prostate cancer, wherein the antigen binding domain that binds a lymphocyte antigen comprises:
the HCDR1 of SEQ ID NO: 116, the HCDR2 of SEQ ID NO: 117, the HCDR3 of SEQ ID NO: 118, the LCDR1 of SEQ ID NO: 119, the LCDR2 of SEQ ID NO: 120 and the LCDR3 of SEQ ID NO: 121;
the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and a LCDR3 of SEQ ID NO: 260; or
the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds a lymphocyte antigen to the subject for a time sufficient to treat the prostate cancer, wherein the antigen binding domain that binds a lymphocyte antigen comprises:
the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 250; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 252; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 253; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 254; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 157.

The disclosure also provides a method of treating breast cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds a lymphocyte antigen to the subject for a time sufficient to treat the breast cancer, wherein the antigen binding domain that binds a lymphocyte antigen comprises:
the HCDR1 of SEQ ID NO: 116, the HCDR2 of SEQ ID NO: 117, the HCDR3 of SEQ ID NO: 118, the LCDR1 of SEQ ID NO: 119, the LCDR2 of SEQ ID NO: 120 and the LCDR3 of SEQ ID NO: 121;
the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and a LCDR3 of SEQ ID NO: 260; or
the HCDR1 of SEQ ID NO: 255, the HCDR2 of SEQ ID NO: 256, the HCDR3 of SEQ ID NO: 257, the LCDR1 of SEQ ID NO: 258, the LCDR2 of SEQ ID NO: 259 and the LCDR3 of SEQ ID NO: 261.

The disclosure also provides a method of treating breast cancer in a subject, comprising administering a therapeutically effective amount of the multispecific protein comprising the antigen binding domain that binds a lymphocyte antigen to the subject for a time sufficient to treat the breast cancer, wherein the antigen binding domain that binds a lymphocyte antigen comprises:
the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 250; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 252; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 253; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 254; or
the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 157.

In one aspect, the present disclosure provides methods of preventing cancer, the methods comprising administering an amount of a lymphocyte expressing one or more of the CARs described to a subject in need thereof.

In one aspect, the present disclosure provides methods of treating a subject having cancer, the methods comprising administering a therapeutically effective amount of a lymphocyte expressing one or more of the CARs described to a subject in need thereof, whereby the lymphocyte induces or modulates killing of cancer cells in the subject.

In another aspect, the present disclosure provides methods of reducing tumor burden in a subject having cancer, the methods comprising administering a therapeutically effective amount of a lymphocyte expressing one or more of the CARs described herein to a subject in need thereof, whereby the lymphocyte induces killing of cancer cells in the subject. In another aspect, the present disclosure provides methods of increasing survival of a subject having cancer, the methods comprising administering a therapeutically effective amount of a lymphocyte expressing one or more of the CARs described to a subject in need thereof, whereby the survival of the subject is lengthened. Generally, the lymphocytes expressing the CAR(s) induce killing of cancer cells in the subject and result in reduction or eradication of the tumors/cancer cells in the subject. A non-limiting list of cancers, inclusive of metastatic lesions, that can be targeted, includes prostate cancer and breast cancer, and combinations thereof. In one embodiment, the cancer being treated in a subject is prostate cancer and breast cancer.

In one aspect, the methods described herein are applicable to treatment of noncancerous conditions that are at risk of developing into a cancerous condition, such as, e.g., enlarged prostate, and high PSA levels in absence of diagnosed prostate cancer.

In one aspect, methods of treating a subject having cancer are provided that comprise administering a therapeutically effective amount of a lymphocyte expressing a CAR, the CAR having an extracellular antigen-binding domain that binds the hK2 antigen, to a subject in need thereof, whereby the lymphocyte induces killing of cancer cells in the subject. In some embodiments, the at least one of the CARs comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 46-61.

In one aspect, a method of targeted killing of a cancer cell is disclosed, the method comprising contacting the cancer cell with a lymphocyte expressing one or more of the CARs described, whereby the lymphocyte induces killing of the cancer cell. A non-limiting list of cancer cells, inclusive of metastatic cancer cells, that can be targeted include prostate cancer, and combinations thereof. In one embodiment, the cancer cell is a prostate cancer cell.

When a therapeutically effective amount is indicated, the precise amount of the CARs or CAR-Ts of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the subject. It can generally be stated that a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of about 10^4 to about 10^{10} cells/kg body weight, in some instances about 10^5 to about 10^6 cells/kg body weight, including all integer values within those ranges. In some embodiments, a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of about 10^6 cells/kg body weight. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., *New Eng. J. of Med.* 319:1676, 1988).

Delivery systems useful in the context of the CAR-T of the invention may include time-released, delayed release, and sustained release delivery systems such that the delivery of the T cell compositions occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the composition, thereby increasing convenience to the subject and the physician, and may be particularly suitable for certain composition embodiments of the invention.

Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polyesteramides, polyorthoesters, polycaprolactones, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Delivery systems also include non-polymer systems that are lipids including sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di- and tri-glycerides; sylastic systems; peptide based systems; hydrogel release systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a)

erosional systems in which the active composition is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775; 4,667,014; 4,748,034; and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480 and 3,832,253. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

In certain aspects, it may be desirable to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate the T cells according to the present disclosure, and reinfuse the subject with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain aspects, T cells can be activated from blood draws of from 10 cc to 400 cc. In certain aspects, T cells are activated from blood draws of 20 cc, 30 cc, 40 cc, 50 cc, 60 cc, 70 cc, 80 cc, 90 cc, or 100 cc.

The administration of the CAR-T cells and compositions may be carried out in any manner, e.g., by parenteral or nonparenteral administration, including by aerosol inhalation, injection, infusions, ingestion, transfusion, implantation or transplantation. For example, the CAR-T cells and compositions described herein may be administered to a patient trans-arterially, intradermally, subcutaneously, intratumorally, intramedullary, intranodally, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one aspect, the compositions of the present disclosure are administered by i.v. injection. In one aspect, the compositions of the present disclosure are administered to a subject by intradermal or subcutaneous injection. The compositions of T cells may be injected, for instance, directly into a tumor, lymph node, tissue, organ, or site of infection.

Administration can be autologous or non-autologous. For example, immunoresponsive cells expressing a human Kallikrein-2 (e.g., hK2)-specific CAR can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived T cells of the present disclosure, or expanded T cells (e.g., *in vivo, ex vivo* or *in vitro* derived) can be administered via, e.g., intravenous injection, localized injection, systemic injection, catheter administration, or parenteral administration.

In particular embodiments, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted *ex vivo* to select and/or isolate the cells of interest, e.g., T cells. These T cell isolates may be expanded by methods known in the art and treated such that one or more CAR constructs of the present disclosure may be introduced, thereby creating a CAR-T cell. Subjects in need thereof may subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain aspects, following or concurrent with the transplant, subjects receive an infusion of the expanded CAR-T cells. In one aspect, expanded cells are administered before or following surgery.

The dosage administered to a patient having a malignancy is sufficient to alleviate or at least partially arrest the disease being treated ("therapeutically effective amount"). The dosage of the above treatments to be administered to a subject will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to practices generally accepted in the art.

The CAR T cells of the invention can undergo *in vivo* T cell expansion and can establish hK2-specific memory cells that persist at high levels for an extended amount of time in blood and bone marrow. In some instances, the CAR T cells of the invention infused into a subject can eliminate cancer cells, e.g., prostate cancer cells, *in vivo* in subjects with advanced chemotherapy-resistant cancer.

In one embodiment, a CAR of the present disclosure is introduced into T cells, e.g., using *in vitro* transcription, and the subject (e.g., human) receives an initial administration of CAR-T cells of the disclosure, and one or more subsequent administrations of the CAR-T cells, wherein the one or more subsequent administrations are administered less than 15 days, e.g., 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration. In one embodiment, more than one administration of the CAR-T cells are administered to the subject (e.g., human) per week, e.g., 2, 3, or 4 administrations of the CAR-T cells are administered per week. In one embodiment, the subject receives more than one administration of the CAR-T cells per week (e.g., 2, 3 or 4 administrations per week) (also referred to herein as a cycle), followed by a week of no CAR-T cell administrations, and then one or more additional administration of the CAR-T cells (e.g., more than one administration of the CAR-T cells per week) is administered to the subject. In another embodiment, the subject receives more than one cycle of CAR-T cells, and the time between each cycle is less than 10, 9, 8, 7, 6, 5, 4, or 3 days. In one embodiment, the CAR-T cells are administered every other day for 3 administrations per week. In one embodiment, the CAR-T cells are administered for at least two, three, four, five, six, seven, eight or more weeks.

In one embodiment, administration may be repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, two months, three months, four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration may be at the same dose or at a different dose.

The CAR-T cells may be administered in the methods of the invention by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

In one embodiment, CAR-T cells are generated using lentiviral viral vectors, such as lentivirus. CAR-T cells generated with such viral vectors will generally have stable CAR expression.

In one embodiment, CAR-T cells transiently express CAR vectors for 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days after transduction. Transient expression of CARs can be affected by RNA CAR vector delivery. In one embodiment, the CAR RNA is transduced into the T cell by electroporation.

If a patient is at high risk of generating an anti-CAR antibody response during the course of transient CAR therapy (such as those generated by RNA transductions), CAR-T infusion breaks should not last more than ten to fourteen days.

Combination therapies

The hK2 binding proteins of the disclosure may be administered in combination with at least one additional therapeutics.

In some embodiments the at least one additional therapeutic is surgery, chemotherapy, androgen deprivation therapy or radiation, or any combination thereof.

In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. I n some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

In one embodiment, other therapeutic agents such as factors may be administered before, after, or at the same time (simultaneous with) as the hK2 binding proteins such as CAR-T cells, including, but not limited to, interleukins, as well as colony stimulating factors, such as G-, M- and GM-CSF, and interferons.

The hK2 binding proteins such as CAR-expressing cell described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the hK2 binding proteins such as CAR-expressing cell

described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

In further embodiments, the hK2 binding proteins such as CAR-expressing cells described herein may be used in a treatment regimen in combination with surgery, radiation, chemotherapy, immunosuppressive agents, antibodies, or other immunoablative agents. In another embodiment, the hK2 binding proteins such as CAR-expressing cell described herein can be used in combination with an anti-androgen treatment. In one embodiment, the subject can be administered an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule.

BISPECIFIC EMBODIMENTS

Provided below are embodiments of bispecific hk2/CD3 antibodies of the present invention.

KLCB91

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 275, 258, 259 and 261, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

KLCB105

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 63, 65, 66, 67, 69 and 71, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 136, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123. According to an

embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 351, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

KLCB95

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

KLCB96

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 141, 142, 143, 144, 145 and 146, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 134, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 352, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

KLCB170

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively. According to an embodiment, the first binding

domain that binds hK2 comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

KLCB80

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360.

KLCB81

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 183, 184, 185, 186 and 187, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 166 and the VL of SEQ ID NO: 444, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 355 a LC1 of SEQ ID NO: 223 and a HC2 of SEQ ID NO: 360.

KLCB113

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 176, 177, 178, 179, 180 and 181, respectively, and the second domain that binds the lymphocyte antigen

comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 164 and the VL of SEQ ID NO: 165, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 356, a LC1 of SEQ ID NO: 222 and a HC2 of SEQ ID NO: 360.

KLCB281

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 188, 189, 190, 191, 192 and 193, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 325, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 353, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

KLCB174

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 116, 117, 118, 119, 120 and 121, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 122 and a VL of SEQ ID NO: 123. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 359 and a LC2 of SEQ ID NO: 272.

KLCB153

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein the first binding domain that binds hK2

comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 206, 207, 208, 182, 470, 209, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259, and 261, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a scFv of SEQ ID NO: 316, and the second binding domain that binds the lymphocyte antigen comprises a VH of SEQ ID NO: 248 and a VL of SEQ ID NO: 151. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 357, a HC2 of SEQ ID NO: 358 and a LC2 of SEQ ID NO: 267.

KLCB245

According to an embodiment, an isolated anti-hK2/anti-CD3 protein comprises a first domain that binds hK2 and a second domain that binds CD3, wherein The first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively. According to an embodiment, the first binding domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second binding domain that binds the lymphocyte antigen comprises a scFv of SEQ ID NO: 331. According to an embodiment, the isolated anti-hK2/anti-CD3 protein comprises a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

The following examples are provided to further describe some of the embodiments disclosed herein. The examples are intended to illustrate, not to limit, the disclosed embodiments.

NUMBERED EMBODIMENTS

The present disclosure also provides the following numbered embodiments:

1. An isolated protein comprising an antigen binding domain that binds kallikrein related peptidase 2 (hK2), wherein said antigen binding domain comprises the HCDR1, the HCDR1, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively.

2. The isolated protein of embodiment 1, wherein the antigen binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VH of SEQ ID NO: 162.
3. The isolated protein of embodiment 2, wherein the antigen binding domain that binds hK2 comprises a VH of SEQ ID NO: 162.
4. The isolated protein of any one of embodiments 1-3, wherein the antigen binding domain that binds hK2 comprises a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VL of SEQ ID NO: 163.
5. The isolated protein of embodiment 4, wherein the antigen binding domain that binds hK2 comprises a VL of SEQ ID NO: 163.
6. The isolated protein of any one of embodiments 1-5, wherein the antigen binding domain that binds hK2 is a Fab.
7. The isolated protein of any one of embodiments 1-6, wherein the protein is conjugated to a half-life extending moiety, wherein the half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, or a fragment of the Ig constant region.
8. The isolated protein of embodiment 7, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR), optionally wherein the mutations that results in reduced binding of the protein to the FcγR are L234A_L235A_D265S.
9. The isolated protein of embodiment 7 or 8, wherein the protein comprises at least one mutation in a CH3 domain of the Ig constant region, optionally wherein the mutations in the CH3 domain of the Ig constant region are T350V_T366L_K392L_T394W.
10. The isolated protein of any one of embodiments 7-9, wherein the IgG heavy chain constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the amino acid sequence of SEQ ID NO: 378.
11. The isolated protein of embodiment 9, wherein the IgG heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 378.
12. The isolated protein of any one of embodiments 7-11, wherein the IgG light chain constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the amino acid sequence of SEQ ID NO: 309.
13. The isolated protein of embodiments 12, wherein the IgG light chain constant region comprises the amino acid sequence of SEQ ID NO: 309.

14. The isolated protein of any one of embodiments 1-13, wherein the isolated protein comprises a HC which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to SEQ ID NO: 354.
15. The isolated protein of embodiment 14, wherein the isolated protein comprises a HC of SEQ ID NO: 354.
16. The isolated protein of any one of embodiments 1-15, wherein the isolated protein comprises a LC which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to SEQ ID NO: 221.
17. The isolated protein of embodiment 16, wherein the isolated protein comprises a LC of SEQ ID NO: 221.
18. The isolated protein of any one of embodiments 1-17, wherein the isolated protein is a multispecific protein.
19. The isolated protein of embodiment 18, wherein the multispecific protein is a bispecific protein.
20. The multispecific protein of embodiment 18 or 19, comprising an antigen binding domain that binds an antigen on a lymphocyte.
21. The multispecific protein of embodiment 20, wherein the lymphocyte is a T cell.
22. The multispecific protein of embodiment 21, wherein the T cell is a $CD8^+$ T cell.
23. The multispecific protein of embodiment 22, wherein the lymphocyte is a natural killer (NK) cell.
24. The multispecific protein of any one of embodiments 20-23, wherein the antigen on the lymphocyte is CD3, CD3 epsilon (CD3ε), CD8, KI2L4, NKG2E, NKG2D, NKG2F, BTNL3, CD186, BTNL8, PD-1, CD195, or NKG2C.
25. The multispecific protein of embodiment 24, wherein the wherein the antigen on the lymphocyte is CD3ε.
26. The multispecific protein of embodiment 25, wherein the antigen binding domain that binds CD3ε comprises
    a. a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 116, a HCDR2 of SEQ ID NO: 117, a HCDR3 of SEQ ID NO: 118, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 119, a LCDR2 of SEQ ID NO: 120 and a LCDR3 of SEQ ID NO: 121; or
    b. the VH of SEQ ID NO: 122 and the VL of SEQ ID NO: 123.
27. The multispecific protein of embodiment 25, wherein the antigen binding domain that binds CD3ε comprises the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 157.
28. The multispecific protein of embodiment 25, wherein the antigen binding domain that binds CD3ε comprises

a. a heavy chain complementarity determining region 1 (HCDR1) of SEQ ID NO: 255, a HCDR2 of SEQ ID NO: 256, a HCDR3 of SEQ ID NO: 257, a light chain complementarity determining region 1 (LCDR1) of SEQ ID NO: 258, a LCDR2 of SEQ ID NO: 259 and a LCDR3 of SEQ ID NO: 260; or
b. the HCDR1 of SEQ ID NO: 255, a HCDR2 of SEQ ID NO: 256, a HCDR3 of SEQ ID NO: 257, a LCDR1 of SEQ ID NO: 258, a LCDR2 of SEQ ID NO: 259 and a LCDR3 of SEQ ID NO: 261; or
c. the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 249; or
d. the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 250; or
e. the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 251; or
f. the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 252; or
g. the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 253; or
h. the VH of SEQ ID NO: 248 and the VL of SEQ ID NO: 254.

29. The multispecific protein of any one of embodiments 25, wherein the antigen binding domain that binds CD3ε comprises a HCDR1 of SEQ ID NO: 255, a HCDR2 of SEQ ID NO: 256, a HCDR3 of SEQ ID NO: 257, a LCDR1 of SEQ ID NO: 258, a LCDR2 of SEQ ID NO: 259 and a LCDR3 of SEQ ID NO: 261.
30. The multispecific protein of embodiment 29, wherein the antigen binding domain that binds CD3ε comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VH of SEQ ID NO: 248.
31. The multispecific protein of embodiment 30, wherein the antigen binding domain that binds CD3ε comprises the VH of SEQ ID NO: 248.
32. The multispecific protein of any one of embodiments 29-31, wherein the antigen binding domain that binds CD3ε comprises a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VL of SEQ ID NO: 251.
33. The multispecific protein of embodiment 32, wherein the antigen binding domain that binds CD3ε comprises the VL of SEQ ID NO: 251.
34. The multispecific protein of any one of embodiments 25-33, wherein the antigen binding domain that binds CD3ε is a scFv.
35. The multispecific protein of embodiment 34, wherein the scFv is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the scFv of SEQ ID NO: 331.
36. The multispecific protein of embodiment 35, wherein the scFv is the scFv of SEQ ID NO: 331.
37. The multispecific protein of any one of embodiments 25-36, wherein the antigen binding domain that binds an antigen on a lymphocyte is conjugated to a half-life extending moiety, wherein the

half-life extending moiety is an immunoglobulin (Ig), a fragment of the Ig, an Ig constant region, or a fragment of the Ig constant region.

38. The multispecific protein of embodiment 37, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fcγ receptor (FcγR), optionally wherein the mutations that results in reduced binding of the protein to the FcγR are L234A_L235A_D265S.
39. The multispecific protein of embodiment 37 or 38, wherein the protein comprises at least one mutation in a CH3 domain of the Ig constant region, optionally wherein the mutations in the CH3 domain of the Ig constant region are T350V_L351Y_F405A_Y407V.
40. The multispecific protein of any one of embodiments 37-39, wherein the IgG constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the amino acid sequence of SEQ ID NO: 109.
41. The multispecific protein of embodiment 40, wherein the IgG constant region comprises the amino acid sequence of SEQ ID NO: 109.
42. The multispecific protein of any one of embodiments 25-41, wherein the protein comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the amino acid sequence of SEQ ID NO: 360.
43. The multispecific protein of embodiment 42, wherein the protein comprises an amino acid sequence of SEQ ID NO: 360.
44. The multispecific protein of any one of embodiments 25-43, wherein the antigen binding domain binds CD3 within epitopes having sequences of SEQ ID NO: 341, 448, and 449.
45. An isolated anti-hK2/anti-CD3 protein, wherein the first binding domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the lymphocyte antigen comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.
46. The isolated anti-hK2/anti-CD3 protein of embodiment 45, wherein the first binding domain that binds hK2 comprises a VH which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VH of SEQ ID NO: 162.
47. The isolated anti-hK2/anti-CD3 protein of embodiment 46, wherein the first binding domain that binds hK2 comprises the VH of SEQ ID NO: 162.
48. The isolated anti-hK2/anti-CD3 protein of any one of embodiments 45-47, wherein the first binding domain that binds hK2 comprises a VL which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VL of SEQ ID NO: 163.

49. The isolated anti-hK2/anti-CD3 protein of embodiment 48, wherein the first binding domain that binds hK2 comprises the VL of SEQ ID NO: 163.
50. The isolated anti-hK2/anti-CD3 protein of any one of embodiments 45-49, wherein the second binding domain that binds the lymphocyte antigen comprises a scFv which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the scFv of SEQ ID NO: 331.
51. The isolated anti-hK2/anti-CD3 protein of embodiment 50, wherein the second binding domain that binds the lymphocyte antigen comprises the scFv of SEQ ID NO: 331.
52. The isolated anti-hK2/anti-CD3 protein of any one of embodiments 45-51, wherein the isolated multispecific protein comprises a HC1 which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC1 of SEQ ID NO: 354.
53. The isolated anti-hK2/anti-CD3 protein of embodiment 52, wherein the isolated multispecific protein comprises the HC1 of SEQ ID NO: 354.
54. The isolated anti-hK2/anti-CD3 protein of any one of embodiments 45-53, wherein the isolated multispecific protein comprises a LC1 which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the LC1 of SEQ ID NO: 221.
55. The isolated anti-hK2/anti-CD3 protein of embodiment 54, wherein the isolated multispecific protein comprises the LC1 of SEQ ID NO: 221.
56. The isolated anti-hK2/anti-CD3 protein of any one of embodiments 45-55, wherein the isolated multispecific protein comprises a HC2 which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC2 of SEQ ID NO: 360.
57. The isolated anti-hK2/anti-CD3 protein of embodiment 56, wherein the isolated multispecific protein comprises the HC2 of SEQ ID NO: 360.
58. The isolated anti-hK2/anti-CD3 protein of any one of embodiments 52-57, wherein the isolated multispecific protein comprises a lysine (e.g. K477) at the C-terminus of HC1 and HC2.
59. The isolated anti-hK2/anti-CD3 protein of embodiment 58, wherein the the isolated multispecific protein comprises a HC1 which comprises is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC1 of SEQ ID NO: 361.
60. The isolated anti-hK2/anti-CD3 protein of embodiment 59, wherein the the isolated multispecific protein comprises the HC1 of SEQ ID NO: 361.
61. The isolated anti-hK2/anti-CD3 protein of any one of embodiments 58-60, wherein the the isolated multispecific protein comprises a HC2 which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC2 of SEQ ID NO: 362.

62. The isolated anti-hK2/anti-CD3 protein of embodiment 61, wherein the the isolated multispecific protein comprises the HC2 of SEQ ID NO: 362.

EXAMPLES

Example 1. Generation of anti-hK2 antibodies and scFvs

Antibody generation from humanization of parental m11B6 antibody.

A parental mouse anti-HK2 antibody, m11B6 has been described in Väisänen et al (Clinical Chemistry 50:9, 1607–1617 (2004)). Humanized 11B6 (referred herein to as hu11B6) has been generated and described in U.S. Pat. No. 9,345,782 and U.S. Pat. No. 10,100,125.

Engineering of hu11B6 were initiated to generate additional anti-HK2 antibodies with improved properties, such as improved thermostability. Residue positions were identified in hu11B6 frameworks which could potentially be altered to improve thermostability of hu11B6 using modeling. The positions identified were residues P41, I49, M70, and A88 in the VH and S80, L82, A88 and Y91 in the VL (residue numbering according to the amino acid sequences of hu11B6_VH of **SEQ ID NO: 5** and hu11B6_VL of **SEQ ID NO: 2**).

Binary combinatorial scFv libraries were generated in the orientation VH-linker-VL in which one of the variable regions represented the combinatorial library and the second one being the parental hu11B6 VH or VL. Linker sequence of **GGSEGKSSGSGSESKSTGGS (SEQ ID NO: 7)** was used to conjugate the VH/VL regions. The engineered scFvs were expressed in E *coli* and the produced scFvs in the supernatants were tested for binding to human hK2 by ELISA and compared to the binding of hu11B6. Any new variants exhibiting binding comparable to hu11B6 were consolidated and further tested for binding to human hK2 after incubation of the supernatants at 55°C, 60°C, and 65°C for 10 minutes. The molecules which retained comparable binding to hu11B6 after incubation at 55°C, 60°C, and 65°C and improved thermostability were matrixed in both orientations (VH-linker-VL; VL-linker-VH) and converted to mammalian scFvs for further characterization. The matrixed scFvs were also incorporated into CAR constructs and further characterized as further described in Example 11.

In addition, another humanization of parental mouse 11B6 was performed following the approach outlined by Singh et al (MAbs. 2015;7(4):778-91). with extensive germ line variation and careful screening of the variants for enhanced thermal stability. Based on sequence conservation, the human heavy chain germline IGHV4-30 and the light chain germline IGKV3D-11, were chosen for framework adaption. A binary scFv library was constructed with residues comprising a select set of somatic hypermutation sites and mouse/human germline variations. The variants were cloned and expressed in E. coli as described above. The supernatants were screened at different temperatures in single point ELISA for enhanced thermal stability. A mouse/human chimeric 11B6 scFv was used as parental control. Clone KL2B359 which maintained binding activity similar to murine 11B6 and a Tm value of 67°C was converted to scFv-Fc and CAR-T for additional profiling. Measured affinity (K_D) of KL2B359 to hK2 by

SPR was ~0.7 – 1nM. HCF3-LCD6, HCG5-LCB7, KL2B357, KL2B358 and KL2B360 also resulted from this campaign and were further characterized for functionality.

Antibody generation using transgenic mice (Ablexis®) and transgenic rats (OmniRat®) expressing human immunoglobulin loci.

The OmniRat® contains a chimeric human/rat IgH locus (comprising 22 human V_Hs, all human D and J_H segments in natural configuration linked to the rat C_H locus) together with fully human IgL loci (12 Vκs linked to Jκ-Cκ and 16 Vλs linked to Jλ-Cλ). (see e.g., Osborn, et al. (2013) J Immunol 190(4): 1481-1490). Accordingly, the rats exhibit reduced expression of rat immunoglobulin, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity chimeric human/rat IgG monoclonal antibodies with fully human variable regions. The preparation and use of OmniRat®, and the genomic modifications carried by such rats, is described in WO14/093908.

Ablexis® mice generate antibodies having human variable domains linked to human CH1 and CL domains, chimeric human/mouse hinge region, and mouse Fc regions. Ablexis Kappa Mouse and Lambda Mouse strains are distinguished by which of their heavy chains are human or mouse as noted below. Antibodies produced by the Kappa Mouse lack sequence derived from mouse V_H, D_H and J_H exons and mouse Vκ, Jκ and Cκ exons. The endogenous mouse Igλ is active in the Kappa Mouse. The human Igκ chains comprise approximately 90-95% of the naïve repertoire and mouse Igλ chains comprise approximately 5-10% of the naïve repertoire in this strain. Antibodies produced by the Lambda Mouse lack sequence derived from mouse V_H, D_H and J_H exons and mouse Vλ, Jλ and Cλ exons. The endogenous mouse Igκ is active in the Lambda Mouse. The human Igλ chains comprise approximately 40% of the naïve repertoire and mouse Igκ chains comprise approximately 60% of the naïve repertoire. The preparation and use of Ablexis®, and the genomic modifications carried by such mice, is described in WO11/123708.

Ablexis mice and OmniRats rats were immunized with soluble full length KLK2 protein (human Kallikrein-2 6-His protein).

human Kallikrein-2 6-His protein

VPLIEGRIVGGWECEKHSQPWQVAVYSHGWAHCGGVLVHPQWVLTAAHCLKKNSQVWLGRH
NLFEPEDTGQRVPVSHSFPHPLYNMSLLKHQSLRPDEDSSHDLMLLRLSEPAKITDVVKVLGLPT
QEPALGTTCYASGWGSIEPEEFLRPRSLQCVSLHYSEKVTEFMLCAGLWTGGKDTCGGDSGGPL
VCNGVLQGITSWGPEPCALPEKPAVYTKVVHYRKWIKDTIAANPHHHHHH (SEQ ID NO: 454)

Lymphocytes from Ablexis mice and OniRats rats were extracted from lymph nodes and fusions performed by cohorts. Cells were combined and sorted for CD138 expression. Hybridoma screening was performed in high throughput miniaturized MSD format using soluble hK2 antigen. Approximately >300 samples were identified to be hK2 binders. The binding of >300 anti-hKLK2 supernatant samples to human KLK2 protein was measured by single cycle kinetics method by Biacore 8K SPR. Additionally the supernatant samples were tested for binding to human KLK3 protein as well. In parallel, supernatants were also tested for binding to KLK2 expressing cell lines VCap and negative cell line DU145 by Flow Cytometry. Selected cell binders were moved forward to scFv conversion in both VH-VL and VL/VH orientation and thermal stability tests as described above. KL2B413, KL2B30, KL2B53 and KL2B242 resulted from the Ablexis mice immunization campaign. KL2B467 and KL2B494 resulted from the OmniRat immunization campaign.

Antibodies generated through the various immunization and humanization campaigns described above were expressed in a fab format, a mAb format, a scFv format in the VH-linker-VL orientation or a scFv format in VL-linker-VH orientation and were further analyzed as described below. The linker sequence of SEQ ID NO: 7 described above was used to conjugate the VH/VL regions.

Example 2. Structural characterization of anti KLK2 antibodies

Sequences of antibody variable domains and scFv antibody fragments which showed highest performance in intracellular assay are provided herein. Variable domains were expressed in a Fab format, a scFv format in the VH-linker-VL orientation or a scFv format in VL-linker-VH orientation.

Variable domains VH, VL and CDRs

Table 3 shows the VH and VL amino acid sequences of selected anti-hK2 antibodies. **Table 4** shows the Kabat HCDR1, HCDR2 and HCDR3 of selected anti-hK2 selected antibodies. **Table 5** shows the Kabat LCDR1, LCDR2 and LCDR3 of the selected anti-hK2 antibodies. **Table 6** shows the AbM HCDR1, HCDR2 and HCDR3 of selected anti-hK2 antibodies. **Table 7** shows the AbM LCDR1, LCDR2 and LCDR3 of the anti-hK2. **Table 8** summarizes the variable domain sequence and SEQ ID NO of selected hK2 antibodies. **Table 9** shows the protein and DNA SEQ ID NOs for the VH and VL regions.

Table 3. VH and VL amino acid sequences of selected anti-hK2 antibodies.

Ab name	VH name	VH amino acid Sequence	VH SEQ ID NO:	VL name	VL amino acid sequence	VL SEQ ID NO:
m11B6	m11B6_VH	DVQLQESGPGLVKPSQSLSLTCTVTGNSITSDYAWNWIRQFPGNRLEWMGYISYSGSTTYSPSLKSRFSITRDTSKNQFFLQLNSVTPEDTATYFCATGYYYGSGFWGQGTLVTVSS	125	m11B6_VL	DIVLTQSPASLAVSLGQRATISCRASESVEYFGTSLMHWYRQKPGQPPKLLIYAASNVESGVPARFSGSGSGTDFSLNIQPVEEDDFSMYFCQQTRKVPYTFGGGTKLEIK	124
h11B6	hu11B6_VH	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWGQGTLVTVSS	5	hu11B6_VL	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEIK	2
HCF3-LCD6	HCF3_VH	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSS	6	LCD6_VL	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEIK	1
HCG5-LCB7	HCG5_VH	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGY	4	LCB7_VL	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTR	3

		YYGSGFWGQGTLVTVSS			KVPYTFGQGTKLEIK	
KL2B357	KL2B357_VH	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSS	159	KL2B357_VL	DIVLTQSPDSLAVSLGERATINCRASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNVESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQTRKVPYTFGGGTKVEIK	160
KL2B358	KL2B358_VH	QVQLQESGPGLVPSQTLSLTCTVSGNTSDYAWNWIRQPPKGLEWIGYISYSGSTYNPSLKSRVTISRTSKNQFSLKLSSVTADTAVYYCATGYYGSGFWGQGTLVTSS	161	KL2B358_VL	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIK	140
KL2B359	KL2B359_VH	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKRLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSS	139	KL2B359_VL	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIK	140
KL2B360	KL2B360_VH	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAA	159	KL2B360_VL	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPE	140

		DTAVYYCATGYY YGSGFWGQGTLV TVSS			DFAVYFCQQTRKV PYTFGGGTKVEIK	
KL2B413	KL2B413_ VH	EVQLVESGGGLV QPGGSLRLSCAAS GFTFSSYWMTWV RQAPGKGLEWV ANIKQDGSERYY VDSVKGRFTISRD NAKNSLYLQMNS LRAEDTAVYYCA RDQNYDILTGHY GMDVWGQGTTV TVSS	137	KL2B413_ VL	EIVLTQSPSFLSASV GDRVTITCRASQGI SSYLSWYQQKPGK APKLLIYATSTLQS GVPSRFSGSGSGTE FTLTISSLQPEDFAT YYCQQLNSYPRTF GQGTKVEIK	138
KL2B30	KL2B30_V H	QVQLQESGPGLV KPSETLSLTCTVS GGSISSYYWSWIR QPPGKGLEWIGYI YYSGSTNYNPSL KSRVTISVDTSKN QFSLKLSSVTAAD TAVYYCAGTTIF GVVTPNFYYGMD VWGQGTTVTVSS	162	KL2B30_V L	DIQMTQSPSFLSAS VGDRVTITCRASQG ISSYLAWYQQKPG KAPKFLIYAASTLQ SGVPSRFSGSGSGT EFTLTISSLQPEDFA TYYCQQLNSYPLTF GGGTKVEIK	163
KL2B53	KL2B53_V H	EVQLVESGGGVV QPGRSLRLSCVAS GFTFSSYDIHWVR QAPGKGLEWVAI ISYDGSKKDYTDS VKGRFTISRDNSK NTLYLQMDSLRV ED SAVYSCARES GWSHYYYYGMD VWGQGTMVTVS S	164	KL2B53_V L	DIVMTQSPSSLSAS VGDRVTITCRASQD ISNYLAWYQQKPG KVPKFLIYAASTLH SGVPSRFSGSGSGT DFTLTISSLQPEDVA TYYCQKYNSAPYT FGQGTRLEIK	165

KL2B242	KL2B242_VH	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQPAGSGLEWIGRLYVSGFTNYNPSLKSRVTLSLDPSRNQLSLKLSSVTAADTAVYYCAGDSGNYWGWFDPWGQGTLVTVSS	166	KL2B242_VL	SYELTQPPSVSVSPGETASITCSGDQLGENYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLTISGTQALDEADYYCQAWDNSIVVFGGGTKLTVL	167
KL2B467	KL2B467_VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSYYGMHWVRQAPGKGLEWVAFISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSYWAFDYWGQGTQVTVSS	168	KL2B467_VL	QSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQQKPGQAPVLVVYDNSDRPSGIPERFSGSNSGTTATLTISRVEAGDEADYYCQVWDSSSDHPVVFGGGTKVTV	169
KL2B494	KL2B494_VH	QVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMSWVRQAPGKGLEWVSTIGGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTALLYDGMDVWGQGTMVTVSS	204	KL2B494_VL	SSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVL	205
KL2B242LC_C33S	KL2B242_VH	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQPAGSGLEWIGRLYVSGFTNYNPSLKSRVTLSLDPSRNQLSLKLSSVTAADTAVYYCAGDSGNYWGWFDPWGQGTLVTVSS	166	KL2B242LC_C33S_VL	SYELTQPPSVSVSPGETASITCSGDQLGENYASWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLTISGTQALDEADYYCQAWDNSIVVFGGGTKLTVL	444

Table 4. Kabat HCDR1, HCDR2 and HCDR3 amino acid sequences of selected anti-KLK2 antibodies.

	Kabat HCDR1		Kabat HCDR2		Kabat HCDR3	
Ab name	Sequence	SEQ ID NO	Sequence	SEQ ID NO	Sequence	SEQ ID NO
m11B6	SDYAWN	63	YISYSGSTTYSPSLKS	64	GYYYGSGF	66
hu11B6	SDYAWN	63	YISYSGSTTYNPSLKS	65	GYYYGSGF	66
HCF3-LCD6	SDYAWN	63	YISYSGSTTYNPSLKS	65	GYYYGSGF	66
HCG5-LCB7	SDYAWN	63	YISYSGSTTYNPSLKS	65	GYYYGSGF	66
KL2B357	SDYAWN	63	YISYSGSTTYNPSLKS	65	GYYYGSGF	66
KL2B358	SDYAWN	63	YISYSGSTTYNPSLKS	65	GYYYGSGF	66
KL2B359	SDYAWN	63	YISYSGSTTYNPSLKS	65	GYYYGSGF	66
KL2B360	SDYAWN	63	YISYSGSTTYNPSLKS	65	GYYYGSGF	66
KL2B413	SYWMT	141	NIKQDGSERYYVDSVKG	142	DQNYDILTGHYGMDV	143
KL2B30	SYYWS	170	YIYYSGSTNYNPSLKS	171	TTIFGVVTPNFYYGMDV	172
KL2B53	SYDIH	176	IISYDGSKKDYTDSVKG	177	ESGWSHYYYYGMDV	178
KL2B242	SYYWS	170	RLYVSGFTNYNPSLKS	183	DSGNYWGWFDP	184
KL2B467	YYGMH	188	FISYDGSNKYYADSVKG	189	LPYSGSYWAFDY	190
KL2B494	HYAMS	206	TIGGSGGSTYYADSVKG	207	PHIVMVTALLYDGMDV	208

Table 5. Kabat LCDR1, LCDR2 and LCDR3 amino acid sequences of selected anti-hK2 antibodies.

	Kabat LCDR1		Kabat LCDR2		Kabat LCDR3	
Ab name	Sequence	SEQ ID NO	Sequence	SEQ ID NO	Sequence	SEQ ID NO
m11B6	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71

hu11B6	KASESVEYFGTSLMH	68	AASNRES	70	QQTRKVPYT	71
HCF3-LCD6	KASESVEYFGTSLMH	68	AASNRES	70	QQTRKVPYT	71
HCG5-LCB7	KASESVEYFGTSLMH	68	AASNRES	70	QQTRKVPYT	71
KL2B357	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
KL2B358	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
KL2B359	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
KL2B360	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
KL2B413	RASQGISSYLS	144	ATSTLQS	145	QQLNSYPRT	146
KL2B30	RASQGISSYLA	173	AASTLQS	174	QQLNSYPLT	175
KL2B53	RASQDISNYLA	179	AASTLHS	180	QKYNSAPYT	181
KL2B242	SGDQLGENYAC	185	QDSKRPS	186	QAWDNSIVV	187
KL2B467	GGDNIGSKSVH	191	DNSDRPS	192	QVWDSSSDHPVV	193
KL2B494	GGNNIGSKSVH	182	DDSDRPS	470	QVWDSSSDHVV	209

Table 6. AbM HCDR1, HCDR2 and HCDR3 amino acid sequences of selected anti-hK2 antibodies.

	AbM HCDR1		AbM HCDR2		AbM HCDR3	
Ab name	Sequence	SEQ ID NO	Sequence	SEQ ID NO	Sequence	SEQ ID NO
m11B6	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66
hu11B6	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66
HCF3-LCD6	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66
HCG5-LCB7	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66
KL2B357	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66
KL2B358	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66

KL2B359	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66
KL2B360	GNSITSDYAWN	72	YISYSGSTT	73	GYYYGSGF	66
KL2B413	GFTFSSYWMT	147	NIKQDGSERY	148	DQNYDILTGHYGMDV	143
KL2B30	GGSISSYYWS	194	YIYYSGSTN	195	TTIFGVVTPNFYYGMDV	172
KL2B53	GFTFSSYDIH	196	IISYDGSKKD	197	ESGWSHYYYYGMDV	178
KL2B242	GGSISSYYWS	198	RLYVSGFTN	199	DSGNYWGWFDP	184
KL2B467	GFTFSYY	200	FISYDGSNKY	201	LPYSGSYWAFDY	190
KL2B494	GFTFSHYAMS	216	TIGGSGGSTYY	217	PHIVMVTALLYDGMDV	218

Table 7. AbM LCDR1, LCDR2 and LCDR3 amino acid sequences of selected anti-hK2 antibodies.

	AbM LCDR1		AbM LCDR2		AbM LCDR3	
Ab name	Sequence	SEQ ID NO	Sequence	SEQ ID NO	Sequence	SEQ ID NO
m11B6	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
hu11B6	KASESVEYFGTSLMH	68	AASNRES	70	QQTRKVPYT	71
HCF3-LCD6	KASESVEYFGTSLMH	68	AASNRES	70	QQTRKVPYT	71
HCG5-LCB7	KASESVEYFGTSLMH	68	AASNRES	70	QQTRKVPYT	71
KL2B357	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71

KL2B358	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
KL2B359	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
KL2B360	RASESVEYFGTSLMH	67	AASNVES	69	QQTRKVPYT	71
KL2B413	RASQGISSYLS	144	ATSTLQS	145	QQLNSYPRT	146
KL2B30	RASQGISSYLA	173	AASTLQS	174	QQLNSYPLT	175
KL2B53	RASQDISNYLA	179	AASTLHS	180	QKYNSAPYT	181
KL2B242	SGDQLGENYAC	185	QDSKRPS	186	QAWDNSIVV	187
KL2B467	GGDNIGSKSVH	191	DNSDRPS	192	QVWDSSSDHPVV	193
KL2B494	GGNNIGSKSVH	182	DDSDRPS	470	QVWDSSSDHVV	209

Table 8. Amino acid sequences and SEQ ID NO summary of the variable domains of selected anti-hK2 antibodies

Antibody name	Region	Amino acid sequence	SEQ ID NO:
m11B6	HCDR1	SDYAWN	63
	HCDR2	YISYSGSTTYSPSLKS	64
	HCDR3	GYYYGSGF	66
	LICDR1	RASESVEYFGTSLMH	67
	LCDR2	AASNVES	69
	LCDR3	QQTRKVPYT	71
	VH (m11B6_VH)	DVQLQESGPGLVKPSQSLSLTCTVTGNSITSDYAWN WIRQFPGNRLEWMGYISYSGSTTYSPSLKSRFSITRD TSKNQFFLQLNSVTPEDTATYFCATGYYYGSGFWG QGTLVTVSS	125

	VL (m11B6_VL)	DIVLTQSPASLAVSLGQRATISCRASESVEYFGTSLM HWYRQKPGQPPKLLIYAASNVESGVPARFSGSGSG TDFSLNIQPVEEDDFSMYFCQQTRKVPYTFGGGTKL EIK	124
h11B6	HCDR1	SDYAWN	63
	HCDR2	YISYSGSTTYNPSLKS	65
	HCDR3	GYYYGSGF	66
	LICDR1	KASESVEYFGTSLMH	68
	LCDR2	AASNRES	70
	LCDR3	QQTRKVPYT	71
	VH (hu11B6_VH)	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQPPGKGLEWIGYISYSGSTTYNPSLKSRVTMSR DTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGF WGQGTLVTVSS	5
	VL (hu11B6_VL)	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKL EIK	2
HCF3-LCD6	HCDR1	SDYAWN	63
	HCDR2	YISYSGSTTYNPSLKS	65
	HCDR3	GYYYGSGF	66
	LICDR1	KASESVEYFGTSLMH	68
	LCDR2	AASNRES	70
	LCDR3	QQTRKVPYT	71
	VH (HCF3_VH)	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRD TSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG QGTLVTVSS	6
	VL (LCD6_VL)	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSG TDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKL EIK	1
	HCDR1	SDYAWN	63

HCG5-LCB7	HCDR2	YISYSGSTTYNPSLKS	65
	HCDR3	GYYYGSGF	66
	LICDR1	KASESVEYFGTSLMH	68
	LCDR2	AASNRES	70
	LCDR3	QQTRKVPYT	71
	VH (HCG5_VH)	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQFPGKGLEWMGYISYSGSTTYNPSLKSRVTISR DTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFW GQGTLVTVSS	4
	VL (LCB7_VL)	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSG TDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTK LEIK	3
KL2B357	HCDR1	SDYAWN	63
	HCDR2	YISYSGSTTYNPSLKS	65
	HCDR3	GYYYGSGF	66
	LICDR1	RASESVEYFGTSLMH	67
	LCDR2	AASNVES	69
	LCDR3	QQTRKVPYT	71
	VH (KL2B357_VH)	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRD TSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFW GQGTLVTVSS	159
	VL (KL2B_357_VL)	DIVLTQSPDSLAVSLGERATINCRASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNVESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYFCQQTRKVPYTFGGGTKV EIK	160
KL2B358	HCDR1	SDYAWN	63
	HCDR2	YISYSGSTTYNPSLKS	65
	HCDR3	GYYYGSGF	66
	LICDR1	RASESVEYFGTSLMH	67
	LCDR2	AASNVES	69
	LCDR3	QQTRKVPYT	71

	VH (KL2B358_VH)	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQPPGKGLEWIGYISYSGSTTYNPSLKSRVTISRD TSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFW GQGTLVTVSS	161
	VL (KL2B_358_VL)	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLM HWYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGT DFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEI K	140
KL2B359	HCDR1	SDYAWN	63
	HCDR2	YISYSGSTTYNPSLKS	65
	HCDR3	GYYYGSGF	66
	LICDR1	RASESVEYFGTSLMH	67
	LCDR2	AASNVES	69
	LCDR3	QQTRKVPYT	71
	VH (KL2B359_VH)	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQFPGKRLEWIGYISYSGSTTYNPSLKSRVTISRD TSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFW GQGTLVTVSS	139
	VL (KL2B_359_VL)	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLM HWYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGT DFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEI K	140
KL2B360	HCDR1	SDYAWN	63
	HCDR2	YISYSGSTTYNPSLKS	65
	HCDR3	GYYYGSGF	66
	LICDR1	RASESVEYFGTSLMH	67
	LCDR2	AASNVES	69
	LCDR3	QQTRKVPYT	71
	VH (KL2B360_VH)	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRD TSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFW GQGTLVTVSS	159

	VL (KL2B_360_VL)	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLM HWYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGT DFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEI K	140
KL2B413	HCDR1	SYWMT	141
	HCDR2	NIKQDGSERYYVDSVKG	142
	HCDR3	DQNYDILTGHYGMDV	143
	LICDR1	RASQGISSYLS	144
	LCDR2	ATSTLQS	145
	LCDR3	QQLNSYPRT	146
	VH (KL2B413_VH)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMT WVRQAPGKGLEWVANIKQDGSERYYVDSVKGRFT ISRDNAKNSLYLQMNSLRAEDTAVYYCARDQNYDI LTGHYGMDVWGQGTTVTVSS	137
	VL (KL2B_413_VL)	EIVLTQSPSFLSASVGDRVTITCRASQGISSYLSWYQ QKPGKAPKLLIYATSTLQSGVPSRFSGSGSGTEFTLT ISSLQPEDFATYYCQQLNSYPRTFGQGTKVEIK	138
KL2B30	HCDR1	SYYWS	170
	HCDR2	YIYYSGSTNYNPSLKS	171
	HCDR3	TTIFGVVTPNFYYGMDV	172
	LICDR1	RASQGISSYLA	173
	LCDR2	AASTLQS	174
	LCDR3	QQLNSYPLT	175
	VH (KL2B30_VH)	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSW IRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDT SKNQFSLKLSSVTAADTAVYYCAGTTIFGVVTPNFY YGMDVWGQGTTVTVSS	162
	VL (KL2B30_VL)	DIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWY QQKPGKAPKFLIYAASTLQSGVPSRFSGSGSGTEFTL TISSLQPEDFATYYCQQLNSYPLTFGGGTKVEIK	163
KL2B53	HCDR1	SYDIH	176
	HCDR2	IISYDGSKKDYTDSVKG	177
	HCDR3	ESGWSHYYYYGMDV	178

	LICDR1	RASQDISNYLA	179
	LCDR2	AASTLHS	180
	LCDR3	QKYNSAPYT	181
	VH (KL2B53_VH)	EVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHW VRQAPGKGLEWVAIISYDGSKKDYTDSVKGRFTISR DNSKNTLYLQMDSLRVED SAVYSCARESGWSHYYYYGMDVWGQGTMVTVSS	164
	VL (KL2B53_VL)	DIVMTQSPSSLSASVGDRVTITCRASQDISNYLAWY QQKPGKVPKFLIYAASTLHSGVPSRFSGSGSGTDFT LTISSLQPEDVATYYCQKYNSAPYTFGQGTRLEIK	165
KL2B242	HCDR1	SYYWS	170
	HCDR2	RLYVSGFTNYNPSLKS	183
	HCDR3	DSGNYWGWFDP	184
	LICDR1	SGDQLGENYAC	185
	LCDR2	QDSKRPS	186
	LCDR3	QAWDNSIVV	187
	VH (KL2B242_VH)	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSW LRQPAGSGLEWIGRLYVSGFTNYNPSLKSRVTLSLD PSRNQLSLKLSSVTAADTAVYYCAGDSGNYWGWF DPWGQGTLVTVSS	166
	VL (KL2B242_VL)	SYELTQPPSVSVSPGETASITCSGDQLGENYACWYQ QKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLT ISGTQALDEADYYCQAWDNSIVVFGGGTKLTVL	167
KL2B467	HCDR1	YYGMH	188
	HCDR2	FISYDGSNKYYADSVKG	189
	HCDR3	LPYSGSYWAFDY	190
	LICDR1	GGDNIGSKSVH	191
	LCDR2	DNSDRPS	192
	LCDR3	QVWDSSSDHPVV	193
	VH (KL2B467_VH)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSYYGMH WVRQAPGKGLEWVAFISYDGSNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSY WAFDYWGQGTQVTVSS	168

	VL (KL2B467_VL)	QSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQ QKPGQAPVLVVYDNSDRPSGIPERFSGSNSGTTATL TISRVEAGDEADYYCQVWDSSSDHPVVFGGGTKVT V	169
KL2B494	HCDR1	HYAMS	206
	HCDR2	TIGGSGGSTYYADSVKG	207
	HCDR3	PHIVMVTALLYDGMDV	208
	LCDR1	GGNNIGSKSVH	182
	LCDR2	DDSDRPS	470
	LCDR3	QVWDSSSDHVV	209
	VH (KL2B494_VH)	QVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMS WVRQAPGKGLEWVSTIGGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMV TALLYDGMDVWGQGTMVTVSS	204
	VL (KL2B494_VL)	SSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQ QKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATL TISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTV L	205

Table 9. SEQ ID NO of Protein and DNA sequences of the VH and VL domains of selected hK2 antibodies.

Antibody	VH Protein SEQ ID NO:	VL Protein SEQ ID NO	VH cDNA SEQ ID NO:	VL cDNA SEQ ID NO:
m11B6	125	124	225	237
hu11B6	5	2	226	238
HCF3-LCD6	6	1	227	239
HCG5-LCB7	4	3	228	240

KL2B357	159	160	229	241
KL2B358	161	140	230	242
KL2B359	139	140	231	242
KL2B360	159	140	229	242
KL2B413	137	138	232	243
KL2B30	162	163	233	244
KL2B53	164	165	234	245
KL2B242	166	167	235	246
KL2B467	168	169	236	247
KL2B494	204	205	263	271

SEQ ID NO:225 (m11B6 VH cDNA)

GATGTGCAGCTTCAGGAGTCTGGACCCGGACTTGTTAAACCAAGTCAGTCTCTGTCCCTGAC
CTGTACCGTCACCGGCAACAGCATCACAAGCGATTACGCATGGAACTGGATCAGGCAGTTCC
CTGGAAATCGACTCGAATGGATGGGCTACATTTCATACTCCGGTTCAACCACTTACTCTCCAT
CCTTGAAATCTAGGTTCAGCATCACCCGTGATACCTCAAAGAACCAATTTTTTCTGCAACTG
AATAGCGTAACTCCAGAGGACACAGCCACATATTTCTGCGCCACTGGGTATTACTATGGCTC
AGGTTTCTGGGGTCAGGGCACTCTCGTCACCGTCAGCAGC

SEQ ID NO: 226 (hu11B6 VH cDNA)

CAGGTCCAACTGCAAGAGAGCGGACCGGGCCTGGTAAAGCCATCCGACACATTGTCCCTGA
CGTGTGCGGTAAGTGGAAACTCTATCACTAGCGACTATGCGTGGAATTGGATAAGACAACC
GCCGGGCAAGGGGCTGGAATGGATAGGATATATCAGCTATTCCGGTTCTACGACATACAATC
CTTCCCTGAAAAGCAGAGTCACTATGTCACGCGACACGTCCAAGAATCAGTTCTCATTGAAA
TTGTCATCCGTAACGGCCGTTGACACTGCGGTTTATTATTGCGCAACCGGATATTACTACGGC
TCTGGTTTTTGGGGACAGGGAACACTTGTTACTGTTAGTTCA

SEQ ID:NO 227 (HCF3-LCD6 VH cDNA)

CAGGTGCAGCTGCAGGAGAGCGGCCCAGGCCTGGTGAAGCCAAGCGACACCCTGAGCCTGA
CCTGCGCCGTGAGCGGCAACAGCATCACCAGCGACTACGCCTGGAACTGGATCCGCCAGTTC
CCAGGCAAGGGCCTGGAGTGGATCGGCTACATCAGCTACAGCGGCAGCACCACCTACAACC
CAAGCCTGAAGAGCCGCGTCACCATCAGCCGCGACACCAGCAAGAACCAGTTCAGCCTGAA
GCTGAGCAGCGTGACCCCTGTGGACACCGCCGTGTACTACTGCGCCACCGGCTACTACTACG
GCAGCGGCTTCTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO: 228 (HCG5-LCB7 VH cDNA)

CAGGTGCAGCTGCAGGAGAGCGGCCCAGGCCTGGTGAAGCCAAGCGACACCCTGAGCCTGA
CCTGCGCCGTGAGCGGCAACAGCATCACCAGCGACTACGCCTGGAACTGGATCCGCCAGTTC
CCAGGCAAGGGCCTGGAGTGGATGGGCTACATCAGCTACAGCGGCAGCACCACCTACAACC
CAAGCCTGAAGAGCCGCGTCACCATCAGCCGCGACACCAGCAAGAACCAGTTCAGCCTGAA
GCTGAGCAGCGTGACCCCTGTGGACACCGCCGTGTACTACTGCGCCACCGGCTACTACTACG
GCAGCGGCTTCTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO: 229 (KL2B357, KL2B360 VH cDNA)

CAGGTTCAGCTGCAAGAGTCTGGACCAGGCCTGGTCAAGCCCTCTCAGACCCTGTCTCTGAC
CTGTACCGTGTCCGGCAACTCCATCACCTCTGACTACGCCTGGAACTGGATTCGGCAGTTCC
CTGGCAAGGGCCTTGAGTGGATCGGCTACATCTCCTACTCCGGTTCCACCACCTACAACCCC
AGCCTGAAGTCCCGGGTCACCATCTCCCGCGACACCTCCAAGAACCAGTTCTCCCTGAAGCT
GTCCTCCGTGACCGCTGCTGATACCGCCGTGTACTACTGTGCCACCGGCTACTACTACGGCTC
CGGCTTTTGGGGACAGGGCACACTGGTTACCGTGTCTAGT

SEQ ID NO: 230 (KL2B358 VH cDNA)

CAGGTTCAGCTGCAAGAGTCTGGACCAGGCCTGGTCAAGCCCTCTCAGACCCTGTCTCTGAC
CTGTACCGTGTCCGGCAACTCCATCACCTCTGACTACGCCTGGAACTGGATTCGGCAGCCAC
CTGGCAAGGGCCTTGAGTGGATCGGCTACATCTCCTACTCCGGTTCCACCACCTACAACCCC
AGCCTGAAGTCCCGGGTCACCATCTCCCGCGACACCTCCAAGAACCAGTTCTCCCTGAAGCT
GTCCTCCGTGACCGCTGCTGATACCGCCGTGTACTACTGTGCCACCGGCTACTACTACGGCTC
CGGCTTTTGGGGACAGGGCACACTGGTTACCGTGTCTAGT

SEQ ID NO: 231 (KL2B359 VH cDNA)

CAGGTTCAGCTGCAAGAGTCTGGACCAGGCCTGGTCAAGCCCTCTCAGACCCTGTCTCTGAC
CTGTACCGTGTCCGGCAACTCCATCACCTCTGACTACGCCTGGAACTGGATTCGGCAGTTCC
CTGGCAAGCGCCTTGAGTGGATCGGCTACATCTCCTACTCCGGTTCCACCACCTACAACCCC
AGCCTGAAGTCCCGGGTCACCATCTCCCGCGACACCTCCAAGAACCAGTTCTCCCTGAAGCT
GTCCTCCGTGACCGCTGCTGATACCGCCGTGTACTACTGTGCCACCGGCTACTACTACGGCTC
CGGCTTTTGGGGACAGGGCACACTGGTTACCGTGTCTAGT

SEQ ID NO: 232 (KL2B413 VH cDNA)

GAGGTGCAACTTGTGGAGAGCGGCGGAGGTCTGGTCCAACCCGGAGGAAGTCTCCGTCTCT
CCTGTGCTGCTAGTGGCTTCACTTTCAGCTCATATTGGATGACATGGGTGAGACAAGCCCCA
GGAAAGGGGCTCGAGTGGGTAGCTAACATTAAACAGGACGGCTCCGAACGGTACTATGTTG
ATTCTGTGAAGGGACGGTTCACTATATCCAGGGATAATGCAAAAAATTCACTCTATCTTCAA
ATGAACTCACTCAGAGCAGAGGACACTGCCGTGTATTATTGCGCCAGGGATCAAAATTATGA
CATACTGACCGGTCATTATGGAATGGATGTTTGGGGCCAGGGAACAACCGTTACCGTCTCAA
GT

SEQ ID NO:233 (KL2B30 VH cDNA)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA
CCTGCACTGTCTCTGGTGGCTCCATCAGTAGTTACTACTGGAGCTGGATCCGGCAGCCCCCA
GGGAAGGGACTGGAGTGGATTGGATATATCTATTACAGTGGGAGCACCAACTACAACCCCT
CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTG
AGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGGGGACTACGATTTTTGGAGT
GGTTACCCCCAACTTCTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCT
CCTCA

SEQ ID NO: 234 (KL2B53 VH cDNA)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGTAGCCTCTGGATTCACCTTCAGTAGTTATGACATACACTGGGTCCGCCAGGCTCCA
GGCAAGGGGCTGGAGTGGGTGGCAATTATTTCATATGATGGAAGTAAAAAAGACTATACAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
ATGGACAGCCTGAGAGTTGAGGACTCGGCTGTGTATTCCTGTGCGAGAGAAAGTGGCTGGTC
CCACTACTACTATTACGGTATGGACGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCA

SEQ ID NO: 235 (KL2B242 VH cDNA)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA
CCTGCACTGTCTCTGGTGGCTCCATCAGTAGTTACTATTGGAGCTGGCTCCGGCAGCCCGCC
GGGTCGGGACTGGAGTGGATTGGGCGTTTATATGTCAGTGGGTTCACCAACTACAACCCCTC
CCTCAAGAGTCGAGTCACCTTGTCACTAGACCCGTCCAGGAACCAGTTGTCCCTGAAACTGA
GTTCTGTGACCGCCGCGGACACGGCCGTATATTATTGTGCGGGAGATAGTGGGAACTACTGG
GGTTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO: 236 (KL2B467 VH cDNA)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTTACTATGGCATGCACTGGGTCCGCCAGGCTCCA
GGCAAGGGGCTGGAGTGGGTGGCATTTATATCATATGATGGAAGTAATAAATACTATGCAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
ATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCCCACCTCCCTTATAGTGG
GAGCTACTGGGCCTTTGACTACTGGGGCCAGGGAACCCAGGTCACCGTCTCTTCA

SEQ ID NO: 263 (KL2B494 VH cDNA)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGTCATTATGCCATGAGCTGGGTCCGCCAGGCTCCAG
GGAAGGGGCTGGAGTGGGTCTCAACTATTGGTGGTAGTGGTGGTAGCACATACTACGCAGA
CTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAACCTCATATTGTAATG
GTGACTGCTCTTCTCTACGACGGTATGGACGTCTGGGGCCAAGGGACAATGGTCACCGTCTC
CTCA

SEQ ID NO: 237 (m11B6 VL cDNA)

GACATTGTGCTGACACAGAGTCCAGCATCCTTGGCAGTATCTTTGGGGCAGCGGGCAACAAT
TTCATGCCGTGCATCTGAAAGTGTGGAGTATTTTGGAACTTCTCTTATGCACTGGTATCGCCA
GAAGCCTGGGCAGCCTCCCAAACTCCTTATATATGCCGCTTCCAACGTGGAGTCCGGAGTAC
CAGCACGCTTTTCCGGCTCTGGGTCCGGCACAGACTTTTCCCTCAATATCCAACCTGTTGAAG
AAGACGATTTTTCCATGTATTTTTGCCAACAGACACGCAAGGTTCCATATACATTCGGCGGC
GGCACTAAACTTGAGATCAAA

SEQ ID NO: 238 (hu11B6 VL cDNA)

GACATAGTCTTGACTCAGAGCCCGGATTCCCTTGCTGTGTCTCTGGGAGAACGAGCTACGAT
CAACTGCAAGGCAAGTGAATCCGTAGAATACTTCGGGACATCATTGATGCATTGGTATCAAC
AGAAACCGGGGCAACCGCCCAAATTGCTGATATATGCGGCTAGTAATAGAGAATCAGGAGT
ACCGGATAGGTTTAGTGGTTCAGGATCAGGTACAGATTTCACCCTGACAATAAGTAGCTTGC
AAGCCGAAGACGTAGCAGTGTATTACTGCCAACAAACCCGAAAGGTGCCATATACGTTTGG
ACAGGGTACAAAGTTGGAAATCAAA

SEQ ID NO: 239 (HCF3-LCD6 VL cDNA)

GACATCGTGCTGACCCAGAGCCCAGACAGCCTGGCCGTGAGCCTGGGCGAGCGCGCCACCA
TCAACTGCAAGGCCAGCGAGAGCGTGGAGTACTTCGGCACCAGCCTGATGCACTGGTACCA
GCAGAAGCCAGGCCAGCCACCAAAGCTGCTGATCTACGCTGCCAGCAACCGCGAGAGCGGC
GTGCCAGACCGCTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCCAGAGCG
TGCAGGCCGAGGACGTCTCCGTGTACTTCTGCCAGCAGACCCGCAAGGTGCCATACACCTTC
GGCCAGGGCACCAAGCTGGAGATCAAG

SEQ ID NO: 240 (HCG5-LCB7 VL cDNA)

GACATCGTGCTGACCCAGAGCCCAGACAGCCTGGCCGTGAGCCTGGGCGAGCGCGCCACCA
TCAACTGCAAGGCCAGCGAGAGCGTGGAGTACTTCGGCACCAGCCTGATGCACTGGTACCA
GCAGAAGCCAGGCCAGCCACCAAAGCTGCTGATCTACGCTGCCAGCAACCGCGAGAGCGGC
GTGCCAGACCGCTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCG
TGCAGGCCGAGGACGTCGCCGTGTACTACTGCCAGCAGACCCGCAAGGTGCCATACACCTTC
GGCCAGGGCACCAAGCTGGAGATCAAG

SEQ ID NO: 241 (KL2B357 VL cDNA)

GACATCGTGCTGACCCAGTCTCCAGACTCTCTGGCTGTGTCTCTGGGCGAGAGAGCCACCAT
CAACTGCAGAGCCTCCGAGTCCGTGGAATACTTCGGCACCTCTCTGATGCACTGGTACCAGC
AGAAGCCCGGCCAGCCTCCTAAGCTGCTGATCTACGCCGCCTCCAACGTGGAATCTGGCGTG
CCCGATAGATTTTCCGGCTCTGGCTCTGGCACCGACTTTACCCTGACCATCAGCTCTCTGCAG
GCCGAGGATGTGGCCGTGTACTTCTGTCAGCAGACCCGGAAGGTGCCCTACACATTTGGCGG
CGGAACAAAGGTGGAAATCAAG

SEQ ID NO: 242 (KL2B358, KL2B359, KL2B360 VL cDNA)

GAGATCGTGCTGACCCAGTCTCCTGCCACACTGTCACTGTCTCCAGGCGAGAGAGCCACCCT
CTCTTGTAGAGCCTCCGAGTCCGTGGAATACTTCGGCACCTCTCTGATGCACTGGTACCAGC

AGAAGCCCGGCCAGCCTCCTAGACTGCTGATCTACGCCGCCTCCAACGTCGAATCTGGCATC
CCCGCTAGATTCTCCGGCTCTGGCTCTGGCACAGACTTTACCCTGACCATCTCCTCCGTGGAA
CCCGAGGATTTCGCTGTGTACTTTTGCCAGCAGACCCGGAAGGTGCCCTACACATTTGGCGG
CGGAACAAAGGTGGAAATCAAG

SEQ ID NO: 243 (KL2B413 VL cDNA)

GAAATCGTACTGACCCAGTCCCCTTCTTTCTTGAGTGCATCAGTTGGGGATAGAGTGACCAT
TACTTGTAGAGCATCTCAAGGTATTTCTTCATACTTGTCTTGGTATCAACAAAAACCTGGCAA
GGCACCCAAACTCTTGATCTACGCCACCTCTACATTGCAAAGTGGGGTTCCTTCTAGGTTTTC
AGGCTCCGGCTCTGGTACCGAGTTCACCCTCACTATAAGCAGTCTCCAACCTGAAGATTTCG
CTACTTATTATTGTCAGCAGCTTAATTCTTATCCCCGAACCTTTGGTCAAGGAACTAAGGTCG
AGATCAAA

SEQ ID NO: 244 (KL2B30 VL cDNA)

GACATCCAGATGACCCAGTCTCCTTCCTTCCTGTCTGCATCTGTAGGAGACAGAGTCACCAT
CACTTGCCGGGCCAGTCAGGGCATTAGCAGTTATTTAGCCTGGTATCAGCAAAAACCAGGGA
AAGCCCCTAAGTTCCTGATCTATGCTGCATCCACTTTGCAAAGTGGGGTCCCATCAAGGTTC
AGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCTGAAGATTT
TGCAACTTATTACTGTCAACAGCTTAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGG
TGGAAATCAAA

SEQ ID NO: 245 (KL2B53 VL cDNA)

GACATCGTGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCAT
CACTTGCCGGGCGAGTCAGGACATTAGCAATTATTTAGCCTGGTATCAGCAGAAACCAGGG
AAAGTTCCTAAGTTCCTGATCTATGCTGCATCCACTTTGCACTCTGGGGTCCCATCTCGGTTC
AGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGT
TGCAACTTATTACTGTCAAAAGTATAACAGTGCCCCGTACACTTTTGGCCAAGGGACACGAC
TGGAGATTAAA

SEQ ID NO: 246 (KL2B242 VL cDNA)

TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGAGAGACAGCCAGCATCAC
CTGCTCTGGAGATCAATTGGGGGAAAATTATGCTTGCTGGTATCAGCAGAAGCCAGGCCAGT
CCCCTGTGTTGGTCATCTATCAAGATAGTAAGCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTCTGGATGAGG

CTGACTATTACTGTCAGGCGTGGGACAACAGTATTGTGGTATTCGGCGGAGGGACCAAGCTG
ACCGTCCTA

SEQ ID NO: 247 (KL2B467 VL cDNA)

CAGTCTGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCCGGGCAGACGGCCAGTATTAC
CTGTGGGGGAGACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCAG
GCCCCTGTGCTGGTCGTCTATGATAATAGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTC
TGGCTCCAACTCTGGGACCACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGGGATGAG
GCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGATCATCCTGTGGTATTCGGCGGAGG
GACCAAGGTCACCGTCCTA

SEQ ID: 271 (KLK2B494_VL DNA)

TCTTCTGAGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATTAC
CTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCAG
GCCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTC
TGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGGGATGAG
GCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGATCATGTGGTATTCGGCGGAGGGAC
CAAGCTGACCGTCCTA

Consensus VH and VL sequences

FIG. 1 shows the sequence alignment of the VH domains of mu11B6, hu11B6, KL2B357, KL2B358, KL2B359, KL2B360, HCF3 and HCG5. FIG.2 shows the sequence alignment of the VL domains of mu11B6, hu11B6, KL2B357, KL2B358, KL2B359, KL2B360, LDC6 and LCB7. Consensus amino acid sequence of SEQ ID NO: 75 and SEQ ID NO:74 were determined for the VH and VL domains, respectively. HCDR and LCDR residues are underlined.

SEQ ID NO: 75

QVQLQESGPGLVKPSX$_1$TLSLTCX$_2$VSGNSITSDYAWNWIRQX$_3$PGKX$_4$LEWX$_5$GYISYSGSTT
YNPSLKSRVTX$_6$SRDTSKNQFSLKLSSVTX$_7$X$_8$DTAVYYCATGYYYGSGFWGQGTLVTVSS

wherein, X$_1$ is D or Q; X$_2$ is A or T; X$_3$ is P or F; X$_4$ is G or R; X$_5$ is I or M; X$_6$ is I or M ; X$_7$ is A or P; or X$_8$ is V or A.

SEQ ID NO: 74

X_1IVLTQSPX$_2$X$_3$LX$_4$X$_5$SX$_6$GERATX$_7$X$_8$CX$_9$ASESVEYFGTSLMHWYQQKPGQPPX$_{10}$LLIYAASNX$_{11}$ESGX$_{12}$PX$_{13}$RFSGSGSGTDFTLTIX$_{14}$SX$_{15}$X$_{16}$QX$_{17}$EDX$_{18}$X$_{19}$VYX$_{20}$CQQTRKVPYTFGX$_{21}$GTKX$_{22}$EIK

wherein, X_1 is D or E; X_2 is D or A; X_3 is S or T; X_4 is A or S; X_5 is V or L; X_6 is L or P; X_7 is I or L; X_8 is N or S; X_9 is R or K; X_{10} is K or R; X_{11} is V or R; X_{12} is V or I; X_{13} is A or D; X_{14} is Q or S; X_{15} is L or V; X_{16} is Q or E; X_{17} is P or A; X_{18} is F or V; X_{19} is A or S, X_{20} is Y or F; X_{21} is Q or G; and X_{22} is L or V

Fab-Fc and scFvs

The hK2 specific VH/VL regions were engineered as VH-CH1-hinge CH2-CH3 and VL-CL and expressed as IgG2 or IgG4 or were engineered as scFvs in either the VH-Linker-VL or VL-linker-VH orientations. The linker that is used in the scFv was the linker of SEQ ID NO: 7 described above. The scFv were used to generate bispecific antibodies as described in Example 7 or to generated CAR as described in Example 11.

Table 10 shows the HC amino acid sequences of selected anti-hK2 antibodies in the mAb format. **Table 11** shows the LC amino acid sequences of selected anti-hK2 antibodies in a mAb. **Table 12** summaries the HC and LC DNA SEQ ID NOs of selected anti-hK2 antibodies in the mAb format. **Table 13** shows the amino acid sequences of selected scFvs in VH-linker-VL or VL-linker-VH orientation.

Table 10. Amino acid sequence of the HC (VH-CH1-hinge CH2-CH3) of selected anti-hK2 antibodies in a mAb format.

HK2 HEAVY CHAIN	HC PROTEIN SEQ ID NO:	HC AMINO ACID SEQUENCE
m11B6_HC	202	DVQLQESGPGLVKPSQSLSLTCTVTGNSITSDYAWNWIRQFP GNRLEWMGYISYSGSTTYSPSLKSRFSITRDTSKNQFFLQLNS VTPEDTATYFCATGYYYGSGFWGQGTLVTVSSAKTTAPSVY PLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVH TFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVD KKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLS

		PIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYN STLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKP KGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVE WTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPGK
h11B6_HC	203	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPP GKGLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSS VTAVDTAVYYCATGYYYGSGFWGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
KL2B30_HC	210	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGK GLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVT AADTAVYYCAGTTIFGVVTPNFYYGMDVWGQGTTVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVD HKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLP SSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
K2B53_HC	211	EVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHWVRQAPG KGLEWVAIISYDGSKKDYTDSVKGRFTISRDNSKNTLYLQM DSLRVEDSAVYSCARESGWSHYYYYGMDVWGQGTMVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA

		KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTV DKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
KL2B242_HC	212	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQPAG SGLEWIGRLYVSGFTNYNPSLKSRVTLSLDPSRNQLSLKLSSV TAADTAVYYCAGDSGNYWGWFDPWGQGTLVTVSSASTKG PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS NTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGK
KL2B467_HC	213	QVQLVESGGGVVQPGRSLRLSCAASGFTFSYYGMHWVRQA PGKGLEWVAFISYDGSNKYYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCAHLPYSGSYWAFDYWGQGTQVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFP PKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
KL2B494_HC	219	QVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMSWVRQAP GKGLEWVSTIGGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKPHIVMVTALLYDGMDVWGQGTMV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK

		VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Table 11. Amino acid sequences of the LC (VL-CL) of selected anti-hK2 antibodies in a mAb (Fab-Fc) format.

HK2 LIGHT CHAIN	LC PROTEIN SEQ ID NO:	LC AMINO ACID SEQUENCE
m11B6_LC	214	DIVLTQSPASLAVSLGQRATISCRASESVEYFGTSLMHWYRQKPGQP PKLLIYAASNVESGVPARFSGSGSGTDFSLNIQPVEEDDFSMYFCQQ TRKVPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLN NFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTK DEYERHNSYTCEATHKTSTSPIVKSFNRNEC
h11B6_LC	215	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQ PPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QTRKVPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
KL2B30_LC	221	DIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKFL IYAASTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYP LTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
KL2B53_LC	222	DIVMTQSPSSLSASVGDRVTITCRASQDISNYLAWYQQKPGKVPKFL IYAASTLHSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAP YTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
KL2B242_LC	223	SYELTQPPSVSVSPGETASITCSGDQLGENYACWYQQKPGQSPVLVI YQDSKRPSGIPERFSGSNSGNTATLTISGTQALDEADYYCQAWDNSI

		VVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYP GAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWK SHRSYSCQVTHEGSTVEKTVAPTECS
KL2B467_LC	224	QSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQQKPGQAPVLV VYDNSDRPSGIPERFSGSNSGTTATLTISRVEAGDEADYYCQVWDSS SDHPVVFGGGTKVTVLGQPKAAPSVTLFPPSSEELQANKATLVCLIS DFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS
KL2B494_LC	220	SSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLV VYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDS SSDHVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLIS DFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS

Table 12. SEQ ID Nos of the cDNA sequences of HC and LC of selected hK2 antibodies

Antibody	HC Protein SEQ ID NO:	LC Protein SEQ ID NO:	HC cDNA SEQ ID NO:	LC cDNA SEQ ID NO:
m11B6	202	214	284	296
hu11B6	203	215	285	297
KL2B30	210	221	292	450
KL2B53	211	222	293	451
KL2B242	212	223	294	305
KL2B467	213	224	295	306
KL2B494	219	220	274	282

SEQ ID NO: 284 (m11B6 HC cDNA)

GATGTGCAGCTTCAGGAGTCTGGACCCGGACTTGTTAAACCAAGTCAGTCTCTGTCCCTGAC
CTGTACCGTCACCGGCAACAGCATCACAAGCGATTACGCATGGAACTGGATCAGGCAGTTCC
CTGGAAATCGACTCGAATGGATGGGCTACATTTCATACTCCGGTTCAACCACTTACTCTCCAT
CCTTGAAATCTAGGTTCAGCATCACCCGTGATACCTCAAAGAACCAATTTTTTCTGCAACTG
AATAGCGTAACTCCAGAGGACACAGCCACATATTTCTGCGCCACTGGGTATTACTATGGCTC
AGGTTTCTGGGGTCAGGGCACTCTCGTCACCGTCAGCAGCGCCAAAACAACAGCACCAAGT
GTCTATCCACTGGCCCCTGTGTGTGGAGATACAACTGGCTCCTCGGTGACTCTAGGATGCCT
GGTCAAGGGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACTCTGGATCCCTGTCCAGTG
GTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACCCTCAGCAGCTCAGTGACTG
TAACCTCGAGCACCTGGCCCAGCCAGTCCATCACCTGCAATGTGGCCCACCCGGCAAGCAGC
ACCAAGGTGGACAAGAAAATTGAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCA
AATGCCCAGCACCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAG
GATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGA
TGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACA
CAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCA
CCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCG
CCCATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTT
GCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACCGAC
TTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACA
AGAACACTGAACCAGTCCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTG
GAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGC
ACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAA

SEQ ID NO: 285 (hu11B6 HC cDNA)

CAGGTCCAACTGCAAGAGAGCGGACCGGGCCTGGTAAAGCCATCCGACACATTGTCCCTGA
CGTGTGCGGTAAGTGGAAACTCTATCACTAGCGACTATGCGTGGAATTGGATAAGACAACC
GCCGGGCAAGGGGCTGGAATGGATAGGATATATCAGCTATTCCGGTTCTACGACATACAATC
CTTCCCTGAAAAGCAGAGTCACTATGTCACGCGACACGTCCAAGAATCAGTTCTCATTGAAA
TTGTCATCCGTAACGGCCGTTGACACTGCGGTTTATTATTGCGCAACCGGATATTACTACGGC
TCTGGTTTTTGGGGACAGGGAACACTTGTTACTGTTAGTTCAGCCTCCACCAAGGGCCCATC
GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC

GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCC
ACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCA
AGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC
GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGA
CAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCT
GCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
GCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACA
CCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA
AGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCAC
CGTGGACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

SEQ ID NO: 292 (KL2B30 HC cDNA)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA
CCTGCACTGTCTCTGGTGGCTCCATCAGTAGTTACTACTGGAGCTGGATCCGGCAGCCCCCA
GGGAAGGGACTGGAGTGGATTGGATATATCTATTACAGTGGGAGCACCAACTACAACCCCT
CCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTG
AGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGGGGACTACGATTTTTGGAGT
GGTTACCCCCAACTTCTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCT
CCTCAGCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCC
GAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTC
GTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAG
GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAAACCTAC
ACTTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAAT
ATGGTCCCCCATGCCCACCATGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTG
TTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGT
GGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCA
GCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTC
CAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA
GAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCC

TGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG
GCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
TCTACAGCAGGCTAACCGTGGACAAGAGCAGATGGCAGGAGGGGAATGTCTTCTCATGCTC
CGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTA
AA

SEQ ID NO: 293 (KL2B53 HC cDNA)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGTAGCCTCTGGATTCACCTTCAGTAGTTATGACATACACTGGGTCCGCCAGGCTCCA
GGCAAGGGGCTGGAGTGGGTGGCAATTATTTCATATGATGGAAGTAAAAAAGACTATACAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
ATGGACAGCCTGAGAGTTGAGGACTCGGCTGTGTATTCCTGTGCGAGAGAAAGTGGCTGGTC
CCACTACTACTATTACGGTATGGACGTCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG
CTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGC
ACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA
CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT
ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAAACCTACACTTGC
AACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTC
CCCCATGCCCACCATGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCC
CCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGA
CGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCC
TCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA
AGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCA
CAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCT
GCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCC
GGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACA
GCAGGCTAACCGTGGACAAGAGCAGATGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGAT
GCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAA

SEQ ID NO: 294 (KL2B242 HC cDNA)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA
CCTGCACTGTCTCTGGTGGCTCCATCAGTAGTTACTATTGGAGCTGGCTCCGGCAGCCCGCC
GGGTCGGGACTGGAGTGGATTGGGCGTTTATATGTCAGTGGGTTCACCAACTACAACCCCTC

CCTCAAGAGTCGAGTCACCTTGTCACTAGACCCGTCCAGGAACCAGTTGTCCCTGAAACTGA
GTTCTGTGACCGCCGCGGACACGGCCGTATATTATTGTGCGGGAGATAGTGGGAACTACTGG
GGTTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCTTCCACCAAGGG
CCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTGG
GCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG
ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAAACCTACACTTGCAACGTAGATCACA
AGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACC
ATGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGG
ACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAA
GACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAA
AGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCAC
CAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCT
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCT
GCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACA
AGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCGTG
GACAAGAGCAGATGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAA

SEQ ID NO: 295 (KL2B467 HC cDNA)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTTACTATGGCATGCACTGGGTCCGCCAGGCTCCA
GGCAAGGGGCTGGAGTGGGTGGCATTTATATCATATGATGGAAGTAATAAATACTATGCAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
ATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCCCACCTCCCTTATAGTGG
GAGCTACTGGGCCTTTGACTACTGGGGCCAGGGAACCCAGGTCACCGTCTCTTCAGCCTCCA
CCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCG
GCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGG
CGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCT
CAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGA
ATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAAC
TCACACATGCCCACCGTGCCCAGCACCTGAAGCCGCCGGGGGACCGTCAGTCTTCCTCTTCC
CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG

AGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGC
ATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGT
CCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGAC
CTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

SEQ ID NO: 274 (KL2B494 HC cDNA)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGTCATTATGCCATGAGCTGGGTCCGCCAGGCTCCAG
GGAAGGGGCTGGAGTGGGTCTCAACTATTGGTGGTAGTGGTGGTAGCACATACTACGCAGA
CTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAA
TGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAACCTCATATTGTAATG
GTGACTGCTCTTCTCTACGACGGTATGGACGTCTGGGGCCAAGGGACAATGGTCACCGTCTC
CTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTG
GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTC
GTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAG
GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC
ATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT
CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGCCGCCGGGGGACCGTC
AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCA
CATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA
CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT
GCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG
GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGA
GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGC
TCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGATGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT
CTCCGGGTAAA

SEQ ID NO: 296 (mu11B6 LC cDNA)

GACATTGTGCTGACACAGAGTCCAGCATCCTTGGCAGTATCTTTGGGGCAGCGGGCAACAAT
TTCATGCCGTGCATCTGAAAGTGTGGAGTATTTTGGAACTTCTCTTATGCACTGGTATCGCCA
GAAGCCTGGGCAGCCTCCCAAACTCCTTATATATGCCGCTTCCAACGTGGAGTCCGGAGTAC
CAGCACGCTTTTCCGGCTCTGGGTCCGGCACAGACTTTTCCCTCAATATCCAACCTGTTGAAG
AAGACGATTTTTCCATGTATTTTTGCCAACAGACACGCAAGGTTCCATATACATTCGGCGGC
GGCACTAAACTTGAGATCAAACGGGCTGATGCTGCACCGACTGTGTCCATCTTCCCACCATC
CAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCA
AAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAG
TTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCACGTTGACC
AAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTT
CACCCATTGTCAAGAGCTTCAACAGGAATGAGTGT

SEQ ID NO: 297 (hu11B6 LC cDNA)

GACATAGTCTTGACTCAGAGCCCGGATTCCCTTGCTGTGTCTCTGGGAGAACGAGCTACGAT
CAACTGCAAGGCAAGTGAATCCGTAGAATACTTCGGGACATCATTGATGCATTGGTATCAAC
AGAAACCGGGGCAACCGCCCAAATTGCTGATATATGCGGCTAGTAATAGAGAATCAGGAGT
ACCGGATAGGTTTAGTGGTTCAGGATCAGGTACAGATTTCACCCTGACAATAAGTAGCTTGC
AAGCCGAAGACGTAGCAGTGTATTACTGCCAACAAACCCGAAAGGTGCCATATACGTTTGG
ACAGGGTACAAAGTTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGC
CATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATC
CCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGA
GAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTG
AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO: 450 (KL2B30 LC cDNA)

GACATCCAGATGACCCAGTCTCCTTCCTTCCTGTCTGCATCTGTAGGAGACAGAGTCACCAT
CACTTGCCGGGCCAGTCAGGGCATTAGCAGTTATTTAGCCTGGTATCAGCAAAAACCAGGGA
AAGCCCCTAAGTTCCTGATCTATGCTGCATCCACTTTGCAAAGTGGGGTCCCATCAAGGTTC
AGCGGCAGTGGATCTGGGACAGAATTCACTCTCACAATCAGCAGCCTGCAGCCTGAAGATTT
TGCAACTTATTACTGTCAACAGCTTAATAGTTACCCTCTCACTTTCGGCGGAGGGACCAAGG
TGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAG

TTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG
CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACT
ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC
AAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO: 451 (KL2B53 LC cDNA)

GACATCGTGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCAT
CACTTGCCGGGCGAGTCAGGACATTAGCAATTATTTAGCCTGGTATCAGCAGAAACCAGGG
AAAGTTCCTAAGTTCCTGATCTATGCTGCATCCACTTTGCACTCTGGGGTCCCATCTCGGTTC
AGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGT
TGCAACTTATTACTGTCAAAAGTATAACAGTGCCCCGTACACTTTTGGCCAAGGGACACGAC
TGGAGATTAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAG
TTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAG
CAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACT
ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC
AAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO: 305 (KL2B242 LC cDNA)

TCCTATGAGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGAGAGACAGCCAGCATCAC
CTGCTCTGGAGATCAATTGGGGGAAAATTATGCTTGCTGGTATCAGCAGAAGCCAGGCCAGT
CCCCTGTGTTGGTCATCTATCAAGATAGTAAGCGGCCCTCAGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTCTGGATGAGG
CTGACTATTACTGTCAGGCGTGGGACAACAGTATTGTGGTATTCGGCGGAGGGACCAAGCTG
ACCGTCCTAGGTCAGCCCAAGGCTGCACCCAGTGTCACTCTGTTCCCGCCCTCCTCTGAGGA
GCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGA
CAGTGGCCTGGAAGGCCGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTC
CAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGTGG
AAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAG
TGGCCCCTACAGAATGTTCA

SEQ ID NO: 306 (KL2B467 LC cDNA)

CAGTCTGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCCGGGCAGACGGCCAGTATTAC
CTGTGGGGGAGACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCAG
GCCCCTGTGCTGGTCGTCTATGATAATAGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTC
TGGCTCCAACTCTGGGACCACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGGGATGAG
GCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGATCATCCTGTGGTATTCGGCGGAGG
GACCAAGGTCACCGTCCTAGGTCAGCCCAAGGCTGCACCCAGTGTCACTCTGTTCCCGCCCT
CCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCG
GGAGCCGTGACAGTGGCCTGGAAGGCCGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCA
CCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCC
TGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTG
GAGAAGACAGTGGCCCCTACAGAATGTTCA

SEQ ID NO: 282 (KL2B494 LC cDNA)

TCTTCTGAGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATTAC
CTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCAG
GCCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGATTCTC
TGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGGGATGAG
GCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGATCATGTGGTATTCGGCGGAGGGAC
CAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCACCCAGTGTCACTCTGTTCCCGCCCTCCT
CTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGA
GCCGTGACAGTGGCCTGGAAGGCCGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA
CACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGA
GCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG
AAGACAGTGGCCCCTACAGAATGTTCA

Table 13. Amino acid sequences of the variable domain of selected anti-hK2 scFvs antibodies in VH-linker-VL (HL) or in VL-linker-VH (LH) format.

scFv name	Acronym	Amino acid sequence of scFv	SEQ ID NO:
scFv1	HCG5_LDC6_HL	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQFPGKGLEWMGYISYSGSTTYNPSLKSRVTISRD	8

		TSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSP DSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSV QAEDVSVYFCQQTRKVPYTFGQGTKLEIK	
scFv2	HCG5_hu11B6_HL	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQFPGKGLEWMGYISYSGSTTYNPSLKSRVTISRD TSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSP DSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSL QAEDVAVYYCQQTRKVPYTFGQGTKLEIK	9
scFv3	HCF3_hu11B6_HL	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRDT SKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQ GTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPD SLAVSLGERATINCKASESVEYFGTSLMHWYQQKPG QPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQ AEDVAVYYCQQTRKVPYTFGQGTKLEIK	10
scFv4	HCG5_LCB7_HL	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQFPGKGLEWMGYISYSGSTTYNPSLKSRVTISRD TSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSP DSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSV QAEDVAVYYCQQTRKVPYTFGQGTKLEIK	11
scFv5	LCD6_HCG5_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEI KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMG	12

		YISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTP VDTAVYYCATGYYYGSGFWGQGTLVTVSS	
scFv6	hu11B6_HCF3_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEI KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYI SYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVD TAVYYCATGYYYGSGFWGQGTLVTVSS	13
scFv7	hu11B6_HCG5_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEI KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMG YISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTP VDTAVYYCATGYYYGSGFWGQGTLVTVSS	14
scFv8	LCB7_HCF3_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLE IKGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYI SYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVD TAVYYCATGYYYGSGFWGQGTLVTVSS	15
scFv9	LCB7_HCG5_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLE IKGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMG YISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTP VDTAVYYCATGYYYGSGFWGQGTLVTVSS	16

scFv10	LCD6_HCF3_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEI KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYI SYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVD TAVYYCATGYYYGSGFWGQGTLVTVSS	17
scFv11	hu11B6_LCB7_HL	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQPPGKGLEWIGYISYSGSTTYNPSLKSRVTMSRD TSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSP DSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSV QAEDVAVYYCQQTRKVPYTFGQGTKLEIK	18
scFv12	hu11B6_LCD6_HL	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQPPGKGLEWIGYISYSGSTTYNPSLKSRVTMSRD TSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSP DSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSV QAEDVSVYFCQQTRKVPYTFGQGTKLEIK	19
scFv13	hu11B6_HL	QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWN WIRQPPGKGLEWIGYISYSGSTTYNPSLKSRVTMSRD TSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSP DSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSL QAEDVAVYYCQQTRKVPYTFGQGTKLEIK	20
scFv14	LCD6_hu11B6_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEI	21

		KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYI SYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAV DTAVYYCATGYYYGSGFWGQGTLVTVSS	
scFv15	hu11B6_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEI KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYI SYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAV DTAVYYCATGYYYGSGFWGQGTLVTVSS	22
scFv16	LCB7_hu11B6_LH	DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGT DFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLE IKGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS DTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYI SYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAV DTAVYYCATGYYYGSGFWGQGTLVTVSS	23
scFv17	KL2B413_HL	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMT WVRQAPGKGLEWVANIKQDGSERYYVDSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCARDQNYDIL TGHYGMDVWGQGTTVTVSSGGSEGKSSGSGSESKS TGGSEIVLTQSPSFLSASVGDRVTITCRASQGISSYLS WYQQKPGKAPKLLIYATSTLQSGVPSRFSGSGSGTEF TLTISSLQPEDFATYYCQQLNSYPRTFGQGTKVEIK	133
scFv18	KL2B413_LH	EIVLTQSPSFLSASVGDRVTITCRASQGISSYLSWYQQ KPGKAPKLLIYATSTLQSGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCQQLNSYPRTFGQGTKVEIKGGSEG KSSGSGSESKSTGGSEVQLVESGGGLVQPGGSLRLSC AASGFTFSSYWMTWVRQAPGKGLEWVANIKQDGSE	134

		RYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTA VYYCARDQNYDILTGHYGMDVWGQGTTVTVSS	
scFv19	KL2B359_HL	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQFPGKRLEWIGYISYSGSTTYNPSLKSRVTISRDT SKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ GTLVTVSSGGSEGKSSGSGSESKSTGGSEIVLTQSPAT LSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQ PPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPE DFAVYFCQQTRKVPYTFGGGTKVEIK	135
scFv20	KL2B359_LH	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMH WYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDF TLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKG GSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSQTL SLTCTVSGNSITSDYAWNWIRQFPGKRLEWIGYISYS GSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTA VYYCATGYYYGSGFWGQGTLVTVSS	136
scFv21	KL2B357_HL	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRDT SKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ GTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPD SLAVSLGERATINCRASESVEYFGTSLMHWYQQKPG QPPKLLIYAASNVESGVPDRFSGSGSGTDFTLTISSLQ AEDVAVYFCQQTRKVPYTFGGGTKVEIK	318
scFv22	KL2B357_LH	DIVLTQSPDSLAVSLGERATINCRASESVEYFGTSLM HWYQQKPGQPPKLLIYAASNVESGVPDRFSGSGSGT DFTLTISSLQAEDVAVYFCQQTRKVPYTFGGGTKVEI KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPS QTLSLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYI SYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAA DTAVYYCATGYYYGSGFWGQGTLVTVSS	319

scFv23	KL2B358_HL	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQPPGKGLEWIGYISYSGSTTYNPSLKSRVTISRDT SKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ GTLVTVSSGGSEGKSSGSGSESKSTGGSEIVLTQSPAT LSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQ PPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPE DFAVYFCQQTRKVPYTFGGGTKVEIK	320
scFv24	KL2B358_LH	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMH WYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDF TLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKG GSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSQTL SLTCTVSGNSITSDYAWNWIRQPPGKGLEWIGYISYS GSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTA VYYCATGYYYGSGFWGQGTLVTVSS	321
scFv25	KL2B360_HL	QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWN WIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISRDT SKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ GTLVTVSSGGSEGKSSGSGSESKSTGGSEIVLTQSPAT LSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQ PPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPE DFAVYFCQQTRKVPYTFGGGTKVEIK	322
scFv26	KL2B360_LH	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMH WYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDF TLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKG GSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSQTL SLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYISYS GSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTA VYYCATGYYYGSGFWGQGTLVTVSS	323
scFv27	KL2B467_HL	QVQLVESGGGVVQPGRSLRLSCAASGFTFSYYGMH WVRQAPGKGLEWVAFISYDGSNKYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSY	324

		WAFDYWGQGTQVTVSSGGSEGKSSGSGSESKSTGG SQSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWY QQKPGQAPVLVVYDNSDRPSGIPERFSGSNSGTTATL TISRVEAGDEADYYCQVWDSSSDHPVVFGGGTKVT V	
scFv28	KL2B467_LH	QSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQ QKPGQAPVLVVYDNSDRPSGIPERFSGSNSGTTATLT ISRVEAGDEADYYCQVWDSSSDHPVVFGGGTKVTV GGSEGKSSGSGSESKSTGGSQVQLVESGGGVVQPGR SLRLSCAASGFTFSYYGMHWVRQAPGKGLEWVAFI SYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAHLPYSGSYWAFDYWGQGTQVTVSS	325
scFv39	KL2B494_HL	QVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMS WVRQAPGKGLEWVSTIGGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTA LLYDGMDVWGQGTMVTVSS GGSEGKSSGSGSESKSTGGSSSELTQPPSVSVAPGQT ARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSD RPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQV WDSSSDHVVFGGGTKLTVL	308
scFv40	KL2B494_LH	SSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQ QKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLT ISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVL GGSEGKSSGSGSESKSTGGSQVQLVESGGGLVQPGG SLRLSCAASGFTFSHYAMSWVRQAPGKGLEWVSTIG GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCAKPHIVMVTALLYDGMDVWGQGTMVT VSS	316
scFv41	KL2B30_HL	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWI RQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVYYCAGTTIFGVVTPNFYY	404

		GMDVWGQGTTVTVSSGGSEGKSSGSGSESKSTGGS DIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWYQ QKPGKAPKFLIYAASTLQSGVPSRFSGSGSGTEFTLTI SSLQPEDFATYYCQQLNSYPLTFGGGTKVEIK	
scFv42	KL2B30_LH	DIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWYQ QKPGKAPKFLIYAASTLQSGVPSRFSGSGSGTEFTLTI SSLQPEDFATYYCQQLNSYPLTFGGGTKVEIKGGSEG KSSGSGSESKSTGGSQVQLQESGPGLVKPSETLSLTC TVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNY NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCA GTTIFGVVTPNFYYGMDVWGQGTTVTVSS	405
scFv43	KL2B53_HL	EVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHW VRQAPGKGLEWVAIISYDGSKKDYTDSVKGRFTISR DNSKNTLYLQMDSLRVEDSAVYSCARESGWSHYYY YGMDVWGQGTMVTVSSGGSEGKSSGSGSESKSTGG SDIVMTQSPSSLSASVGDRVTITCRASQDISNYLAWY QQKPGKVPKFLIYAASTLHSGVPSRFSGSGSGTDFTL TISSLQPEDVATYYCQKYNSAPYTFGQGTRLEIK	406
scFv44	KL2B53_LH	DIVMTQSPSSLSASVGDRVTITCRASQDISNYLAWYQ QKPGKVPKFLIYAASTLHSGVPSRFSGSGSGTDFTLTI SSLQPEDVATYYCQKYNSAPYTFGQGTRLEIKGGSE GKSSGSGSESKSTGGSEVQLVESGGGVVQPGRSLRL SCVASGFTFSSYDIHWVRQAPGKGLEWVAIISYDGS KKDYTDSVKGRFTISRDNSKNTLYLQMDSLRVEDSA VYSCARESGWSHYYYYGMDVWGQGTMVTVSS	407
scFv45	KL2B242_HL	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWL RQPAGSGLEWIGRLYVSGFTNYNPSLKSRVTLSLDPS RNQLSLKLSSVTAADTAVYYCAGDSGNYWGWFDP WGQGTLVTVSSGGSEGKSSGSGSESKSTGGSSYELT QPPSVSVSPGETASITCSGDQLGENYACWYQQKPGQ	408

		SPVLVIYQDSKRPSGIPERFSGSNSGNTATLTISGTQA LDEADYYCQAWDNSIVVFGGGTKLTVL	
scFv46	KL2B242_LH	SYELTQPPSVSVSPGETASITCSGDQLGENYACWYQ QKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLTI SGTQALDEADYYCQAWDNSIVVFGGGTKLTVLGGS EGKSSGSGSESKSTGGSQVQLQESGPGLVKPSETLSL TCTVSGGSISSYYWSWLRQPAGSGLEWIGRLYVSGF TNYNPSLKSRVTLSLDPSRNQLSLKLSSVTAADTAV YYCAGDSGNYWGWFDPWGQGTLVTVSS	409

Example 3. Biophysical characterization of anti-hK2 antibodies

Affinity and thermal stability of anti-hK2 antibodies.

Affinity of selected hK2 antibodies for soluble hK2 was measured by surface plasmon resonance (SPR). SPR is a label-free technique to study the strength of an interaction between two binding partners by measuring the change in mass upon complex formation and dissociation. Antibodies were captured on a sensor chip coated with an anti-Fc antibody followed by injection of soluble hK2 at various concentrations and specified association and dissociation times. Post dissociation, the surface was regenerated with an appropriate solution to prepare for the next interaction. Kinetic information (on-rate and off-rate constants) were extracted by fitting sensorgrams to the 1:1 Langmuir model. Binding affinity (K_D) are reported as the ratio of rate constants (k_{off}/k_{on}). K_D values of selected hK2 antibodies are listed in **Table 14.**

Thermal stability was determined by Differential Scanning Fluorimetry (NanoDSF) using an automated Prometheus instrument. NanoDSF was used to measure T_m of molecules at a concentration of 0.5 mg/mL in Phosphate Buffered Saline, pH 7.4. Measurements were made by loading samples into 24 well capillary from a 384 well sample plate. Duplicate runs were performed for each sample. The thermal scans span from 20°C to 95°C at a rate of 1.0°C/minute. Intrinsic tryptophan and tyrosine fluorescence were monitored at the emission wavelengths of 330 nm and 350 nm, and the F350/F330 nm ratio were plotted against temperature to generate unfolding curves. Measured Tm values are listed in **Table 14**.

Table 14. K_D and Tm of selected molecules

Molecule	K_D (nM)	Tm (°C)
KL2B413 (scFv-LH-Fc)	34.3	67
KL2B359 (scFv-LH-Fc)	0.7 – 1	67
KL2B30 (Fab)	0.460	>70
KL2B242 (Fab)	0.040	>70
KL2B53 (Fab)	0.080	>70
KL2B467 (Fab)	0.078	>70
KL2B494 (Fab)	0.053	>70

KL2B413 scFv generated from the Ablexis immunization campaign had a thermal stability (Tm) of 67°C as measured by Nano DSF and a binding affinity (K_D) to human hK2 of about 34 nM. Clone KL2B359 obtained for the re-humanization campaign and which had maintained a binding affinity similar to murine 11B6 was converted to scFv-Fc and CAR-T for additional profiling. KL2B359 scFv shows a Tm of 67°C and a binding affinity (K_D) to hK2 of ~0.7 – 1nM. KL2B30, KL2B242, KL2B53, KL2B467 and KL2B494 Fab showed binding affinities below 0.5 nM and Tm values above 70°C.

Epitope mapping

The epitope and paratope of selected anti-hK2 antibodies and anti-hK2/CD3 bispecific antibodies were determined by hydrogen-deuterium exchange mass spectrometry (HDX-MS). Human KLK2 antigen was used for epitope and paratope mapping experiment.

Briefly, purified KLK2 antigen was incubated with and without anti-hK2 antibodies or anti-hK2/CD3 bispecific antibodies in deuterium oxide labeling buffer. The hydrogen-deuterium exchange (HDX) mixture was quenched at different time point by the addition of 8 M urea, 1M TCEP, pH 3.0. The quenched sample was passed over an immobilized pepsin/FPXIII column at 600 µL/min equilibrated with buffer A (1% acetonitrile, 0.1% FA in H2O) at room temperature. Peptic fragments were loaded onto a

reverse phase trap column at 600 µL/min with buffer A and desalted for 1 min (600 µL buffer A). The desalted fragments were separated by a C18 column with a linear gradient of 8% to 35% buffer B (95% acetonitrile, 5% H2O, 0.0025% TFA) at 100 µL/min over 20 min and analyzed by mass spectrometry. Mass spectrometric analyses were carried out using an LTQ™ Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific) with the capillary temperature at 275 °C, resolution 150,000, and mass range (m/z) 300 – 1,800. BioPharma Finder 3.0 (Thermo Fisher Scientific) was used for the peptide identification of non-deuterated samples prior to the HDX experiments. HDExaminer version 2.5 (Sierra Analytics, Modesto, CA) was used to extract centroid values from the MS raw data files for the HDX experiments.

Incubation of hK2 antibodies, hu11B6, KL2B494, KL2B467, KL2B30, KL2B413 and KL2B53 with soluble hK2 protein resulted in different patterns of hydrogen exchange and overall protection. The protected segments were mapped onto the sequence of hK2 antigen to visualize the binding epitopes (**FIG 3**). KL2B494, KL2B467 and KL2B30 bound to common sequences of (i) residues 174-178 (**SEQ ID NO: 111**, KVTEF) (e.g., KL2B494, KL2B467 and KL2B30 bound at least three of the residues of SEQ ID NO: 111, namely, the KVT residues at 174-176) and (ii) residues 230-234 (**SEQ ID NO: 112**, HYRKW) (e.g., KL2B494, KL2B467 and KL2B30 bound at least three of the residues of SEQ ID NO: 112, namely, the HYR residues at 230-232). KL2B413 also bound all residues of SEQ ID NO: 111 and the KW residues of SEQ ID NO: 112, as shown in **FIG. 3**. An embodiment of the present invention provides an isolated protein comprising an antigen binding domain that binds hK2, wherein said antigen binding domain binds to hK2 within epitopes having sequences of SEQ ID NO: 111 and SEQ ID NO: 112; for example, said antigen binding domain binds to all residues, or at least four residues, or at least three residues of SEQ ID NO: 111 and binds to all residues, or at least four residues, or at least three residues of SEQ ID NO: 112.

KL2B53 showed a different pattern of protection and bound to a sequence consisting of residues 27-32 (Seq ID NO: **113**, SHGWAH), 60-75 (**SEQ ID NO: 114**, RHNLFEPEDTGQRVP) and 138-147 (**SEQ ID NO: 115**, GWGSIEPEE).

According to an embodiment, an isolated anti-hK2/anti-CD3 protein (e.g., hu11B6, KL2B494, KL2B467, KL2B30, KL2B413, or KL2B53) comprises an hk2-specific antigen binding domain that specifically binds to a discontinuous epitope (i.e., epitopes whose residues are distantly placed in the sequence) of hK2 comprising one or more amino acid sequences selected from the group consisting of SEQ ID NO: 111, 112, 113, 114, and 115.

The paratope of anti-hK2 antibodies hu11B6, KL2B494, KL2B467, KL2B413 and anti-hK2/CD3 bispecific antibodies KLCB113 and KLCB80 were identified based on significant differences in deuterium uptake from the HDExaminer residue plots. KL2BB494 comprises three paratope regions two of which are located in the KL2B494 heavy chain variable domain (GFTFSH (SEQ ID NO: 455) and TAVYYCAKPHIVMVTAL (SEQ ID NO: 456)) and a single paratope region located within the light chain variable domain (YDDSDRPSGIPER (SEQ ID NO: 457)). KL2B467 comprises three paratope regions, two of which are located in the KL2B467 heavy chain variable domain (FTFSY (SEQ ID NO: 458) and GSYWAFDY (SEQ ID NO: 459)) and a single paratope region within the light chain variable domain (DNSD (SEQ ID NO: 460)). Hu11B6 comprises a single epitope region located in the heavy chain (GNSITSDYA (SEQ ID NO: 461)). KL2B413 comprises two paratope regions located in the heavy chain variable domain (GFTF (SEQ ID NO: 462) and ARDQNYDIL (SEQ ID NO: 463)). KL2B30 of bispecific KLCB80 comprise a paratope region locate in the heavy chain (comprising amino acid residues TIF and VTPNF (SEQ ID NO: 464)) and a paratope region located in the light chain (YAASTLQSG (SEQ ID NO: 465)). KL2B53 of bispecific KLCB113 comprise a single paratope region locate in the heavy chain (comprising amino acid residues ESGWSHY (SEQ ID NO: 466)). **FIGS. 38A-38F** show the binding paratope of these anti-hK2 antibodies and anti-hK2/CD3 bispecific antibodies (underlined sequences indicate CDR regions and highlighted sequences indicate paratope regions).

Example 4: Generation of anti-CD3 antibodies

Immunization

The generation of anti-CD3 antibody CD3B376 has been described in US20200048349.

Anti-CD3 antibodies were generated using Ablexis transgenic mouse platform. Ablexis mice were immunized with TRCW5 **(SEQ ID NO: 338)**, including 13 Kappa mice and 12 Lambda mice. TRCW5 is comprised of the extracellular region of CD3δ fused by a 26 amino acid linker to the extracellular region of CD3ε as reported in Kim et al, JMB (2000) 302(4): 899-916. This polypeptide had at its C-terminus a human IgG1 Fc domain with a C-terminal Avi-tag used for site-specific biotinylation (Fairhead & Howarth, Methods Mol Biol (2015); 1266: 171–184).

TRCW5 (SEQ ID NO: 338):

FKIPIEELEDRVFVNCNTSITWVEGTVGTLLSDITRLDLGKRILDPRGIYRCNGTDIYKDKE STVQVHYRMGSADDAKKDAAKKDDAKKDDAKKDGSDGNEEMGGITQTPYKVSISGTTVILTCP QYPGSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANFYLYL

RARVSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGLNDIFEAQKIEWHE

Mice were immunized twice weekly for the duration of 7 weeks. On day 42, mice were boosted for hybridoma fusion by administration of 50 µg TRCW5 and 50 µg CD40 mAb spread over 8 sites, including 6 subcoutaneous and 2 intradermal injections. For a final boost, mice received 20 µL injections of Jurkat cells, a T cell line which endogenously expresses the T cell receptor complex, including CD3ε (Schneider et al (1977) Int. J. Cancer, 19 (5): 621-6), at 4.74x107 cells/mL.

Lymph nodes and spleens were extracted from mice and fusions performed by cohorts. Lymph node cells were counted and combined in a 1:1 ratio with FO myeloma cells (ATCC (CRL-1646)) and incubated for 10 d at 37 °C prior to antibody screening. Supernatants from hybridoma fusion cells were then assayed by ELISA for binding to TRCW5 using TRCW5 either non-specifically immobilized on the plate (ELISA, Thermo cat. # 34022) or by streptavidin conjugation to biotinylated-TRCW5 (SPARCL ELISA, Lumigen), according to manufacturers' instructions. ELISA assays were performed by coating plates with 0.5 ug/mL TRCW5 and 0.5 ug/mL HVEM-Fc (R&D cat. # 365-HV) overnight @ 4 °C. Plates were blocked by addition of 0.4 % (w/v) bovine serum albumin (BSA) in phosphate-buffered saline (PBS) overnight @ 4 °C. Plates were washed with 1 X PBS supplemented with 0.02 % (v/v) Tween 20. To each well, 50 uL of hybridoma supernatant was applied and incubated for 1 hr at room temperature. Bound antibody was detected by addition of goat anti-mouse IgG Fc conjugated to horseradish peroxidase (Jackson cat. # 115-036-071) diluted 1:10,000 in blocking buffer followed by incubation for 30 min at room temperature. 3, 3′ , 5, 5′ -tetramethylbenzidine (TMB) substrate buffer (Thermo cat. # 34022) was added at 25 uL / well and incubated for 10 min in the dark. Reactions were stopped by addition of 25 uL / well of 4 M H2SO4. Luminescence was read at 450 nm using BioTek® Epoch2 Microplate Reader. Hits were selected having signal at least 3-fold higher than background.

The two assay formats resulted in 426 hits (264 hits from ELISA, 194 from SPARCL ELISA, 70 hits were identified in both assays). Of these 426 initial hits, 49 ELISA and 32 SPARCL ELISA hits were confirmed. The hyriboma fusions corresponding to the positive binders were refed and tested for their abilities to bind Jurkat cells, using flow cytometry. The results suggested that three antibodies, including clone 003_F12, clone 036_E10 and clone 065_D03, showed significant binding to Jurkat cells, endogenously expressing CD3, based on mean fluorescence index (MFI, see Table 4). While clones 003_F12 and 036_E10 (from human kappa mice) were confirmed positive for human kappa light chain by

ELISA, clone 065_D03 (from human lambda mouse) was negative for human lambda. The variable genes of these three clones were then sequenced.

Table 15. Mean fluorescence index (MFI) for binding of selected clones to Jurkat cells

Clone ID	MFI (arbitrary units)
003_F12	176147
036_E10	43133
065_D03	136269
No Ab	2075.61
10 nM UCHT1	89214.29

Next, these three clones were screened for their abilities to bind primary human and cyno T cells. Briefly, primary human and cyno pan T cells were resuspended at 1 X 10^6 cells/mL in flow staining buffer and cells were plated at 50,000 cells/well. To each well, 50 uL of hybridoma supernatant were added and the mixture was incubated on ice for 30 min. After incubation, 200 µL of staining buffer was added and cells were pelleted by centrifugation at 300 X G for 5 min. Anti-mouse IgG conjugated to Alexa-647 was added at 2 µg/mL in staining buffer in 50 µL total volume and incubated for 30 min on ice. 150 µL of staining buffer was added and cells were pelleted by centrifugation at 300 X G for 5 min. Cells were resuspended in 30 µL of running buffer containing 1:1,000-diluated Sytox green dead cell stain and run on iQue Screener. Cells were gated on FCS vs SCS to eliminate debris. Singlets were gated on SCS-A vs SCS-H, and from singlet population, live cells were chosen using BL1 channel for low-negative with Sytox green. CD3 binding was assessed by comparing test articles to negative control by RL1 (Alexa-647) geomeans. In this assay, clone 065_D03 showed the highest cell binding signal **(FIG. 4A and FIG.4B).**

The variable region of the Clone 065_D03 was then cloned into an IgG1 backbone, resulting in the antibody termed CD3B815 (sequences are shown in **Table 16**). CD3B815 was screened again for binding to Jurkat cells and showed positive binding to Jurkat cells.

Table 16. CD3B815 amino acid sequences.

Protein	SEQ ID NO:	Amino acid sequences

CD3B815 Heavy Chain	262	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKGL EWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGLRAEDT AIYYCTRGWGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
CD3B815 Light Chain	265	DILLTQSPGILSVSPGERVSFSCRARQSIGTAIHWYQQRTNGSPRLLI KYASESISGIPSRFSGSGSGTDFTLTINSVESEDIADYYCQQSNSWPY TFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC

Humanization and scFv formatting of CD3 binding domains

The light chain (LC) of the v-region of CD3B815 was humanized in scFv format. Briefly, the LC from CD3B815 was grafted onto the human IGKV1-39*01-IGKJ2*01 germline and two positions (Y49K and L78V) were identified for human to mouse back mutations. This resulted in variants, having either Y49K, L78V, or both Y49K and L78V. The LC from CD3B815 also contained an NS motif which presents a risk for deamidation at positions 92-93. Therefore, several variants generated also contained N92G. These variants and associated mutations are described in **Table 17**.

Table 17. Mutations in humanized scFv variants, defined according to Kabat numbering system.

scFv identification	Description	VL mutations
CD3W234	CD3B815-HL-scFV, Contains mouse VL	none
CD3W238	CDR of CD3B815 grafted into IGKV1D-39*01	none
CD3W241	CDR of CD3B815 grafted into IGKV1D-39*01	L78V
CD3W242	CDR of CD3B815 grafted into IGKV1D-39*01	Y49K
CD3W243	CDR of CD3B815 grafted into IGKV1D-39*01	Y49K, L78V

CD3W244	CDR of CD3B815 grafted into IGKV1D-39*01	L78V, N92G
CD3W245	CDR of CD3B815 grafted into IGKV1D-39*01	Y49K, N92G
CD3W246	CDR of CD3B815 grafted into IGKV1D-39*01	Y49K, L78V, N92G
CD3W247	CDR of CD3B815 grafted into IGKV1D-39*01	N92G
CD3W248	CD3B815-HL-scFV, Contains mouse VL	N92G

Table 18 shows the VH and the VL amino acid sequences of selected anti-CD3 antibodies. **Table 19** shows the VH and the VL DNA sequences of selected anti-CD3 antibodies. **Table 20** shows the Kabat HCDR1, HCDR2 and the HCDR3 amino acid sequences of selected anti-CD3 antibodies in Kabat delineation. **Table 21** shows the Kabat LCDR1, LCDR2 and the LCDR3 amino acid sequences of selected anti-CD3 antibodies in Kabat delineation. Table 22 summarizes the CDRs, VH and VL sequences of selected CD3 antibodies. **FIG. 6** shows the alignment of the VL region of CD3B815, CD3W244, CD3W245, CD3W246, and CD3W247.

Table 18. VH and VL amino acid sequences of selected anti-CD3 scFv variants.

mAb	VH name	VH sequence	VH SEQ ID NO:	VL name	VL sequence	VL SEQ ID NO:
CD3B815	CD3H488	EVQLVESGGGL VKPGGSLRLSCA ASGFTFSRYNMN WVRQAPGKGLE WVSSISTSSNYIY YADSVKGRFTFS RDNAKNSLDLQ MSGLRAEDTAIY YCTRGWGPFDY WGQGTLVTVSS	248	CD3L372	DILLTQSPGILSVSP GERVSFSCRARQSI GTAIHWYQQRTN GSPRLLIKYASESIS GIPSRFSGSGSGTD FTLTINSVESEDIA DYYCQQSNSWPY TFGGGTKLEIK	249
CD3W244	CD3H488	EVQLVESGGGL VKPGGSLRLSCA	248	CD3L394	DIQMTQSPSSLSAS VGDRVTITCRARQ	250

		ASGFTFSRYNMN WVRQAPGKGLE WVSSISTSSNYIY YADSVKGRFTFS RDNAKNSLDLQ MSGLRAEDTAIY YCTRGWGPFDY WGQGTLVTVSS			SIGTAIHWYQQKP GKAPKLLIYYASE SISGVPSRFSGSGS GTDFTLTISSVQPE DFATYYCQQSGS WPYTFGQGTKLEI K	
CD3W245	CD3H488	EVQLVESGGGL VKPGGSLRLSCA ASGFTFSRYNMN WVRQAPGKGLE WVSSISTSSNYIY YADSVKGRFTFS RDNAKNSLDLQ MSGLRAEDTAIY YCTRGWGPFDY WGQGTLVTVSS	248	CD3L395	DIQMTQSPSSLSAS VGDRVTITCRARQ SIGTAIHWYQQKP GKAPKLLIKYASE SISGVPSRFSGSGS GTDFTLTISSLQPE DFATYYCQQSGS WPYTFGQGTKLEI K	251
CD3W246	CD3H488	EVQLVESGGGL VKPGGSLRLSCA ASGFTFSRYNMN WVRQAPGKGLE WVSSISTSSNYIY YADSVKGRFTFS RDNAKNSLDLQ MSGLRAEDTAIY YCTRGWGPFDY WGQGTLVTVSS	248	CD3L396	DIQMTQSPSSLSAS VGDRVTITCRARQ SIGTAIHWYQQKP GKAPKLLIKYASE SISGVPSRFSGSGS GTDFTLTISSVQPE DFATYYCQQSGS WPYTFGQGTKLEI K	252
CD3W247	CD3H488	EVQLVESGGGL VKPGGSLRLSCA ASGFTFSRYNMN WVRQAPGKGLE WVSSISTSSNYIY YADSVKGRFTFS	248	CD3L397	DIQMTQSPSSLSAS VGDRVTITCRARQ SIGTAIHWYQQKP GKAPKLLIYYASE SISGVPSRFSGSGS GTDFTLTISSLQPE	253

		RDNAKNSLDLQ MSGLRAEDTAIY YCTRGWGPFDY WGQGTLVTVSS			DFATYYCQQSGS WPYTFGQGTKLEI K	
CD3W248	CD3H488	EVQLVESGGGL VKPGGSLRLSCA ASGFTFSRYNMN WVRQAPGKGLE WVSSISTSSNYIY YADSVKGRFTFS RDNAKNSLDLQ MSGLRAEDTAIY YCTRGWGPFDY WGQGTLVTVSS	248	CD3L398	DILLTQSPGILSVSP GERVSFSCRARQSI GTAIHWYQQRTN GSPRLLIKYASESIS GIPSRFSGSGSGTD FTLTINSVESEDIA DYYCQQSGSWPY TFGGGTKLEIK	254
CD3B376	CD3H219	QVQLQQSGPRL VRPSQTLSLTCAI SGDSVFNNNAA WSWIRQSPSRGL EWLGRTYYRSK WLYDYAVSVKS RITVNPDTSRNQ FTLQLNSVTPED TALYYCARGYSS SFDYWGQGTLV TVSS	122	CD3L150	QSALTQPASVSGS PGQSITISCTGTSSN IGTYKFVSWYQQH PDKAPKVLLYEVS KRPSGVSSRFSGS KSGNTASLTISGLQ AEDQADYHCVSY AGSGTLLFGGGTK LTVL	123

Table 19. VH and VL nucleic acid sequences of the humanized scFv variants.

Binding domain name	VH nucleic acid Sequence	VH SEQ ID NO:	VL nucleic acid sequence	VL SEQ ID NO:

CD3B815	GAGGTGCAACTGGTGG AGTCTGGGGGAGGCCT GGTCAAGCCTGGGGGG TCCCTGAGACTCTCCTG TGCAGCCTCTGGATTCA CCTTCAGTAGATATAAC ATGAACTGGGTCCGCCA GGCTCCAGGGAAGGGG CTGGAGTGGGTCTCATC CATTAGTACTAGTAGTA ATTACATATACTACGCA GACTCAGTGAAGGGCC GATTCACCTTCTCCAGA GACAACGCCAAGAACT CACTGGATCTGCAAATG AGCGGCCTGAGAGCCG AGGACACGGCTATTTAT TACTGTACGAGAGGCTG GGGGCCTTTTGACTACT GGGGCCAGGGAACCCT GGTCACCGTCTCCTCA	273	GATATACTTCTTACCCAGA GTCCCGGCATCCTCTCCGT TAGCCCTGGGGAGAGAGT CTCATTCTCATGCCGAGCC AGACAGTCAATTGGTACC GCAATACACTGGTATCAA CAGCGGACCAATGGTTCT CCCCGACTTCTGATAAAGT ACGCATCAGAATCAATTA GTGGAATACCATCAAGAT TTAGTGGCTCAGGGAGTG GAACCGATTTTACTCTGAC CATCAACTCAGTGGAATCT GAGGACATTGCCGACTAC TACTGTCAACAAAGCAAT AGTTGGCCATATACCTTCG GAGGCGGAACTAAATTGG AGATAAAA	276
CD3W244	GAGGTGCAACTGGTGG AGTCTGGGGGAGGCCT GGTCAAGCCTGGGGGG TCCCTGAGACTCTCCTG TGCAGCCTCTGGATTCA CCTTCAGTAGATATAAC ATGAACTGGGTCCGCCA GGCTCCAGGGAAGGGG CTGGAGTGGGTCTCATC CATTAGTACTAGTAGTA ATTACATATACTACGCA GACTCAGTGAAGGGCC GATTCACCTTCTCCAGA	273	GACATCCAGATGACACAG TCACCTTCTAGTTTGTCTG CTTCTGTAGGCGACCGTGT AACTATCACCTGTCGAGCC CGTCAAAGTATTGGTACTG CCATTCACTGGTACCAACA AAAACCTGGCAAAGCTCC AAAACTCTTGATCTACTAT GCCTCCGAAAGCATATCA GGGGTCCCAAGCAGATTC TCAGGCAGTGGCAGTGGC ACTGACTTCACTCTCACCA TTTCTAGCGTGCAACCAGA	277

	GACAACGCCAAGAACT CACTGGATCTGCAAATG AGCGGCCTGAGAGCCG AGGACACGGCTATTTAT TACTGTACGAGAGGCTG GGGGCCTTTTGACTACT GGGGCCAGGGAACCCT GGTCACCGTCTCCTCA		GGACTTCGCCACTTATTAC TGCCAACAGTCAGGGAGC TGGCCCTACACCTTCGGCC AAGGTACAAAACTGGAGA TCAAA	
CD3W245	GAGGTGCAACTGGTGG AGTCTGGGGGAGGCCT GGTCAAGCCTGGGGGG TCCCTGAGACTCTCCTG TGCAGCCTCTGGATTCA CCTTCAGTAGATATAAC ATGAACTGGGTCCGCCA GGCTCCAGGGAAGGGG CTGGAGTGGGTCTCATC CATTAGTACTAGTAGTA ATTACATATACTACGCA GACTCAGTGAAGGGCC GATTCACCTTCTCCAGA GACAACGCCAAGAACT CACTGGATCTGCAAATG AGCGGCCTGAGAGCCG AGGACACGGCTATTTAT TACTGTACGAGAGGCTG GGGGCCTTTTGACTACT GGGGCCAGGGAACCCT GGTCACCGTCTCCTCA	273	GACATACAAATGACACAA TCACCCTCTTCTCTTTCTG CAAGCGTTGGCGACCGTG TCACTATCACTTGTCGAGC CCGCCAGTCCATAGGTACT GCCATTCACTGGTATCAAC AGAAGCCTGGCAAGGCTC CCAAACTCCTGATTAAGTA TGCCAGCGAGAGCATTTC CGGCGTACCTTCAAGATTT TCCGGCTCCGGTAGTGGG ACAGATTTCACTCTCACTA TATCTAGCCTCCAACCAGA AGATTTCGCCACTTACTAC TGTCAACAATCAGGTTCAT GGCCTTACACTTTCGGCCA GGGGACAAAATTGGAGAT CAAG	278
CD3W246	GAGGTGCAACTGGTGG AGTCTGGGGGAGGCCT GGTCAAGCCTGGGGGG TCCCTGAGACTCTCCTG TGCAGCCTCTGGATTCA	273	GACATCCAAATGACTCAA TCACCTAGCAGCCTCTCCG CCTCCGTTGGAGATAGAG TGACAATAACTTGCCGAG CCCGGCAAAGTATCGGAA	279

	CCTTCAGTAGATATAAC ATGAACTGGGTCCGCCA GGCTCCAGGGAAGGGG CTGGAGTGGGTCTCATC CATTAGTACTAGTAGTA ATTACATATACTACGCA GACTCAGTGAAGGGCC GATTCACCTTCTCCAGA GACAACGCCAAGAACT CACTGGATCTGCAAATG AGCGGCCTGAGAGCCG AGGACACGGCTATTTAT TACTGTACGAGAGGCTG GGGGCCTTTTGACTACT GGGGCCAGGGAACCCT GGTCACCGTCTCCTCA		CTGCTATTCACTGGTATCA ACAAAAACCTGGAAAGGC ACCTAAGCTCTTGATTAAA TACGCTTCTGAGTCCATCT CCGGCGTGCCTTCACGATT CAGCGGCAGCGGTAGTGG TACTGACTTTACCCTCACT ATTAGTTCTGTTCAGCCAG AGGACTTCGCAACTTATTA CTGCCAACAGAGTGGTTC CTGGCCATACACTTTTGGC CAGGGGACTAAATTGGAA ATCAAA	
CD3W247	GAGGTGCAACTGGTGG AGTCTGGGGGAGGCCT GGTCAAGCCTGGGGGG TCCCTGAGACTCTCCTG TGCAGCCTCTGGATTCA CCTTCAGTAGATATAAC ATGAACTGGGTCCGCCA GGCTCCAGGGAAGGGG CTGGAGTGGGTCTCATC CATTAGTACTAGTAGTA ATTACATATACTACGCA GACTCAGTGAAGGGCC GATTCACCTTCTCCAGA GACAACGCCAAGAACT CACTGGATCTGCAAATG AGCGGCCTGAGAGCCG AGGACACGGCTATTTAT TACTGTACGAGAGGCTG	273	GACATCCAAATGACTCAA AGCCCCTCTAGTTTGAGTG CATCTGTAGGTGACCGGG TAACAATCACCTGCCGTGC CCGGCAAAGTATAGGTAC TGCAATCCACTGGTACCA GCAAAAACCCGGCAAAGC ACCAAAGCTGCTCATATA CTATGCTAGTGAGAGCATT TCTGGCGTTCCTAGTCGAT TTTCTGGATCAGGGAGTG GAACTGATTTTACACTGAC AATCAGCAGCCTCCAACC CGAAGACTTCGCCACCTA CTATTGTCAGCAGTCTGGG TCCTGGCCTTACACATTCG GTCAAGGAACTAAATTGG AGATCAAA	280

	GGGGCCTTTTGACTACT GGGGCCAGGGAACCCT GGTCACCGTCTCCTCA			
CD3W248	GAGGTGCAACTGGTGG AGTCTGGGGGAGGCCT GGTCAAGCCTGGGGGG TCCCTGAGACTCTCCTG TGCAGCCTCTGGATTCA CCTTCAGTAGATATAAC ATGAACTGGGTCCGCCA GGCTCCAGGGAAGGGG CTGGAGTGGGTCTCATC CATTAGTACTAGTAGTA ATTACATATACTACGCA GACTCAGTGAAGGGCC GATTCACCTTCTCCAGA GACAACGCCAAGAACT CACTGGATCTGCAAATG AGCGGCCTGAGAGCCG AGGACACGGCTATTTAT TACTGTACGAGAGGCTG GGGGCCTTTTGACTACT GGGGCCAGGGAACCCT GGTCACCGTCTCCTCA	273	GACATTTTGCTGACACAG AGCCCTGGTATCCTCTCAG TCAGTCCAGGGGAACGCG TTTCATTTAGCTGCCGTGC TCGACAGAGCATTGGGAC CGCAATCCACTGGTACCA ACAAAGAACTAACGGTTC ACCACGGCTTTTGATTAAG TATGCCTCCGAATCCATCA GTGGCATTCCTAGTCGTTT TTCTGGATCAGGATCAGG CACCGACTTTACTCTCACA ATTAATAGTGTCGAAAGT GAGGACATTGCAGACTAT TATTGTCAGCAATCCGGTT CCTGGCCCTATACTTTTGG TGGTGGTACTAAGTTGGA AATTAAA	281
CD3B376	CAGGTGCAGCTGCAGC AGTCTGGCCCTAGACTC GTGCGGCCTTCCCAGAC CCTGTCTCTGACCTGTG CCATCTCCGGCGACTCC GTGTTCAACAACAACGC CGCCTGGTCCTGGATCC GGCAGAGCCCTTCTAGA GGCCTGGAATGGCTGG	275	AGTCTGCTCTGACCCAGCC TGCCTCCGTGTCTGGCTCT CCCGGCCAGTCCATCACC ATCAGCTGTACCGGCACCT CCTCCAACATCGGCACCTA CAAGTTCGTGTCCTGGTAT CAGCAGCACCCCGACAAG GCCCCCAAAGTGCTGCTGT ACGAGGTGTCCAAGCGGC	283

	GCCGGACCTACTACCGG TCCAAGTGGCTGTACGA CTACGCCGTGTCCGTGA AGTCCCGGATCACCGTG AACCCTGACACCTCCCG GAACCAGTTCACCCTGC AGCTGAACTCCGTGACC CCTGAGGACACCGCCCT GTACTACTGCGCCAGAG GCTACTCCTCCTCCTTC GACTATTGGGGCCAGG GCACCCTCGTGACCGTG TCCTCT		CCTCTGGCGTGTCCTCCAG ATTCTCCGGCTCCAAGTCT GGCAACACCGCCTCCCTG ACCATCAGCGGACTGCAG GCTGAGGACCAGGCCGAC TACCACTGTGTGTCCTACG CTGGCTCTGGCACCCTGCT GTTTGGCGGAGGCACCAA GCTGACCGTGCTG	

Table 20. HCDR1, HCDR2 and HCDR3 amino acid sequences of selected anti-CD3 scFv antibodies using Kabat delineation.

mAb	HCDR1	SEQ ID NO:	HCDR2	SEQ ID NO:	HCDR3	SEQ ID NO:
CD3B815	RYNMN	255	SISTSSNYIYYADSVKG	256	GWGPFDY	257
CD3W244	RYNMN	255	SISTSSNYIYYADSVKG	256	GWGPFDY	257
CD3W245	RYNMN	255	SISTSSNYIYYADSVKG	256	GWGPFDY	257
CD3W246	RYNMN	255	SISTSSNYIYYADSVKG	256	GWGPFDY	257
CD3W247	RYNMN	255	SISTSSNYIYYADSVKG	256	GWGPFDY	257
CD3W248	RYNMN	255	SISTSSNYIYYADSVKG	256	GWGPFDY	257
CD3B376	NNNAAWS	116	RTYYRSKWLYDYAVS VKS	117	GYSSSFDY	118

Table 21. LCDR1, LCDR2 and LCDR3 amino acid sequences of selected anti-CD3 scFv antibodies using Kabat delineation.

mAb	LCDR1	SEQ ID NO:	LCDR2	SEQ ID NO:	LCDR3	SEQ ID NO:
CD3B815	RARQSIGTAIH	258	YASESIS	259	QQSNSWPYT	260
CD3W244	RARQSIGTAIH	258	YASESIS	259	QQSGSWPYT	261
CD3W245	RARQSIGTAIH	258	YASESIS	259	QQSGSWPYT	261
CD3W246	RARQSIGTAIH	258	YASESIS	259	QQSGSWPYT	261
CD3W247	RARQSIGTAIH	258	YASESIS	259	QQSGSWPYT	261
CD3W248	RARQSIGTAIH	258	YASESIS	259	QQSGSWPYT	261
CD3B376	TGTSSNIGTYKFVS	119	EVSKRPS	120	VSYAGSGTLL	121

Table 22. HCDR1, HCDR2, HCD3, LCD1, LCD2, LCD3, VH and VL of anti-CD3 antibodies

Antibody	Region	Amino Acid sequence	SEQ ID NO:
CD3B815	HCDR1	RYNMN	255
	HCDR2	SISTSSNYIYYADSVKG	256
	HCDR3	GWGPFDY	257
	LCDR1	RARQSIGTAIH	258
	LCDR2	YASESIS	259
	LCDR3	QQSNSWPYT	260
	VH (CD3H488)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNW VRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDN AKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGT LVTVSS	248

	VL (CD3L372)	DILLTQSPGILSVSPGERVSFSCRARQSIGTAIHWYQQR TNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLTINSVES EDIADYYCQQSNSWPYTFGGGTKLEIK	249
CD3W244	HCDR1	RYNMN	255
	HCDR2	SISTSSNYIYYADSVKG	256
	HCDR3	GWGPFDY	257
	LCDR1	RARQSIGTAIH	258
	LCDR2	YASESIS	259
	LCDR3	QQSGSWPYT	261
	VH (CD3H488)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNW VRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDN AKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGT LVTVSS	248
	VL (CD3L394)	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQ KPGKAPKLLIYYASESISGVPSRFSGSGSGTDFTLTISSV QPEDFATYYCQQSGSWPYTFGQGTKLEIK	250
CD3BW245	HCDR1	RYNMN	255
	HCDR2	SISTSSNYIYYADSVKG	256
	HCDR3	GWGPFDY	257
	LCDR1	RARQSIGTAIH	258
	LCDR2	YASESIS	259
	LCDR3	QQSGSWPYT	261

	VH (CD3H488)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNW VRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDN AKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGT LVTVSS	248
	VL (CD3L395)	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQ KPGKAPKLLIKYASESISGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSGSWPYTFGQGTKLEIK	251
CD3BW246	HCDR1	RYNMN	255
	HCDR2	SISTSSNYIYYADSVKG	256
	HCDR3	GWGPFDY	257
	LCDR1	RARQSIGTAIH	258
	LCDR2	YASESIS	259
	LCDR3	QQSGSWPYT	261
	VH (CD3H488)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNW VRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDN AKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGT LVTVSS	248
	VL (CD3L396)	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQ KPGKAPKLLIKYASESISGVPSRFSGSGSGTDFTLTISSV QPEDFATYYCQQSGSWPYTFGQGTKLEIK	252
CD3BW247	HCDR1	RYNMN	255
	HCDR2	SISTSSNYIYYADSVKG	256
	HCDR3	GWGPFDY	257
	LCDR1	RARQSIGTAIH	258

	LCDR2	YASESIS	259
	LCDR3	QQSGSWPYT	261
	VH (CD3H488)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNW VRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDN AKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGT LVTVSS	248
	VL (CD3L397)	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQ KPGKAPKLLIYYASESISGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSGSWPYTFGQGTKLEIK	253
CD3BW248	HCDR1	RYNMN	255
	HCDR2	SISTSSNYIYYADSVKG	256
	HCDR3	GWGPFDY	257
	LCDR1	RARQSIGTAIH	258
	LCDR2	YASESIS	259
	LCDR3	QQSGSWPYT	261
	VH (CD3H488)	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNW VRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDN AKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGT LVTVSS	248
	VL (CD3L398)	DILLTQSPGILSVSPGERVSFSCRARQSIGTAIHWYQQR TNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLTINSVES EDIADYYCQQSGSWPYTFGGGTKLEIK	254
CD3B376	HCDR1	NNNAAWS	116
	HCDR2	RTYYRSKWLYDYAVSVKS	117

	HCDR3	GYSSSFDY	118
	LCDR1	TGTSSNIGTYKFVS	119
	LCDR2	EVSKRPS	120
	LCDR3	VSYAGSGTLL	121
	VH (CD3H219)	QVQLQQSGPRLVRPSQTLSLTCAISGDSVFNNNAAWS WIRQSPSRGLEWLGRTYYRSKWLYDYAVSVKSRITVN PDTSRNQFTLQLNSVTPEDTALYYCARGYSSSFDYWG QGTLVTVSS	122
	VL (CD3L150)	QSALTQPASVSGSPGQSITISCTGTSSNIGTYKFVSWYQ QHPDKAPKVLLYEVSKRPSGVSSRFSGSKSGNTASLTI SGLQAEDQADYHCVSYAGSGTLLFGGGTKLTVL	123

Consensus sequence

FIG. 6 shows the alignment of the VL regions of CD3B815, CD3W244, CD3W245, CD3W246, and CD3W247. HCDR and LCDR residues are underlined. A consensus amino acid sequence of **SEQ ID NO: 157** was determined for the VL region and.

SEQ ID NO: 157

DIQX$_1$TQSPX$_2$X$_3$LSX$_4$SX$_5$GX$_6$RVX$_7$X$_8$X$_9$CRARQSIGTAIHWYQQKX$_{10}$X$_{11}$X$_{12}$X$_{13}$PX$_{14}$LLIX$_{15}$YASESI SGX$_{16}$PSRFSGSGSGTDFTLTIX$_{17}$SX$_{18}$QX$_{19}$EDX$_{20}$AX$_{21}$YYCQQSX$_{22}$SWPYTFGX$_{23}$GTKLEIK

wherein, X$_1$ is L or M; X$_2$ is G or S; X$_3$ is I or S; X$_4$ is V or A; X$_5$ is P or V; X$_6$ is E or D; X$_7$ is S or T; X$_8$ is F or I; X$_9$ is S or T; X$_{10}$ is T or P; X$_{11}$ is N or G; X$_{12}$ is G or K; X$_{13}$ is S or A; X$_{14}$ is R or K; X$_{15}$ is K or Y; X$_{16}$ is I or V; X$_{17}$ is N or S; X$_{18}$ is V or L; X$_{19}$ is S or P; X$_{20}$ is I or F; X$_{21}$ is D or T; X$_{22}$ is N or G; or X$_{23}$ is G or Q.

Binding of humanized anti-CD3 scFv variants to CD3 after heat shock.

The variable regions of the anti-CD3 molecules were formatted as scFv in VH-linker-VL orientation using linker GTEGKSSGSGSESKST **(SEQ ID NO: 108)** for expression in E.coli. , and then screened for binding to recombinant CD3 (homodimeric CD3εγ-Fc, CD3W147, **SEQ ID NO: 339**), binding to T cells, and thermostability.

CD3W147 (SEQ ID NO: 339):

QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDH LSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVGSADDAKKDAAKKDDAKKDDAKKDG SQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGFLTEDKKKWNLGSNAKDPRG MYQCKGSQNKSKPLQVYYRMGSGSLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS KAKGQPREPQVYTFPPSQEEMTKNQVSLRCLVKGFYPSDIAVEWESNGQPENNYKTTKPVLDSD GSFRLESRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSGGHHHHHH

Briefly, scFv-coding sequences were cloned into a pADL™-22c vector having a PelB leader sequence for secretion. E. coli cells were transformed with plasmid and grown overnight at 37 °C in 2xYT microbial growth medium supplemented with 100 µg/mL Carbenicillin. Protein expression was induced by addition of 1 mM IPTG and cultures were grown overnight. After expression, cells were pelleted by centrifugation at 2,200 X g for 5 min and supernatants were collected and tested directly for binding to biotinylated CD3W147 by ELISA.

The sequences of the scFv -HL proteins expressed in E.coli are shown in **Table 24.**

Table 24. scFv-HL E.coli amino acid sequences.

scFv	SEQ ID NO:	Amino acid sequence
CD3W234-HL-E.c.	342	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDILLTQSPGILSVSPGERV SFSCRARQSIGTAIHWYQQRTNGSPRLLIKYASESISGIP SRFSGSGSGTDFTLTINSVESEDIADYYCQQSNSWPYTF GGGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS

CD3W238-HL-E.c.	343	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDIQMTQSPSSLSASVGDR VTITCRARQSIGTAIHWYQQKPGKAPKLLIYYASESISG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSNSWPY TFGQGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS
CD3W242-HL-E.c.	344	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDIQMTQSPSSLSASVGDR VTITCRARQSIGTAIHWYQQKPGKAPKLLIKYASESISG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSNSWPY TFGQGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS
CD3W243-HL-E.c.	345	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDIQMTQSPSSLSASVGDR VTITCRARQSIGTAIHWYQQKPGKAPKLLIKYASESISG VPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQSNSWPY TFGQGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS
CD3W244-HL-E.c.	346	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDIQMTQSPSSLSASVGDR VTITCRARQSIGTAIHWYQQKPGKAPKLLIYYASESISG VPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQSGSWPY TFGQGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS
CD3W245-HL-E.c.	347	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDIQMTQSPSSLSASVGDR VTITCRARQSIGTAIHWYQQKPGKAPKLLIKYASESISG

		VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGSWPY TFGQGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS
CD3W246-HL-E.c.	348	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDIQMTQSPSSLSASVGDR VTITCRARQSIGTAIHWYQQKPGKAPKLLIKYASESISG VPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQSGSWPY TFGQGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS
CD3W247-HL-E.c.	349	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDIQMTQSPSSLSASVGDR VTITCRARQSIGTAIHWYQQKPGKAPKLLIYYASESISG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGSWPY TFGQGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS
CD3W248-HL-E.c.	350	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWV RQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNA KNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL VTVSSGTEGKSSGSGSESKSTDILLTQSPGILSVSPGERV SFSCRARQSIGTAIHWYQQRTNGSPRLLIKYASESISGIP SRFSGSGSGTDFTLTINSVESEDIADYYCQQSGSWPYTF GGGTKLEIKGPGGQHHHHHHGAYPYDVPDYAS

The binding of the anti-CD3 scFv variants to CD3 was determined by ELISA. Biotinylated CD3W147 (**SEQ ID NO: 339**) was immobilized on the plate in concentrations ranging from 0.039 µg/mL to 2.5 µg/mL in 2-fold dilutions followed by incubation at room temperature for 45 min. Bound scFv was detected using chicken anti-HA-horseradish peroxidase and then detected with chemiluminescence substrate. All tested scFv molecules derived from CD3B815 bound CD3ε (**FIG. 5**).

The scFv molecules were then tested for their abilities to bind T cells, using flow cytometry. Briefly, human T cells were thawed and resuspended into flow staining buffer at 1 X 10^6 cells/mL and plated at 50,000 cells/well. A positive control, CD3W36 comprised of an anti-CD3 antibody SP34 formatted as \scFv-LH, and a negative control, B23, an scFv targeted against the F-glycoprotein from

respiratory syncytial virus, were used for comparison. E. coli supernatants were added at 150 µL/well and incubated at 4 °C for 1 hr. After incubation, plates were washed with staining buffer and detected with anti-His antibody conjugated to Alexa-647 in staining buffer. After incubation, 200 µL of IntelliCyt running buffer was added to the mixture, and cells were resuspended in 30 µL running buffer containing 1:1,000 Sytox Green dead cell stain and analyzed on iQue Screener. Gating and analysis were performed as above. All scFv molecules derived from CD3B815 displayed mean fluorescence indices consistent with T cell binding (**Table 25**).

Table 25. T cell-based binding of humanized scFv molecules.

Protein	**MFI (n=2)**
CD3W245-HL-E.c.	178140.0
CD3W244-HL-E.c.	165631.0
CD3W246-HL-E.c.	153895.8
CD3W238-HL-E.c.	137380.4
CD3W242-HL-E.c.	126105.9
CD3W243-HL-E.c.	111347.6
CD3W241-HL-E.c.	120793.8
CD3W247-HL-E.c.	110932.3
CD3W248-HL-E.c.	60437.1
CD3W234-HL-E.c.	66790.3
B23	51.8
CD3W36	99451.6

Epitope Identification

The epitope on CD3 was determined by hydrogen-deuterium exchange mass spectrometry (HDX-MS). The antibody clone OKT3 was used as a control for the HDX experiment, since its epitope on CD3ε was known from crystal structure (PDB ID 1SY6) (Kjer-Nielsen, L. *et al.; Proc Natl Acad Sci U S A* **101**, 7675-7680).

On-Exchange Experiment for HDX-MS. On-exchange reaction was initiated by mixing 10 µL of 10 µM CD3W220 (**SEQ ID NO: 340**), which was comprised of CD3εγ fused with a 26-aa linker region fused onto a serum albumin domain, with or without 1.2 molar-excess of ligand and 30 µL of H_2O or a

deuterated buffer (20 mM MES, pH 6.4, 150 mM NaCl in 95% D2O or 20 mM Tris, pH 8.4, 150 mM NaCl in 95% D2O). The reaction mixture was incubated for 15, 50, 150, 500, or 1,500 s at 1.2 °C. The on-exchanged solution was quenched by the addition of chilled 40 μL of 8 M urea, 1 M TCEP, pH 3.0 and immediately analyzed.

CD3W220 (CD3εγ-HSA-6xHis) (SEQ ID NO: 340):

QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQHNDKNIGGDEDDKNIGSDEDH
LSLKEFSELEQSGYYVCYPRGSKPEDANFYLYLRARVGSADDAKKDAAKKDDAKKDDAKKDG
SQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNITWFKDGKMIGFLTEDKKKWNLGSNAKDPRG
MYQCKGSQNKSKPLQVYYRMGGGSDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFEDH
VKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECF
LQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFT
ECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFA
EVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVE
NDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTL
EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTP
TLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNR
RPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMD
DFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGSHHHHHHHH

General Procedure for HDX-MS Data Acquisition. HDX-MS sample preparation was performed with automated HDx system (LEAP Technologies, Morrisville, NC). The columns and pump were; protease, protease type XIII (protease from Aspergillus saitoi, type XIII) /pepsin column (w/w, 1:1; 2.1 x 30 mm) (NovaBioAssays Inc., Woburn, MA); trap, ACQUITY UPLC BEH C18 VanGuard Pre-column (2.1 x 5 mm) (Waters, Milford, MA), analytical, Accucore C18 (2.1 x 100 mm) (Thermo Fisher Scientific, Waltham, MA); and LC pump, VH-P10-A (Thermo Fisher Scientific). The loading pump (from the protease column to the trap column) was set at 600 μL/min with 99% water, 1% acetonitrile, 0.1% formic acid. The gradient pump (from the trap column to the analytical column) was set from 8% to 28% acetonitrile in 0.1% aqueous formic acid in 20 min at 100 μL/min.

MS Data Acquisition. Mass spectrometric analyses were carried out using an LTQ™ Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific) with the capillary temperature at 275 °C, resolution 150,000, and mass range (m/z) 300 – 1,800.

HDX-MS Data Extraction. BioPharma Finder 3.0 (Thermo Fisher Scientific) was used for the peptide identification of non-deuterated samples prior to the HDX experiments. HDExaminer version 2.5

(Sierra Analytics, Modesto, CA) was used to extract centroid values from the MS raw data files for the HDX experiments.

HDX-MS Data Analysis. The extracted HDX-MS data were further analyzed in Excel. All exchange time points (at pH 6.4 or pH 8.4 at 1.2 °C) were converted to the equivalent time points at pH 7.4 and 23 °C (e.g., 15 s at pH 6.4 at 1.2 °C is equivalent of 0.15 s at pH 7.4 at 23 °C; **Table 23**).

Table 23. HDX reaction conditions and exchange times versus exchange times corrected to pH 7.4 and 23 °C.

Time adjusted to pH 7.4, 23 °C (s)	pH 6.4 1.2 °C (s)	pH 8.4 1.2 °C (s)
0.015	-----	-----
0.05	-----	-----
0.15	15	-----
0.5	50	-----
1.5	150	-----
5	500	-----
15	1,500	15
50	-----	50
150	-----	150
500	-----	500
1,500	-----	1,500

Results. Incubation of KLCB91, the bispecific antibodies comprising CD3W245 as an anti-CD3 arm described in Example 5, with recombinant CD3ε (SEQ ID NO: 340) resulted in different patterns of overall protection and degrees of protection at specific segments of the antigen. KLCB91 and OKT3 both protected non-continuous segments (**FIG. 7**) indicating conformational non-identical epitopes. The protected segments were mapped onto the crystal structure of CD3ε (PDB 1SY6) to visualize the binding epitopes in three dimensions.

Consistent with the crystal structure of OKT3 bound to CD3ε (Uniprot ID P07766), the epitope of OKT3 was found to consist of peptides covering spanning residues 29-37, 79-84, and 87-89 of CD3ε (SEQ ID NO: 340 and FIG. 7). CD3W245 bound to an epitope partially overlapping with that of OKT3, and included amino acid residues 29-37 (PQYPGSEIL, SEQ ID NO 341), 55-63 (GSDEDHLSL, SEQ ID NO:448), and 79-84 (PRGSKP, SEQ ID NO: 449) of CD3ε SEQ ID NO: 340 (FIG. 7).

Example 5: Generation of bispecific hK2xCD3 antibodies

The VH/VL regions of the anti-hK2 antibodies generated in Example 1-3 and the VH/VL regions of the anti-CD3 antibody of Example 4 were engineered into bispecific format and expressed as IgG1.

Engineering of CD3 scFvs for hK2/CD3 bispecific generation

CD3 VH/VL regions were engineered as scFvs in either VH-Linker-VL or VL-linker-VH orientations using the linker of SEQ ID NO: 7 **(Table 1)**. Amino acid sequences of anti-CD3 molecules in scFv format are shown in **Table 26**. The VH-Linker-VL or VL-linker-VH scFv molecules binding CD3 were further engineered into a scFv-hinge-CH2-CH3 format comprising Fc silencing mutation (L234A/L235A/D265S) and the T350V/L351Y/F405A/Y407V mutations designed to promote selective heterodimerization. The polypeptide of SEQ ID NO: 109 was used as the constant domain hinge-CH2-CH3. Amino acid sequences of anti-CD3 molecules in scFv-hinge-CH2-CH3 format (scFv-Fc) are shown in **Table 27.** DNA SEQ ID NO: of anti-CD3 molecules in scFv format and scFv-hinge-CH2-CH3 format (scFv-Fc) are shown in **Table 28**.

SEQ ID NO: 109 (huIgG1_G1m(17)-hinge-Fc_C220S_AAS_ZWA)
EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYVYPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALV
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Table 26. ScFv sequences of selected anti-CD3 antibodies

scFv name	Acronym	Amino acid Sequence of scFv	SEQ ID NO:
scFv29	CD3W244_HL	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQ APGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLD LQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGS EGKSSGSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRA RQSIGTAIHWYQQKPGKAPKLLIYYASESISGVPSRFSGSGS GTDFTLTISSVQPEDFATYYCQQSGSWPYTFGQGTKLEIK	328

scFv30	CD3W244_LH	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPG KAPKLLIYYASESISGVPSRFSGSGSGTDFTLTISSVQPEDFA TYYCQQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTG GSEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVR QAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSL DLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSS	329
scFv31	CD3W245_HL	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQ APGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLD LQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGS EGKSSGSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRA RQSIGTAIHWYQQKPGKAPKLLIKYASESISGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCQQSGSWPYTFGQGTKLEIK	330
scFv32	CD3W245_LH	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPG KAPKLLIKYASESISGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTG GSEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVR QAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSL DLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSS	331
scFv33	CD3W246_HL	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQ APGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLD LQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGS EGKSSGSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRA RQSIGTAIHWYQQKPGKAPKLLIKYASESISGVPSRFSGSGS GTDFTLTISSVQPEDFATYYCQQSGSWPYTFGQGTKLEIK	332
scFv34	CD3W246_LH	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPG KAPKLLIKYASESISGVPSRFSGSGSGTDFTLTISSVQPEDFA TYYCQQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTG GSEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVR QAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSL DLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSS	333
scFv35	CD3W247_HL	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQ APGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLD LQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGS	334

		EGKSSGSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRA RQSIGTAIHWYQQKPGKAPKLLIYYASESISGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCQQSGSWPYTFGQGTKLEIK	
scFv36	CD3W247_LH	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPG KAPKLLIYYASESISGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTG GSEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVR QAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSL DLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSS	335
scFv37	CD3W248_HL	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQ APGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLD LQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGS EGKSSGSGSESKSTGGSDILLTQSPGILSVSPGERVSFSCRAR QSIGTAIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGT DFTLTINSVESEDIADYYCQQSGSWPYTFGGGTKLEIK	336
scFv38	CD3W248_LH	DILLTQSPGILSVSPGERVSFSCRARQSIGTAIHWYQQRTNG SPRLLIKYASESISGIPSRFSGSGSGTDFTLTINSVESEDIADY YCQQSGSWPYTFGGGTKLEIKGGSEGKSSGSGSESKSTGGS EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQ APGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLD LQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSS	337

Table 27. SEQ ID NOs and amino acid sequences of scFv-Fc (scFv-hinge CH2-CH3) anti-CD3 antibodies

scFv name	Antibody Name	scFv-Fc (scFv-hinge CH2-CH3) amino acid sequences	SEQ ID NO:
scFv29	CD3W244-HL-Fc	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAP GKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQM SGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGSEGKSS GSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRARQSIGT AIHWYQQKPGKAPKLLIYYASESISGVPSRFSGSGSGTDFTLTI	326

		SSVQPEDFATYYCQQSGSWPYTFGQGTKLEIKEPKSSDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	
scFv30	CD3W244-LH-Fc	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKA PKLLIYYASESISGVPSRFSGSGSGTDFTLTISSVQPEDFATYYC QQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGSEVQ LVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKG LEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGL RAEDTAIYYCTRGWGPFDYWGQGTLVTVSSEPKSSDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	327
scFv31	CD3W245-HL-Fc	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAP GKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQM SGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGSEGKSS GSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRARQSIGT AIHWYQQKPGKAPKLLIKYASESISGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQSGSWPYTFGQGTKLEIKEPKSSDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	375
scFv32	CD3W245-LH-Fc	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKA PKLLIKYASESISGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGSEVQ LVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKG	376

		LEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGL RAEDTAIYYCTRGWGPFDYWGQGTLVTVSSEPKSSDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	
scFv33	CD3W246-HL-Fc	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAP GKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQM SGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGSEGKSS GSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRARQSIGT AIHWYQQKPGKAPKLLIKYASESISGVPSRFSGSGSGTDFTLTI SSVQPEDFATYYCQQSGSWPYTFGQGTKLEIKEPKSSDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	377
scFv34	CD3W246-LH-Fc	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKA PKLLIKYASESISGVPSRFSGSGSGTDFTLTISSVQPEDFATYYC QQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGSEVQ LVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKG LEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGL RAEDTAIYYCTRGWGPFDYWGQGTLVTVSSEPKSSDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	453
scFv35	CD3W247-HL-Fc	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAP GKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQM SGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGSEGKSS	379

		GSGSESKSTGGSDIQMTQSPSSLSASVGDRVTITCRARQSIGT AIHWYQQKPGKAPKLLIYYASESISGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQSGSWPYTFGQGTKLEIKEPKSSDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	
scFv36	CD3W247-LH-Fc	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKA PKLLIYYASESISGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGSEVQ LVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKG LEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGL RAEDTAIYYCTRGWGPFDYWGQGTLVTVSSEPKSSDKTHTC PPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV YPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK	380
scFv37	CD3W248-HL-Fc	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAP GKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQM SGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSGGSEGKSS GSGSESKSTGGSDILLTQSPGILSVSPGERVSFSCRARQSIGTAI HWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLTINS VESEDIADYYCQQSGSWPYTFGGGTKLEIKEPKSSDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK	381

scFv38	CD3W248-LH-Fc	DILLTQSPGILSVSPGERVSFSCRARQSIGTAIHWYQQRTNGSP RLLIKYASESISGIPSRFSGSGSGTDFTLTINSVESEDIADYYCQ QSGSWPYTFGGGTKLEIKGGSEGKSSGSGSESKSTGGSEVQL VESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKGL EWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGLR AEDTAIYYCTRGWGPFDYWGQGTLVTVSSEPKSSDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK	382

Table 28. DNA SEQ ID NOs of anti-CD3 antibodies in scFv and scFv-hinge-CH2-CH3 (scFv-Fc) format

Antibody Name	scFv DNA SEQ ID NO	scFv-Fc DNA SEQ ID NO
CD3W244_HL	384	394
CD3W244_LH	385	395
CD3W245_HL	386	396
CD3W245_LH	387	397
CD3W246_HL	388	398
CD3W246_LH	389	399
CD3W247_HL	390	400
CD3W247_LH	391	401

CD3W248_HL	392	402
CD3W248_LH	393	403

SEQ ID NO: 384 (CD3W244_HL scFv)

GAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGCAGCCTGCGCCTGA
GCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGTGCGCCAAGCCCCA
GGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTACATCTACTACGCCG
ACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACAGCCTGGACCTGCA
GATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGCGGTTGGGGCCCAT
TCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGGCGGATCTGAGGGAAAGTC
CAGCGGCTCCGGCAGCGAAAGCAAGTCCACCGGCGGAAGCGACATCCAGATGACCCAGAGC
CCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCATCACCTGTCGTGCCCGCCAGA
GCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGCAAGGCCCCAAAGCTGCTGAT
CTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCTTCAGCGGCAGCGGCAGCGGC
ACCGACTTCACCCTGACCATCAGCAGCGTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA
GCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG

SEQ ID NO: 385 (CD3W244_LH scFv)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCGTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTC
CACCGGCGGAAGCGAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGC
AGCCTGCGCCTGAGCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGT
GCGCCAAGCCCCAGGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTAC
ATCTACTACGCCGACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACA
GCCTGGACCTGCAGATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGC
GGTTGGGGCCCATTCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO: 386 (CD3W245_HL scFv)

GAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGCAGCCTGCGCCTGA
GCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGTGCGCCAAGCCCCA
GGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTACATCTACTACGCCG
ACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACAGCCTGGACCTGCA
GATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGCGGTTGGGGCCCAT
TCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGGCGGATCTGAGGGAAAGTC
CAGCGGCTCCGGCAGCGAAAGCAAGTCCACCGGCGGAAGCGACATCCAGATGACCCAGAGC
CCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCATCACCTGTCGTGCCCGCCAGA
GCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGCAAGGCCCCAAAGCTGCTGAT
CAAGTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCTTCAGCGGCAGCGGCAGCGGC
ACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA
GCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG

SEQ ID NO: 387 (CD3W245_LH scFv)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCAAGTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTC
CACCGGCGGAAGCGAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGC
AGCCTGCGCCTGAGCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGT
GCGCCAAGCCCCAGGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTAC
ATCTACTACGCCGACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACA
GCCTGGACCTGCAGATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGC
GGTTGGGGCCCATTCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO: 388 (CD3W246_HL scFv)

GAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGCAGCCTGCGCCTGA
GCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGTGCGCCAAGCCCCA
GGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTACATCTACTACGCCG
ACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACAGCCTGGACCTGCA

GATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGCGGTTGGGGCCCAT
TCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGGCGGATCTGAGGGAAAGTC
CAGCGGCTCCGGCAGCGAAAGCAAGTCCACCGGCGGAAGCGACATCCAGATGACCCAGAGC
CCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCATCACCTGTCGTGCCCGCCAGA
GCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGCAAGGCCCCAAAGCTGCTGAT
CAAGTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCTTCAGCGGCAGCGGCAGCGGC
ACCGACTTCACCCTGACCATCAGCAGCGTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA
GCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG

SEQ ID NO: 389 (CD3W246_LH scFv)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCAAGTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCGTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTC
CACCGGCGGAAGCGAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGC
AGCCTGCGCCTGAGCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGT
GCGCCAAGCCCCAGGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTAC
ATCTACTACGCCGACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACA
GCCTGGACCTGCAGATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGC
GGTTGGGGCCCATTCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO: 390 (CD3W247_HL scFv)

GAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGCAGCCTGCGCCTGA
GCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGTGCGCCAAGCCCCA
GGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTACATCTACTACGCCG
ACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACAGCCTGGACCTGCA
GATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGCGGTTGGGGCCCAT
TCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGGCGGATCTGAGGGAAAGTC
CAGCGGCTCCGGCAGCGAAAGCAAGTCCACCGGCGGAAGCGACATCCAGATGACCCAGAGC
CCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCATCACCTGTCGTGCCCGCCAGA
GCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGCAAGGCCCCAAAGCTGCTGAT

CTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCTTCAGCGGCAGCGGCAGCGGC
ACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCAGAGGACTTCGCCACCTACTACTGCCA
GCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG

SEQ ID NO: 391 (CD3W247_LH scFv)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTC
CACCGGCGGAAGCGAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGC
AGCCTGCGCCTGAGCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGT
GCGCCAAGCCCCAGGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTAC
ATCTACTACGCCGACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACA
GCCTGGACCTGCAGATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGC
GGTTGGGGCCCATTCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

SEQ ID NO: 392 (CD3W248_HL scFv)

GAGGTGCAACTGGTGGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGATATAACATGAACTGGGTCCGCCAGGCTCCA
GGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTACTAGTAGTAATTACATATACTACGCAGA
CTCAGTGAAGGGCCGATTCACCTTCTCCAGAGACAACGCCAAGAACTCACTGGATCTGCAAA
TGAGCGGCCTGAGAGCCGAGGACACGGCTATTTATTACTGTACGAGAGGCTGGGGGCCTTTT
GACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGCGGATCTGAGGGAAAGTCCA
GCGGCTCCGGCAGCGAAAGCAAGTCCACCGGCGGAAGCGACATCTTGCTGACTCAGTCTCC
AGGCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCAGTTTCTCCTGCAGGGCCAGACAGAGC
ATTGGCACAGCCATACACTGGTATCAGCAAAGAACAAATGGTTCTCCAAGGCTTCTCATAAA
GTATGCTTCTGAGTCTATCTCTGGGATCCCTTCCAGGTTTAGCGGCAGTGGATCAGGGACAG
ATTTTACTCTTACCATCAACAGTGTGGAGTCTGAAGATATTGCAGATTATTACTGTCAACAA
AGTGGGAGCTGGCCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA

SEQ ID NO: 393 (CD3W248_LH scFv)

GACATCTTGCTGACTCAGTCTCCAGGCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCAGTTT
CTCCTGCAGGGCCAGACAGAGCATTGGCACAGCCATACACTGGTATCAGCAAAGAACAAAT
GGTTCTCCAAGGCTTCTCATAAAGTATGCTTCTGAGTCTATCTCTGGGATCCCTTCCAGGTTT
AGCGGCAGTGGATCAGGGACAGATTTTACTCTTACCATCAACAGTGTGGAGTCTGAAGATAT
TGCAGATTATTACTGTCAACAAAGTGGGAGCTGGCCGTACACGTTCGGAGGGGGGACCAAG
CTGGAAATAAAAGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTCCA
CCGGCGGAAGCGAGGTGCAACTGGTGGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGATATAACATGAACTGGGTCC
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTACTAGTAGTAATTACATA
TACTACGCAGACTCAGTGAAGGGCCGATTCACCTTCTCCAGAGACAACGCCAAGAACTCACT
GGATCTGCAAATGAGCGGCCTGAGAGCCGAGGACACGGCTATTTATTACTGTACGAGAGGC
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GAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGCAGCCTGCGCCTGA
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GGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTACATCTACTACGCCG
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GATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGCGGTTGGGGCCCAT
TCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGGCGGATCTGAGGGAAAGTC
CAGCGGCTCCGGCAGCGAAAGCAAGTCCACCGGCGGAAGCGACATCCAGATGACCCAGAGC
CCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCATCACCTGTCGTGCCCGCCAGA
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CTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCTTCAGCGGCAGCGGCAGCGGC
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GCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCAAGCTGGAGATCAAGGAGCCC
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SEQ ID NO: 395 (CD3W244_LH-scFv-Fc)

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AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA
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AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG
GGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGAC
GGCTCCTTCGCCCTCGTGAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAACG
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SEQ ID NO: 397 (CD3W245_LH-scFv-Fc)

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CTGTCTCCGGGTAAA

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GGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGA
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AGCTGGAGATCAAGGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTC
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GATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGCGGTTGGGGCCCAT
TCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGGCGGATCTGAGGGAAAGTC
CAGCGGCTCCGGCAGCGAAAGCAAGTCCACCGGCGGAAGCGACATCCAGATGACCCAGAGC
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GCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGCAAGGCCCCAAAGCTGCTGAT
CTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCTTCAGCGGCAGCGGCAGCGGC
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TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
CGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC
AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCA
AAGGGCAGCCCCGAGAACCACAGGTGTACGTGTACCCCCCATCCCGGGAGGAGATGACCAA
GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGT
GGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGA
CGGCTCCTTCGCCCTCGTGAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAAC
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CCTGTCTCCGGGTAAA

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CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTC

CACCGGCGGAAGCGAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGC
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CTAGCGACAAAACTCACACATGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTC
AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCA

CATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
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CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT
GCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG
GCAGCCCCGAGAACCACAGGTGTACGTGTACCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGA
GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGC
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SEQ ID NO: 403 (CD3W248_LH-scFv-Fc)

GACATCTTGCTGACTCAGTCTCCAGGCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCAGTTT
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AGCGGCAGTGGATCAGGGACAGATTTTACTCTTACCATCAACAGTGTGGAGTCTGAAGATAT
TGCAGATTATTACTGTCAACAAAGTGGGAGCTGGCCGTACACGTTCGGAGGGGGGACCAAG
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CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
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CAGCCCCGAGAACCACAGGTGTACGTGTACCCCCCATCCCGGGAGGAGATGACCAAGAACC
AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT
CCTTCGCCCTCGTGAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTTC

TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC
TCCGGGTAAA

Engineering of CD3 Fabs for hK2/CD3 bispecific generation

The CD3 specific VH and VL regions were engineered in VH-CH1-hinge-CH2-CH3 and VL-CL formats respectively and expressed as IgG1. The polypeptide of **SEQ ID NO: 158** comprising the Fc silencing mutation L234A/L235A/D265S and the CH3 mutation T350V/L351Y/F405A/Y407V designed to promote selective heterodimerization was used to generate the CD3 specific VH-CH1-hinge-CH2-CH3 (**Table 29**).

SEQ ID NO: 158 (huIgG1_G1m(17)_AAS_ZWA)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK
DTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSREEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

The polypeptides of SEQ ID NO: 309 or 447 were used to generate the CD3 specific VL-CL (**Table 30**)

SEQ ID NO: 309 (human kappa light chain)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 447 (human lambda light chain)

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNK
YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

SEQ ID NO: of DNA sequences of anti-CD3 molecules in VH-CH1-hinge-CH2-CH3 and Vl-CL format are shown in **Table 31**.

Table 29. VH-CH1-hinge-CH2-CH3. heavy chain amino acid sequences of selected anti CD3 antibodies

HC protein	SEQ ID NO:	HC amino acid sequence
CD3W244 HC, CD3W245 HC, CD3W246 HC, CD3W247 HC, CD3W248 HC,	264	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKGL EWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGLRAEDT AIYYCTRGWGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYVYPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
CD3B376 HC	359	QVQLQQSGPRLVRPSQTLSLTCAISGDSVFNNNAAWSWIRQSPSRG LEWLGRTYYRSKWLYDYAVSVKSRITVNPDTSRNQFTLQLNSVTPE DTALYYCARGYSSSFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Table 30. VL-CL light chain amino acid sequences of selected anti-CD3 antibodies

HC protein	SEQ ID NO:	LC amino acid sequence
CD3W244 LC	266	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKAPKLL IYYASESISGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQSGSWP YTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
CD3W245 LC	267	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKAPKLL IKYASESISGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGSWP YTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC

CD3W246 LC	268	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKAPKLL IKYASESISGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQQSGSWP YTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
CD3W247 LC	269	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKPGKAPKLL IYYASESISGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSGSWP YTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
CD3W248 LC	270	DILLTQSPGILSVSPGERVSFSCRARQSIGTAIHWYQQRTNGSPRLLIK YASESISGIPSRFSGSGSGTDFTLTINSVESEDIADYYCQQSGSWPYTF GGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
CD3B376 LC	272	QSALTQPASVSGSPGQSITISCTGTSSNIGTYKFVSWYQQHPDKAPK VLLYEVSKRPSGVSSRFSGSKSGNTASLTISGLQAEDQADYHCVSYA GSGTLLFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLIS DFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECS

Table 31. cDNA SEQ ID NOs of anti-CD3 antibodies HC in VH-CH1-hinge-CH2-CH3 (HC) format and LC in VL-CL (LC) format.

Antibody	HC cDNA SEQ ID NO:	LC cDNA SEQ ID NO:
CD3W244	471	311
CD3W245	471	452
CD3W246	471	313
CD3W247	471	314
CD3W248	471	315

CD3B376	471	317

SEQ ID NO: 471 (CD3W244, CDRW245, CD3W246, CD3W247, CD3W248 HC cDNA)

GAGGTGCAGCTGGTGGAGAGCGGTGGCGGTCTGGTGAAGCCAGGTGGCAGCCTGCGCCTGA
GCTGTGCCGCCAGCGGTTTCACCTTCAGCCGCTACAACATGAACTGGGTGCGCCAAGCCCCA
GGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGCACCAGCAGCAACTACATCTACTACGCCG
ACAGCGTGAAGGGCCGCTTCACCTTCAGCCGCGACAACGCCAAGAACAGCCTGGACCTGCA
GATGAGCGGTCTGCGCGCCGAGGACACCGCCATCTACTACTGCACCCGCGGTTGGGGCCCAT
TCGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGCGCCTCCACCAAGGGCCCATC
GGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGTCC
ACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCA
AGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCCAC
GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGA
CAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCT
GCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
GCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACG
TGTACCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA
AGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCGCCCTCGTGAGCAAGCTCAC
CGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTC
TGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

SEQ ID NO: 311 (CD3W244 LC cDNA)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCGTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAG

CAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGC
CAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAG
ACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO: 452 (CD3W245 LC cDNA)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCAAGTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAG
CAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGC
CAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAG
ACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO: 313 (CD3W246 LC cDNA)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCAAGTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCGTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAG
CAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGC
CAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAG
ACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO: 314 (CD3W247 LC cDNA)

GACATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCCAGCGTCGGCGACCGCGTGACCA
TCACCTGTCGTGCCCGCCAGAGCATCGGCACCGCCATCCACTGGTACCAGCAGAAGCCAGGC
AAGGCCCCAAAGCTGCTGATCTACTACGCCAGCGAGAGCATCAGCGGTGTGCCAAGCCGCT
TCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCAGAGGA
CTTCGCCACCTACTACTGCCAGCAGAGCGGCAGCTGGCCATACACCTTCGGCCAGGGCACCA
AGCTGGAGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAG
CAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGC
CAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAG
ACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO: 315 (CD3W248 LC cDNA)

GACATCTTGCTGACTCAGTCTCCAGGCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCAGTTT
CTCCTGCAGGGCCAGACAGAGCATTGGCACAGCCATACACTGGTATCAGCAAAGAACAAAT
GGTTCTCCAAGGCTTCTCATAAAGTATGCTTCTGAGTCTATCTCTGGGATCCCTTCCAGGTTT
AGCGGCAGTGGATCAGGGACAGATTTTACTCTTACCATCAACAGTGTGGAGTCTGAAGATAT
TGCAGATTATTACTGTCAACAAAGTGGGAGCTGGCCGTACACGTTCGGAGGGGGGACCAAG
CTGGAAATAAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCA
GTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA
AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGA
GCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGAC
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGT

Engineering of hK2 scFvs-Fc for hK2/CD3 bispecific generation

hK2 VH/VL regions engineered as scFvs in either VH-Linker-VL or VL-linker-VH orientations using the linker of SEQ ID NO: 7 (**Table 1**) as described in Example 2 were further engineered into a scFv-hinge-CH2-CH3 format comprising the Fc silencing mutation (L234A/L235A/D265S) and the T350V/T366L/K392L/T394W mutations designed to promote selective heterodimerization and expressed as IgG1. The polypeptide of **SEQ ID NO: 110** was used as the constant domain hinge-CH2-CH3.

SEQ ID NO: 110 (huIgG1_G1m(17)-hinge-Fc_C220S_AAS_ZWB)

EPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYVLPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Amino acid sequences of anti-hK2 molecules in scFv-hinge-CH2-CH3 format (scFv-Fc) are shown in **Table 32.**

Table 32. Amino acid sequences of anti-hK2 molecules in scFvs-Fc format used for hK2/CD3 bispecific generation

Protein	SEQ ID NO:	Amino acid sequence
KL2B359-LH-scFv-Fc	351	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWY QQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSV EPEDFAVYFCQQTRKVPYTFGGGTKVEIKGGSEGKSSGSG SESKSTGGSQVQLQESGPGLVKPSQTLSLTCTVSGNSITSD YAWNWIRQFPGKRLEWIGYISYSGSTTYNPSLKSRVTISR DTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQG TLVTVSSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYVLPPSREEMTKNQVSLLC LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
KL2B413-LH-scFv-Fc	352	EIVLTQSPSFLSASVGDRVTITCRASQGISSYLSWYQQKPG KAPKLLIYATSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDF ATYYCQQLNSYPRTFGQGTKVEIKGGSEGKSSGSGSESKS TGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMT WVRQAPGKGLEWVANIKQDGSERYYVDSVKGRFTISRD NAKNSLYLQMNSLRAEDTAVYYCARDQNYDILTGHYGM DVWGQGTTVTVSSEPKSSDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY

		KCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSREEMT KNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
KL2B467-LH-scFv-Fc	353	QSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQQKPG QAPVLVVYDNSDRPSGIPERFSGSNSGTTATLTISRVEAGD EADYYCQVWDSSSDHPVVFGGGTKVTVLGGSEGKSSGS GSESKSTGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFS YYGMHWVRQAPGKGLEWVAFISYDGSNKYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSYW AFDYWGQGTQVTVSSEPKSSDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSREE MTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG
KL2B494-LH-scFv-Fc	357	SSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPG QAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGD EADYYCQVWDSSSDHVVFGGGTKLTVLGGSEGKSSGSGS ESKSTGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSHY AMSWVRQAPGKGLEWVSTIGGSGGSTYYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTALLY DGMDVWGQGTMVTVSSEPKSSDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSR EEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTW PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG

Engineering of hK2 Fab-Fc for hK2/CD3 bispecific generation

The hK2 specific VH and VL regions were engineered in VH-CH1-hinge-CH2-CH3 and VL-CL formats respectively. The polypeptide of **SEQ ID NO: 378** comprising the Fc silencing mutation L234A/L235A/D265S and the CH3 mutation T350V/T366L/K392L/T394W designed to promote selective heterodimerization was used to generate the KLK2 specific VH-CH1-hinge-CH2-CH3).

SEQ ID NO: 378 (huIgG1_G1m(17)_AAS_ZWB)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK
DTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSREEMTKNQVSLLCLVKGFYPS
DIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

The polypeptides of SEQ ID NO: 309 or 447 were used to generate the hK2 specific VL-CL.

SEQ ID NO: 309 (human kappa light chain)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 447 (human lambda light chain)

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNK
YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

The amino acid sequences of hK2 Fab-Fc HC are shown in **Table 33.**

Table 33. Amino acid sequences of hK2 Fab-Fc HC (VH-CH1-hinge-CH2-CH3) for hK2/CD3 bispecific generation

Protein	SEQ ID NO:	Amino acid sequence
KL2B30 Fab HC	354	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPP GKGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKL SSVTAADTAVYYCAGTTIFGVVTPNFYYGMDVWGQGTT VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP

		VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV LPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENN YLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG
KL2B242 Fab HC	355	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQP AGSGLEWIGRLYVSGFTNYNPSLKSRVTLSLDPSRNQLSL KLSSVTAADTAVYYCAGDSGNYWGWFDPWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPP SREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYL TWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
KL2B53 Fab HC	356	EVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHWVRQ APGKGLEWVAIISYDGSKKDYTDSVKGRFTISRDNSKNTL YLQMDSLRVEDSAVYSCARESGWSHYYYYGMDVWGQG TMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YVLPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPE NNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG
KL2B30 Fab w/K477	361	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPP GKGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKL

		SSVTAADTAVYYCAGTTIFGVVTPNFYYGMDVWGQGTT VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV LPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENN YLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK

hK2/CD3 bispecifics

CD3B376 and CD3W245, engineered as Fabs and the hK2 VH/VL regions of KL2B359, KL2B413, KL2B467 and KL2B494 engineered as scFvs in both orientation as described above, were expressed to generate bispecific antibodies, yielding hK2/CD3 bispecific antibodies with a hK2 binding arm in a format scFv-hinge-CH2-CH3 and a CD3 binding arm in a format of : heavy chain: VH-CH1-hinge-CH2-CH3 and light chain: VL-CL. Alternatively, the VH/VL regions of the anti-CD3 antibodies CD3W245 engineered as scFvs in the LH-linker-VH orientation and the VH/VL regions of the anti-hK2 antibodies KL2B30, KL2B242 and KL2B53 engineered as Fabs as described above, were expressed to generate bispecific antibodies, yielding hK2/CD3 bispecific antibodies with a hK2 binding arm in the format of a heavy chain VH-CH1-hinge-CH2-CH3 and light chain VL-CL and a CD3 binding arm in a format scFv-hinge-CH2-CH3. The linker used to generate the anti- scFv is the linker of **SEQ ID NO:** 7.

T350V_L351Y_F405A_Y407V CH3 mutations were engineered into one heavy chain and T350V_T366L_K392L_T394W CH3 mutations were engineered into the other heavy chain as described above. In addition, both HK2 and CD3 binding arms were engineered to contain Fc effector silencing mutations L234A_L235A_D265S as described above.

The KL2B242 light chain, which belonged to the human lamda-3 germline, contained one free Cys residue which was mutated into a Ser (C33S) to eliminate the unpaired free Cys and prevent potential manufacturability issues.

The engineered chains were expressed, and the resulting bispecific antibodies purified using standard methods. The bispecific antibodies were characterized for their binding to hK2 and CD3, and their *in vitro* and *in vivo* cytotoxicity as described in Example 8 and 9. **Table 34** shows the CDR SEQ ID NOs: of selected anti hKL2/CD3 bispecific antibodies. **Table 35** shows the VH, VL and scFv SEQ ID NOs: of selected anti hKL2/CD3 bispecific antibodies. **Table 36** shows the HC1, HC2, LC1 and LC2 SEQ ID NOs of selected anti hKL2/CD3 bispecific antibodies. HC1 and LC1 refer to the heavy and light chain of the hKL2 binding arm. Alternatively, HC1 can also refer to the scFv-hinge-CH2-CH3 of the hKl2 binding arm. HC2 and LC2 refer to the heavy and light chain of the CD3 binding arm. Alternatively, HC2 can also refer to the scFv-hinge-CH2-CH3 of the CD3 binding arm. **Table 37** the amino acid sequences of HC1, LC1, HC2 and LC2. Table 37 the cDNA sequences of HC1, LC1, HC2 and LC2.

Table 34. Kabat CDR SEQ ID NOs of bispecific hK2/CD3 antibodies

Bispecific antibody	Parental (hK2 arm/CD3 arm)	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
KLCB91	KL2B359-LH-scFv	63	65	66	67	69	71
	CD3W245 Fab	255	256	257	258	259	261
KLCB105	KL2B359-LH-scFv	63	65	66	67	69	71
	CD3B376 Fab	116	117	118	119	120	121
KLCB95	KL2B413-LHscFv	141	142	143	144	145	146
	CD3W245 Fab	255	256	257	258	259	261
KLCB96	KL2B413-LH-scFv	141	142	143	144	145	146
	CD3B376 Fab	116	117	118	119	120	121
KLCB170	KL2B467-LH-scFv	188	189	190	191	192	193
	CD3W245 Fab	255	256	257	258	259	261
KLCB80	KL2B30 Fab	170	171	172	173	174	175

	CD3W245-LH-scFv	255	256	257	258	259	261
KLCB81	KL2B242 LC_C33S Fab	170	183	184	185	186	187
	CD3W245-LH-scFv	255	256	257	258	259	261
KLCB113	KL2B53 Fab	176	177	178	179	180	181
	CD3W245-LH-scFv	255	256	257	258	259	261
KLCB281	KL2B467-LH-scFv	188	189	190	191	192	193
	CD3B376-Fab	116	117	118	119	120	121
KLCB174	KL2B494-LH-scFv	206	207	208	182	470	209
	CD3B376-Fab	116	117	118	119	120	121
KLCB153	KL2B494-LH-scFv	206	207	208	182	470	209
	CD3W245-Fab	255	256	257	258	259	261
KLCB245	KL2B30-Fab w/ K447	170	171	172	173	174	175
	CD3W245-LH-scFv w/ K447	255	256	257	258	259	261

Table 35. SEQ ID NOs of the variable region of the hK2 arm and the CD3 arm of selected KL2/CD3 bispecific antibodies.

Bispecific Name	hK2 arm				CD3 arm			
	Name	VH1 SEQ	VL1 SEQ	scFv SEQ	Name	VH2 SEQ	VL2 SEQ	scFv SEQ

		ID NO:	ID NO:	ID NO		ID NO:	ID NO:	ID NO:
KLCB91	KL2B359-LH-scFv(scFv20)			136	CD3W245 Fab	248	251	
KLCB105	KL2B359-LHscFv (scFv20)			136	CD3B376 Fab	122	123	
KLCB95	KL2B413-LH-scFv (scFv18)			134	CD3W245 Fab	248	251	
KLCB96	KL2B413-LH-scFv(scFv18)			134	CD3B376 Fab	122	123	
KLCB170	KL2B467-LH-scFv(scFv28)			325	CD3W245 Fab	248	251	
KLCB80	KL2B30 Fab	162	163		CD3W245-LH-scFv (scFv32)			331
KLCB81	KL2B242LC_C33S Fab	166	444		CD3W245-LH-scFv (scFv32)			331
KLCB113	KL2B53 Fab	164	165		CD3W245-LH-scFv (scFv32)			331
KLCB281	KL2B467-LH-scFv (scFv28)			325	CD3B376 Fab	122	123	
KLCB174	KL2B494-LH-scFv			316	CD3B376-Fab	122	123	
KLCB153	KL2B494-LH-scFv			316	CD3W245-Fab	248	251	
KLCB245	KL2B30-Fab w/ K447	162	163		CD3W245-LH-scFv w/ K447			331

Table 36. HC and LC amino acid SEQ ID NOs of hK2/CD3 bispecific antibodies

Bispecific Name	hK2 arm			CD3 arm		
	Name	HC1 or scFv -Fc SEQ ID NO:	LC1 SEQ ID NO:	Name	HC2 or scFv - Fc SEQ ID NO:	LC2 SEQ ID NO:

KLCB91	KL2B359 LH-Fc	351		CD3W245 Fab	358	267
KLCB105	KL2B359-LH-Fc	351		CD3B376 Fab	359	272
KLCB95	KL2B413-LH-Fc	352		CD3W245 Fab	358	267
KLCB96	KL2B413-LH-Fc	352		CD3B376 Fab	359	272
KLCB170	KL2B467-LH-Fc	353		CD3W245 Fab	358	267
KLCB80	KL2B30 Fab	354	221	CD3W245- LH-scFv-Fc	360	
KLCB81	KL2B242LC_C33S Fab	355	445	CD3W245- LH-scFv-Fc	360	
KLCB113	KL2B53 Fab	356	222	CD3W245- LH-scFv-Fc	360	
KLCB281	KL2B467-LH-scFv (scFv28)	353		CD3B376 Fab	359	272
KLCB174	KL2B494-LH-scFv	357		CD3B376-Fab	359	272
KLCB153	KL2B494-LH-scFv	357		CD3W245-Fab	358	267
KLCB245	KL2B30-Fab w/ K447	361	221	CD3W245-LH-scFv w/ K447	362	

Table 37. Bispecific HC1 and HC2 amino acid sequences

Protein	SEQ ID NO:	Amino acid sequence
KL2B359-LH-scFv-Fc	351	EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWY QQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSV EPEDFAVYFCQQTRKVPYTFGGGTKVEIKGGSEGKSSGSG SESKSTGGSQVQLQESGPGLVKPSQTLSLTCTVSGNSITSD YAWNWIRQFPGKRLEWIGYISYSGSTTYNPSLKSRVTISR DTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQG TLVTVSSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYVLPPSREEMTKNQVSLLC LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

KL2B413-LH-scFv-Fc	352	EIVLTQSPSFLSASVGDRVTITCRASQGISSYLSWYQQKPG KAPKLLIYATSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDF ATYYCQQLNSYPRTFGQGTKVEIKGGSEGKSSGSGSESKS TGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMT WVRQAPGKGLEWVANIKQDGSERYYVDSVKGRFTISRD NAKNSLYLQMNSLRAEDTAVYYCARDQNYDILTGHYGM DVWGQGTTVTVSSEPKSSDKTHTCPPCPAPEAAGGPSVFL FPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSREEMT KNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
KL2B467-LH-scFv-Fc	353	QSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQQKPG QAPVLVVYDNSDRPSGIPERFSGSNSGTTATLTISRVEAGD EADYYCQVWDSSSDHPVVFGGGTKVTVLGGSEGKSSGS GSESKSTGGSQVQLVESGGGVVQPGRSLRLSCAASGFTFS YYGMHWVRQAPGKGLEWVAFISYDGSNKYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSYW AFDYWGQGTQVTVSSEPKSSDKTHTCPPCPAPEAAGGPS VFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSREE MTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG
KL2B30 Fab HC	354	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPP GKGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKL SSVTAADTAVYYCAGTTIFGVVTPNFYYGMDVWGQGTT VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ

		DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYV LPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENN YLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG
KL2B242 Fab HC	355	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQP AGSGLEWIGRLYVSGFTNYNPSLKSRVTLSLDPSRNQLSL KLSSVTAADTAVYYCAGDSGNYWGWFDPWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPP SREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYL TWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
KL2B242LC_C33S _Fab LC	445	SYELTQPPSVSVSPGETASITCSGDQLGENYASWYQQKPG QSPVLVIYQDSKRPSGIPERFSGSNSGNTATLTISGTQALDE ADYYCQAWDNSIVVFGGGTKLTVLGQPKAAPSVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVE TTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGS TVEKTVAPTECS
KL2B53 Fab HC	356	EVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHWVRQ APGKGLEWVAIISYDGSKKDYTDSVKGRFTISRDNSKNTL YLQMDSLRVEDSAVYSCARESGWSHYYYYGMDVWGQG TMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YVLPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPE NNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG

KL2B494-LH-scfV-Fc	357	SSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMSWVRQAPGKGLEWVSTIGGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTALLYDGMDVWGQGTMVTVSSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
KL2B30 Fab w/K477	361	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAGTTIFGVVTPNFYYGMDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSREEMTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
CD3W245 Fab HC	358	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNMNWVRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRDNAKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRE

		EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG
CD3B376 Fab	359	QVQLQQSGPRLVRPSQTLSLTCAISGDSVFNNNAAWSWIR QSPSRGLEWLGRTYYRSKWLYDYAVSVKSRITVNPDTSR NQFTLQLNSVTPEDTALYYCARGYSSSFDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAA GGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG
CD3W245-LH-scfv-Fc	360	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKP GKAPKLLIKYASESISGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSES KSTGGSEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYN MNWVRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSR DNAKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQG TLVTVSSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYVYPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPG
CD3W245-LH-scfv-Fc w/K447	362	DIQMTQSPSSLSASVGDRVTITCRARQSIGTAIHWYQQKP GKAPKLLIKYASESISGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSGSWPYTFGQGTKLEIKGGSEGKSSGSGSESK STGGSEVQLVESGGGLVKPGGSLRLSCAASGFTFSRYNM NWVRQAPGKGLEWVSSISTSSNYIYYADSVKGRFTFSRD NAKNSLDLQMSGLRAEDTAIYYCTRGWGPFDYWGQGTL

		VTVSSEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYVYPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K

Table 38. HC and LC DNA SEQ ID NOs of hK2/CD3 bispecific antibodies

Bispecific Name	hK2 arm			CD3 arm		
	Name	HC1 or scFv - Fc DNA SEQ ID NO:	LC1 DNA SEQ ID NO:	Name	HC2 or scFv - Fc DNA SEQ ID NO:	LC2 DNA SEQ ID NO:
KLCB91	KL2B359 LH - scFv - Fc	363		CD3W245 Fab	371	312
KLCB105	KL2B359 – LH – scFv - Fc	363		CD3B376 Fab	372	317
KLCB95	KL2B413 – LH – scFv - Fc	364		CD3W245 Fab	371	312
KLCB96	KL2B413-LH-scFv-Fc	364		CD3B376 Fab	372	317
KLCB170	KL2B467-LH-scFv-Fc	365		CD3W245 Fab	371	312
KLCB80	KL2B30 Fab	367	303	CD3W245-LH-scFv-Fc	373	
KLCB81	KL2B242LC_C33S Fab	369	446	CD3W245-LH-scFv-Fc	373	

KLCB113	KL2B53 Fab	368	304	CD3W245-LH-scFv-Fc	373	
KLCB281	KL2B467-LH-scFv-Fc	365		CD3B376 Fab	372	317
KLCB174	KL2B494-LH-scFv	366		CD3B376-Fab	372	317
KLCB153	KL2B494-LH-scFv	366		CD3W245-Fab	371	312
KLCB245	KL2B30-Fab w/ K447	370	303	CD3W245-LH-scFv-Fc w/ K447	374	

SEQ ID NO: 363 (KL2B359-LH-scFv-Fc)

GAGATTGTTCTCACCCAATCCCCAGCTACTCTCTCTCTTTCACCCGGTGAGCGGGCAACCCTC
TCCTGTAGAGCCAGCGAGAGCGTGGAGTATTTTGGCACATCCCTGATGCACTGGTATCAGCA
AAAACCAGGACAACCCCCCAGACTCCTCATATATGCCGCCTCAAATGTCGAGAGTGGGATA
CCTGCACGGTTTTCAGGAAGCGGCAGCGGTACTGACTTCACATTGACTATATCCTCTGTAGA
GCCAGAGGATTTTGCAGTCTACTTCTGCCAGCAAACTAGGAAGGTTCCATATACTTTTGGGG
GCGGTACAAAAGTTGAGATAAAGGGCGGCTCCGAGGGCAAGAGCAGCGGCAGCGGCAGCG
AGAGCAAGAGCACCGGCGGCAGCCAAGTACAGCTCCAGGAGTCAGGACCTGGGCTCGTCAA
ACCATCTCAGACATTGTCCCTGACATGCACAGTTTCCGGCAACAGTATTACTTCCGACTATGC
TTGGAATTGGATCAGGCAATTCCCAGGAAAGCGGCTCGAGTGGATAGGTTATATTTCTTACT
CTGGATCTACTACCTACAATCCCAGTTTGAAGTCTCGCGTGACAATTAGCCGGGACACATCA
AAAAATCAATTCTCACTTAAACTTAGTTCTGTAACCGCTGCCGATACAGCCGTGTACTACTG
CGCCACTGGTTATTATTATGGAAGCGGATTTTGGGGGCAAGGAACTTTGGTGACCGTCTCTT
CCGAGCCCAAATCTAGCGACAAAACTCACACATGTCCACCGTGCCCAGCACCTGAAGCAGC
AGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGA
CCCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAA
CTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAA
GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACGTGCTGCCCCCATCCCGGGAGGAGA
TGACCAAGAACCAGGTCAGCCTGCTGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACCTCACCTGGCCTCCCGTGCTGG
ACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAG
GGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAG
CCTCTCCCTGTCTCCGGGT

SEQ ID NO: 364 (KL2B413 – LH – scFv – Fc)

GAGGTACAACTTGTCGAAAGTGGCGGTGGAGTCGTCCAGCCTGGGCGATCACTTCGCCTCTC
CTGTGTAGCCTCTGGTTTCACTTTCTCATCTTACGACATACACTGGGTCCGCCAGGCACCTGG
TAAGGGGCTGGAGTGGGTTGCCATCATTAGTTACGATGGCTCCAAAAAAGATTACACCGATA
GCGTAAAGGGCAGATTTACCATTTCCAGGGATAATTCAAAGAACACCCTGTATCTGCAAATG
GACAGCCTCCGCGTCGAAGACTCTGCAGTTTATAGCTGTGCCAGGGAGTCAGGCTGGTCCCA
TTATTACTATTATGGTATGGACGTTTGGGGCCAGGGAACCATGGTCACTGTTAGTTCAGCCTC
CACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAG
CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCA
GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC
CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAA
AACTCACACATGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCCTCT
TCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
GTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAG
CGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCA
ACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGA
ACCACAGGTGTACGTGCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG
CTGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
AGCCGGAGAACAACTACCTCACCTGGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCT
ACAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT

SEQ ID NO: 365 (KL2B467-LH-scFv-Fc)

CAGAGCGTACTTACCCAGCCTCCCAGCGTGTCTGTAGCCCCAGGACAGACAGCCAGTATTAC
ATGCGGTGGTGACAATATAGGTTCCAAATCCGTGCATTGGTACCAGCAGAAGCCAGGGCAA
GCTCCCGTGCTCGTGGTATATGATAATTCCGACCGCCCTTCCGGCATTCCCGAACGGTTTAGT
GGTTCAAATTCAGGCACCACAGCAACTCTGACCATAAGCAGAGTCGAAGCTGGAGACGAAG
CCGACTACTACTGTCAGGTATGGGACTCTAGTAGTGACCACCCTGTCGTCTTCGGTGGGGGA
ACCAAAGTGACCGTTCTGGGCGGCTCCGAGGGCAAGAGCAGCGGCAGCGGCAGCGAGAGC
AAGAGCACCGGCGGCAGCCAGGTCCAGCTCGTAGAAAGTGGGGGCGGCGTAGTTCAGCCAG
GCAGGAGTCTCCGGCTGAGTTGTGCAGCCAGCGGCTTTACTTTTTCCTACTATGGAATGCACT
GGGTACGTCAGGCACCCGGCAAAGGTTTGGAGTGGGTCGCATTCATTTCTTATGATGGATCA

AATAAGTATTATGCCGATAGTGTAAAGGGCAGATTTACAATAAGTCGAGACAACTCAAAGA
ACACTCTCTACCTCCAAATGAATAGTCTTCGGGCAGAGGATACTGCAGTGTACTATTGTGCT
CATCTTCCTTATTCCGGTTCTTACTGGGCATTCGATTATTGGGGGCAAGGGACACAAGTTACC
GTGTCTAGCGAGCCCAAATCTAGCGACAAAACTCACACATGTCCACCGTGCCCAGCACCTGA
AGCAGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT
CCCGGACCCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAA
GTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG
CAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAA
TGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACGTGCTGCCCCCATCCCGGG
AGGAGATGACCAAGAACCAGGTCAGCCTGCTGTGCCTGGTCAAAGGCTTCTATCCCAGCGA
CATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACCTCACCTGGCCTCCC
GTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGTCTAGATG
GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC
AGAAGAGCCTCTCCCTGTCTCCGGGT

SEQ ID NO: 366 (KL2B494-LH-scFv-Fc)

AGCAGCGAATTGACCCAACCACCTTCCGTCAGCGTCGCACCAGGGCAAACCGCCCGCATCA
CATGCGGTGGGAACAATATAGGAAGCAAATCTGTCCACTGGTACCAGCAAAAACCAGGACA
AGCCCCTGTTCTGGTCGTCTATGATGACAGCGACAGACCAAGTGGTATTCCCGAGAGATTCT
CCGGTAGCAACTCTGGAAATACAGCTACTTTGACCATCTCCAGAGTTGAGGCTGGTGACGAG
GCAGATTACTATTGCCAGGTCTGGGACAGCTCCAGCGACCACGTCGTATTCGGTGGCGGGAC
CAAGCTGACTGTGCTGGGCGGCTCCGAGGGCAAGAGCAGCGGCAGCGGCAGCGAGAGCAA
GAGCACCGGCGGCAGCCAGGTGCAGTTGGTAGAGTCAGGAGGGGGCCTCGTTCAACCTGGT
GGCAGCCTCCGTTTGTCTTGTGCTGCCAGTGGATTTACTTTCAGTCACTACGCAATGAGCTGG
GTGAGACAAGCACCTGGCAAGGGCCTTGAGTGGGTCTCCACTATCGGCGGTTCAGGGGGGA
GCACTTACTACGCTGACTCTGTAAAAGGTCGCTTTACTATATCTAGAGATAACTCTAAAAAC
ACACTCTACTTGCAGATGAACAGCCTGCGAGCCGAAGATACAGCCGTGTACTACTGCGCCAA
GCCTCATATTGTAATGGTCACTGCCCTCTTGTATGATGGCATGGATGTTTGGGGCCAAGGGA
CAATGGTGACAGTCTCAAGCGAGCCCAAATCTAGCGACAAAACTCACACATGTCCACCGTG
CCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACA
CCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCCACGAAGAC
CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGC
CGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCA

GGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCC
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACGTGCTGC
CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGCTGTGCCTGGTCAAAGGCTT
CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACCTC
ACCTGGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGAC
AAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT

SEQ ID NO: 367 (KLK2B30 Fab HC cDNA)

CAGGTTCAACTTCAAGAATCCGGGCCAGGTCTGGTCAAGCCTTCAGAGACTTTGTCCCTTAC
TTGCACAGTGAGCGGTGGCTCTATCTCAAGTTACTACTGGTCATGGATACGGCAGCCCCCAG
GAAAGGGGCTTGAGTGGATTGGGTACATTTATTACTCAGGGTCAACAAACTACAATCCCTCC
CTCAAATCCCGAGTGACAATTAGTGTCGATACATCTAAAAACCAGTTTTCCCTGAAATTGAG
CTCAGTCACCGCAGCTGATACTGCAGTCTATTATTGTGCTGGCACAACAATCTTCGGGGTAG
TAACTCCAAACTTCTACTACGGGATGGACGTGTGGGGGCAAGGAACAACCGTAACAGTAAG
TAGTGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTG
GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTC
GTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAG
GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC
ATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT
CTTGTGACAAAACTCACACATGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTC
AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCA
CATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA
CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT
GCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG
GCAGCCCCGAGAACCACAGGTGTACGTGCTGCCCCCATCCCGGGAGGAGATGACCAAGAAC
CAGGTCAGCCTGCTGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGA
GAGCAATGGGCAGCCGGAGAACAACTACCTCACCTGGCCTCCCGTGCTGGACTCCGACGGC
TCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT
CTCCGGGT

SEQ ID NO: 303 (KLK2B30 Fab LC cDNA)

GATATTCAAATGACCCAGTCACCATCATTCCTGTCCGCCTCAGTGGGAGATCGCGTCACTAT
TACTTGTCGTGCTAGCCAGGGGATATCATCATATTTGGCTTGGTATCAACAAAAGCCAGGAA
AGGCCCCAAAATTCCTTATATATGCAGCTAGTACACTCCAGAGTGGTGTTCCTAGCCGGTTC
TCTGGCAGCGGCTCAGGGACCGAGTTCACCCTGACAATCTCCAGCTTGCAGCCCGAAGACTT
TGCAACCTACTATTGCCAGCAACTGAACTCCTATCCTCTGACTTTCGGGGGAGGAACCAAGG
TTGAGATTAAACGGACAGTGGCCGCTCCTTCCGTGTTCATCTTCCCACCTTCCGACGAGCAG
CTGAAGTCCGGCACAGCTTCTGTCGTGTGCCTGCTGAACAACTTCTACCCTCGGGAAGCCAA
GGTGCAGTGGAAGGTGGACAATGCCCTGCAGTCCGGCAACTCCCAAGAGTCTGTGACCGAG
CAGGACTCCAAGGACAGCACCTACAGCCTGTCCTCCACACTGACCCTGTCCAAGGCCGACTA
CGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCATCAGGGCCTGTCTAGCCCTGTGACC
AAGTCTTTCAACCGGGGCGAGTGT

SEQ ID NO: 368 (KL2B53 Fab HC cDNA)

GAGGTACAACTTGTCGAAAGTGGCGGTGGAGTCGTCCAGCCTGGGCGATCACTTCGCCTCTC
CTGTGTAGCCTCTGGTTTCACTTTCTCATCTTACGACATACACTGGGTCCGCCAGGCACCTGG
TAAGGGGCTGGAGTGGGTTGCCATCATTAGTTACGATGGCTCCAAAAAAGATTACACCGATA
GCGTAAAGGGCAGATTTACCATTTCCAGGGATAATTCAAAGAACACCCTGTATCTGCAAATG
GACAGCCTCCGCGTCGAAGACTCTGCAGTTTATAGCTGTGCCAGGGAGTCAGGCTGGTCCCA
TTATTACTATTATGGTATGGACGTTTGGGGCCAGGGAACCATGGTCACTGTTAGTTCAGCCTC
CACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAG
CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCA
GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC
CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAA
AACTCACACATGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCCTCT
TCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
GTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAG
CGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCA
ACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGA
ACCACAGGTGTACGTGCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG
CTGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC
AGCCGGAGAACAACTACCTCACCTGGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCT

ACAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT

SEQ ID NO: 304 (KL2B53 Fab LC cDNA)

GATATTGTAATGACTCAGTCACCCTCTTCACTGAGTGCATCAGTAGGTGATCGCGTTACCATC
ACTTGCCGTGCCAGTCAAGACATTTCAAATTACCTTGCATGGTACCAACAAAAGCCCGGAAA
AGTGCCAAAGTTTTTGATTTATGCCGCTTCAACACTCCATTCAGGAGTGCCCTCTCGTTTCAG
TGGATCTGGCAGTGGCACCGATTTTACTCTCACAATAAGCAGTCTCCAGCCTGAGGATGTAG
CCACCTATTATTGCCAAAAATATAATTCAGCCCCCTATACTTTTGGACAGGGCACACGCCTT
GAGATTAAACGGACAGTGGCCGCTCCTTCCGTGTTCATCTTCCCACCTTCCGACGAGCAGCT
GAAGTCCGGCACAGCTTCTGTCGTGTGCCTGCTGAACAACTTCTACCCTCGGGAAGCCAAGG
TGCAGTGGAAGGTGGACAATGCCCTGCAGTCCGGCAACTCCCAAGAGTCTGTGACCGAGCA
GGACTCCAAGGACAGCACCTACAGCCTGTCCTCCACACTGACCCTGTCCAAGGCCGACTACG
AGAAGCACAAGGTGTACGCCTGCGAAGTGACCCATCAGGGCCTGTCTAGCCCTGTGACCAA
GTCTTTCAACCGGGGCGAGTGT

SEQ ID NO: 369 (KLK2B242 Fab HC cDNA and KL2B242LC_C33S Fab HC)

CAAGTACAACTTCAAGAGTCTGGCCCTGGGCTTGTTAAGCCCTCAGAGACCTTGTCACTGAC
CTGTACCGTATCAGGCGGGTCAATTTCATCTTACTACTGGAGTTGGCTTCGTCAGCCTGCCGG
ATCTGGACTGGAGTGGATAGGTAGACTGTATGTTTCCGGCTTTACAAATTACAACCCATCTTT
GAAAAGCCGTGTGACTCTCAGCCTCGACCCTTCTCGGAATCAACTTTCACTTAAATTGTCTTC
TGTTACAGCTGCCGACACTGCAGTATATTATTGTGCAGGGGACTCAGGCAACTATTGGGGAT
GGTTTGATCCTTGGGGGCAGGGGACCCTGGTAACCGTGAGTTCTGCCTCCACCAAGGGCCCA
TCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTG
CCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCA
GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG
GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGT
CCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACC
CAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCC
ACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA
GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC
CTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCC
CAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA

CGTGCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGCTGTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
CTACCTCACCTGGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCAC
CGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTC
TGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT

SEQ ID NO: 446 (KLK2B242LC_C33S Fab LC cDNA)

AGTTATGAGCTGACTCAACCACCCAGTGTCAGCGTATCCCCAGGAGAAACTGCCTCTATAAC
ATGCAGCGGAGACCAGTTGGGAGAAAATTACGCCTCCTGGTACCAACAGAAGCCTGGACAA
AGTCCTGTCCTCGTTATTTATCAAGATTCTAAACGTCCCTCTGGGATCCCCGAACGATTCTCC
GGCTCTAACTCTGGGAATACCGCTACCTTGACAATAAGTGGTACACAGGCACTTGATGAAGC
TGATTATTACTGCCAGGCATGGGATAACAGCATTGTGGTTTTCGGGGGCGGCACCAAACTCA
CAGTTCTCGGTCAGCCCAAGGCTGCACCCAGTGTCACTCTGTTCCCGCCCTCCTCTGAGGAG
CTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGAC
AGTGGCCTGGAAGGCCGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCC
AAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGA
AGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGT
GGCCCCTACAGAATGTTCA

SEQ ID NO: 370 (KLK2B30 wK477 Fab HC cDNA)

CAGGTTCAGCTGCAAGAGTCTGGCCCTGGCCTGGTCAAGCCTTCCGAGACACTGTCTCTGAC
CTGCACCGTGTCTGGCGGCTCCATCTCCTCCTACTACTGGTCCTGGATCAGACAGCCTCCTGG
CAAAGGCCTGGAATGGATCGGCTACATCTACTACTCCGGCTCCACCAACTACAACCCCAGCC
TGAAGTCCAGAGTGACCATCTCCGTGGACACCTCCAAGAACCAGTTCTCCCTGAAGCTGTCC
TCCGTGACCGCTGCTGATACCGCCGTGTACTATTGTGCTGGCACCACCATCTTCGGCGTGGTC
ACCCCTAACTTCTACTACGGCATGGACGTGTGGGGCCAAGGCACAACAGTGACAGTCTCTTC
TGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
TGACAAAACTCACACTTGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCT
TCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
GTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG

TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGT
GGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
GTGTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC
CCCGAGAACCACAGGTGTACGTGCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGT
CAGCCTGCTGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCA
ATGGGCAGCCGGAGAACAACTACCTCACCTGGCCTCCCGTGCTGGACTCCGACGGCTCCTTC
TTCCTCTACAGCAAGCTCACCGTGGACAAGTCCAGATGGCAGCAGGGGAACGTCTTCTCATG
CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGTCTCTCTCCCTGTCTCCGG
GAAAA

SEQ ID NO: 373 (CD3W245 – LH-scFv-Fc cDNA)

GACATACAAATGACACAATCACCCTCTTCTCTTTCTGCAAGCGTTGGCGACCGTGTCACTATC
ACTTGTCGAGCCCGCCAGTCCATAGGTACTGCCATTCACTGGTATCAACAGAAGCCTGGCAA
GGCTCCCAAACTCCTGATTAAGTATGCCAGCGAGAGCATTTCCGGCGTACCTTCAAGATTTT
CCGGCTCCGGTAGTGGGACAGATTTCACTCTCACTATATCTAGCCTCCAACCAGAAGATTTC
GCCACTTACTACTGTCAACAATCAGGTTCATGGCCTTACACTTTCGGCCAGGGGACAAAATT
GGAGATCAAGGGCGGCTCCGAGGGCAAGAGCAGCGGCAGCGGCAGCGAGAGCAAGAGCAC
CGGCGGCAGCGAGGTGCAACTGGTGGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCC
CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGATATAACATGAACTGGGTCCG
CCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTACTAGTAGTAATTACATAT
ACTACGCAGACTCAGTGAAGGGCCGATTCACCTTCTCCAGAGACAACGCCAAGAACTCACT
GGATCTGCAAATGAGCGGCCTGAGAGCCGAGGACACGGCTATTTATTACTGTACGAGAGGC
TGGGGGCCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGAGCCCAAATC
TAGCGACAAAACTCACACATGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCA
GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC
ATGCGTGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTG
CAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
CAGCCCCGAGAACCACAGGTGTACGTGTACCCCCCATCCCGGGAGGAGATGACCAAGAACC
AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
AGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT
CCTTCGCCCTCGTGAGCAAGCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTTC

TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTC
TCCGGGT

SEQ ID NO: 374 (CD3W245-LH-scFv-Fc w/ K447)

GACATCCAGATGACCCAGTCTCCATCCTCTCTGTCCGCCTCTGTGGGCGACAGAGTGACCAT
TACCTGCCGGGCCAGACAGTCTATCGGCACCGCTATCCACTGGTATCAGCAGAAGCCTGGCA
AGGCCCCTAAGCTGCTGATTAAGTACGCCTCCGAGTCCATCTCCGGCGTGCCCTCCAGATTTT
CTGGCTCTGGATCTGGCACCGACTTTACCCTGACAATCTCCAGCCTGCAGCCTGAGGACTTC
GCCACCTACTACTGTCAGCAGTCCGGCTCTTGGCCTTACACCTTTGGTCAGGGCACCAAGCT
GGAAATCAAGGGCGGATCTGAGGGAAAGTCCAGCGGCTCCGGCAGCGAAAGCAAGTCCACC
GGCGGAAGCGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTAAGCCTGGCGGCTCTC
TGAGACTGTCTTGTGCTGCTTCTGGCTTCACCTTCAGCCGGTACAACATGAACTGGGTCCGAC
AGGCTCCTGGCAAAGGCCTGGAATGGGTGTCCTCCATCTCCACCTCCAGCAACTACATCTAC
TACGCCGACTCCGTGAAGGGCAGATTCACCTTCTCCAGAGACAACGCCAAGAACTCCCTGGA
CCTGCAGATGTCTGGCCTGAGAGCTGAGGACACCGCTATCTACTACTGCACCAGAGGCTGGG
GACCCTTCGATTATTGGGGCCAGGGAACCCTGGTCACCGTGTCATCTGAGCCCAAATCTAGC
GACAAAACTCACACTTGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTT
CCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG
TGGTGGTGAGCGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTG
GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
TGTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCC
CCGAGAACCACAGGTGTACGTGTACCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCA
ATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTC
GCCCTCGTGAGCAAGCTCACCGTGGACAAGTCCAGATGGCAGCAGGGGAACGTCTTCTCAT
GCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGTCTCTCTCCCTGTCTCCG
GGAAAA

SEQ ID NO: 371 (CD3W245 Fab-HC-Fc)

GAGGTGCAACTGGTGGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGATATAACATGAACTGGGTCCGCCAGGCTCCA
GGGAAGGGGCTGGAGTGGGTCTCATCCATTAGTACTAGTAGTAATTACATATACTACGCAGA
CTCAGTGAAGGGCCGATTCACCTTCTCCAGAGACAACGCCAAGAACTCACTGGATCTGCAAA

TGAGCGGCCTGAGAGCCGAGGACACGGCTATTTATTACTGTACGAGAGGCTGGGGGCCTTTT
GACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGT
CTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG
TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGC
GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGAC
CGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGTCCACC
GTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGG
ACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGAGCGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAA
AGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCA
CCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCC
CCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACGTGT
ACCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGG
CTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCGCCCTCGTGAGCAAGCTCACCGT
GGACAAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC
ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT

SEQ ID NO: 312 (CD3W245 Fab-LC-Fc)

GACATACAAATGACACAATCACCCTCTTCTCTTTCTGCAAGCGTTGGCGACCGTGTCACTATC
ACTTGTCGAGCCCGCCAGTCCATAGGTACTGCCATTCACTGGTATCAACAGAAGCCTGGCAA
GGCTCCCAAACTCCTGATTAAGTATGCCAGCGAGAGCATTTCCGGCGTACCTTCAAGATTTT
CCGGCTCCGGTAGTGGGACAGATTTCACTCTCACTATATCTAGCCTCCAACCAGAAGATTTC
GCCACTTACTACTGTCAACAATCAGGTTCATGGCCTTACACTTTCGGCCAGGGGACAAAATT
GGAGATCAAGCGGACAGTGGCCGCTCCTTCCGTGTTCATCTTCCCACCTTCCGACGAGCAGC
TGAAGTCCGGCACAGCTTCTGTCGTGTGCCTGCTGAACAACTTCTACCCTCGGGAAGCCAAG
GTGCAGTGGAAGGTGGACAATGCCCTGCAGTCCGGCAACTCCCAAGAGTCTGTGACCGAGC
AGGACTCCAAGGACAGCACCTACAGCCTGTCCTCCACACTGACCCTGTCCAAGGCCGACTAC
GAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCATCAGGGCCTGTCTAGCCCTGTGACCA
AGTCTTTCAACCGGGGCGAGTGT

SEQ ID NO: 372 (CD3B376 Fab-HC-Fc)

CAGGTGCAGCTCCAACAGAGTGGTCCCAGACTCGTGAGACCCTCTCAAACACTCAGTTTGAC
TTGTGCCATCTCAGGCGATTCAGTTTTCAACAACAATGCAGCTTGGAGCTGGATTAGGCAGT
CACCTAGTCGCGGTCTTGAATGGCTTGGGCGTACATACTATCGCTCTAAATGGTTGTATGATT
ACGCTGTGTCCGTGAAGAGCCGAATCACCGTAAACCCTGATACCTCCAGGAATCAGTTCACA
TTGCAACTGAATAGTGTGACTCCCGAGGATACTGCACTCTATTATTGTGCCCGAGGATATAG
CAGTAGCTTCGACTATTGGGGACAAGGGACACTCGTTACCGTTAGTTCAGCCTCCACCAAGG
GCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCA
GCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCAC
AAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACA
CATGTCCACCGTGCCCAGCACCTGAAGCAGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCA
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGAGCGT
GAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAAT
GCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCA
CCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACGTGTACCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCT
GGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCGCCCTCGTGAGCAA
GCTCACCGTGGACAAGTCTAGATGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATG
AGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT

SEQ ID NO: 317 (CD3B376 Fab-LC-Fc)

CAGTCTGCTCTGACCCAGCCTGCCTCCGTGTCTGGCTCTCCCGGCCAGTCCATCACCATCAGC
TGTACCGGCACCTCCTCCAACATCGGCACCTACAAGTTCGTGTCCTGGTATCAGCAGCACCC
CGACAAGGCCCCCAAAGTGCTGCTGTACGAGGTGTCCAAGCGGCCCTCTGGCGTGTCCTCCA
GATTCTCCGGCTCCAAGTCTGGCAACACCGCCTCCCTGACCATCAGCGGACTGCAGGCTGAG
GACCAGGCCGACTACCACTGTGTGTCCTACGCTGGCTCTGGCACCCTGCTGTTTGGCGGAGG
CACCAAGCTGACCGTGCTGGGTCAGCCCAAGGCTGCACCCAGTGTCACTCTGTTCCCGCCCT
CCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCG
GGAGCCGTGACAGTGGCCTGGAAGGCCGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCA
CCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCC

TGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTG
GAGAAGACAGTGGCCCCTACAGAATGTTCA

Example 6: Biophysical characterization of hK2xCD3 bi-specific antibodies

Affinity of selected hK2 x CD3 bispecific antibodies

Affinity of selected hK2xCD3 bispecific antibodies to hK2 or human CD3 was measured by surface plasmon resonance (SPR). SPR is a label-free technique to study the strength of an interaction between two binding partners by measuring the change in mass upon complex formation and dissociation. Antibodies were captured on a sensor chip coated with an anti-Fc antibody followed by injection of soluble hK2 or soluble recombinant human CD3 at various concentrations and specified association and dissociation times. Post dissociation, the surface was regenerated with an appropriate solution to prepare for the next interaction. Kinetic information (on-rate and off-rate constants) were extracted by fitting sensorgrams to the 1:1 Langmuir model. Binding affinity (KD) are reported as the ratio of rate constants (koff/kon). KD values of selected hK2/CD3 bispecific antibodies are listed in **Table 39.**

Table 39. K_D values of selected hK2/CD3 bispecific antibodies for the respective binding arms

KLK2 arm	K_D (nM)
KL2B467 Fab	0.09
KL2B494 Fab	0.06
KL2B359-LH-scFv	0.63
KL2B413-LH-scFv	16.4
CD3B376 Fab	20-40
CD3W245 Fab	0.14
CD3W245 LH scFv	20-30
KL2B30 Fab	2

KL2B53 Fab	0.1
KL2B242 Fab	0.14

Thermal stability of selected hK2 x CD3 bispecific antibodies

Thermal stability of the bispecific antibody samples was determined by NanoDSF method using an automated Prometheus instrument. Measurements were made by loading sample into 24 well capillary from a 384 well sample plate. Duplicate runs were performed for each sample. Prometheus NanoDSF user interface (Melting Scan tab) was used to set up the experimental parameters for the run. The thermal scans for the samples span from 20°C to 95°C at a rate of 1.0°C/minute. Dual-UV technology monitors intrinsic tryptophan and tyrosine fluorescence at the emission wavelengths of 330 nm and 350 nm, and this ratio (F350 nm/F330 nm) is plotted against temperature to generate an unfolding curve. Nano DSF is used for measuring Tm of all molecules at 0.5mg/mL concentration in Phosphate Buffered Saline, pH 7.4. Measured Tm values are listed in **Table 40**.

Table 40. Tm values for KLK2 or CD3 binding arms of selected hK2 x CD3 bispecific antibodies.

Molecule	**Tm (°C) by DSF**
KL2B413 (scFv)	67
KL2B359 (scFv)	67
KL2B30 (Fab)	>70
KL2B242 (Fab)	>70
KL2B53 (Fab)	>70
KL2B467 (Fab)	>70
KL2B494 (Fab)	>70
CD3B376 (Fab)	61
CD3W245 LH scFv	66

Self-Association Potential by AC-SINS (Affinity Capture-Self Interaction Nanoparticle Spectroscopy)

A high throughput screening assay was used to measure the propensity of an Ab candidate to self-interact. Propensity for self-interaction usually translates into poor Ab solubility and challenges in downstream Ab manufacturing. In this assay, gold nanoparticles (AuNPs) are coated with goat anti-human IgG (H+L) capture antibody and later incubated with candidate Abs in the presence of polyclonal goat IgG. Any candidate Ab that self-associates brings the AuNPs into proximity, resulting in a shift of the nanoparticles' plasmon wavelength (λ_p), also referred to as the wavelength at maximum absorbance (λ_{max}). The magnitude of the shift ($\Delta\lambda$max) for each candidate Ab is indicative of the strength of its self-association. Proper control antibodies are used in this assay that show none to high self-association potential. All molecules tested in this assay showed none to low risks for self-association.

Example 7: In vitro and in vivo characterization of bispecific hK2xCD3 antibodies

In vitro cytotoxicity of hK2xCD3 bi-specific antibodies

The cytotoxicity potential of the generated bispecific antibodies was measured *in vitro* by T-cell-mediated cytotoxicity assay using live-time lapse imaging on the Incucyte platform. The bispecific antibodies were tested in hK2 positive cell line VCaP cells, in the presence of isolated pan human CD3+ T cells from healthy donors at a Effector:Target ratio (E:T ratio) of 3:1. Cell death by apoptosis was monitored by measuring the fluorescence signal from a dye which is stably expressed by target VCaP cells.

Normal donor pan T cells were co-incubated with KLK2+ VCaP cells. KLK2xCD3 bispecific antibodies were dosed from 0 to 100nM for 96 hours. 3:1 Effector-to-Target (ET) ratio was used. **FIG. 8A** shows in vitro target cytotoxicity of KL2BxCD3 bi-specific molecules measured by incuCyte imaging system in real-time for quantifying target cell death. Target cells were stably expressing a red nuclear dye which was measured by IncuCyte imaging system in real-time for quantifying target cell death. Overall tumor cell lysis was graphed based on AUC of real-time kinetic killing curve of VCaP cells (**FIG 8A**). **FIG. 8B** shows in vitro target cytotoxicity of KL2BxCD3 bi-specific molecules measured by fluorescent

caspase 3/7 reagent to measure apoptosis signal from target cell death. Green fluorescent Caspase 3/7 reagent was used to measure apoptosis signal from target cell death. Total Caspase 3/7 activity was graphed based on AUC of real-time caspase 3/7 activity curve (**FIG. 8B**). The data shows that bispecific hK2/CD3 antibodies promote a dose-dependent reduction of viable VCaP cells with increasing time and hence induce T cell mediated death of the VCaP tumor cells. bispecific hK2xCD3 antibodies are therefore effective at mediating T cell activation and show dose-dependent KLK2+ tumor cell killing.

In vitro T cell activation and proliferation by hK2xCD3 bi-specific molecules

hK2xCD3 bispecific antibodies were tested for their ability to promote T cell activation and proliferation. Normal donor pan T cells were labelled with CFSE (5uM) and co-cultured with KLK2 (+) VCap cells. KLK2xCD3 bispecific antibodies were dosed from 0 to 100nM for 96 hours. 3:1 Effector-to-Target (ET) ratio was used. After 96 hours co-incubation, cells were harvested and stained with CD25, live/dead Dye. Flow cytometric analysis was performed on a Fortessa flow cytometer with Flowjo software. The frequencies of CTV dye dilution and activation marker CD25 were determined. The frequency of CD25 positive cells in different doses were used to graph in vitro T activation (**FIG. 9A**). The proliferation gate was determined using the 0nM treatment group. The frequency of cells entering into proliferation gate was used to graph in vitro T cell proliferation (**FIG. 9B**). The data confirms dose dependent activation and proliferation of T cells by various KLK2xCD3 bi-specific antibodies.

In vitro T cell cytokine release by hK2xCD3 bi-specific molecules.

The effect of anti-hK2xCD3 antibodies on T-cell cytokines release was measured in vitro. Supernatant samples were collected from the in vitro cytotoxicity experiment described above. A 13-plex cytokine Luminex assay was carried out to quantify IFN-γ and TNF-α concentrations at different doses of hK2xCD3 bispecific antibodies. **FIG. 10A** and **FIG. 10B** shows functional cytokine release by T cells activated triggered by KLK2xCD3 bi-specific antibodies in a dose-dependent manner.

Efficacy of bispecific hK2xCD3 antibodies in established subcutaneous (SC) human prostate xenograph model in T cell humanized mice

The purpose of this study is to evaluate KLK2xCD3 bispecific in vivo efficacy in human prostate tumor VCaP s.c. mouse xenograft model. In vivo efficacy of KLK2xCD3 bispecific antibodies was evaluated in human prostate tumor VCaP s.c. mouse xenograft model. The antitumor efficacy of KLK2xCD3

molecules was evaluated in established SC human prostate VCaP xenografts. Intact male NSG mice were used to provide a suitable host for engrafting human tumors and human T cells. The human prostate cell line VCaP was obtained from American Type Culture Collection (ATCC). VCaP cells were harvested during exponential growth and mice were injected with 1×10^7 cells SC in a volume of 0.2 mL in the right flank. 20e6 human T cells were injected i.p for each animal. Three dose levels were evaluated with 5-fold escalation: 0.2mg/kg, 1mg/kg and 5mg/kg (**FIG, 39A**). Bispecific antibodies were dosed twice a week via i.p. Eye blood was sampled at 6 hours post first i.p dosing and functional cytokine levels were measured using Luminex based assays (**FIG. 39B**). Tumor volume and body weight measurements were collected twice weekly throughout all studies. The percent delta tumor growth inhibition (ΔTGI) was defined as the difference between mean tumor burden of the treated and control groups, calculated as % ΔTGI = ([(TVc-TVc0)-(TVt-TVt0)]/(TVc-TVc0))×100; where 'TVc' is the mean tumor burden of a given control group, 'TVc0' is the mean initial tumor burden of a given control group, 'TVt' is the mean tumor burden of the treated group, and 'TVt0' is the mean initial tumor burden of the treated group. %TGI was defined as ([TVc-TVt]/TVc)×100.

At least one KLK2xCD3 compound of the present invention showed dose-dependent anti-tumor effect, i.e., at 1 mg/kg, KLCB80 showed marginal tumor growth inhibition and at 5 mg/kg showed robust anti-tumor effect. Cytokine assessment at 6 hours post first dosing showed above-background functional cytokine release of the active KLCB80, which is consistent with in vivo efficacy.

Example 8: Generation and characterization of a CAR comprising 11B6.

Construction and expression of hK2 11B6 scFv CARs

hK2 CAR constructs (CAR13: HL_HCHumanized_LCHumanized_20AA and CAR15: LH_LCHumanized_HCHumanized_20AA) comprising an scFv derived from the hK2-targeting antibody 11B6 were constructed as illustrated in **FIG. 11**. Dynabeads Human T-Expander CD3/CD28 stimulated T cells were subjected to electroporation, then washed three times with OPTI-MEM (Invitrogen), and resuspended in OPTI-MEM at the final concentration of $50x10^6$/ml. Subsequently, 0.1 ml of the cells (5E6) was mixed with 10 µg of IVT CAR encoding RNA and electroporated. Post electroporation, the T cells were transferred to a 6-well plate and immediately put back into a 37°C incubator. Primary human T cells were electroporated with no mRNA (MOCK) or 10 µg of mRNA expressing either an hK2 scFv CAR or irrelevant control CAR. 24 hours post-electroporation CAR surface expression was measured by flow

cytometry following staining with 2 µg/ml biotinylated L-protein and streptavidin-conjugated PE (**FIG. 12** top panel) or biotinylated hK2 (1 µg/ml) and streptavidin-conjugated PE (**FIG. 12** bottom panel).

Twenty-four hours post electroporation, the T cells were counted. 1E5 T cells were collected for each. The cells were washed with FACS buffer twice using 200 µL/well of FACS buffer for microtiter plates, with the supernatant discarded. All wells were stained with 100 µl staining buffer containing Protein L (Genscript, Cat. No. M000971:500;2ug/ml), incubated for at least 30 minutes at 4°C while being protected from light. The cells were washed by adding FACS Buffer twice, using 150 µL/well for microtiter plates with FACS buffer. Centrifugation at 400x g was performed for 4 minutes at room temperature. The supernatant was then discarded. All wells were stained with 100 µl Streptavidin-R-Phycoerythrin (SA-PE;1:250) and Live/dead Fixable Near-IR Dead Cell Stain dye (1:1000), incubated for at least 30 minutes at 4°C while being protected from light. The cells were then ready for flow cytometry analysis.

As shown in **FIG. 12**, **top panel**, protein L staining was observed on both KLK2 HL & LH CARs (41.4% and 66.3%, respectively), whereas only the background staining (~5.5%) was seen in the control T cells that were T cells without mRNA electroporation. As shown in **FIG. 12**, **bottom panel,** CAR expression on primary human T cells also could be detected via J&J internal biotin-labeled recombinant KLK2 protein (Biotin-Hk2 protein,Lot:20180810, CBIS ID:KL2W12.CB.003, 2.4mg/ml, 1mM Benzamidine) followed by SA-PE. As shown, T cells efficiently expressed KLK2 LH CAR (82.7%) as measured by flow cytometry, whereas only the background staining (~1.73% or 1.19%) was seen in the control T cells that were T cells without mRNA electroporation or undisclosed control CAR (non-KLK2 specific). Results are shown for representative donor T cells and were reproducible in multiple donors (at least n = 3).

Table 41 shows the SEQ ID numbers of CAR13 and 15.

Tumor cell killing by hK2 11B6 CAR-T cells

Co-culture for CellTrace Violet (CTV, Thermo Fisher Scientific) based cytotoxicity assay using flow cytometer was performed to study cytotoxicity of 11B6 CAR-T cells.

T cells were prepared as follows. Twenty-four hours post EP, T cells were counted and resuspended at the concentration needed for the most concentrated/desired E:T. The T cells were added

at 100 µl/well of assay ($2x10^6$ cells/ml; plated 100 µl in a 10:1 E:T ratio, i.e., 2E5 T cells per 2E4 target cells). A stock of the 10:1 E:T concentration was made, with two-fold serial dilutions made with complete T cell media (Optimizer w/CTS, 5% Human Serum, 1% GlutaMax) to 0.3125:1. The T cells were plated (100ul/well) in triplicate using a 96 well round bottom tissue culture treated plate.

CTV labeled target cells were prepared as follows. 20 µL DMSO was added to a vial of CTV staining solution. This stock solution was diluted into 20 mL of PBS (warmed to 37°C) for a 5 µM staining solution. 10E6 tumor cells were collected, washed with PBS twice and resuspended in 4E6/ml (2.5ml). An equal volume (2.5ml) of CTV staining solution was added. The cells were incubated for 20 minutes in a 37°C incubator. 40 ml PRMI+20% FBS was added to the cells to absorb any unbound dye. The cells were incubated for 5 minutes. The cells were centrifuged for 5 minutes at 400 x g. The cell pellet was resuspended in pre-warmed RPMI+10%FBS medium. In the meantime, T cells were seeded at the desired E/T ratio described above. The tumor cell lines Vcap (KLK2+) and DU145(KLK2-) were recounted, and then the cells were resuspended in 2E5/ml and 100ul in duplicate. The cells were co-incubated with labelled tumor cell lines in a flat-bottom 96-well plate.

A cytotoxicity assay was performed as follows using a flow cytometer. After 20 hours of co-culture, all of the cells were transferred to a U-bottom 96-well plate and washed. After 20 hours of co-culture all of the cells were collected from a flat-bottom 96-well plate and transferred to a U-bottom 96-well plate, and then washed. 30 µl of 0.25% trypsin was added to all the wells and incubated for 5 minutes in a 37°C incubator. After 5 minutes, all of the tumor cells were collected to a U-bottom 96-well plate. The cells were centrifuged and washed for 5 minutes at 400 x g twice. The cell pellet was then resuspended in diluted (1:1000) LIVE/DEAD™ Fixable Near-IR staining dye (100 µl). The cells were incubated for 30 mins at 4 °C, and washed with FACS buffer twice by centrifuging the cells for 5 minutes at 400 x g. After washing, all of the cells were fixed for 10 minutes using 100 µl of BD Cytofix™ Fixation Buffer (50ul FACS buffer +50ulFixation Buffer). The cells were centrifuged and washed for 5 minutes at 400 x g once. The cell pellet was resuspended in FACS buffer. Stained samples were analyzed by multicolor flow cytometry after the end of the incubation period.

The percentage of cytotoxic activity was calculated using the following equation:

% specific death = % Near IR+CTV+(dead) cells - % spontaneous Near IR +CTV+ / (100% - % spontaneous Near IR +CTV+ (dead) cells) x 100%.

Twenty-four hours after transient transfection, target cells (hK2 positive Vcap and hK2 negative DU145 cells) were labeled with Cell Trace Violet (CTV) fluorescent dye and then co-cultured

with hK2 CAR-T cells. Mock T cells served as negative effector controls. Cells were co-cultured for 20 hours at the effector-to-target cell (E/T) ratios ranging from 0.3125:1 to 10:1 as shown in **FIG. 13**. Percent killing was measured as the ratio of the absolute number of live (viability dye negative) target (CTV positive) cells remaining in the co-culture relative to the number of live target cells cultured without CAR-T cells. As shown, hK2 CAR LH T cells specifically and efficiently lysed the hK2(+) human prostate cancer cell lines VCap cells (**FIG. 13**, top panel) but not K2 (-) DU145 cells (**FIG. 13**, bottom panel) at E/T ratios of 10:1 to 0.3125:1, whereas only the background cytotoxicity was seen in the T cells that were Mock or hK2 HL CAR.

hK2 CAR-T cells were also tested for real-time cytotoxicity using xCELLigence as a real-time cell analysis system as a potency assay for immune cell-mediated cytolysis of target cells.

50 µL of target cancer cell culturing media was added to each well of the 96-well E-Plates (ACEA Biosciences), and the background impedance was measured and displayed as a Cell Index. Then, adherent target cells VCap and DU145 were dissociated and seeded at a density of 5E4 (VCap), 5E3 (DU145) cells/well of the E-Plate in a volume of 100 µL, and allowed to passively adhere on the electrode surface. Post seeding, the E-Plate was kept at ambient temperature inside a laminar flow hood for 30 minutes and then transferred to the RTCA MP instrument inside a cell culture incubator. Data recording was initiated immediately at 15-minute intervals for the entire duration (96 hours) of the experiment.

At the time treatment was applied (24 hours post cancer cells seeding), data acquisition was paused, 50 µL of media was removed from each well, and effector CAR-T cells were added at different effector to target (E:T) ratios in a volume of 50 µL. hK2 CAR-T and undisclosed control CAR (non-hK2 specific) T cells were resuspended. Two-fold dilutions were then performed in duplicate in a 96-well plate (from 5:1 to 0.156:1 E/T ratio). Target plus Mock effector controls (no RNA electroporation T cells) were also added to the target cells.

Target cells VCap (5E4) and DU145 (5E3) incubated with Mock, 10µg mRNA electroporated (24 hours post transfection) hK2 11B6 CAR LH or control CAR-T cells at different E/T ratios for approximately 72 hours. Normalized cell index (CI) plots for VCap and DU145 are shown in **FIG. 14** and **FIG. 15**, respectively. When seeded alone, target cells adhered to the plate and proliferated, increasing the CI readout. When T cells were added to target cells, hK2 CAR and control CAR-T cells mediated hK2 (+) VCap cell cytolysis and subsequent progressive decrease in CI at an E/T ratio from 5:1 to 0.156:1.

Cytokine production by hK2 11B6 CAR-T cells

IFN-γ produced by cytotoxic T cells allows for exertion of immune surveillance of tumors, which can directly inhibit proliferation and induce apoptosis of some malignancies *in vivo* and *in vitro*. To determine whether hK2 CAR-T cells were able to recognize and be activated by hK2 (+) tumor cells, the supernatant was collected from xCELLigence-based killing assay, as described in Example 3. After about 70 hours co-culture, the supernatant was collected and assayed by ELISA according to the directions provided with the ELISA kit (Human IFN-γ ELISA MAX™ Deluxe, BioLegend, Cat#:430106).

IFN-γ production of antigen-stimulated CAR-T cells is shown in **FIG. 16**. hK2 CAR LH and control CAR-T cells secreted IFN-γ during co-culture with hK2-expressing VCap cells in a E:T ratio-dependent manner, but not during co-culture with hK2-negative DU145 cells. Undisclosed control CAR secreted much higher amount of IFN- γ due to the much higher antigen expression level than hK2. Mean IFN-γ concentration ± SD (pg/ml) from duplicate cultures was recorded.

Example 9: Generation and characterization of additional scFvs CAR constructs

As described in Example 1, the ectodomain of 11B6 cloned as scFv did not retain binding at elevated temperature (55°C) and hence additional campaigns were initiated to generate new humanized antibodies from the parental 11B6.

hK2 CARs containing thermally stabilized scFvs derived from the antibody 11B6 were generated (CAR1-CAR12, CAR14, CAR16). The description and SEQ ID NOs: of the CAR constructs containing thermally stabilized scFvs are provided in **Table 13**. The names in the description (HCG5, LCD6, HCF3, LCB7) refer back to the VH or the VL chains identified in Example 1, 20AA refers to the 20 amino acid long linker between the VH/VL pairs in the scFv. The scFv in the CARs were cloned in either VH-L-VL or VL-L-VH orientation.

The generated scFvs also incorporated into CAR constructs (CAR17-CAR36) and further characterized. **Table 41** shows the generated CAR constructs and their SEQ ID NOs. The signal sequence used is MAWVWTLLFLMAAAQSIQA (SEQ ID NO: 24)

Table 41.

Name of CAR construct	Description of scFv in the CAR	SEQ ID NO: of scFv amino acid sequence of the CAR	SEQ ID NO: of full ECD domain of CAR (including signal sequence)	SEQ ID NO: of CAR amino acid sequence
CAR1	HL_HCG5_LCD6_20AA (scFv1)	8	29	46
CAR2	HL_HCG5_LCHumanized_20AA (scFv2)	9	30	47
CAR3	HL_HCF3_LCB7_20AA (scFv3)	10	31	48
CAR4	HL_HCG5_LCB7_20AA (scFv4)	11	32	49
CAR5	LH_LCD6_HCG5_20AA (scFv5)	12	33	50
CAR6	LH_LCHumanized_HCF3_20AA (scFv6)	13	34	51
CAR7	LH_LCHumanized_HCG5_20AA (scFv7)	14	35	52
CAR8	LH_LCB7_HCF3_20AA (scFv8)	15	36	53
CAR9	LH_LCB7_HCG5_20AA (scFv9)	16	37	54
CAR10	LH_LCD6_HCF3_20AA (scFv10)	17	38	55
CAR11	HL_HCHumanized_LCB7_20AA (scFv11)	18	39	56
CAR12	HL_HCHumanized_LCD6_20AA (scFv12)	19	40	57
CAR13	HL_HCHumanized_LCHumanized_20AA (scFv13)	20	41	58
CAR14	LH_LCD6_HCHumanized_20AA (scFv14)	21	42	59

CAR15	LH_LCHumanized_HCHumanized_20AA (scFv15)	22	43	60
CAR16	LH_LCB7_HCHumanized_20AA (scFv16)	23	44	61
CAR17	KL2B413_HL (scFv17)	133	149	153
CAR18	KL2B413_LH (scFv18)	134	150	154
CAR19	KL2B359_HL (scFv19)	135	151	155
CAR20	KL2B359_LH (scFv20)	136	152	156
CAR21	KL2B357_HL	318	410	426
CAR22	KL2B357_LH	319	411	427
CAR23	KL2B358_HL	320	412	428
CAR24	KL2B358_LH	321	413	429
CAR25	KL2B360_HL	322	414	430
CAR26	KL2B360_LH	323	415	431
CAR27	KL2B30_HL	404	416	432
CAR28	KL2B30_LH	405	417	433
CAR29	KL2B53_HL	406	418	434
CAR30	KL2B53_LH	407	419	435
CAR31	KL2B242_HL	408	420	436
CAR32	KL2B242_LH	409	421	437
CAR33	KL2B467_HL	324	422	438
CAR34	KL2B467_LH	325	423	439
CAR35	KL2B494_HL	308	424	440
CAR36	KL2B494_LH	316	425	441
LCHumanized refers to hu11B6_VL. HCHumanized refers to hu11B6_VH				

The scFv amino acid sequences of the CARs are shown in table 13 of Example 2.

The CARs full-length ECD domain and full-length amino acid sequences are listed below.

SEQ ID NO: 29 - Extracellular antigen-binding domain 1 (HL_HCG5_LCD6_20AA)

MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK

GLEWMGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG

QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM
HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTF
GQGTKLEIK

SEQ ID NO: 30 - Extracellular antigen-binding domain 2 (HL_HCG5_LCHumanized_20AA)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWMGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG
QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM
HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTF
GQGTKLEIK

SEQ ID NO: 31 - Extracellular antigen-binding domain 3 (HL_HCF3_LCB7_20AA)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQ
GTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMH
WYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTF
GQGTKLEIK

SEQ ID NO: 32 - Extracellular antigen-binding domain 4 (HL_HCG5_LCB7_20AA)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWMGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG
QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM
HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYT
FGQGTKLEIK

SEQ ID NO: 33 - Extracellular antigen-binding domain 5 (LH_LCD6_HCG5_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWMGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG
QGTLVTVSS

SEQ ID NO: 34 - Extracellular antigen-binding domain 6 (LH_LCHumanized_HCF3_20AA)

MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQ
GTLVTVSS

SEQ ID NO: 35 - Extracellular antigen-binding domain 7 (LH_LCHumanized_HCG5_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWMGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG
QGTLVTVSS

SEQ ID NO: 36 - Extracellular antigen-binding domain 8 (LH_LCB7_HCF3_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQ
GTLVTVSS

SEQ ID NO: 37 - Extracellular antigen-binding domain 9 (LH_LCB7_HCG5_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWMGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWG
QGTLVTVSS

SEQ ID NO: 38 - Extracellular antigen-binding domain 10 (LH_LCD6_HCF3_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQ
GTLVTVSS

SEQ ID NO: 39 - Extracellular antigen-binding domain 11 (HL_HCHumanized_LCB7_20AA)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGK
GLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG
QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM
HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYT
FGQGTKLEIK

SEQ ID NO: 40 - Extracellular antigen-binding domain 12 (HL_HCHumanized_LCD6_20AA)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGK
GLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG
QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM
HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTF
GQGTKLEIK

SEQ ID NO: 41 - Extracellular antigen-binding domain 13 (HL_HCHumanized_LCHumanized_20AA)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGK
GLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG
QGTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLM
HWYQQKPGQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTF
GQGTKLEIK

SEQ ID NO: 42 - Extracellular antigen-binding domain 14 (LH_LCD6_HCHumanized_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGK
GLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG
QGTLVTVSS

SEQ ID NO: 43 - Extracellular antigen-binding domain 15 (LH_LCHumanized_HCHumanized_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGK
GLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG
QGTLVTVSS

SEQ ID NO: 44 - Extracellular antigen-binding domain 16 (LH_LCB7_HCHumanized_20AA)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGK
GLEWIGYISYSGSTTYNPSLKSRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWG
QGTLVTVSS

SEQ ID NO: 149 (KL2B413_HL)
MAWVWTLLFLMAAAQSIQAEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMTWVRQAPGK
GLEWVANIKQDGSERYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDQNYDILT
GHYGMDVWGQGTTVTVSSGGSEGKSSGSGSESKSTGGSEIVLTQSPSFLSASVGDRVTITCRASQ
GISSYLSWYQQKPGKAPKLLIYATSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSY
PRTFGQGTKVEIK

SEQ ID NO: 150 (KL2B413_LH)
MAWVWTLLFLMAAAQSIQAEIVLTQSPSFLSASVGDRVTITCRASQGISSYLSWYQQKPGKAPK
LLIYATSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPRTFGQGTKVEIKGGSEG
KSSGSGSESKSTGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMTWVRQAPGKGLEWV
ANIKQDGSERYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDQNYDILTGHYGM
DVWGQGTTVTVSS

SEQ ID NO: 151 (KL2B359_HL)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGK
RLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ
GTLVTVSSGGSEGKSSGSGSESKSTGGSEIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMH
WYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGG
GTKVEIK

SEQ ID NO: 152 (KL2B359_LH)
MAWVWTLLFLMAAAQSIQAEIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPG
QPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKG
GSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKRL

EWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGT
LVTVSS

SEQ ID NO: 410 (KL2B357_HL)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ
GTLVTVSSGGSEGKSSGSGSESKSTGGSDIVLTQSPDSLAVSLGERATINCRASESVEYFGTSLMH
WYQQKPGQPPKLLIYAASNVESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQTRKVPYTFG
GGTKVEIK

SEQ ID NO: 411 (KL2B357_LH)
MAWVWTLLFLMAAAQSIQADIVLTQSPDSLAVSLGERATINCRASESVEYFGTSLMHWYQQKP
GQPPKLLIYAASNVESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQTRKVPYTFGGGTKVEI
KGGSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ
GTLVTVSS

SEQ ID NO: 412 (KL2B358_HL)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQPPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ
GTLVTVSSGGSEGKSSGSGSESKSTGGSEIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMH
WYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGG
GTKVEIK

SEQ ID NO: 413 (KL2B358_LH)
MAWVWTLLFLMAAAQSIQAEIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPG
QPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKG
GSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQPPGKGL
EWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGT
LVTVSS

SEQ ID NO: 414 (KL2B360_HL)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGK
GLEWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQ

GTLVTVSSGGSEGKSSGSGSESKSTGGSEIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMH
WYQQKPGQPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGG
GTKVEIK

SEQ ID NO: 415 (KL2B360_LH)
MAWVWTLLFLMAAAQSIQAEIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPG
QPPRLLIYAASNVESGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKG
GSEGKSSGSGSESKSTGGSQVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKGL
EWIGYISYSGSTTYNPSLKSRVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGT
LVTVSS

SEQ ID NO: 416 (KL2B30_HL)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGL
EWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAGTTIFGVVTPNFYY
GMDVWGQGTTVTVSSGGSEGKSSGSGSESKSTGGSDIQMTQSPSFLSASVGDRVTITCRASQGIS
SYLAWYQQKPGKAPKFLIYAASTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPL
TFGGGTKVEIK

SEQ ID NO: 417 (KL2B30_LH)
MAWVWTLLFLMAAAQSIQADIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPK
FLIYAASTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPLTFGGGTKVEIKGGSEG
KSSGSGSESKSTGGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYI
YYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAGTTIFGVVTPNFYYGMDVW
GQGTTVTVSS

SEQ ID NO: 418 (KL2B53_HL)
MAWVWTLLFLMAAAQSIQAEVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHWVRQAPGKG
LEWVAIISYDGSKKDYTDSVKGRFTISRDNSKNTLYLQMDSLRVEDSAVYSCARESGWSHYYYY
GMDVWGQGTMVTVSSGGSEGKSSGSGSESKSTGGSDIVMTQSPSSLSASVGDRVTITCRASQDIS
NYLAWYQQKPGKVPKFLIYAASTLHSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAP
YTFGQGTRLEIK

SEQ ID NO: 419 (KL2B53_LH)

MAWVWTLLFLMAAAQSIQADIVMTQSPSSLSASVGDRVTITCRASQDISNYLAWYQQKPGKVP
KFLIYAASTLHSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPYTFGQGTRLEIKGGS
EGKSSGSGSESKSTGGSEVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHWVRQAPGKGLEW
VAIISYDGSKKDYTDSVKGRFTISRDNSKNTLYLQMDSLRVEDSAVYSCARESGWSHYYYYGM
DVWGQGTMVTVSS

SEQ ID NO: 420 (KL2B242_HL)
MAWVWTLLFLMAAAQSIQAQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQPAGSG
LEWIGRLYVSGFTNYNPSLKSRVTLSLDPSRNQLSLKLSSVTAADTAVYYCAGDSGNYWGWFDP
WGQGTLVTVSSGGSEGKSSGSGSESKSTGGSSYELTQPPSVSVSPGETASITCSGDQLGENYACW
YQQKPGQSPVLVIYQDSKRPSGIPERFSGSNSGNTATLTISGTQALDEADYYCQAWDNSIVVFGG
GTKLTVL

SEQ ID NO: 421 (KL2B242_LH)
MAWVWTLLFLMAAAQSIQASYELTQPPSVSVSPGETASITCSGDQLGENYACWYQQKPGQSPV
LVIYQDSKRPSGIPERFSGSNSGNTATLTISGTQALDEADYYCQAWDNSIVVFGGGTKLTVLGGS
EGKSSGSGSESKSTGGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQPAGSGLEWI
GRLYVSGFTNYNPSLKSRVTLSLDPSRNQLSLKLSSVTAADTAVYYCAGDSGNYWGWFDPWGQ
GTLVTVSS

SEQ ID NO: 422 (KL2B467_HL)
MAWVWTLLFLMAAAQSIQAQVQLVESGGGVVQPGRSLRLSCAASGFTFSYYGMHWVRQAPGK
GLEWVAFISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSYW
AFDYWGQGTQVTVSSggsegkssgsgseskstggsQSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWY
QQKPGQAPVLVVYDNSDRPSGIPERFSGSNSGTTATLTISRVEAGDEADYYCQVWDSSSDHPVVF
GGGTKVTV

SEQ ID NO: 423 (KL2B467_LH)
MAWVWTLLFLMAAAQSIQAQSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQQKPGQAPV
LVVYDNSDRPSGIPERFSGSNSGTTATLTISRVEAGDEADYYCQVWDSSSDHPVVFGGGTKVTVg
gsegkssgsgseskstggsMAWVWTLLFLMAAAQSIQAQVQLVESGGGVVQPGRSLRLSCAASGFTFSY
YGMHWVRQAPGKGLEWVAFISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV
YYCAHLPYSGSYWAFDYWGQGTQVTVSS

SEQ ID NO: 424 (KL2B494_HL)
QVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMSWVRQAPGKGLEWVSTIGGSGGSTYYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTALLYDGMDVWGQGTMVTVSS
GGSEGKSSGSGSESKSTGGSSSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVL
VVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVL

SEQ ID NO: 425 (KL2B494_LH)
MAWVWTLLFLMAAAQSIQASSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPV
LVVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVL
GGSEGKSSGSGSESKSTGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMSWVRQAPGKG
LEWVSTIGGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTALL
YDGMDVWGQGTMVTVSS

SEQ ID NO: 46 - CAR 1 (HL_HCG5_LCD6_20AA; pDR000083431)
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMGYISYSGSTTYNPSL
KSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSG
SESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAAS
NRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEIKTSTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 47 - CAR 2 (HL_HCG5_LCHumanized_20AA; pDR000083432)
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMGYISYSGSTTYNPSL
KSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSG
SESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAAS
NRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEIKTSTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 48 - CAR 3 (HL_HCF3_LCB7_20AA; pDR000083436)

QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLK
SRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSGSE
SKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNR
ESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEIKTSTPAPRPPTPAP
TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY
DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPR

SEQ ID NO: 49 - CAR 4 (HL_HCG5_LCB7_20AA; pDR000083437)
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMGYISYSGSTTYNPSL
KSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSG
SESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAAS
NRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEIKTSTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 50 - CAR 5 (LH_LCD6_HCG5_20AA; pDR000083438)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMGYISYSGSTTYNPSL
KSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 51 - CAR 6 (LH_LCHumanized_HCF3_20AA; pDR000083440)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLK
SRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPAP

TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY
DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPR

SEQ ID NO: 52 - CAR 7 (LH_LCHumanized_HCG5_20AA; pDR000083441)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMGYISYSGSTTYNPSL
KSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 53 - CAR 8 (LH_LCB7_HCF3_20AA; pDR000083443)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLK
SRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPAP
TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY
DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPR

SEQ ID NO: 54 - CAR 9 (LH_LCB7_HCG5_20AA; pDR000083444)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWMGYISYSGSTTYNPSL
KSRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 55 - CAR 10 (LH_LCD6_HCF3_20AA; pDR000083446)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLK
SRVTISRDTSKNQFSLKLSSVTPVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPAP
TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY
DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPR

SEQ ID NO: 56 - CAR 11 (HL_HCHumanized_LCB7_20AA; pDR000083433)
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLK
SRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSG
SESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAAS
NRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEIKTSTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 57 - CAR 12 (HL_HCHumanized_LCD6_20AA; pDR000083434)
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLK
SRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSG
SESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAAS
NRESGVPDRFSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEIKTSTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 58 - CAR 13 (HL_HCHumanized_LCHumanized_20AA; pDR000083435)
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLK
SRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSG

SESKSTGGSDIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAAS
NRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEIKTSTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 59 - CAR 14 (LH_LCD6_HCHumanized_20AA; pDR000083439)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTIQSVQAEDVSVYFCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLK
SRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 60 - CAR 15 (LH_LCHumanized_HCHumanized_20AA; pDR000083442)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTISSLQAEDVAVYYCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLK
SRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 61 - CAR 16 (LH_LCB7_HCHumanized_20AA; pDR000083445)
DIVLTQSPDSLAVSLGERATINCKASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNRESGVPDR
FSGSGSGTDFTLTISSVQAEDVAVYYCQQTRKVPYTFGQGTKLEIKGGSEGKSSGSGSESKSTGGS
QVQLQESGPGLVKPSDTLSLTCAVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLK
SRVTMSRDTSKNQFSLKLSSVTAVDTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE

EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

>SEQ ID NO: 153 – CAR17 (KL2B413_HL; PBD000091628)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMTWVRQAPGKGLEWVANIKQDGSERYYVDS
VKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDQNYDILTGHYGMDVWGQGTTVTVSSG
GSEGKSSGSGSESKSTGGSEIVLTQSPSFLSASVGDRVTITCRASQGISSYLSWYQQKPGKAPKLLI
YATSTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPRTFGQGTKVEIKTSTPAPRP
PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR

>SEQ ID NO: 154 – CAR18 (KL2B413_LH; PBD000091623)
EIVLTQSPSFLSASVGDRVTITCRASQGISSYLSWYQQKPGKAPKLLIYATSTLQSGVPSRFSGSGS
GTEFTLTISSLQPEDFATYYCQQLNSYPRTFGQGTKVEIKGGSEGKSSGSGSESKSTGGSEVQLVE
SGGGLVQPGGSLRLSCAASGFTFSSYWMTWVRQAPGKGLEWVANIKQDGSERYYVDSVKGRFT
ISRDNAKNSLYLQMNSLRAEDTAVYYCARDQNYDILTGHYGMDVWGQGTTVTVSSTSTPAPRP
PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLG
RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR

>SEQ ID NO: 155 - CAR19 (KL2B359_HL; PBD000091576)
QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKRLEWIGYISYSGSTTYNPSLKS
RVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSGGSEGKSSGSGSE
SKSTGGSEIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVE
SGIPARFSGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKTSTPAPRPPTPAPTIA
SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFK
QPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA
TKDTYDALHMQALPPR

>SEQ ID NO: 156 – CAR20 (KL2B359_LH; PBD000091577)

EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARF
SGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKGGSEGKSSGSGSESKSTGGSQ
VQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKRLEWIGYISYSGSTTYNPSLKSR
VTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIF
KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPR

SEQ ID NO: 426 (CAR21)
QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLKS
RVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSggsegkssgsgseskstggs
DIVLTQSPDSLAVSLGERATINCRASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNVESGVPDR
FSGSGSGTDFTLTISSLQAEDVAVYFCQQTRKVPYTFGGGTKVEIKTSTPAPRPPTPAPTIASQPLS
LRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR
PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRR
GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY
DALHMQALPPR

SEQ ID NO: 427 (CAR22)
DIVLTQSPDSLAVSLGERATINCRASESVEYFGTSLMHWYQQKPGQPPKLLIYAASNVESGVPDR
FSGSGSGTDFTLTISSLQAEDVAVYFCQQTRKVPYTFGGGTKVEIKggsegkssgsgseskstggsQVQLQE
SGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLKSRVTISR
DTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSTSTPAPRPPTPAPTIASQPL
SLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM
RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPR

SEQ ID NO: 428 (CAR23)
QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLKS
RVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSggsegkssgsgseskstggs
EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARF
SGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKTSTPAPRPPTPAPTIASQPLSLR

PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV
QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

SEQ ID NO: 429 (CAR24)
QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQPPGKGLEWIGYISYSGSTTYNPSLKS
RVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSggsegkssgsgseskstggs
EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARF
SGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKTSTPAPRPPTPAPTIASQPLSLR
PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV
QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

SEQ ID NO: 430 (CAR25)
QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLKS
RVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSggsegkssgsgseskstggs
EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARF
SGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKTSTPAPRPPTPAPTIASQPLSLR
PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV
QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

SEQ ID NO: 431 (CAR26)
QVQLQESGPGLVKPSQTLSLTCTVSGNSITSDYAWNWIRQFPGKGLEWIGYISYSGSTTYNPSLKS
RVTISRDTSKNQFSLKLSSVTAADTAVYYCATGYYYGSGFWGQGTLVTVSSggsegkssgsgseskstggs
EIVLTQSPATLSLSPGERATLSCRASESVEYFGTSLMHWYQQKPGQPPRLLIYAASNVESGIPARF
SGSGSGTDFTLTISSVEPEDFAVYFCQQTRKVPYTFGGGTKVEIKTSTPAPRPPTPAPTIASQPLSLR
PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV
QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRG
RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

SEQ ID NO: 432 (CAR27)
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSR
VTISVDTSKNQFSLKLSSVTAADTAVYYCAGTTIFGVVTPNFYYGMDVWGQGTTVTVSSGGSEG
KSSGSGSESKSTGGSDIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKFLIYAA
STLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPLTFGGGTKVEIKTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 433 (CAR28)
DIQMTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKFLIYAASTLQSGVPSRFSGSG
SGTEFTLTISSLQPEDFATYYCQQLNSYPLTFGGGTKVEIKGGSEGKSSGSGSESKSTGGSQVQLQ
ESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISVD
TSKNQFSLKLSSVTAADTAVYYCAGTTIFGVVTPNFYYGMDVWGQGTTVTVSSTSTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 434 (CAR29)
EVQLVESGGGVVQPGRSLRLSCVASGFTFSSYDIHWVRQAPGKGLEWVAIISYDGSKKDYTDSV
KGRFTISRDNSKNTLYLQMDSLRVEDSAVYSCARESGWSHYYYYGMDVWGQGTMVTVSSGGS
EGKSSGSGSESKSTGGSDIVMTQSPSSLSASVGDRVTITCRASQDISNYLAWYQQKPGKVPKFLIY
AASTLHSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPYTFGQGTRLEIKTSTPAPRPP
TPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGR
REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR

SEQ ID NO: 435 (CAR30)
DIVMTQSPSSLSASVGDRVTITCRASQDISNYLAWYQQKPGKVPKFLIYAASTLHSGVPSRFSGSG
SGTDFTLTISSLQPEDVATYYCQKYNSAPYTFGQGTRLEIKGGSEGKSSGSGSESKSTGGSEVQLV

ESGGGVVQPGRSLRLSCVASGFTFSSYDIHWVRQAPGKGLEWVAIISYDGSKKDYTDSVKGRFTI
SRDNSKNTLYLQMDSLRVEDSAVYSCARESGWSHYYYYGMDVWGQGTMVTVSSTSTPAPRPP
TPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGR
REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR

SEQ ID NO: 436 (CAR31)
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQPAGSGLEWIGRLYVSGFTNYNPSLKS
RVTLSLDPSRNQLSLKLSSVTAADTAVYYCAGDSGNYWGWFDPWGQGTLVTVSSGGSEGKSSG
SGSESKSTGGSSYELTQPPSVSVSPGETASITCSGDQLGENYACWYQQKPGQSPVLVIYQDSKRPS
GIPERFSGSNSGNTATLTISGTQALDEADYYCQAWDNSIVVFGGGTKLTVLTSTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIF
KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPR

SEQ ID NO: 437 (CAR32)
SYELTQPPSVSVSPGETASITCSGDQLGENYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSGSNS
GNTATLTISGTQALDEADYYCQAWDNSIVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQVQL
QESGPGLVKPSETLSLTCTVSGGSISSYYWSWLRQPAGSGLEWIGRLYVSGFTNYNPSLKSRVTL
SLDPSRNQLSLKLSSVTAADTAVYYCAGDSGNYWGWFDPWGQGTLVTVSSTSTPAPRPPTPAPT
IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIF
KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYD
VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPR

SEQ ID NO: 438 (CAR33)
QVQLVESGGGVVQPGRSLRLSCAASGFTFSYYGMHWVRQAPGKGLEWVAFISYDGSNKYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSYWAFDYWGQGTQVTVSSggsegk
ssgsgseskstggsQSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQQKPGQAPVLVVYDNSDRPS
GIPERFSGSNSGTTATLTISRVEAGDEADYYCQVWDSSSDHPVVFGGGTKVTVTSTPAPRPPTPAP
TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY

DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPR

SEQ ID NO: 439 (CAR34)
QSVLTQPPSVSVAPGQTASITCGGDNIGSKSVHWYQQKPGQAPVLVVYDNSDRPSGIPERFSGSN
SGTTATLTISRVEAGDEADYYCQVWDSSSDHPVVFGGGTKVTVggsegkssgsgseskstggsQVQLVES
GGGVVQPGRSLRLSCAASGFTFSYYGMHWVRQAPGKGLEWVAFISYDGSNKYYADSVKGRFTI
SRDNSKNTLYLQMNSLRAEDTAVYYCAHLPYSGSYWAFDYWGQGTQVTVSSTSTPAPRPPTPA
PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPR

SEQ ID NO: 440 (CAR35)
QVQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMSWVRQAPGKGLEWVSTIGGSGGSTYYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTALLYDGMDVWGQGTMVTVSS
GGSEGKSSGSGSESKSTGGSSSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVL
VVYDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLTS
TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 441 (CAR36)
SSELTQPPSVSVAPGQTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSN
SGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLGGSEGKSSGSGSESKSTGGSQ
VQLVESGGGLVQPGGSLRLSCAASGFTFSHYAMSWVRQAPGKGLEWVSTIGGSGGSTYYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPHIVMVTALLYDGMDVWGQGTMVTVSST
STPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY
CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLY
NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR

To identify different scFv based CARs, their antigen-dependent activation via Jurkat-Lucia™ NFAT Cells (JNLs) was assessed. Nuclear factor of activated T-cells (NFAT) is a family of transcription factors first identified as a regulator of immune cells. T cell activation leads to calcium influx, activating calcineurin that dephosphorylates serine rich nuclear localization signal at the N-terminus of NFAT, leading to nuclear import of NFAT. Without wishing to be bound by theory, tonic signaling due to scFv clustering could be subsequently detected by Firefly luciferase driven by NFAT promoter in JNL reporter cell line.

Lentiviral transduction was performed as follows. JNL cells were harvested and re-suspended to 1E6/ml. 500 µl of JNL cells and lentiviral virus were added at a MOI of 3, and mixed by pipetting up and down. The mixture was incubated in a 37°C incubator for 24 hours. 500 µl JNL media was added to each well, with culturing continued in a 37°C incubator. The cells were transferred to a T25 flask on day 4. At days 5-6, transduction validation was performed. 150 µl of cells was harvested to examine CAR expression via appropriate detection reagent for the specific CAR used (e.g. biotin-hK2 protein to detect CAR). The JNL cells were maintained at $5x10^5$/ml until enough cells were obtained to either freeze down or use in the assay.

The thermally stabilized scFv CARs were evaluated in a JNL reporter assay for antigen-dependent activity. Jurkat cells containing the luciferase gene driven by the signaling-responsive NFAT promoter were transduced with the various hK2 CAR constructs. The assay was performed as follows. Co-culturing with target cell lines was performed at an effector to target ratio of 2:1. The JNL cells were spun down to remove any secreted luciferase in the medium; the JNL cells were then resuspend in fresh media at $4x10^5$/ml. The cells were harvested. Then, both antigen-positive and antigen-negative target cell lines were prepared at 2E5/ml. 100 µl JNL was added to 100 µl target cells. For the JNL-only control, 100 µl media was added instead of target cells. For positive control, JNL cells only or CAR JNL cells were added to 1x Cell Stimulation Cocktail and incubate in a 37°C incubator for 24 hours. 150 µl of the supernatant was harvested into a 96-well plate and centrifuged to remove cells. 100 µl of supernatant was transferred from the plate to a solid bottom black plate. Then, 100 µl QUANTI-luc lucia detection reagent was added. The mixture was incubated at room temperature for 5 minutes before reading with Envision multiplate reader.

CAR expression (CARs 1-10) on JNL cells was determined by biotinylated hK2 followed by streptavidin-conjugated PE, with the results shown in **FIG. 17**. CAR expression in transduced JNL cells was confirmed for all of the selected clones with 60–75% of cells showing detectable expression across the different clones and parental 11B6 CARs. JNL cells containing the indicated CAR clones and un-

transduced JNL cells (UTD) were co-cultured with target cells lines (VCap or DU145 cells) and luciferase activity was measured as luminescence intensity. Clones were considered active when the luminescence intensity exceeded 1.5-fold the level of UTD cells in the presence of antigen-expressing cells.

The binding between the CAR construct and its cognate cellular antigen (hK2 on target cells) lead to luciferase expression in the JNL cells (**FIG. 18**). JNL cells containing the indicated CAR clones and JNL cells (untransduced, hereinafter "UTD") were co-cultured with target cells lines (VCap or DU145 cells) and luciferase activity was measured as luminescence intensity. Clones were considered active when the luminescence intensity exceeded 1.5-fold the level of UTD cells in the presence of antigen-expressing cells. No antigen-dependent activation was found for all the 10 clones and parental 11B6 CAR HL and LH.

Example 10: Expression of the additional scFvs CARs on T cells

To evaluate the function of thermally stabilized 11B6 CARs T cells, 11B6 thermally stabilized and parental scFv transduced CAR -T cells were generated using lentiviral transduction. Primary human T cells were transduced with 11B6 thermally stabilized and parental scFvs CAR lentivirus with a multiplicity of infection (MOI) of 3. CAR expression was determined by biotinylated hK2 (1μg/ml) followed by streptavidin-conjugated PE 14 days post transduction.

The experimental protocol was as follows.

Lentiviral packaging was undertaken. 14E6 Lenti-X™ 293T cells were seeded per 150 mm^2 round-bottomed Corning™ BioCoat™ Collagen I coated culture dish, and incubated at 37°C overnight using DMEM+10% FBS medium.

With Lenti-X™ 293T cells at the ideal confluency of 80-85%, the following was prepared. Culture media was replaced with 21ml 293T growth media. Lipofectamine 2000 was diluted at room temperature and equilibrated OptiMEM. Diluted Lipofectamine 2000 was added to mixed plasmids drop-wise, and mixed well. The mixture was incubated at room temperature for 25 minutes. The transfection mixture was added to Lenti-X 293T cells dropwise, with the plate gently swirled to mix. The mixture was incubated in a 37°C incubator. Lentivirus was harvested 24 and 48 hours post transfection. The above reagents are summarized in the **Table 42**.

Table 42.

Reagents	Per 15cm dish
pMDLg/p	18 µg
pRSV.Rev	18 µg
pMD2.G	7 µg
Transfer (CAR) plasmid	15 µg
OptiMEM	1500ul

Lentiviral concentration was performed as follows. Lentivirus-containing supernatants were harvested and centrifuged at 500 x g for 10 min if excessive 293T cells are floating around before filtering through 0.45 µm cellulose acetate filters. Supernatant was transferred to a sterile container, combined with 1/3 volume of Lenti-X Concentrator (Clontech, Cat. # 631232), and mixed by gentle inversion. The mixture was incubated at 4°C overnight. The sample was centrifuged at 1,500 x g for 45 minutes at 4°C, with a white pellet visible after centrifugation. The supernatant was carefully removed without disturbing the pellet. Residual supernatants were removed with a pipette after a brief centrifugation at 1,500 x g. The pellet was gently resuspended in 1/10 of the original volume using cold T cell growth. The virus was aliquoted in 200 µl and immediately stored at –80°C.

Viral titering was performed as follows. Log-phase SupT1 was grown in complete RPMI. 100 µl 20,000 SupT1 cells/ml were seeded per well in a flat 96-well plate. Virus titration was then performed in 96-well round bottom plates). 100 µl media was added to all the wells in the plate. A virus aliquot was thawed from -80°C freezer with one's hands. The aliquot was pipetted up and down to mix well, with 50 µl virus added to the first well, followed by thorough mixing. A serial 1:3 dilution was performed by pipetting 50 µl virus to the next well. Then, 50 µl media was added to primary Ab only, secondary Ab only, and un-transduced controls. 50 µl of each was transferred from the titration plate to 100 µl SupT1 cells in the assay plate with a multi-channel pipette, and incubated at 37°C overnight. 100 µl of pre-warmed media was added to each sample, and incubated another two days. The transduction efficiency was determined by flow cytometry. Samples were transferred to a round bottom 96 well plate and spun at 1400 RPM for 3 minutes. The supernatant was discarded quickly, followed by blotting to remove excessive media in the hood. The wash was repeated with 150 µl FACS buffer. A staining protocol appropriate for CAR constructs was then undertaken. Samples were resuspended in 150 µl FACS buffer before flow cytometry analysis.

Viral titer calculation: Titer = (% CAR positive/100) x 2x104 x 20 x dilution factor

To exclude multiple copy integrations, titers with the closest transduction to 20% is used.

T-cell activation, transduction and expansion was performed as follows. A vial of naïve T cells was thawed in a water bath and added drop-wise to a 15 ml conical tube containing 9 ml of pre-warmed T-cell media (TCM CTS OpTmizer T Cell Expansion SFM+5% human serum +IL-2 100IU/ml). The cryovial was washed with 1 ml of media to recover maximum number, and spun at 300 x g for 8 minutes at room temperature. The pellet was resuspended in fresh 10 ml TCM, counted, and resuspended to 1E6 cells per milliner. 5E5 cells were added per well in 24-well plate. $1.5x10^6$ CD3/CD28 beads were added per well in 500 µl volume such that the total volume was 1 ml per well. Twenty-four hours post activation, virus was thawed at room temperature. Virus was added at a multiplicity of infection (MOI) of 3, gently swirled to mix, and returned to the plate at the incubator. (The lentiviral functional titer was previously determined with SupT1 titer assay.) One well was reserved for Untransduced (UTD) in which is no virus added.

Twenty four hours post-transduction, 1 ml TCM was added to each well. Penicillin-Streptomycin was added from this point onward (Day 2). On days 3-5, 4 ml TCM was added for each 2 ml of cells and transferred to a T25 tissue culture treated flask. The flasks were placed horizontally in the incubator (Day 3). Equal volume of TCM was added for T-cell culture (Day 4). On days 5-14, the cells were checked every other day, with the viability, size and total cell count recorded. The cell density was adjusted to one million cells per mL. The ideal time of harvest is donor dependent, and determined by the cell size and the fold of expansion. Cells were generally frozen when the cell size was less than 8 µm based on the MOXI flow cell counter with the 100 to 200-fold of expansion.

Lentiviral transduction efficiency was checked on harvest day as follows. Cultures were mixed well. 100 µl of T-cells was harvested to a corresponding well in 96-well plate with 100 µl FACS Buffer, mixed, and spun at 1300 RPM for 3 minutes at room temperature. The cells were resuspended in 200 µl FACS buffer and centrifugation repeated at 4°C.

14 days post-transduction, the cells were resuspended in 100 µl of Biotin labeled hK2 (1 µg/ml diluted in FACS buffer) and incubated on ice for 30 minutes. 100 µl of FACS buffer was added, and spun washed at 4°C. The spin wash was repeated after adding another 200 µl FACS buffer. Staining with 100 µl of master solution containing secondary antibody SA-PE (1:250), live dead fixable stain (1:500), αCD3, αCD4 and αCD8 antibodies was performed on ice for 30 minutes. 100 µl FACS buffer

was added and spun wash at 4°C, discarded and re-suspended in 200 µl FACS buffer before spin washing at 4°C. The samples were resuspended in 100 µl FACS Buffer before analyzing by flow cytometry.

Cell harvest and freezing was performed as follows. The final cell count was determined. The culture was harvested and centrifuged at 300 x g for 10 minutes at room temperature. The culture was resuspended in a smaller volume of media to fit in a 50 ml conical tube. The tubes were placed in magnets at 4°C for 10 minutes to "de-bead". The cells were gently transferred from the tube to minimize disturbing the beads / magnet, and the exact volume recorded. Counting was repeated. Centrifugation was performed at 300 x g for 10 minutes, with the supernatant discarded. Cells were frozen in CS-10 CryoStor® cell cryopreservation media in a cooling container. The containers were immediately transferred to -80°C for 24-48 hours before permanent storage in liquid Nitrogen. The results are shown in **FIG. 27**, and confirmed hK2 CAR expression on the surface of the transduced T cells. Summary of the percentage of hK2 CAR + T cells (% positive) detected by each of the 10 thermally stabilized clones and parental 11B6 HL & LH analyzed were provided in the Figure. The results of the surface expression of CAR17 (KL2B413_HL), CAR18 (KL2B413_LH), CAR19 (KL2B359_HL) and CAR20 (HK2B359_LH) are shown in **FIG. 27.** As shown, different clones have different CAR expression level which ranged from 17.9% to 42.7%. The percentage of CAR positive CD3+ T cells was 56.0% for CAR17, 51.2% for CAR18, 45.1% for CAR19 and 59.9% for CAR20, and 0.065%s for untransduced cells (UTD in the Figure). All CAR T cells were normalized to the equal CAR+ T cells for subsequent functional assays.

Example 11: CAR-T cells mediate tumor cell killing

scFv CAR-T cells were evaluated in the real-time IncuCyte tumor killing assay for antigen-dependent cytotoxicity. Thermally stabilized hK2 CAR-T cells and parental 11B6 CAR-T cells were co-incubated with VCaP cells (**FIG. 20**) or PC3 cells (**FIG. 21**) for 88 hours at effector:target ratio of 1:1 or 0.5:1 which was calculated based on CAR expression data. CAR-T cells transduced with CAR17, CAR18, CAR19 and CAR20 were co-incubated with hK2 positive VCaP cells and hK2 negative DU145 cells for 96 hours at effector-to-target (ET) ratio of 1:1 or 0.5:1 which was calculated based on CAR expression on T cells. The percent tumor cell growth inhibition over time of CAR-T cells transduced with CAR17, CAR18, CAR19 and CAR20 is shown in **FIG. 30** for VCap cells and in **FIG. 31** for DU145 cells. Target cells were stably expressing a red nuclear dye which was measured by IncuCyte imaging system in a real-time fashion. Tumor cell growth inhibition (%) =(Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%). Tested CAR-T

cells achieved approximately 100% TGI whereas the untransduced control did not demonstrate any TGI. No TGI was observed with the tested CAR-T cells in hK2 negative DU145 cells.

Example 12: Cytokine production by thermally stabilized scFv CAR-T cells

Supernatant was collected from overnight (approximately 20 hours) co-culture of hK2 CAR-T cells with VCaP cells at 1:1 of E/T ratio and was analyzed using 13-plex Milliplex Human High Sensitivity T cell kit (HSTCMAG28SPMX13). hK2 CAR modified T cells secreted cytokines during co-culture with hK2-expressing VCap cells, but minimal for un-transduced T cells (UTD). The results of cytokine release by hK2 CAR-T cells are shown in **FIG. 22.**

Supernatant was collected from overnight (approximately 20 hours) co-culture of hK2 CAR-T cells with VCap, DU145 (5E4 cells) cells at 1:1 of E/T ratio. hK2 CAR-T cells secreted IFN-γ during co-culture with hK2-expressing VCap cells, but not during co-culture with hK2-negative DU145 cells. CD3/28 beads stimulated T cells and T cells only were used as positive and negative controls, respectively. IFN-γ release by hK2 CAR-T cells is shown in **FIG. 23.** Mean IFN-γ concentration ± SD (pg/ml) from duplicate cultures is shown. Different thermally stabilized CAR-T cells produced different amount of IFN- γ when co-culture with hK2 (+) VCap cells.

Example 13: Proliferation of hK2 CAR-T cells

hK2 CAR-T cells were evaluated in a proliferation assay. T-cell proliferation is an important *in vitro* parameter of *in vivo* immune function. To further evaluate the function of thermally stabilized 11B6 CAR-T cells, 11B6 thermally stabilized and parental scFvs CAR -T cells were labeled with CTV to assess T cells proliferation.

hK2 CAR-T and un-transduced (UTD) T cells were labelled with CellTrace Violet (CTV; 5μM) and co-cultured with hK2 (+) VCap and hK2 (-) DU145 cells. Five days post co-culture, cells were harvested and stained with CD3, CD25, NearIR live/dead Dye and hK2 CAR. Flow cytometric analysis was performed on a Fortessa flow cytometer with Flowjo software. Lymphocytes were identified by live CD3, and the frequencies of CAR-T cells with CTV dye dilution and activation marker CD25 were determined. By gating on hK2 CAR+ T cells, as shown, the hK2(+) Vcap cells but not hK2(-) DU145 cells promoted the all CAR constructs engineered T cells proliferation (**FIG. 33**) and upregulation of activation marker CD25 (**FIG. 34**). CD3/28 beads stimulated T cells and T cells only were used as positive and negative controls, respectively. T cells only without any stimulation did not proliferate and

CD3/28 beads stimulated T cells displayed equivalent proliferation pattern. As shown in **FIG. 35**, hK2 CAR-T cells proliferated more robustly than CD3/28 beads positive control after 5 days of co-culture with VCap cells. Different CAR constructs engineered T cells have different proliferation activity and displayed different CAR-T cells counts. The CAR-T cells counts based on mean absolute cell count +/- SEM from three technical replicates.

The protocol was as follows. The tumor cells Vcap and DU145 were collected, washed twice with PBS, and resuspended in 10E6/ml in PBS containing 100ug/ml Mitomycin C (MMC) for 1.5 hours in a 37°C incubator so as to block tumor cells proliferation. 20 µL of DMSO was added to a vial of CTV staining solution. 5 µl of the solution was diluted into 5mL (1:1000) PBS (warmed to 37°C) to provide a 5 µM staining solution. The 2E6 T cells were counted, collected, washed with PBS twice, and resuspended in 4E6/ml (0.5ml). An equal volume (0.5 ml) of CTV staining solution was added. The cells were incubated for 20 minutes at 37°C. Then, 4 ml PRMI+20% FBS was added to the cells to absorb any unbound dye. The cells were incubated for 5 minutes, and centrifuged for 5 minutes at 400 x g. The cell pellet was resuspended in pre-warmed RPMI+10%FBS medium. The T cells were counted, and 1E5 cells(100ul) were seeded in 96-wells flat bottom-plate.

In the meantime, MMC-treated tumor cells hK2(+) VCap and HK2(-) DU145 were collected and counted after 1.5 hours, and then resuspended at 1E6/ml. 1E5 of the cells (100 µl) were cocultured with T cells in a 96-well plate. T cells alone, and T cells added 3:1 CD3/28 beads to cells ratio, were used as negative and positive controls, respectively.

After 5 days of co-culture, all of the cells were collected from each well. The cells were centrifuged and washed for 5 minutes at 400 x g twice, then stained hK2CAR, CD3, CD8 and CD25, live/dead (Near-IR) in 96-well U bottom plate. After washing, all cells were fixed for 10 minutes using 100 µl BD Cytofix™ Fixation Buffer (50ul FACS buffer +50ulFixation Buffer). The stained samples were analyzed by multicolor flow cytometry after the end of the incubation period.

Data analysis was performed as follows. A CTV histogram was prepared. The CTV undiluted gate was set to encompass the far-right peak (CTV bright) of T cells cultured alone, and the CTV diluted gate to capture the rest of the population. This was applied to all samples.

Example 14. Characterization of CAR-T cells transduced with CAR17, CAR18, CAR19 or CAR20.

Activation of CAR-T cells is antigen-dependent

The generated CAR-T cells were evaluated in the JNL reporter assay for antigen-dependent activity as described in Example 5. Briefly, Jurkat cells containing the luciferase gene driven by the signaling-responsive NFAT promoter (termed JNL cells) were transduced with CAR17 (KL2B413_HL), CAR18 (KL2B413_LH), CAR19 (KL2B359_HL) or CAR20 (KL2B359_LH) constructs. Expression of each CAR was determined by biotinylated hK2 followed by streptavidin-conjugated PE. CAR expression in transduced JNL cells was confirmed for the selected clones with 46–50% of cells expressing KL2B359-based CARs and 73.7-96% of cells expressing KL2B413-based CARs as shown in **FIG. 28**. The percent JNL cells expressing each CAR was following: CAR17 (KL2B413_HL): 73.7%. CAR18 (KL2B413_LH): 93.6%, CAR19 (KL2B359_HL): 46%, CAR20 (KL2B359_LH): 50.8%.

Binding between the hK2 CAR construct and its cognate cellular antigen (hK2 on target cells) leads to luciferase expression in the JNL cells. To that end, JNL cells transduced with the test CAR constructs or untransduced JNL cells (UTD) were co-cultured with target cells lines (VCap, LNCap/hK2 (LNCaP cells recombinantly expressing hK2), LNCaP, C4-2B, 22Rv1 or DU145 cells) and luciferase activity was measured as luminescence intensity. Constructs were considered active when the luminescence intensity exceeded 1.5-fold the level of UTD cells in the presence of antigen-expressing cells. No antigen-dependent activation was found for the tested CAR constructs. **FIG. 29** shows the RLU (relative light units) resulting from binding of CAR-T cells to test target cells ad indicated in the Figure.

CAR-T cells mediate tumor cell killing in antigen-dependent manner

CAR-T cells transduced with CAR17, CAR18, CAR19 and CAR20 were co-incubated with hK2 positive VCaP cells and hK2 negative DU145 cells for 96 hours at effector-to-target (ET) ratio of 1:1 or 0.5:1 which was calculated based on CAR expression on T cells (Example 11). Target cells were stably expressing a red nuclear dye, which was measured by IncuCyte imaging system in a real-time fashion. The percent tumor cell growth inhibition over time is shown in **FIG. 30** for VCap cells and in **FIG. 31** for DU145 cells. Tumor cell growth inhibition (TGI) (%) = (Initial Viable Target Cell Number-Current Viable Target Cell Number)/Initial Viable Cell Number*100 (%). Tested CAR-T cells achieved approximately 100% TGI whereas the untransduced control did not demonstrate any TGI. No TGI was observed with the tested CAR-T cells in hK2 negative DU145 cells.

CAR-T cells produce cytokines upon antigen stimulation

IFN-γ produced by cytotoxic T cells is critical for exerting immune surveillance of tumors, which can directly inhibit proliferation and induce apoptosis of some malignancies *in vivo* and *in vitro*. To determine whether hK2 CAR-modified human T cells were able to recognize and become activated by hK2 positive tumor cells, primary T cells transduced with indicated CAR clones and control untransduced T cells (UTD) were co-cultured with target cells lines (LNCaP/hK2, LNCaP, C4-2B, 22Rv1 or DU145 cells) and supernatant were collected for IFN-γ concentration measurement. As shown in **FIG. 32**, CAR-T cells transduced with hK2 CARs cells secreted IFN-γ during co-culture with LNCaP cells recombinantly expressing hK2 (LNCap/hK2) cells and also during co-culture with very low hK2-expressing C4-2B and LNCap cells but not hK2-negative DU145 cells. Mean IFN-γ concentration ± SD (pg/ml) from duplicate cultures is shown in the Figure.

CAR-T cells are activated and upregulate markers of degranulation in antigen-dependent manner

Tumor cells can be recognized and killed by cytotoxic lymphocytes, such as CD8+ T lymphocytes and natural killer (NK) cells mainly through the immune secretion of lytic granules that kill the target tumor cells. This process involves the fusion of the granule membrane with the cytoplasmic membrane of the immune effector cell, resulting in surface exposure of lysosomal-associated proteins that are typically present on the lipid bilayer surrounding lytic granules, such as CD107a. Therefore, membrane expression of CD107a constitutes a marker of immune cell activation and cytotoxic degranulation.

The degranulation assay was performed as described below. Target cells (5×10^4) were co-cultured with an equal number of effector cells in 0.1ml per well in a 96-well plate. Control wells contained T cells alone. Anti-CD107a (5µl per well) were added in addition to 1µl/sample of monensin (BD Biosciences) and incubated for 4 hours at 37°C. Cells were washed two times with PBS, stained for expression of the hK2 CAR, CD3, and CD8 and analyzed on a flow cytometer BD Fortessa.

The results of the degranulation assay are shown in **FIG. 33**. An increase in CD107A+ hK2 CAR-T cells was evident in co-cultures with hK2 positive Vcap cells, whereas co-culture with hK2 negative DU145 had no effect. Only background staining(<2%) was seen in the CAR-T cells only without tumor cells stimulation and in the UTD control. Phorbol 12-myristate 13-acetate (PMA) in combination with ionomycin(eBioscience™ Cell Stimulation Cocktail ;500X) was a consistent inducer of

CD107a cell surface expression in this 4-hour cell culture model, which was used as a positive control. Results are shown for representative donor T cells and were reproducible in multiple donors.

CAR-T cells proliferate in antigen-dependent manner

CAR-T cells were evaluated for their proliferation using T-cell proliferation assay protocol described in Example 9. hK2 CAR-T and untransduced (UTD) T cells were labelled with CellTrace Violet (CTV; 5μM) and co-cultured with hK2 positive VCap and hK2 negative DU145 cells. Five days post co-culture, cells were harvested and stained with CD3, CD25, NearIR live/dead Dye and hK2 CAR. Flow cytometric analysis was performed on a Fortessa flow cytometer with Flowjo software. Lymphocytes were identified by live CD3, and the frequencies of CAR-T cells with CTV dye dilution and activation marker CD25 were determined. By gating on CD3+ T cells, the hK2 positive Vcap cells but not hK2 negative DU145 cells promoted proliferation of each tested CAR-T cell line, as shown in **FIG. 34 and FIG. 35,** and upregulation of activation marker CD25 as shown in **FIG. 36** and **FIG. 37**. T cells only without any stimulation did not proliferate and CD3/28 beads stimulated T cells displayed equivalent proliferation pattern. hK2 CAR+ T cells proliferated more robustly than CD3/28 beads positive control after 5 days of coculture with VCap cells. Different tested CAR-T cells had different proliferation activity and displayed different CAR-T cells counts. The percentage of proliferating T cells and CD25 expressing T cells was based on mean absolute cell count +/- SEM from duplicate.

Example 15: Generation and characterization of ADC

Antibodies or antigen binding domains that bind hK2 are be labeled with various radiometals such as In-111, Zr-89, or Lu-177 Ac-225 and in vivo tumor biodistribution or tumor efficacy is evaluated in established subcutaneous (SC) human prostate models including LNCaP, VCaP or C4-2B in male NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG, The Jackson Laboratory, Bar Harbor, ME) or in Balb-c or SHO Nude mice. Various doses of antibodies or antigen binding domains that bind hK2 or isotype control antibodies are labeled with different amounts of radioactivity (10-1000 nCi) and tumor uptake or efficacy is measured.

CLAIMS

1. An isolated anti-hK2/anti-CD3 antibody comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
    a. the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the CD3 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
    b. the first domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second domain that binds the CD3 comprises a scFv of SEQ ID NO: 331; and/or
    c. the isolated anti-hK2/anti-CD3 antibody comprises a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360.
2. An isolated anti-hK2/anti-CD3 antibody comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
    a. the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the CD3 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively.
3. The isolated anti-hK2/anti-CD3 antibody of claim 2, wherein:
    a. the first domain that binds hK2 comprises a VH which is at least 80% (e.g. at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VH of SEQ ID NO: 162 and a VL which is at least 80% (e.g. at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the VL of SEQ ID NO: 163, and the second domain that binds the CD3 comprises a scFv which is at least 80% (e.g. at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the scFv of SEQ ID NO: 331; and/or
    b. the isolated anti-hK2/anti-CD3 antibody comprises a HC1 which is at least 80% (e.g. at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC1 of SEQ ID NO: 354, a LC1 which is at least 80% (e.g. at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the LC1 of SEQ ID NO: 221 and a HC2 which is at least 80% (e.g. at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC2 of SEQ ID NO: 360.

4. The isolated anti-hK2/anti-CD3 antibody of claim 2 or claim 3, wherein the isolated anti-hK2/anti-CD3 antibody comprises a lysine at the C-terminus of HC1 and HC2, and wherein the the isolated anti-hK2/anti-CD3 antibody comprises a HC1 which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC1 of SEQ ID NO: 361 and a HC2 which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to the HC2 of SEQ ID NO: 362.
5. An isolated anti-hK2/anti-CD3 antibody comprising a first domain that binds hK2 and a second domain that binds CD3, wherein
    a. the first domain that binds hK2 comprises a HCDR1, a HCDR2, a HCDR3, a LCDR1, a LCDR2 and a LCDR3 of SEQ ID NOs: 170, 171, 172, 173, 174 and 175, respectively, and the second domain that binds the CD3 comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 255, 256, 257, 258, 259 and 261, respectively;
    b. the first domain that binds hK2 comprises a VH of SEQ ID NO: 162 and the VL of SEQ ID NO: 163, and the second domain that binds the CD3 comprises a scFv of SEQ ID NO: 331; and/or
    c. the isolated anti-hK2/anti-CD3 antibody comprises a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.
6. The isolated anti-hK2/anti-CD3 antibody of any one of claims 1-5, wherein the first domain that binds hK2 and/or the second domain that binds CD3 comprise a scFv, a $(scFv)_2$, a Fv, a Fab, or a $F(ab')_2$.
7. The isolated anti-hK2/anti-CD3 antibody of claim 6, wherein the first domain that binds hK2 comprises a Fab and the second domain that binds CD3 comprises a scFv.
8. The isolated anti-hK2/anti-CD3 antibody of claim 7, wherein the scFv comprises, from N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).
9. The isolated anti-hK2/anti-CD3 antibody of claim 8, wherein the L1 comprises:
    a. 5-50 amino acids;
    b. 5-40 amino acids;
    c. 10-30 amino acids; or
    d. 10-20 amino acids.
10. The isolated anti-hK2/anti-CD3 antibody of claim 9, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 7, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, or 108.

11. The isolated anti-hK2/anti-CD3 antibody of claim 2, wherein:

    a. the first domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second domain that binds CD3 comprises a scFv of SEQ ID NO: 331;

    b. the isolated anti-hK2/anti-CD3 antibody comprises a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360; and/or

    c. the isolated anti-hK2/anti-CD3 antibody comprises a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

12. An isolated anti-hK2/anti-CD3 antibody comprising a first domain that binds hK2 and a second domain that binds CD3, wherein the first domain that binds hK2 comprises a VH of SEQ ID NO: 162 and a VL of SEQ ID NO: 163, and the second domain that binds CD3 comprises a scFv of SEQ ID NO: 331.

13. The isolated anti-hK2/anti-CD3 antibody of claim 12, wherein the isolated anti-hK2/anti-CD3 antibody comprises:

    a. a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360; and/or

    b. a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.

14. The isolated anti-hK2/anti-CD3 antibody of any one of claims 1-13, wherein:
    the first domain that binds hK2 is conjugated to a first immunoglobulin (Ig) heavy chain constant region, or a fragment of the first Ig heavy chain constant region, and
    the second domain that binds CD3 is conjugated to a second immunoglobulin (Ig) heavy chain constant region, or a fragment of the second Ig heavy chain constant region.

15. The isolated anti-hK2/anti-CD3 antibody of claim 14, wherein the first Ig heavy chain constant region, or the fragment of the first Ig heavy chain constant region, and the second Ig heavy chain constant region, or the fragment of the second Ig heavy chain constant region, is an IgG1 isotype.

16. The isolated anti-hK2/anti-CD3 antibody of claim 14 or 15, wherein the antibody comprises the following amino acid mutations:
    L234A_L235A_D265S_T350V_ T366L_K392L_T394W in the first Ig heavy chain constant region, or the fragment of the first Ig heavy chain constant region, and
    L234A_L235A_D265S_T350V_ L351Y_F405A_Y407V in the second Ig heavy chain constant region, or the fragment of the second Ig heavy chain constant region,
    wherein numbering of the amino acid mutations is according to the EU index.

17. The isolated anti-hK2/anti-CD3 antibody of claim 16, wherein the first Ig heavy chain constant region, or the fragment of the first Ig heavy chain constant region, comprises an amino acid sequence of SEQ ID NO: 378.
18. The isolated anti-hK2/anti-CD3 antibody of claim 16 or claim 17, wherein the second Ig heavy chain constant region, or the fragment of the second Ig heavy chain constant region, comprises an amino acid sequence of SEQ ID NO: 109.
19. The isolated anti-hK2/anti-CD3 antibody of any one of claims 14-18, wherein:
the first Ig heavy chain constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to SEQ ID NO: 378 and the second Ig heavy chain constant region comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to SEQ ID NO: 109.
20. The isolated anti-hK2/anti-CD3 antibody of claim 19, wherein:
the first domain that binds hK2 is conjugated to a first immunoglobulin (Ig) light chain constant region, or a fragment of the first Ig light chain constant region, and
the first immunoglobulin (Ig) light chain constant region, or the fragment of the first Ig light chain constant region, comprises an amino acid sequence which is at least 80% (*e.g.* at least 85%, at least 90%, at least 95%, at least 99% or 100%) identical to SEQ ID NO: 309.
21. An isolated anti-hK2/anti-CD3 antibody comprising a HC1 of SEQ ID NO: 354, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 360.
22. An isolated anti-hK2/anti-CD3 antibody comprising a HC1 of SEQ ID NO: 361, a LC1 of SEQ ID NO: 221 and a HC2 of SEQ ID NO: 362.
23. An immunoconjugate comprising the isolated anti-hK2/anti-CD3 antibody of any one of claims 1-22 conjugated to a therapeutic agent or an imaging agent.
24. A pharmaceutical composition comprising the isolated anti-hK2/anti-CD3 antibody of any one of claims 1-22 and a pharmaceutically acceptable carrier.
25. A polynucleotide encoding the isolated anti-hK2/anti-CD3 antibody of any one of claims 1-22.
26. A vector comprising the polynucleotide of claim 25.
27. A host cell comprising the vector of claim 26.
28. A method of producing the isolated anti-hK2/anti-CD3 antibody of any one of claims 1-22, comprising culturing the host cell of claim 27 in conditions that the isolated anti-hK2/anti-CD3 antibody is expressed, and recovering the isolated anti-hK2/anti-CD3 antibody produced by the host cell.

29. Use of the isolated anti-hK2/anti-CD3 antibody of any one of claims 1-22, the immunoconjugate of claim 23, or the pharmaceutical composition of claim 24 for the manufacture of a medicament for the treatment of a hK2 expressing cancer in a subject.
30. The use according to claim 29, wherein the hK2 expressing cancer is a prostate cancer.
31. The use according to claim 30 wherein the prostate cancer is relapsed, refractory, malignant or castration resistant prostate cancer, or any combination thereof.
32. The use according to any one of claims 29-31, wherein the antibody is to be administered in combination with at least one additional therapeutic agent.
33. The use according to claim 32 wherein the additional therapeutic agent is surgery, chemotherapy, androgen deprivation therapy or radiation, or any combination thereof.
34. A kit comprising the isolated anti-hK2/anti-CD3 antibody of any one of claims 1-22, the immunoconjugate of claim 23, or the pharmaceutical composition of claim 24.