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(54) Title: ANTI-HLA-A2 ANTIBODIES AND METHODS OF USING THE SAME

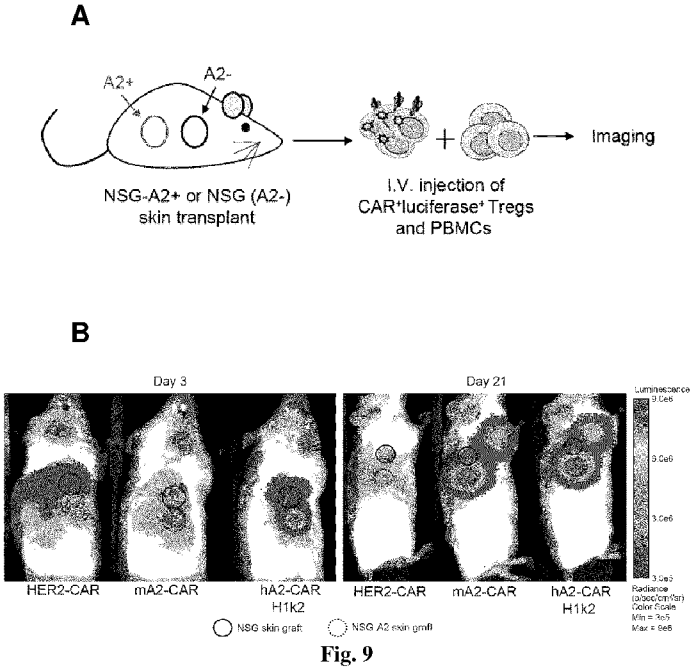


Fig. 9

(57) Abstract: Provided are humanized anti-HLA-A2 antibodies. In certain aspects, the humanized anti-HLA-A2 antibodies are capable of constituting an antigen binding domain of a chimeric antigen receptor (CAR), where the CAR is capable of being expressed in a human cell such that the CAR specifically binds to HLA-A2. Also provided are CARs that include the humanized anti-HLA-A2 antibodies. Modified cells including the antibodies and CARs, as well as methods of using such modified cells are also provided.

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ANTI-HLA-A2 ANTIBODIES AND METHODS OF USING THE SAME

Cross-Reference to Related Applications

This application claims the benefit of United States Provisional Patent Application Serial No. 62/560,574, filed September 19, 2017, and United States Provisional Patent Application Serial No. 62/692,386, filed June 29, 2018, which applications are incorporated herein by reference in their entireties.

Field of the Invention

The present invention relates in some aspects to HLA-A2 binding molecules, in particular, to humanized anti-HLA-A2 antibodies. The present invention further relates to recombinant receptors containing such antibodies, including chimeric antigen receptors (CARs), which contain such antibodies. The disclosure further relates to genetically engineered cells expressing such receptors and antibodies, and use thereof in cell therapy.

Background of the Invention

Class I HLA antigens are polymorphic proteins expressed on all nucleated cells and are critical targets for immune recognition in the context of transplantation. Indeed, the development of HLA class I specific T cells and/or antibodies are major risk factors for acute and chronic rejection and allograft, and the presence of pre-formed anti-donor HLA Class I antibodies can result in hyper-acute rejection (Konvalinka et al., 2015). Thus finding ways to control the immune response to HLA Class I proteins would be a major breakthrough in transplantation.

Classical HLA Class I molecules are polymorphic and encoded by many different alleles which have evolved in response to evolutionary pressure from infections. There are three loci that encode the classical HLA Class I proteins, which are named the A, B and C loci. Within the HLA-A locus, the HLA-A2 family of alleles is the largest and most diverse family, with at least 31 different HLA-A2 alleles known to exist in humans. Interestingly, contrary to many other HLA allele families, HLA-A2 is frequent in all ethnic groups, and is found in 50% of Caucasians and 35% of African-Americans (Ellis et al., 2000). Many HLA-A2 alleles differ by only 1 to 9 amino acids, with the majority of the polymorphism centered around the peptide

binding groove (Hilton et al., 2013). HLA-A2 alleles are sub-grouped into two main branches: those derived via interallelic gene conversion events from A*0201 or A*0205 (Ellis et al., 2000).

Adoptive immunotherapy with T regulatory (Treg) cells as a way to control unwanted immunity to HLA proteins and other antigens that drive transplant rejection is a promising treatment for allograft rejection and graft-versus-host disease (GVHD). The use of polyclonal Treg cell transfer in the prevention of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) has been reported (Brunstein et al., 2011; Di Ianni et al., 2011; Trzonkowski et al., 2009). The use of Treg cell transfer in the maintenance of c-peptide levels in type 1 diabetes has also been reported (Bluestone et al., 2015; Marek-Trzonkowska et al., 2012). Notably, it has been reported that there may be a transient risk of generalized immunosuppression associated with the use of polyclonal Treg cells for such cell therapy (Brunstein et al., 2013).

Data from animal studies indicate that the potency and specificity of cell therapy with Treg cells can be significantly enhanced by the use of antigen-specific cells. For example, in models of autoimmunity, antigen-specific Treg cells are superior to polyclonal Treg cells in reducing disease: Treg cells isolated from pancreatic lymph nodes or pulsed with islet antigen are significantly better at preventing or curing type 1 diabetes than are polyclonal Treg cells (Green et al., 2002; Masteller et al., 2005; Tang et al., 2004; Tarbell et al., 2007; Tarbell et al., 2004), and Treg cells expressing an autoantigen-specific transgenic T cell receptor (TCR) are superior to polyclonal Treg cells at suppressing central nervous system inflammation in a model of experimental autoimmune encephalomyelitis (EAE) (Stephens et al., 2009). Similarly, alloantigen-specific Treg cells, enriched by alloantigen-stimulated expansion *in vitro*, or engineered to express a TCR transgene, are more effective than polyclonal Treg cells at preventing rejection of organ and tissue grafts (Golshayan et al., 2007; Joffre et al., 2008; Nishimura et al., 2004; Sanchez-Fueyo et al., 2006; Tsang et al., 2008). There is some evidence that Treg cells expanded with alloantigens effectively prevent GVHD (Trenado et al., 2006) and that *in vivo* induction of antigen-specific Treg cells promotes acceptance of hematopoietic allografts without GVHD (Verginis et al., 2008). Humanized mouse models have shown similar results: alloantigen-expanded human Treg cells are more potent suppressors of skin

graft rejection than are polyclonal Treg cells (Putnam et al., 2013; Sagoo et al., 2011).

An alternate approach to over-expressing transgenic TCRs or antigen-stimulated expansion to enrich for antigen-specific T cells is the use of chimeric antigen receptors (CARs). In cell-based adoptive immunotherapy, immune cells isolated from a patient can be modified to express synthetic proteins that enable the cells to perform new therapeutic functions after they are subsequently transferred back into the patient. An example of such a synthetic protein is a CAR. An example of a currently used CAR is a fusion of an extracellular recognition domain (e.g., an antigen-binding domain), a transmembrane domain, and one or more intracellular signaling domains. Upon antigen engagement, the intracellular signaling portion of the CAR can initiate an activation-related response in an immune cell. For example, T cells may be genetically engineered to express extracellular single-chain antibody (scFv) antigen binding domains fused to intracellular signaling domains (Gill and June, 2015; June et al., 2015). In particular, Treg cells expressing CARs specific for model antigens have been reported (Blat et al., 2014; Elinav et al., 2009; Elinav et al., 2008; Fransson et al., 2012; Hombach et al., 2009, Boardman et al., 2016; MacDonald et al., 2016; Noyan et al., 2016).

SUMMARY

Aspects of the present disclosure include anti-HLA-A2 antibodies. Chimeric antigen receptors (CARs) including an extracellular domain including any of the anti-HLA-A2 antibodies of the present disclosure are also provided. Nucleic acids encoding the anti-HLA-A2 antibodies and CARs of the present disclosure, expression vectors including same, and host cells including such expression vectors are also provided. Aspects of the present disclosure also include humanized anti-HLA-A2 antibodies. Chimeric antigen receptors (CARs) including an extracellular domain including any of the humanized anti-HLA-A2 antibodies of the present disclosure are also provided. Nucleic acids encoding the humanized anti-HLA-A2 antibodies and CARs of the present disclosure, expression vectors including same, and host cells including such expression vectors are also provided. Also provided are immune cells, e.g., immune regulatory cells, which include the CARs and/or expression vectors of the present disclosure, compositions and pharmaceutical compositions including such immune cells, kits of parts including such immune cells and/or reagents (e.g., a nucleic acid or vector encoding an anti-HLA-A antibody or CAR of the present disclosure) for making such immune cells, and

methods of making such immune cells. Methods of using the anti-HLA-A2 antibodies, CARs, immune cells, and pharmaceutical compositions of the present disclosure are also provided. For example, the subject anti-HLA-A2 antibodies, CARs, immune cells (e.g., immune regulatory cells), and pharmaceutical compositions find use, *e.g.*, in promoting immune tolerance in a subject, preventing or treating graft versus host disease (GVHD) in a subject, preventing or treating organ or tissue transplant rejection in a subject, and the like.

In some embodiments, provided is a humanized anti-HLA-A2 antibody, where the antibody is capable of constituting an antigen binding domain of a chimeric antigen receptor (CAR), where the CAR is capable of being expressed in a human cell (*e.g.*, a human immune cell, such as a human immune regulatory cell) such that the CAR specifically binds to HLA-A2. In certain aspects, such antibodies compete for binding to HLA-A2 with an antibody including: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190.

In certain aspects, provided is a humanized anti-HLA-A2 antibody, where the antibody competes for binding to HLA-A2 with an antibody including: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190.

According to certain embodiments, a humanized anti-HLA-A2 antibody as set forth above binds to the same HLA-A2 epitope as an antibody including: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190.

In some embodiments, a humanized anti-HLA-A2 antibody of the present disclosure has less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, and any combination thereof, as compared to a BB7.2 antibody. For example, in some embodiments, a humanized anti-HLA-A2 antibody of the present disclosure has less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, and any combination thereof, as compared to a BB7.2 antibody.

In certain aspects, a humanized anti-HLA-A2 antibody of the present disclosure includes a heavy chain variable region including an amino acid sequence selected from the group consisting of: SYHIQ (SEQ ID NO: 1) and GYTFTSY (SEQ ID NO: 2).

According to certain embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a heavy chain variable region including an amino acid sequence selected from the group consisting of: YPGDGS (SEQ ID NO: 4) and WIYPGDGSTX^{10}YX^{12}X^{13}KFX^{16}G (SEQ ID NO: 10), where X^{10} is Q or K, X^{12} is N or S, X^{13} is E or Q, and X^{16} is K or Q. Such an antibody may include, *e.g.*, a heavy chain variable region including an amino acid sequence selected from the group consisting of: WIYPGDGSTQYNEKFKG (SEQ ID NO: 3) and YPGDGS (SEQ ID NO: 4). Also by way of example, such an antibody may include, *e.g.*, a heavy chain variable region including the amino acid sequence WIYPGDGSTKYSQKFQG (SEQ ID NO: 5). In certain aspects, a humanized

antibody of the present disclosure includes a heavy chain variable region including the amino acid sequence EGTYYAMDY (SEQ ID NO: 6).

In some embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a light chain variable region including the amino acid sequence RSSQSIVHSNGNTYLE (SEQ ID NO: 7). In certain aspects, a humanized antibody of the present disclosure includes a light chain variable region including the amino acid sequence KVSNRFS (SEQ ID NO: 8). According to some embodiments, a humanized antibody of the present disclosure includes a light chain variable region including the amino acid sequence FQGSHVPRT (SEQ ID NO: 9).

In certain aspects, a humanized anti-HLA-A2 antibody of the present disclosure includes a heavy chain variable region including a framework region 1 (VH FR1) including an amino acid sequence selected from the group consisting of:
QVQLVQSGAEVKKPGASVKVSCKAS (SEQ ID NO: 11) and
QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 12).

According to certain embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a heavy chain variable region including a framework region 2 (VH FR2) including an amino acid sequence selected from the group consisting of:

WVRQAPGQX^{9}LEWMGX^{15} (SEQ ID NO: 13),

WVRQAPGQX^{9}LEWMGX^{15}WI (SEQ ID NO: 17),

HIQWVRQAPGQX^{12}LEWMGX^{18}WI (SEQ ID NO: 21), and

HIQWVRQAPGQX^{12}LEWMGX^{18} (SEQ ID NO: 25), where:

X^{9} is R or G and X^{15} is I or absent in SEQ ID NO: 13;

X^{9} is R or G, and X^{15} is I or absent in SEQ ID NO: 17;

X^{12} is R or G, and X^{18} is I or absent in SEQ ID NO: 21; and

X^{12} is R or G, and X^{18} is I or absent in SEQ ID NO: 25.

In some embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a heavy chain variable region including a framework region 3 (VH FR3) including an amino acid sequence selected from the group consisting of:

$X^1VTX^4TX^6DTSX^{10}STAYMX^{16}LSX^{19}LRSX^{23}DX^{25}AVYYCAR$ (SEQ ID NO: 29),

$TX^2YX^4X^5KFX^8GX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}$

AVYYCAR (SEQ ID NO: 35),

$TQYNEKFKGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}$

AVYYCAR (SEQ ID NO: 36), and

$TKYSQKFQGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}AVYYCAR$ (SEQ ID NO: 37), where:

X^1 is R or absent, X^4 is I or M, X^6 is R or A, X^{10} is A, T or I, X^{16} is E or L, X^{19} is S or R, X^{23} is E or D, and X^{25} is T or M in SEQ ID NO: 29;

X^2 is Q or K, X^4 is N or S, X^5 is E or Q, X^8 is K or Q, X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 35;

X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 36; and

X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 37.

In certain aspects, a humanized anti-HLA-A2 antibody of the present disclosure includes a heavy chain variable region including a framework region 4 (VH FR4) including the amino acid sequence WGQGTTVTVSS (SEQ ID NO: 44). According to certain embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a heavy chain variable region including an amino acid sequence selected from the group consisting of SEQ ID NOs: 61-66.

According to certain embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a light chain variable region including a framework region 1 (VL FR1)

including the amino acid sequence $DX^2VMTQX^7PLSX^{11}X^{12}VTX^{15}GQPASISX^{23}$ (SEQ ID NO: 46), where X^2 is V or I, X^7 is S or T, X^{11} is L or S, X^{12} is P or S, X^{15} is L or P, and X^{23} is C or F.

In some embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a light chain variable region including a framework region 2 (VL FR2) including the amino acid sequence $WX^2X^3QX^5PGQX^9PX^{11}X^{12}LIY$ (SEQ ID NO: 51), where X^2 is F or Y, X^3 is Q or L, X^5 is R or K, X^9 is S or P, X^{11} is R or Q, and X^{12} is R or L.

In certain aspects, a humanized anti-HLA-A2 antibody of the present disclosure includes a light chain variable region including a framework region 3 (VL FR3) including the amino acid sequence $GVPDRFSGSGX^{11}GTDFTLKISRVEAEDVGVYYC$ (SEQ ID NO: 56), where X^{11} is S or A. According to certain embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a light chain variable region including a framework region 4 (VL FR4) including the amino acid sequence FGGGTKVEIK (SEQ ID NO: 59). In some embodiments, a humanized anti-HLA-A2 antibody of the present disclosure includes a light chain variable region including an amino acid sequence selected from the group consisting of SEQ ID NOs: 67-71. In some embodiments, a humanized anti-HLA-A2 antibody of the present disclosure is a whole antibody, a single chain antibody, a dimeric single chain antibody, a Fv, a scFv, a Fab, a $F(ab)'_2$, a defucosylated antibody, a bi-specific antibody, a diabody, a triabody, a tetrabody, an antibody fragment selected from the group consisting of a unibody, a domain antibody, and a nanobody or an antibody mimetic selected from the group consisting of an affibody, an alphabody, an armadillo repeat protein based scaffold, a knottin, a kunitz domain peptide, an affilin, an affitin, an adnectin, an atrimer, an evasin, a DARPin, an anticalin, an avimer, a fynomer, a versabody or a duocalin.

According to certain embodiments, a humanized anti-HLA-A2 antibody of the present disclosure is an scFv. For example, a humanized anti-HLA-A2 antibody of the present disclosure may be an scFv including an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-91.

In some embodiments, provided is a humanized anti-HLA-A2 antibody as set forth above, where the antibody is capable of constituting an antigen binding domain of a chimeric antigen receptor (CAR), where the CAR is capable of being expressed in an immune cell (*e.g.*,

a T regulatory cell (Treg)) such that the CAR specifically binds to HLA-A2. In certain aspects, provided is a humanized anti-HLA-A2 antibody as set forth above, where the antibody is capable of constituting an antigen binding domain of a chimeric antigen receptor (CAR), where the CAR is capable of being expressed in an immune cell (*e.g.*, a T regulatory cell (Treg)) such that the immune cell is activated by HLA-A2.

In certain aspects, provided is a nucleic acid encoding any of the humanized anti-HLA-A2 antibodies set forth above. Also provided is an expression vector and gene therapy vectors that include such a nucleic acid. A host cell including such an expression vector or a gene therapy vector is also provided.

Aspects of the present disclosure further include chimeric antigen receptors (CARs). For example, provided is a CAR including: (i) an extracellular domain including any of the humanized anti-HLA-A2 antibodies set forth above; (ii) a transmembrane domain; and (iii) a cytoplasmic domain including an intracellular signaling domain; where the CAR is capable of being expressed in an immune cell such that the CAR specifically binds to HLA-A2. Such a CAR may have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, and any combination thereof, as compared to a CAR including a BB7.2 antibody. For example, in some embodiments, such a CAR has less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, and any combination thereof, as compared to a CAR including a BB7.2 antibody. A CAR of the present disclosure may be capable of being expressed in an immune cell (*e.g.*, a T regulatory cell (Treg)) such that the immune cell is activated by HLA-A2. A CAR of the present disclosure may include a hinge region. In certain aspects, the hinge region includes a stalk region of CD8α.

A CAR of the present disclosure may include a transmembrane domain that includes a transmembrane domain of a protein selected from the group consisting of: CD3 gamma, CD3 delta, CD3 epsilon, CD3 zeta, the alpha chain of the T-cell receptor, the beta chain of the T-cell receptor, the gamma chain of the T-cell receptor, the delta chain of the T-cell receptor, CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, and any combination thereof. In some embodiments, the transmembrane domain includes a transmembrane domain of CD28.

According to certain embodiments, a CAR of the present disclosure includes an intracellular signaling domain that includes a functional signaling domain of a protein selected from the group consisting of: CD3 gamma, CD3 delta, CD3 epsilon, CD3 zeta, FcR gamma, FcR alpha, FcR epsilon, CD5, CD22, CD79a, CD79b, and CD66d, and any combination thereof. In some embodiments, the intracellular signaling domain includes a functional signaling domain of CD3 zeta. In certain aspects, the intracellular signaling domain further includes a costimulatory domain. Such a costimulatory domain may include a functional signaling domain of a protein selected from the group consisting of OX40, CD27, CD28, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18), TNFR1 (CD120a/TNFRSF1A), TNFR2 (CD120b/TNFRSF1B), CTLA-4 (CD152), CD95, ICOS (CD278), 4-1BB (CD137), CD2, CD30, CD40, PD-1, CD7, LIGHT, NKG2C, B7-H3, ICAM-1, a ligand that specifically binds with CD83, IL2ra (CD25), IL6Ra (CD126), IL-7Ra (CD127), IL-13RA1, IL-13RA2, IL-33R(IL1RL1), IL-10RA, IL-10RB, IL-4R, IL-5R (CSF2RB), ARHR, BAFF receptor, IL-21R, TGFbR1, TGFbR2, TGFbR3, common gamma chain, and any combination thereof. According to certain embodiments, the costimulatory domain includes a functional signaling domain of a protein selected from CD28 and 4-1BB. For example, the costimulatory domain may include a functional signaling domain of CD28.

Also provided are modified immune cells including any of the CARs of the present disclosure. In some embodiments, the modified immune cell is a T regulatory cell (Treg).

The present disclosure provides nucleic acids encoding any of the CARs of the present disclosure. Expression vectors including such nucleic acids are also provided, as are immune cells (e.g., T regulatory cells (Tregs)) including such expression vectors.

Compositions (e.g., pharmaceutical compositions) are also provided. In certain aspects, provided is a pharmaceutical composition including a plurality of modified immune cells or immune cells of the present disclosure. Kits of parts are also provided. In some embodiments, said kits of parts comprise in a first part immune cells of the present disclosure, and in a second part another therapeutic agent, such as, for example, an immunosuppressive agent. In some embodiments, said kits of parts comprise one or more reagents (e.g., a nucleic acid or expression vector encoding an anti-HLA-A antibody or CAR of the present disclosure) for making the cells of the present disclosure. Methods of making modified immune cells of the

present disclosure are also provided. In some embodiments, such methods include transducing an immune cell with an expression vector of the present disclosure, thereby generating the modified immune cell.

Methods of using the antibodies, CARs, immune cells, modified immune cells, and pharmaceutical compositions of the present disclosure are also provided. In certain aspects, provided are methods of promoting immune tolerance in a subject, the methods including administering to the subject a pharmaceutical composition of the present disclosure, *e.g.*, a pharmaceutical composition including a plurality of the modified immune cells or immune cells of the present disclosure. In some embodiments, the immune tolerance is tolerance to a transplanted organ or tissue. According to certain embodiments, provided are methods of preventing or treating graft versus host disease (GVHD) in a subject, the methods including administering to the subject a pharmaceutical composition of the present disclosure, *e.g.*, a pharmaceutical composition including a plurality of the modified immune cells or immune cells of the present disclosure. In certain aspects, the subject is undergoing or has undergone a hematopoietic stem cell transplant. Also provided are methods of preventing or treating organ or tissue transplant rejection in a subject, the methods including administering to the subject a pharmaceutical composition of the present disclosure, *e.g.*, a pharmaceutical composition including a plurality of the modified immune cells or immune cells of the present disclosure. In some embodiments, the subject is further receiving an immunosuppressive agent. According to certain embodiments, provided are methods of preventing or treating organ or tissue transplant rejection or graft versus host disease (GVHD) in a subject, the methods including administering to the subject a combination of an immune cell of the present disclosure with at least one immunosuppressive agent for inducing immune tolerance. In any of the methods of using the antibodies, CARs, immune cells, modified immune cells, and pharmaceutical compositions of the present disclosure, the subject may be human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Construction of humanized anti-HLA-A2 CARs. Schematic representation of the lentiviral constructs. Top: truncated NGFR control construct (no CAR); Bottom: humanized anti-HLA-A2 CAR construct. *"SP": signal peptide; "GS": Glycine-Serine linker; "TM": transmembrane region; "hs": humanized.*

Figure 2. Cell surface expression and specificity of humanized anti-HLA-A2 CARs. 293T cells were transiently transfected with the indicated construct and after 48 hours expression and antigen specificity was measured by flow cytometric staining with anti-ΔNGFR mAbs and HLA-A2 tetramers. **A & B** show dot plots for constructs which do, or do not, retain their ability to bind to HLA-A2, respectively. Data are representative of two independent experiments.

Figure 3. Comparison of humanized anti-HLA-A2 CAR strength of binding. 293T cells were transfected with the indicated humanized anti-HLA-A2 CAR constructs and stained with the indicated dilutions of HLA-A2 tetramer. A, B & C show graphs depicting the geometric mean fluorescence intensity of HLA-A2 tetramer binding within gated ΔNGFR+ cells, with constructs grouped according to light chain usage.

Figure 4. Expression and specificity of humanized anti-HLA-A2 CARs on Tregs. $CD4^+CD25^{hi}CD127^{lo}$ Tregs were activated, one day later transduced with the indicated lenti virus, then allowed to expand. Seven days after activation, ΔNGFR-expressing cells were selected by magnetic-bead based separation. Transduction efficiency and HLA-A2 binding was determined by flow cytometry before and after separation of ΔNGFR+ cells (**A, B, C & D**). Numbers represent the proportion of $\Delta NGFR^+tetramer^+$ cells. Data are representative of independent experiments. (**E**) summarized data of percent or mean fluorescence intensity of A*02:01-tetramer binding.

Figure 5. HLA-A2 CAR-mediated activation of Tregs. $CD4^+CD25^{hi}CD127^{lo}$ Tregs were activated, transduced with the indicated lentivirus and allowed to expand. After 7 days, the Tregs were rested with 100U/mL IL-2 overnight then left unstimulated or stimulated by co-culture with a 2:1 (Tregs: K562 cells) ratio of anti-CD3/28-loaded CD64-K562 cells (TCR), or HLA-A2-K562 cells (CAR). After 24 hours, expression of CD69, CD154, CTLA-4 and LAP was measured by flow cytometry on live CD4+ cells. **(A & B)** show representative histograms and (**C & D**) show averaged data from two independent experiments (**E**) ΔNGFR control/CAR Tregs were co-cultured with a 2:1 (Tregs: K562) ratio of HLA-A2-expressing K562 cells. After 16 hours, expression of CD69, CD71, CTLA-4 and LAP were measured by flow cytometry. Percent positive and fold increase over baseline (no K562) expression of CD69 and CD71. **(F)** Percent positive and fold increase over baseline (no K562) expression of CTLA-4 and LAP. Data are n=2-4 for each construct from at least two independent experiments. One-way

ANOVA and Holm-Sidak's post-test comparing all constructs to mA2-CAR Tregs. Mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure 6. Determination of cross-reactivity of humanized anti-HLA-A2 CARs with common HLA-A and HLA-B allelic variants. (**A**) show the schematic diagram of the experimental set up and gating strategy for the FlowPRT cell assay. ΔNGFR$^+$ Tregs expressing the indicated humanized CARs were incubated with Flow Panel Reactive Single Antigen beads and a fixable viability dye for 30 minutes at room temperature. Samples were then washed, fixed and analyzed via flow cytometry. (**B**) Binding to HLA-A*02:01-coated beads for each m/hA2-CAR Treg relative to binding of a ΔNGFR Treg control. (**C & D**) Correlation between the mean of HLA-A*02:01 binding measured by the FlowPRT cell assay and either (**C**) HLA-A*02:01-tetramer MFI evaluated by flow cytometry or **(D)** the increase in proportion of CD69+ cells 16h after co-culture with HLA-A*02:01 versus negative control HLA-A*24:01 K562 cells. Data in **E, F, G & H** show the percent binding relative to control Tregs expressing only truncated NGFR, normalized for the number of HLA negative beads collected by the cytometer. Data points above the shaded grey area (**E**, **F** & **G**) or the horizontal dotted line (**H**) represent values that were more than two standard deviations from the mean of the bead-only control and thus statistically significant ($p < 0.05$). Data are the average of three independent experiments. (**I**) ΔNGFR or m/hA2-CAR Tregs were co-cultured with the indicated K562 cells transduced to express selected HLA-A alleles. After 16 hours, expression of CD69, CD71, LAP and CTLA-4 was measured on live CD4$^+$ T cells. n=2-6 from at least 2 independent experiments. Statistical significance determined by one-way ANOVA and Holm-Sidak post-test comparing to mA2-CAR. mean ± SEM, ** $p < 0.01$.

Figure 7. Tregs expressing a humanized HLA-A2 CAR potently suppress T cell proliferation stimulated by HLA-A2$^+$ dendritic cells. **(A)** Schematic diagram of experiment setup. Matured HLA-A2$^+$ dendritic cells were used to stimulate with Cell Proliferation Dye (CPD)-e450-labelled HLA-A2^{neg} CD4+ "responder" T cells. CPD-e660-labelled Tregs which were either untransduced, or transduced with a control lenti virus encoding ΔNGFR, or Humanized A2 CAR-expressing Tregs. (**B, C, D & E**) The indicated ratios of cells were co-cultured for six days, then the amount of proliferation of the responder CPD-e450-labelled responder CD4$^+$ T cells was measured by flow cytometry. **B** shows representative dot plots and

C, D and E show graphed data for multiple cell ratios. **C, D and E** show average data for n=3-7 from at least 3 independent experiments. Statistics were performed using a two-way ANOVA with Holm-Sidak post-test versus a ΔNGFR Treg control. * $p < 0.05$, mean ± SEM.

Figure 8. Tregs expressing a humanized HLA-A2 CAR potently suppress xenogeneic graft-versus-host disease. Irradiated NSG mice were injected with PBS (n=3), 8 x10^6 HLA-$A2^+$ PBMCs alone (n=5) or with 4 x10^6 H1k2 CAR-expressing Tregs (n=6). Human cell engraftment in the blood was monitored every 7 days. (**A**) Survival curve and (**B**) percent of weight change relative to the start of experiment. (**C**) Proportion of total mononuclear cells (live singlets) expressing human CD45 in blood. (**D**) Gating strategy to discriminate overall human $CD45^+$ and CAR Treg ($hCD45^+hCD4^+HLA\text{-}A2^-$) cell engraftment. (**E**) In-vivo cell engraftment after adoptive transfer in a xenogeneic GVHD mouse model as shown by the absolute number of PBMC and CAR Treg engraftment per µL of blood over time. The number of PBMC were calculated as hCD45+ minus total CAR Treg count as gated in **D**.

Figure 9. Expression of m/hA2 CARs endows Tregs with rapid and persistent homing to HLA-A2:01$^+$ skin allografts. Tregs were co-transduced with lentivirus encoding luciferase and either a control HER2-CAR, mA2-CAR or hA2-CAR (H1k2). Dual transduced cells were FACS-sorted, expanded for 5 days, then injected to NSG mice which had previously been transplanted with juxtaposed skin transplants from both NSG and NSG-HLA-A*02:01 transgenic mice. **(A)** Schematic representation of the experimental setup. **(B)** Representative luciferase imaging of skin grafts (left) 72 hours or (right) 21 days after Treg injection. Amount of luciferase radiance was quantified using the average amount of photons/sec/cm^2/steradian and plotted as a ratio between **(C)** the HLA-A*02:01-NSG and NSG skin grafts 72 hours after Treg injection or **(D)** over time. n=2-3 per group from three independents experiments, mean ± SEM. Repeated measures ANOVA with Bonferroni correction.

Figure 10. Flow cytometric tracking of m/hA2 CAR Tregs with rapid and persistent homing to HLA-A2:01$^+$ skin allografts. Tregs were co-transduced with lentivirus containing luciferase and either HER2-CAR, mA2-CAR or hA2-CAR constructs, expanded and injected into transplanted NSG mice as shown in Figure 9. **(A)** Pre-gating for flow cytometry plots were based on cells from the spleen of control HER2-CAR. **(B)** hCD4/hCD8 flow cytometry profile for the indicated constructs. Plots were pre-gated on FvD^-hCD45^+ as in (A). (**C**) Flow

cytometry plots showing staining for m/hA2-CAR Tregs in the spleen and draining lymph node upon experiment endpoint n=1 per group from one independent experiment. * $p < 0.05$.

Figure 11. hA2-CAR-Tregs diminish human skin allograft rejection. NSG mice were transplanted with HLA-A*02:01^{+} human skin and injected three weeks later with either: PBS (n=3); HLA-A*02:01^{neg} PBMCs alone (n=4) or with a 2:1 ratio of autologous H1k2 CAR Tregs (n=6). PBMC/hA2-CAR Tregs were from two individual donors, tested in one experiment. **(A)** Body weight was monitored thrice weekly and **(B)** the proportion of human CD45^{+} cells in the blood (left) and spleen (right) was measured upon the experimental endpoint. **(C)** Cumulative histological score of transplanted skin sections as determined by H&E stain. (**D**) Transplanted skin grafts were immunostained at experiment endpoint to quantify the amount of involucrin expression and proportion of Ki-67^{+} cells in the epidermis. (**E**) mRNA expression of the indicated genes within transplanted skin sections was determined by qRT-PCR. **(F)** Transplanted skin grafts were immunostained at experiment endpoint to quantify the proportion of FOXP3+ cells within human CD45+ cells. **(G)** Transplanted skin grafts, intestine, lung and liver sections were immunostained at the experiment endpoint to show the proportion of FOXP3+ cells within human CD45+ cells in each tissue. Each data point represents one mouse. Box-whisker plots show mean ± range. Statistical significance determined by two-tailed Mann-Whitney test comparing PBMC to H1k2. * $p < 0.05$.

Figure 12: Flow cytometric tracking of hA2 CAR Tregs in blood in the human skin transplant model. NSG mice were transplanted with human HLA-A*02^{+} skin and injected with cells as described in Figure 11. **(A)** Gating strategy to discriminate overall human CD45^{+} (PBMC) and CAR Treg (hCD45+hCD4+NGFR+) cell engraftment. **(B)** Absolute number of PBMCs and CAR Treg engraftment per µL of blood over time. Number of PBMCs were calculated as hCD45+ minus total CAR Treg count, as gated in (A).

Figure 13: Activation of hA2 CAR Tregs using artificial antigen presenting cells. ΔNGFR control/CAR Tregs were co-cultured for 16 hours with either no stimulation or a 2:1 (Tregs: K562) ratio of CD64-expressing K562 cells loaded with anti-CD3 and anti-CD28 monoclonal antibodies (TCR stimulation). **(A)** Example gating strategy. **(B)** Expression of CD69, CD71, CTLA-4 and LAP were measured by flow cytometry (left). Fold increase of each activation marker over baseline (no stimulation) was calculated (right). Data are n=2-4 for each construct

from at least two independent experiments. Mean ± SEM. One-way ANOVA and Holm-Sidak's multiple comparisons test comparing all constructs to mA2-CAR Tregs. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

DETAILED DESCRIPTION

I. GENERAL TECHNIQUES

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (M. J. Gait, ed., 1984); "Animal Cell Culture" (R. I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Current Protocols in Molecular Biology" (F. M. Ausubel et al., eds., 1987, and periodic updates); "PCR: The Polymerase Chain Reaction", (Mullis et al., ed., 1994); "A Practical Guide to Molecular Cloning" (Perbal Bernard V., 1988); "Phage Display: A Laboratory Manual" (Barbas et al., 2001).

It is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. In

the event that any definition set forth conflicts with any document incorporated herein by reference, the definition set forth below shall control.

II. DEFINITIONS

The terms "a" and "an" refer to one or to more than one of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or in some instances ±10%, or in some instances ±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

The terms "HLA-A2" and "A2" as used herein each refer to human leukocyte antigen (HLA) proteins including cell surface proteins, encoded by the HLA-A*02 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A2" and "A2" include HLA proteins identified as belonging to the HLA-A*02 antigen type by serological testing or genotyping. Additional names for the HLA-A*02 antigen type include "HLA-A2", HLA-A02" and "HLA-A*2". Different naming systems have been developed which identify HLA proteins encoded by this family of alleles including the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System. The terms "HLA-A2" and "A2" refer to HLA proteins encoded by alleles having designations according to this naming system which begin with "HLA-A*02:", including but not limited to designations which begin with "HLA-A*02:01", "HLA-A*02:02", "HLA-A*02:03", "HLA-A*02:04", "HLA-A*02:05", "HLA-A*02:06", "HLA-A*02:07", "HLA-A*02:08", "HLA-A*02:09", "HLA-A*02:10", and "HLA-A*02:11". In addition to the numerical digits which

follow "HLA-A*02:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*02:01P or HLA-A*02:01:01G). The allele designations which begin with "HLA-A*02:" followed by 2, 3, or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A2" and "A2" also refer to HLA proteins identified with designations which begin with "HLA-A*02:" according to this naming system, including but not limited to the designations "HLA-A*02:01", "HLA-A*02:02", "HLA-A*02:03", "HLA-A*02:04", "HLA-A*02:05", "HLA-A*02:06", "HLA-A*02:07", "HLA-A*02:08", "HLA-A*02:09", "HLA-A*02:10", and "HLA-A*02:11".

An "HLA-A subtype" as used herein refers to a protein encoded by an allele of the HLA-A gene.

The term "HLA-A*03" as used herein refers to HLA proteins including cell surface proteins, encoded by the HLA-A*03 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the term "HLA-A*03" include HLA proteins identified as belonging to the HLA-A*03 antigen type by serological testing or genotyping. Additional names for the HLA-A*03 antigen type include "HLA-A03" and "HLA-A3". The term "HLA-A*03" refers to HLA proteins encoded by alleles having designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*03:", including but not limited to designations which begin with "HLA-A*03:01", "HLA-A*03:02", "HLA-A*03:04", "HLA-A*03:05", "HLA-A*03:06", "HLA-A*03:07", "HLA-A*03:08", "HLA-A*03:09", "HLA-A*03:10", and "HLA-A*03:12". In addition to the numerical digits which follow "HLA-A*03:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*03:01P or HLA-A*03:01:01G). The allele designations which begin with "HLA-A*03:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The term "HLA-A*03" also refers to HLA proteins identified with designations which begin with "HLA-A*03:" according to this naming system, including but not limited to the designations "HLA-A*03:01", "HLA-A*03:02", "HLA-A*03:04", "HLA-A*03:05", "HLA-

A*03:06", "HLA-A*03:07", "HLA-A*03:08", "HLA-A*03:09", "HLA-A*03:10", and "HLA-A*03:12".

The terms "HLA-A*25", "HLA-A25" and "A25" as used herein each refer to HLA proteins including cell surface proteins, encoded by the HLA-A*25 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A*25", "HLA-A25" and "A25" include HLA proteins identified as belonging to the HLA-A*25 antigen type by serological testing or genotyping. Additional names for the HLA-A*25 antigen type include "HLA-A25". The terms "HLA-A*25", "HLA-A25" and "A25" refer to HLA proteins encoded by alleles having designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*25:", including but not limited to designations which begin with "HLA-A*25:01", "HLA-A*25:02", "HLA-A*25:03", "HLA-A*25:04", "HLA-A*25:05", "HLA-A*25:06", "HLA-A*25:07", "HLA-A*25:08", "HLA-A*25:09", "HLA-A*25:10", and "HLA-A*25:11". In addition to the numerical digits which follow "HLA-A*25:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*25:01P or HLA-A*25:01:01G). The allele designations which begin with "HLA-A*25:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A*25", "HLA-A25" and "A25" also refer to HLA proteins identified with designations which begin with "HLA-A*25:" according to this naming system, including but not limited to the designations "HLA-A*25:01", "HLA-A*25:02", "HLA-A*25:03", "HLA-A*25:04", "HLA-A*25:05", "HLA-A*25:06", "HLA-A*25:07", "HLA-A*25:08", "HLA-A*25:09", "HLA-A*25:10", and "HLA-A*25:11".

The terms "HLA-A*29", "HLA-A29" and "A29" as used herein each refer to HLA proteins including cell surface proteins, encoded by the HLA-A*29 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A*29", "HLA-A29" and "A29" include HLA proteins identified as belonging to the HLA-A*29 antigen type by serological testing or genotyping. Additional names for the HLA-A*29 antigen type include "HLA-A29". The terms "HLA-A*29", "HLA-A29" and "A29" refer to HLA proteins encoded by alleles having designations according to the HLA naming system

developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*29:", including but not limited to designations which begin with "HLA-A*29:01", "HLA-A*29:02", "HLA-A*29:03", "HLA-A*29:04", "HLA-A*29:05", "HLA-A*29:06", "HLA-A*29:07", HLA-A*29:09", "HLA-A*29:10", and "HLA-A*29:11". In addition to the numerical digits which follow "HLA-A*29:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*29:02P or HLA-A*29:02:01G). The allele designations which begin with "HLA-A*29:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A*29", "HLA-A29" and "A29" also refer to HLA proteins identified with designations which begin with "HLA-A*29:" according to this naming system, including but not limited to the designations "HLA-A*29:01", "HLA-A*29:02", "HLA-A*29:03", "HLA-A*29:04", "HLA-A*29:05", "HLA-A*29:06", "HLA-A*29:07", "HLA-A*29:09", "HLA-A*29:10", and "HLA-A*29:11".

The terms "HLA-A*30", "HLA-A30" and "A30" as used herein each refer to HLA proteins including cell surface proteins, encoded by the HLA-A*30 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A*30", "HLA-A30" and "A30" include HLA proteins identified as belonging to the HLA-A*30 antigen type by serological testing or genotyping. Additional names for the HLA-A*30 antigen type include "HLA-A30". The terms "HLA-A*30", "HLA-A30" and "A30" refer to HLA proteins encoded by alleles having designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*30:", including but not limited to designations which begin with "HLA-A*30:01", "HLA-A*30:02", "HLA-A*30:03", "HLA-A*30:04", "HLA-A*30:06", "HLA-A*30:07", "HLA-A*30:08", "HLA-A*30:09", "HLA-A*30:10", and "HLA-A*30:11". In addition to the numerical digits which follow "HLA-A*30:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*30:01P, HLA-A*30:02P, HLA-A*30:04P, HLA-A*30:01:01G, HLA-A*30:02:01G or HLA-A*30:04:01G). The allele designations which begin with "HLA-A*30:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A*30", "HLA-A30" and "A30" also refer to HLA proteins identified with designations which begin with

"HLA-A*30:" according to this naming system, including but not limited to the designations "HLA-A*30:01", "HLA-A*30:02", "HLA-A*30:03", "HLA-A*30:04", "HLA-A*30:06", "HLA-A*30:07", "HLA-A*30:08", "HLA-A*30:09", "HLA-A*30:10", and "HLA-A*30:11".

The terms "HLA-A*31", "HLA-A31" and "A31" as used herein each refer to HLA proteins including cell surface proteins, encoded by the HLA-A*31 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A*31", "HLA-A31" and "A31" include HLA proteins identified as belonging to the HLA-A*31 antigen type by serological testing or genotyping. Additional names for the HLA-A*31 antigen type include "HLA-A31". The terms "HLA-A*31", "HLA-A31" and "A31" refer to HLA proteins encoded by alleles having designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*31:", including but not limited to designations which begin with "HLA-A*31:01", "HLA-A*31:02", "HLA-A*31:03", "HLA-A*31:04", "HLA-A*31:05", "HLA-A*31:06", "HLA-A*31:07", "HLA-A*31:08", "HLA-A*31:09", "HLA-A*31:10", and "HLA-A*31:11". In addition to the numerical digits which follow "HLA-A*31:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*31:01P or HLA-A*31:01:02G). The allele designations which begin with "HLA-A*31:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A*31", "HLA-A31" and "A31" also refer to HLA proteins identified with designations which begin with "HLA-A*31:" according to this naming system, including but not limited to the designations "HLA-A*31:01", "HLA-A*31:02", "HLA-A*31:03", "HLA-A*31:04", "HLA-A*31:05", "HLA-A*31:06", "HLA-A*31:07", "HLA-A*31:08", "HLA-A*31:09", "HLA-A*31:10", and "HLA-A*31:11".

The terms "HLA-A*33", "HLA-A33" and "A33" as used herein each refer to HLA proteins including cell surface proteins, encoded by the HLA-A*33 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A*33", "HLA-A33" and "A33" include HLA proteins identified as belonging to the HLA-A*33 antigen type by serological testing or genotyping. Additional names for the HLA-A*33 antigen type include "HLA-A33". The terms "HLA-A*33", "HLA-A33" and "A33" refer to HLA

proteins encoded by alleles having designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*33:", including but not limited to designations which begin with "HLA-A*33:01", "HLA-A*33:03", "HLA-A*33:04", "HLA-A*33:05", "HLA-A*33:06", "HLA-A*33:07", "HLA-A*33:08", "HLA-A*33:09", "HLA-A*33:10", and "HLA-A*33:11". In addition to the numerical digits which follow "HLA-A*33:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*33:01P or HLA-A*33:01:01G). The allele designations which begin with "HLA-A*33:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A*33", "HLA-A33" and "A33" also refer to HLA proteins identified with designations which begin with "HLA-A*33:" according to this naming system, including but not limited to the designations "HLA-A*33:01", "HLA-A*33:03", "HLA-A*33:04", "HLA-A*33:05", "HLA-A*33:06", "HLA-A*33:07", "HLA-A*33:08", "HLA-A*33:09", "HLA-A*33:10", and "HLA-A*33:11".

The terms "HLA-A*36", "HLA-A36" and "A36" as used herein each refer to HLA proteins including cell surface proteins, encoded by the HLA-A*36 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A*36", "HLA-A36" and "A36" include HLA proteins identified as belonging to the HLA-A*36 antigen type by serological testing or genotyping. Additional names for the HLA-A*36 antigen type include "HLA-A36". The terms "HLA-A*36", "HLA-A36" and "A36" refer to HLA proteins encoded by alleles having designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*36:", including but not limited to designations which begin with "HLA-A*36:01", "HLA-A*36:02", "HLA-A*36:03", "HLA-A*36:04", and "HLA-A*36:05". In addition to the numerical digits which follow "HLA-A*36:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G". The allele designations which begin with "HLA-A*36:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A*36", "HLA-A36" and "A36" also refer to HLA proteins identified with designations which begin with "HLA-A*36:" according to this

naming system, including but not limited to the designations "HLA-A*36:01", "HLA-A*36:02", "HLA-A*36:03", "HLA-A*36:04", "HLA-A*36:05", and "HLA-A*36:06".

The terms "HLA-A*68", "HLA-A68" and "A68" as used herein each refer to HLA proteins including cell surface proteins, encoded by the HLA-A*68 allele family at the HLA-A locus of the HLA gene complex. HLA proteins encompassed by the terms "HLA-A*68", "HLA-A68" and "A68" include HLA proteins identified as belonging to the HLA-A*68 antigen type by serological testing or genotyping. Additional names for the HLA-A*68 antigen type include "HLA-A68". The terms "HLA-A*68", "HLA-A68" and "A68" refer to HLA proteins encoded by alleles having designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System which begin with "HLA-A*68:", including but not limited to designations which begin with "HLA-A*68:01", "HLA-A*68:02", "HLA-A*68:03", "HLA-A*68:04", "HLA-A*68:05", "HLA-A*68:06", "HLA-A*68:07", "HLA-A*68:08", "HLA-A*68:09", and "HLA-A*68:10". In addition to the numerical digits which follow "HLA-A*68:", the allele designations may also contain an upper case letter, including but not limited to upper case letters "P" and "G" (e.g., HLA-A*68:01P, HLA-A*68:01:01G or HLA-A*68:01:02G). The allele designations which begin with "HLA-A*68:" followed by 2, 3 or 4 additional numerical digits may constitute the complete designation or a beginning portion of the designation. The allele designations may be italicized. The terms "HLA-A*68", "HLA-A68" and "A68" also refer to HLA proteins identified with designations which begin with "HLA-A*68:" according to this naming system, including but not limited to the designations "HLA-A*68:01", "HLA-A*68:02", "HLA-A*68:03", "HLA-A*68:04", "HLA-A*68:05", "HLA-A*68:06", "HLA-A*68:07", "HLA-A*68:08", "HLA-A*68:09", and "HLA-A*68:10".

Specific HLA proteins may be referred to herein using protein designations according to the HLA naming system developed in 2010 by the WHO Committee for Factors of the HLA System. For example, the term "HLA-A*02:01" as used herein refers to an HLA protein with the designation "HLA-A*02:01" according to this naming system. Similarly, the terms "HLA-A*03:01", "HLA-A*25:01", "HLA-A*29:02", "HLA-A*30:01", "HLA-A*31:01", "HLA-A*33:01", "HLA-A*36:01" and "HLA-A*68:01" refer to HLA proteins with designations

"HLA-A*03:01", "HLA-A*25:01", "HLA-A*29:02", "HLA-A*30:01", "HLA-A*31:01", "HLA-A*33:01", "HLA-A*36:01" and "HLA-A*68:01", respectively.

The term "anti-HLA-A2 antibody" as used herein refers to an antibody that preferentially or specifically binds to HLA-A2.

The term "BB7.2" as used herein, refers to a murine hybridoma identified as ATCC Deposit HB-82. The BB7.2 hybridoma cells secrete a murine monoclonal antibody of IgG2b kappa isotype, which has been characterized by Parham, P. et al. and Hilton et al. (Parham, P. et al, 1981; Hilton et al., 2013). The amino acid sequences of the six complementarity determining regions (CDRs) of the monoclonal antibody secreted by BB7.2 are as follows:

Heavy chain CDR1 (HCDR1): SYHIQ (SEQ ID NO: 183);

Heavy chain CDR2 (HCDR2): WIYPGDGSTQYNEKFKG (SEQ ID NO: 185);

Heavy chain CDR3 (HCDR3): EGTYYAMDY (SEQ ID NO: 187);

Light chain CDR1 (LCDR1): RSSQSIVHSNGNTYLE (SEQ ID NO: 188);

Light chain CDR2 (LCDR2): KVSNRFS (SEQ ID NO: 189);

Light chain CDR3 (LCDR3): FQGSHVPRT (SEQ ID NO: 190).

As used herein, a "BB7.2 antibody" is an antibody having the VH (SEQ ID NO: 191) and VL (SEQ ID NO: 192) of the monoclonal antibody secreted by BB7.2. A BB7.2 antibody may be a whole antibody or a fragment thereof having the VH and VL of the monoclonal antibody secreted by BB7.2, such as an scFv having the VH and VL of the monoclonal antibody secreted by BB7.2.

The terms "antibodies" and "immunoglobulin" include antibodies or immunoglobulins of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to, Fab, Fab', $F(ab')_2$, Fv, scFv, Fd, diabodies, single domain antibodies (sdAbs), linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments, chimeric antibodies, humanized antibodies, single-chain antibodies, and fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein.

Antibodies can be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules. The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to a H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the α and γ chains and four CH domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (VL) followed by a constant domain (CL) at its other end. The VL is aligned with the VH and the CL is aligned with the first constant domain of the heavy chain (CH1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. The five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM have heavy chains designated α, δ, ε, γ, and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in CH sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "VH", "V_H" or “H”. The variable domain of the light chain may be referred to as "VL", "V_L" or “L”. These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110- to 130-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)).

An “intact” antibody is one which comprises an antigen-binding site as well as a CL and at least heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variants thereof. In an embodiment, the intact antibody may have one or more effector functions.

The term “antibody fragment” refers to at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen binding domain, e.g., an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antibody fragments include Fab, Fab', F(ab')2, Fv fragments, scFv fragments; diabodies; single domain antibodies (sdAbs); linear antibodies (see U.S. Patent No. 5,641 ,870, Example 2; Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. Also included among anti-HLA-A2 antibody fragments are portions of anti-HLA-A2 antibodies (and combinations of portions of anti-HLA-A2 antibodies, for example, scFv) that may be used as targeting arms, directed to an HLA-A2 antigen, in chimeric antigenic receptors

of CAR-modified immune cells. Such fragments are not necessarily proteolytic fragments but rather portions of polypeptide sequences that can confer affinity for a target. Further included among anti-HLA-A2 antibody fragments are single domain antibodies (sdAbs) (see, for example, Li et al. (2017); Jamnani et al. (2014)). Such single domain antibodies may be used as targeting arms in the CAR-modified immune cells of the present invention.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (CH1). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')2 fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having additional few residues at the carboxy terminus of the CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part recognized by Fc receptors (FcR) found on certain types of cells.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This fragment consists of a dimer of one heavy-chain variable region domain and one light-chain variable region domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy-chain variable domain and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding

specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. The scFv polypeptide may further comprise a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. Unless specified, as used herein an 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. For a review of scFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Antibody Engineering, ed. Borrebaeck, Oxford University Press, New York (1995). In one embodiment, an anti-HLA-A2 antibody derived scFv may be used as the targeting arm of a CAR-modified immune cell disclosed herein.

The term “adnectin”, also known as monobody, is well known in the art and refers to proteins designed to bind with high affinity and specificity to antigens. They belong to the class of molecules collectively called “antibody mimetics”.

The term “alphabody”, refers to as Cell-Penetrating Alphabodies, refers to a type of antibody mimetics consisting of small 10 kDa proteins engineered to bind to a variety of antigens. Alphabodies are able to reach and bind to intracellular protein targets.

The term “affibody” is well known in the art and refers to affinity proteins based on a 58 amino acid residue protein domain, derived from one of the IgG binding domain of staphylococcal protein A.

The term “anticalin” is well known in the art and refers to an antibody mimetic technology, wherein the binding specificity is derived from lipocalin. Anticalin may also be formatted as dual targeting protein, called Duocalin.

The term “armadillo repeat protein-based scaffold” refers to a type of antibody mimetics corresponding to artificial peptide binding scaffolds based on armadillo repeat proteins. Armadillo repeat proteins are characterized by an armadillo domain, composed of

tandem armadillo repeats of approximately 42 amino acids, which mediates interactions with peptides or proteins.

The term "avimers" is well known in the art and refers to an antibody mimetic technology. The term "DARPins" (Designed Ankyrin Repeat Proteins) is well known in the art and refers to an antibody mimetic DRP (designed repeat protein) technology developed to exploit the binding abilities of non-antibody polypeptides.

The term "diabodies" refers to small antibody fragments prepared by constructing scFv fragments with short linkers (about 5-10 residues) between the VH and VL domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, *i.e.*, fragment having two antigen binding sites. Bispecific diabodies are heterodimers of two "crossover" scFv fragments in which the VH and VL domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 0404097; WO 93/11161; and Holliger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

The term "evasin" is well known in the art and refers to a class of chemokine-binding proteins.

The term "**f**ynomer" is well known in the art and refers to proteins that belong to the class of antibody mimetic. They are attractive binding molecules due to their high thermal stability and reduced immunogenicity.

The term "knottin" (that may also be referred to as inhibitor cystine not) refers to an antibody mimetic comprising a protein structural motif containing three disulfide bridges.

The term "kunitz domain peptide" refers to a type of antibody mimetics, and is based on the active domains of proteins inhibiting the function of proteases.

The term "nanobody" is well known in the art and refers to an antibody-derived therapeutic protein that contains the unique structural and functional properties of naturally-occurring heavy chain antibodies. These heavy chain antibodies contain a single variable domain (VHH) and two constant domains (CH2 and CH3).

The term "unibody" is well known in the art and refers to an antibody fragment lacking the hinge region of IgG4 antibodies. The deletion of the hinge region results in a molecule that

is essentially half the size of traditional IgG4 antibodies and has a univalent binding region rather than the bivalent biding region of IgG4 antibodies.

The term “versabody” is well known in the art and refers to another antibody mimetic technology. They are small proteins of 3-5 kDa with >15% cysteines, which form a high disulfide density scaffold, replacing the hydrophobic core the typical proteins have.

The term "flexible polypeptide linker" or "linker" as used in the context of an scFv refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together. In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence $(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3, n=4, n=5, n=6, n=7, n=8, n=9 and n=10. In another embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3, n=4, n=5, n=6, n=7, n=8, n=9 and n=10. In one embodiment, the flexible polypeptide linkers include, but are not limited to, $(Gly_4\ Ser)_4$ or $(Gly_4\ Ser)_3$. In another embodiment, the linkers include multiple repeats of (Gly_2Ser), (GlySer) or (Gly_3Ser). Also included within the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference).

The term "heavy chain," refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

The term "light chain," refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (K) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

The term “hypervariable region”, “HVR”, or “HV”, when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region delineations are in use and are encompassed herein. The Kabat Complementarity Determining

Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., 1991). Chothia refers instead to the location of the structural loops (Chothia et al., 1987). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions are noted below. The residues from each of these hypervariable regions are noted below.

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

The terms "hypervariable region" and "complementarity determining region" and their respective abbreviations (HVR, HV, CDR) are used interchangeably herein. Further, the following pairs of terms are also used interchangeably herein:

"VH CDR1" and "HCDR1";

"VH CDR2" and "HCDR2";

“VH CDR3” and “HCDR3”;

“VL CDR1” and “LCDR1”;

“VL CDR2” and “LCDR2”; and

“VL CDR3” and “LCDR3”.

Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 (L3) in the VL and 26-35B (HI), 50-65, 47-65 or 49-65 (H2) and 93-102, 94-102 or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat et al. (Kabat et al., 1991) for each of these definitions.

"Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues herein defined.

The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat", and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., (Kabat et al., 1991). Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g, Kabat et al., 1991). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., supra). The "EU index as in Kabat" refers to the residue numbering of the human IgGl EU antibody. Unless stated otherwise herein, references to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system.

The term "specifically binds," refers to a ligand (e.g., a humanized anti-HLA-A2 antibody) which recognizes and binds with a cognate binding partner (e.g., HLA-A2) protein present in a sample, but which ligand does not substantially recognize or bind other molecules in the sample. Non-specific binding would refer to binding with an affinity of less than 10^{-7} M, e.g., binding with an affinity of 10^{-6} M, 10^{-5} M, 10^{-4} M, etc.

An antibody that "specifically binds" an antigen or epitope of interest is one that binds the antigen or epitope with sufficient affinity that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule.

The term "recombinant antibody" refers to an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage or yeast expression system. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology first described by Kohler et al., Nature, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described

in Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991), for example.

The term "antigen" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated, synthesized or can be derived from a biological sample, or might be a macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

As used herein, the term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as a dissociation constant (Kd). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1000-fold greater, or more, than the affinity of an antibody for unrelated amino acid sequences. Affinity of an antibody to a target protein can be, for example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM) or more. As used herein, the term "avidity" refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms "immunoreactive", "preferentially binds" and “specifically binds” are used interchangeably herein with respect to antibodies and fragments

thereof. Anti-HLA-A2 antibodies of the invention, including humanized anti-HLA-A2 antibodies, as well as fragments thereof as such term is used herein, specifically bind to HLA-A2.

The term "binding" refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. Non-specific binding would refer to binding with an affinity of less than 10^{-7} M, e.g., binding with an affinity of 10^{-6} M, 10^{-5} M, 10^{-4} M, etc.

The term “reactivity” as used herein refers to the ability of an antibody to react with (that is, bind to) a molecule (e.g., specifically bind to the molecule). A first antibody has “less reactivity” to a molecule (e.g., an HLA molecule) than a second antibody when the first antibody exhibits reduced binding to the molecule as compared to the second antibody. Approaches for readily comparing the reactivities of first and second antibodies to one or more particular HLA molecules are known. One example approach is provided in the Examples section herein, where FlowPRA® Single Antigen beads (One Lambda) were employed to interrogate antibodies for the ability to react with (or bind to) particular HLA molecules. Such flow cytometric approaches are amenable to high-throughput antibody reactivity analyses.

As used herein, the term "hinge region" refers to a flexible polypeptide connector region (also referred to herein as "hinge" or "spacer") providing structural flexibility and spacing to flanking polypeptide regions and can consist of natural or synthetic polypeptides. A hinge region may influence the potency of an immune cell expressing a CAR (see for example Watanabe et al. (2016)). A "hinge region" derived from an immunoglobulin (e.g., IgG1) is generally defined as stretching from Glu_{216} to Pro_{230} of human IgG1 (Burton (1985) Molec. Immunol., 22:161-206). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain disulfide (S--S) bonds in the same positions. The hinge region may be of natural occurrence or non-natural occurrence, including but not limited to an altered hinge region as described in U.S. Pat. No. 5,677,425. The hinge region can include complete hinge region derived from an antibody of a different class or subclass from that of the CH1 domain. The term "hinge region" can also

include regions derived from CD8 and other receptors that provide a similar function in providing flexibility and spacing to flanking regions.

As used herein, the term "immune cells" generally includes white blood cells (leukocytes) which are derived from hematopoietic stem cells (HSC) produced in the bone marrow. "Immune cells" includes, e.g., lymphocytes (T cells, B cells, natural killer (NK) cells) and myeloid-derived cells (neutrophil, eosinophil, basophil, monocyte, macrophage, dendritic cells).

"T cell" includes all types of immune cells expressing CD3 including T-helper cells ($CD4^+$ cells), $CD8^+$ T-cells (e.g., cytotoxic $CD8^+$ T cell, regulatory $CD8^+$ T cell), T-regulatory cells (Treg), gamma-delta T cells, and double negative T cells.

A "cytotoxic cell" includes cytotoxic $CD8^+$ T cells, natural-killer (NK) cells, and neutrophils, which cells are capable of mediating cytotoxicity responses.

As used herein, the term “regulatory immune cell” refers to an immune cell that acts in a "regulatory" way to suppress activation of the immune system and thereby maintains immune system homeostasis and tolerance to self-antigens. “Regulatory immune cells” may also have effects on non-immune cells that result in an improved clinical state such as promoting tissue repair or regeneration. Regulatory immune cells may include regulatory T cells, $CD4^+$ regulatory T cells, $CD8^+$ regulatory T cells, regulatory γδ T cells, regulatory DN T cells, regulatory B cells, regulatory NK cells, regulatory macrophages, and regulatory dendritic cells.

“Regulatory T lymphocyte”, “regulatory T cell,”, "T regulatory cell", “Treg cell” or “Treg,” as used in the present specification and claims are synonymous and are intended to have its standard definition as used in the art. Treg cells are a specialized subpopulation of T cells that act in a "regulatory" way to suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens. Tregs have sometimes been referred to as suppressor T-cells. Treg cells are often, but not always, characterized by expression of the forkhead family transcription factor Foxp3 (forkhead box p3). They may also express CD4 or CD8 surface proteins. They usually also express CD25. As used in the present specification and claims, and unless otherwise specified, Tregs include "natural" Tregs which develop in the thymus, induced/adaptive/peripheral Tregs that arise via a differentiation process

which takes place outside the thymus (e.g. in tissues or secondary lymphoid organs, or in the laboratory setting under defined culture conditions), and Tregs that have been created using recombinant DNA technology, for example by engineered expression of FOXP3. Naturally-occurring Treg cells (CD4+CD25+Foxp3+) arise like all other T cells in the thymus. In contrast, induced/adaptive/peripheral Treg cells (which include CD4+CD25+Foxp3+ Tregs, Tr1 cells, Th3 cells and others) arise outside the thymus. One way to induce Tregs is by exposure of T effector cells to IL-10 or TGF-β. T-cells may also be converted to Treg cells by transfection or transduction of the Foxp3 gene into a mixed population of T-cells. A T-cell that is caused to express Foxp3 adopts the Treg phenotype and such recombinant Tregs are also defined herein as "Tregs".

As used herein, the term “immune effector cell” refers to a cell of the immune system which is in a form that is capable of mounting a specific immune response.

As used herein, the term "immune response" includes T cell mediated and/or B cell mediated immune responses. Exemplary immune responses include T cell responses, e.g., cytokine production and cellular cytotoxicity. In addition, the term immune response includes immune responses that are indirectly effected by T cell activation, e.g., antibody production (humoral responses) and activation of cytokine responsive cells, e.g., macrophages. Immune cells involved in the immune response include lymphocytes, such as B cells and T cells (CD4+, CD8+, Thl and Th2 cells); antigen presenting cells (e.g., professional antigen presenting cells such as dendritic cells, macrophages, B lymphocytes, Langerhans cells, and non-professional antigen presenting cells such as keratinocytes, endothelial cells, astrocytes, fibroblasts, oligodendrocytes); natural killer cells; myeloid cells, such as macrophages, eosinophils, mast cells, basophils, and granulocytes.

The term "rejection" refers to a state in which a transplanted organ or tissue is not accepted by the body of the recipient. Rejection results from the recipient's immune system attacking the transplanted organ or tissue. Rejection can occur days to weeks after transplantation (acute) or months to years after transplantation (chronic).

The term “graft-versus-host disease” or “GVHD” as used herein refers to a medical complication following the receipt of transplanted tissue from a genetically different person. Immune cells in the donated tissue (the graft) recognize the recipient (the host) as foreign. The

transplanted immune cells then attack the host's body cells. GVHD is commonly associated with stem cell transplant; however, the term includes GVHD arising from other forms of tissue graft. GVHD may also occur after a blood transfusion.

As used herein, the term "immunological tolerance" or “immune tolerance” refers to methods performed on a proportion of treated subjects in comparison with untreated subjects where: a) a decreased level of a specific immunological response (thought to be mediated at least in part by antigen-specific effector T lymphocytes, B lymphocytes, antibody, or their equivalents); b) a delay in the onset or progression of a specific immunological response; or c) a reduced risk of the onset or progression of a specific immunological response. "Specific" immunological or immune tolerance occurs when immunological or immune tolerance is preferentially invoked against certain antigens in comparison with others.

As used herein, the term “operational tolerance” refers to a clinical situation where there is a stable graft function lacking histological signs of rejection, including acute or chronic rejection, in the absence of any immunosuppressive drug therapies for at least 1 year, in an immunocompetent host capable of responding to other challenges including infections.

As used herein, the term “immune accommodation” refers to a condition of a transplant recipient in which an organ or tissue transplant functions normally despite the presence of antibodies in the recipient which are specific for the organ or tissue transplant.

As used herein, the term "stem cell" generally includes pluripotent or multipotent stem cells. "Stem cells" includes, e.g., embryonic stem cells (ES); mesenchymal stem cells (MSC); induced-pluripotent stem cells (iPS); and committed progenitor cells (hematopoeitic stem cells (HSC); bone marrow derived cells, etc.).

As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, e.g., in a human, and includes relieving the disease, i.e., causing regression of the disease and/or amelioration of one or more symptoms of the disease.

As used herein, the terms “prevention, "prevent," “preventing,” and the like, mean to provide prophylactic or protective treatment for a disease or disease state. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof. "Prevention," as used herein, covers any prophylactic effect on a disease in a mammal, e.g., in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; and (b) inhibiting the disease, i.e., arresting its development.

The terms "patient," "subject," "individual," “host,” and the like are used interchangeably herein, and refer to any animal, or cells thereof whether *in vitro* or *in situ*, amenable to the methods described herein. The terms "patient," "subject," "individual," “host,” and the like are intended to include living organisms in which an immune response can be elicited (e.g., mammals). Examples of "patient," "subject," "individual," “host,” include murines (e.g., rats, mice), lagomorphs (e.g., rabbits), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc. and transgenic species thereof. In certain non-limiting embodiments, the patient, subject, host, or individual is a human.

The term "effective amount" or "therapeutically effective amount" are used interchangeably herein, and refers to the amount of a therapeutic agent, or combined amounts of more than one therapeutic agent, that will elicit the biological or medical response of a tissue, system, or subject that is being sought by the researcher, veterinarian, medical doctor or other clinician. The term "therapeutically effective amount" includes that amount of a therapeutic agent that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the signs or symptoms of the disorder or disease being treated. The therapeutically effective amount will vary depending on the therapeutic agent, the disease and its severity and the age, weight, etc., of the subject to be treated.

The term “activation” as used herein, refers to the state of a T cell (e.g., a regulatory T cell) that has been sufficiently stimulated to induce a detectable cellular response. Activation can also be associated with detectable effector function(s) such as cytokine production or suppressive activity. The term “activated” regulatory T cells refers to, among other things, regulatory T cells that are capable of suppressing an immune response.

The term “chimeric antigen receptor” or alternatively a “CAR” refers to a recombinant polypeptide construct comprising an extracellular domain comprising an antigen binding domain; a transmembrane domain; and a cytoplasmic domain comprising an intracellular signaling domain. In one embodiment, the CAR optionally comprises a hinge. The terms “chimeric receptor” or “chimeric antigen receptor” or “CAR” may in particular refer to one polypeptide or to a set of polypeptides, typically two in the simplest embodiments, which when in an immune cell, provides the cell with specificity for a target ligand and with intracellular signal generation. In some embodiments, the set of polypeptides are contiguous with each other. In some embodiments, the chimeric receptor is a chimeric fusion protein comprising the set of polypeptides. In some embodiments, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, *e.g.*, can couple a ligand binding domain to an intracellular signaling domain. In one embodiment, the chimeric receptor comprises an optional leader sequence at the amino-terminus (N-ter) of the chimeric receptor fusion protein. In one embodiment, the chimeric receptor further comprises a leader sequence at the N-terminus of the extracellular ligand binding domain, wherein the leader sequence is optionally cleaved from the ligand binding domain during cellular processing and localization of the chimeric receptor to the cellular membrane.

The term “signaling domain” refers to the functional portion of a 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 term "autologous" refers to any material derived from the same subject to whom it is later to be re-introduced into the subject.

The term "allogeneic" refers to any material derived from a different subject of the same species as the subject to whom the material is introduced. Two or more subjects are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from subjects of the same species may be sufficiently unlike genetically to interact antigenically. The term “allograft” refers to a graft derived from a different subject of the same species.

The term "xenogeneic" refers to any material derived from a subject of a different species. The term “xenograft” refers to a graft derived from a subject of a different species.

As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compositions and methods of the invention. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the nucleic acid, peptide, cell, and/or composition of the invention or be shipped together with a container which contains the nucleic acid, peptide, cell and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the nucleic acid, peptide, cell and/or composition be used cooperatively by the recipient.

A "modification" of an amino acid residue/position, as used herein, refers to a change of a primary amino acid sequence as compared to a starting amino acid sequence, wherein the change results from a sequence alteration involving said amino acid residue/positions. For example, typical modifications include substitution of the residue (or at said position) with another amino acid (e.g., a conservative or non-conservative substitution), insertion of one or more (generally fewer than 5 or 3) amino acids adjacent to said residue/position, and deletion of said residue/position. An "amino acid substitution", or variation thereof, refers to the replacement of an existing amino acid residue in a predetermined (starting) amino acid sequence with a different amino acid residue. Generally and preferably, the modification results in alteration in at least one physicobiochemical activity of the variant polypeptide compared to a polypeptide comprising the starting (or "wild type") amino acid sequence. For example, in the case of an antibody, a physicobiochemical activity that is altered can be binding affinity, binding capability and/or binding effect upon a target molecule.

The term "conservative sequence modification" refers to an amino acid modification that does not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions

are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested using the functional assays described herein.

The term "stimulation," refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules, such as upregulation or downregulation of cytokines and cell surface proteins, and/or reorganization of cytoskeletal structures, and the like.

The term "stimulatory molecule," refers to a molecule expressed by a T cell that provides the primary cytoplasmic signaling sequence(s) that regulate primary activation of the TCR complex in a stimulatory way for at least some aspect of the T cell signaling pathway. In one aspect, the primary signal is initiated by, for example, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a "primary signaling domain") that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif (ITAM).

The term "antigen presenting cell" or "APC" refers to an immune system cell such as an accessory cell (e.g., a B cell, a dendritic cell, macrophages, Langerhans cells and the like) that can display an antigen complexed with major histocompatibility complexes (MHCs) on its surface for recognition by certain lymphocytes such as T cells. T cells may recognize these

complexes using their T cell receptors (TCRs). Antigen presenting cells may process antigens for display in conjunction with MHCs. The term “antigen presenting cell” or “APC” as used herein includes states where the APCs are displaying an antigen and states where the APCs are not displaying an antigen. In some instances, APCs process antigens and present them to T cells. In other instances, T cells may recognize APCs in the absence of antigen presentation where the TCR directly binds to the MHC protein. For example, in the context of transplantation, APCs may directly stimulate T cells via expression of foreign MHC proteins.

An "intracellular signaling domain," as the term is used herein, refers to an intracellular portion of a CAR. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CAR Treg cell. Examples of immune effector function, e.g., in a CAR Treg cell, may include suppression or downregulation of the effector function of other immune cells. Other immune cells includes any type of leukocytes, for example (but not limited to) T cells, B cells, NK cells. In addition, the immune effector function of Tregs may include effects on non-immune cells that result in an improved clinical state such as promoting tissue repair or regeneration.

The term "zeta" or alternatively "zeta chain", or "CD3-zeta" is defined as the protein provided as GenBan Acc. No. BAG36664.1, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, and a "zeta stimulatory domain" or a "CD3-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.

"Co-stimulatory ligand," as the term is used herein, includes a molecule on an antigen presenting cell (e.g., an APC, dendritic cell, B cell, and the like) that specifically binds a cognate co-stimulatory molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A co-stimulatory ligand can include, but is not limited to, CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible

costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, a ligand, including an antibody, that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited to, an MHC class I molecule, BTLA, a Toll ligand receptor, OX40, CD27, CD28, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18), TNFR1 (CD120a/TNFRSF1A), TNFR2 (CD120b/TNFRSF1B), CTLA-4 (CD152), CD95, ICOS (CD278), 4-1BB (CD137), CD2, CD30, CD40, PD-1, CD7, LIGHT, NKG2C, B7-H3, ICAM-1, GITR, HVEM, SLAMF7, NKp80, CD160, IL2ra, IL6Ra, IL-7Ra, IL-13RA1/RA2, IL-33R(IL1RL1), IL-10RA/RB, IL-4R, IL-5R (CSF2RB), ARHR, BAFF receptor, IL-21R, TGFbR1/2/3, common gamma chain, a ligand that specifically binds with CD83, and any combination thereof.

The term "costimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. A costimulatory 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. Costimulatory molecules include, but are not limited to an MHC class I molecule, BTLA, a Toll ligand receptor, OX40, CD27, CD28, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18), TNFR1 (CD120a/TNFRSF1A), TNFR2 (CD120b/TNFRSF1B), CTLA-4 (CD152), CD95, ICOS (CD278), 4-1BB (CD137), CD2, CD30, CD40, PD-1, CD7, LIGHT, NKG2C, B7-H3, ICAM-1, GITR, HVEM, SLAMF7, NKp80, CD160, IL2ra, IL6Ra, IL-7Ra, IL-13RA1/RA2, IL-33R(IL1RL1), IL-10RA/RB, IL-4R, IL-5R (CSF2RB), ARHR, BAFF receptor, IL-21R, TGFbR1/2/3, common gamma chain, a ligand that specifically binds with CD83, and any combination thereof.

A “costimulatory intracellular signaling domain” or “costimulatory domain” can be the intracellular portion of a costimulatory molecule. Examples of such molecules include an MHC

class I molecule, BTLA, a Toll ligand receptor, OX40, CD27, CD28, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18), TNFR1 (CD120a/TNFRSF1A), TNFR2 (CD120b/TNFRSF1B), CTLA-4 (CD152), CD95, ICOS (CD278), 4-1BB (CD137), CD2, CD30, CD40, PD-1, CD7, LIGHT, NKG2C, B7-H3, ICAM-1, GITR, HVEM, SLAMF7, NKp80, CD160, IL2ra, IL6Ra, IL-7Ra, IL-13RA1/RA2, IL-33R(IL1RL1), IL-10RA/RB, IL-4R, IL-5R (CSF2RB), ARHR, BAFF receptor, IL-21R, TGFbR1/2/3, common gamma chain, a ligand that specifically binds with CD83, and the like.

A "co-stimulatory signal," as used herein, refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell proliferation and/or upregulation or downregulation of key molecules.

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.

A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

The term "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 is usually provided in sequence listings, 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.

Unless otherwise specified, a nucleotide sequence or nucleic acid sequence encoding an amino acid sequence includes all nucleotide or nucleic acid sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence or nucleic acid sequence that encodes a protein or a RNA may also include introns to the extent that the nucleotide or nucleic acid sequence encoding the protein may in some version contain an intron(s).

A "transplant," as used herein, refers to cells, tissue, or an organ that is introduced into a subject. The source of the transplanted material can be cultured cells, cells from another subject, or cells from the same subject (e.g., after the cells are cultured *in vitro*). Exemplary organ transplants are kidney, liver, heart, lung, and pancreas. An exemplary tissue transplant is islets. An exemplary cell transplant is allogeneic hematopoietic stem cell transplantation.

The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter.

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 term "lentivirus" refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses.

The term "lentiviral vector" refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in

Milone et al., Mol. Ther. 17(8): 1453-1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include but are not limited to, e.g., the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

The term "homologous" or "identity" refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, scFv, Fab, scFab, sdAb, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof are human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, a humanized antibody/antibody fragment can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications can further refine and optimize antibody or antibody fragment performance. In general, the humanized antibody or antibody fragment thereof will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human

immunoglobulin and all or a significant portion of the FR regions are those of a human immunoglobulin sequence. The humanized antibody or antibody fragment can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature, 321: 522-525, 1986; Reichmann et al., Nature, 332: 323-329, 1988; Presta, Curr. Op. Struct. Biol., 2: 593-596, 1992.

A “human" immunoglobulin, antibody or antibody fragment refers to an immunoglobulin, such as an antibody or antibody fragment, where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody or immunoglobulin.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell. An "isolated antibody" is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

The term "parenteral" administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intradermal, intranodal, intramedullary, intraperitoneal, intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or infusion techniques.

The terms "nucleic acid" or "polynucleotide", used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. Thus, unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

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

polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term "promoter/regulatory sequence" refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term "constitutive" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term "inducible" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

As used herein, "transient" refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

The term, a "substantially purified" cell refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some aspects, the cells are cultured in vitro. In other aspects, the cells are not cultured in vitro.

The term "transfected" or "transformed" or "transduced" refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or

transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

Various aspects of the invention can be presented throughout this disclosure in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

III. ANTI-HLA-A2 ANTIBODIES

In one aspect, the present invention provides anti-HLA-A2 antibodies. Exemplary antibodies include monoclonal antibodies, polyclonal antibodies, recombinant antibodies, chimeric antibodies, human antibodies, humanized antibodies, and antigen binding fragments thereof.

In one embodiment, the invention provides humanized anti-HLA-A2 antibodies. The humanized anti-HLA-A2 antibodies provided herein bind specifically to HLA-A2. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein bind specifically to HLA-A*02:01. As would be appreciated by one skilled in the art, the ability of an antibody to bind to HLA-A2 may be detected through the use of techniques known in the art. For example, binding of an antibody to HLA-A2 may be detected through the use of an HLA-A2 tetramer as exemplified herein. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein compete for binding to HLA-A2 with an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3

(HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein bind to the same HLA-A2 epitope as an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein compete for binding to HLA-A2 with a BB7.2 antibody.

In one embodiment, the humanized anti-HLA-A2 antibodies provided herein bind to the same HLA-A2 epitope as a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from two or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33,HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from three or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from four or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from five or more of HLA-A*03, HLA-

A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from six or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from seven or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from each of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from two or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from each of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least two of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least three of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least four of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein

have less reactivity to at least five of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least six of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least seven of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least two of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*25:01 as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*29:02 as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*30:01 as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*03:01 as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*31:01 as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*33:01 as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*36:01 as compared to a BB7.2 antibody. In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*68:01

as compared to a BB7.2 antibody. The BB7.2 antibody may be isolated from the BB7.2 hybridoma (ATCC Deposit No. HB-82).

Techniques for determining the reactivity of the humanized anti-HLA-A2 antibodies to HLA-A subtypes would be known to those of ordinary skill in the art. For example, the reactivity of the humanized anti-HLA-A2 antibodies to HLA-A subtypes may be determined by a single antigen bead assay. Such single antigen bead assays are commercially available (e.g., FlowPRA Single Antigen Antibody; ONE LAMBDA).

In one embodiment, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, and any combination thereof, as compared to a BB7.2 antibody, e.g., as compared to a BB7.2 scFv when measured in the conditions of Test A. For example, in some embodiments, the humanized anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, and any combination thereof, as compared to a BB7.2 antibody, e.g., as compared to a BB7.2 scFv when measured in the conditions of Test A.

Test A:

$0.25.10^6$ T cells expressing a CAR comprising the humanized anti-HLA-A2 antibody or a BB7.2 antibody, e.g., a BB7.2 scFv (mA2 CAR)) are incubated with FlowPRA single antigen antibody beads panel (FL1HD01, FL1HD02, FL1HD03, FL1HD04, FL1HD06 and FL1HD08, One Lambda) and fixable viability dye (FVD, ThermoFisher, 65-0865-14, eBioscience) for 30 minutes at room temperature. Samples are washed, fixed with 0.5% formaldehyde and analyzed via flow cytometry. Two hundred negative control beads are acquired per sample. Beads alone were used as a negative control. For analysis, dead cells are first eliminated using the fixable viability dye. Single antigen beads are then gated after exclusion of dead cells and doublets. Then, the number of beads per HLA is determined by their respective PE intensity peak. Data are normalized by multiplying the number of beads of interest in each HLA-peak by 200, divided by the number of negative beads in the sample. For each HLA-peak the percent relative binding of CAR Tregs compared to control (non-CAR-expressing cells) is determined by subtracting the number of beads in the CAR-Treg from the number of beads in the control

sample then dividing the average number of beads in the non-CAR-expressing control, times 100.

In one embodiment, the humanized anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 statistically inferior to a BB7.2 antibody, e.g., when measured in the conditions of Test A.

In one embodiment, the anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 statistically inferior to a BB7.2 antibody, e.g., when measured in the conditions of Test A.

In one embodiment, the term "statistically inferior" means that the reactivity (for example, the relative binding in the conditions of Test A) measured for the anti-HLA-A2 antibody of the invention is inferior to the reactivity measured for a BB7.2 antibody with a *p* value of at most about 0.05, preferably of at most about 0.01, more preferably of at most about 0.005, and even more preferably of at most about 0.001, in particular when analyzed by 2-way ANOVA, Dunnett post-test.

In one embodiment, the anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 inferior to a BB7.2 antibody In some embodiments, such an anti-HLA-A2 antibody has a relative binding for at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 inferior to a BB7.2 antibody when measured in the conditions of Test A. In certain aspects, the relative binding measured for such a anti-HLA-A2 antibody is at most about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less of the relative binding measured for a BB7.2 antibody.

In one embodiment, the anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-

A*30 inferior to a BB7.2 antibody In some embodiments, such an anti-HLA-A2 antibody has a relative binding for at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 inferior to a BB7.2 antibody when measured in the conditions of Test A. In certain aspects, the relative binding measured for such an anti-HLA-A2 antibody is at most about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less of the relative binding measured for a BB7.2 antibody.

Further, humanized anti-HLA-A2 antibodies provided herein with antigen binding activity, are capable of constituting antigen binding domains of chimeric antigen receptors (CARs), wherein such CARs are capable of being expressed in human cells such that the CARs specifically bind to HLA-A2. In one embodiment, the CARs specifically bind to HLA-A*02:01. As would be appreciated by one skilled in the art, the ability of a CAR to bind to HLA-A2 may be detected through the use of techniques known in the art. For example, binding of a CAR to HLA-A2 may be detected through the use of an HLA-A2 tetramer as exemplified herein. In one embodiment, the human cell is an immune cell. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg. Further, humanized anti-HLA-A2 antibodies provided herein with antigen binding activity, are capable of constituting antigen binding domains of chimeric antigen receptors (CARs), wherein such CARs are capable of being expressed in a T regulatory cell (Treg) such that the CARs specifically bind to HLA-A2. In one embodiment, the CARs specifically bind to HLA-A*02:01. In one embodiment, the Treg is a human Treg.

In one embodiment, the humanized anti-HLA-A2 antibody is capable of constituting an antigen binding domain of a CAR, wherein such CAR is capable of being expressed in an immune cell such that the immune cell is activated by HLA-A2. In one embodiment, the immune cell is activated by HLA-A*02:01. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg. In one embodiment, the immune cell is a human immune cell. In one embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the T cell is a human T cell. In one embodiment, the Treg is a human Treg.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: SYHIQ (SEQ ID NO: 1) and GYTFTSY (SEQ ID NO: 2).

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 1 (VH CDR1) selected from SEQ ID NOs: 1-2. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a VH CDR1 set forth by SEQ ID NO: 1. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a VH CDR1 set forth by SEQ ID NO: 2.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: YPGDGS (SEQ ID NO: 4) and WIYPGDGSTX^{10}YX^{12}X^{13}KFX^{16}G (SEQ ID NO: 10), wherein in SEQ ID NO: 10, the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising the amino acid sequence WIYPGDGSTX^{10}YX^{12}X^{13}KFX^{16}G (SEQ ID NO: 10), wherein the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 2 (VH CDR2) comprising an amino acid sequence selected from the group consisting of: YPGDGS (SEQ ID NO: 4) and WIYPGDGSTX^{10}YX^{12}X^{13}KFX^{16}G (SEQ ID NO: 10), wherein in SEQ ID NO: 10, the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 2 (VH CDR2) comprising the amino acid sequence WIYPGDGSTX^{10}YX^{12}X^{13}KFX^{16}G (SEQ ID NO: 10), wherein the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at

position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is Q or K, the amino acid at position 12 is S, the amino acid at position 13 is Q, and the amino acid at position 16 is Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is K, the amino acid at position 12 is N or S, the amino acid at position 13 is Q, and the amino acid at position 16 is Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is K, the amino acid at position 12 is S, the amino acid at position 13 is E or Q, and the amino acid at position 16 is Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is K, the amino acid at position 12 is S, the amino acid at position 13 is Q, and the amino acid at position 16 is K or Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is Q or K, the amino acid at position 12 is N or S, the amino acid at position 13 is Q, and the amino acid at position 16 is Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is Q or K, the amino acid at position 12 is S, the amino acid at position 13 is E or Q, and the amino acid at position 16 is Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is Q or K, the amino acid at position 12 is S, the amino acid at position 13 is Q, and the amino acid at position 16 is K or Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is K, the amino acid at position 12 is N or S, the amino acid at position 13 is E or Q, and the amino acid at position 16 is Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is K, the amino acid at position 12 is N or S, the amino acid at position 13 is Q, and the amino acid at position 16 is K or Q. In one embodiment, the heavy chain variable region comprises a VH

CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is K, the amino acid at position 12 is S, the amino acid at position 13 is E or Q, and the amino acid at position 16 is K or Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is Q or K, the amino acid at position 12 is N or S, the amino acid at position 13 is E or Q, and the amino acid at position 16 is Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is Q or K, the amino acid at position 12 is N or S, the amino acid at position 13 is Q, and the amino acid at position 16 is K or Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is Q or K, the amino acid at position 12 is S, the amino acid at position 13 is E or Q, and the amino acid at position 16 is K or Q. In one embodiment, the heavy chain variable region comprises a VH CDR2 comprising an amino acid sequence set forth in SEQ ID NO: 10, wherein the amino acid at position 10 is K, the amino acid at position 12 is N or S, the amino acid at position 13 is E or Q, and the amino acid at position 16 is K or Q.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: WIYPGDGSTQYNEKFKG (SEQ ID NO: 3), YPGDGS (SEQ ID NO: 4), and WIYPGDGSTKYSQKFQG (SEQ ID NO: 5). In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: WIYPGDGSTQYNEKFKG (SEQ ID NO: 3) and YPGDGS (SEQ ID NO: 4). In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: YPGDGS (SEQ ID NO: 4) and WIYPGDGSTKYSQKFQG (SEQ ID NO: 5).

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence set forth by SEQ ID NO: 3. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence set forth by SEQ ID NO: 4. In one embodiment, the

humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence set forth by SEQ ID NO: 5.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 2 (VH CDR2) selected from SEQ ID NOs: 3-5. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 2 (VH CDR2) selected from SEQ ID NOs: 3-4. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 2 (VH CDR2) selected from SEQ ID NOs: 4-5. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 2 (VH CDR2) set forth by SEQ ID NO: 5. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a VH CDR2 set forth by SEQ ID NO: 3. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a VH CDR2 set forth by SEQ ID NO: 4.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising the amino acid sequence EGTYYAMDY (SEQ ID NO: 6).

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 3 (VH CDR3) set forth by SEQ ID NO: 6.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising at least one of the following CDRs:

a VH CDR1 set forth by SEQ ID NO: 1; or

a VH CDR2 set forth by SEQ ID NO: 10, wherein the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q; or

a VH CDR3 set forth by SEQ ID NO: 6.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising at least one of the following CDRs:

a VH CDR1 set forth by SEQ ID NO: 1; or

a VH CDR2 set forth by SEQ ID NO: 3; or

a VH CDR3 set forth by SEQ ID NO: 6.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising at least one of the following CDRs:

a VH CDR1 set forth by SEQ ID NO: 1; or

a VH CDR2 set forth by SEQ ID NO: 5; or

a VH CDR3 set forth by SEQ ID NO: 6.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR1 set forth by SEQ ID NO: 1; one VH CDR2 set forth by SEQ ID NO: 10 wherein the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q; and one VH CDR3 set forth by SEQ ID NO: 6.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR1 set forth by SEQ ID NO: 1; one VH CDR2 set forth by SEQ ID NO: 3; and one VH CDR3 set forth by SEQ ID NO: 6.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR1 set forth by SEQ ID NO: 1; one VH CDR2 set forth by SEQ ID NO: 5; and one VH CDR3 set forth by SEQ ID NO: 6.

According to the present invention, any of the CDRs 1, 2 or 3 of the heavy chain may be characterized as having an amino acid sequence that shares at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the particular sets of CDRs listed in the corresponding SEQ ID NOs: 1, 3, 5, 6 and 10.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising at least one of the following CDRs:

a VH CDR1 set forth by SEQ ID NO: 2; or

a VH CDR2 set forth by SEQ ID NO: 4; or

a VH CDR3 set forth by SEQ ID NO: 6

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR1 set forth by SEQ ID NO: 2; one VH CDR2 set forth by SEQ ID NO: 4; and one VH CDR3 set forth by SEQ ID NO: 6.

According to the present invention, any of the CDRs 1, 2 or 3 of the heavy chain may be characterized as having an amino acid sequence that shares at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the particular sets of CDRs listed in the corresponding SEQ ID NOs: 2, 4 and 6.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising the amino acid sequence RSSQSIVHSNGNTYLE (SEQ ID NO: 7).

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a complementarity determining region 1 (VL CDR1) set forth by SEQ ID NO: 7.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising the amino acid sequence KVSNRFS (SEQ ID NO: 8).

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a complementarity determining region 2 (VL CDR2) set forth by SEQ ID NO: 8.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising the amino acid sequence FQGSHVPRT (SEQ ID NO: 9).

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a complementarity determining region 3 (VL CDR3) set forth by SEQ ID NO: 9.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising at least one of the following CDRs:

a VL CDR1 set forth by SEQ ID NO: 7; or

a VL CDR2 set forth by SEQ ID NO: 8; or

a VL CDR3 set forth by SEQ ID NO: 9.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising one VL CDR1 set forth by SEQ ID NO: 7; one VL CDR2 set forth by SEQ ID NO: 8; and one VL CDR3 set forth by SEQ ID NO: 9.

According to the present invention, any of the CDRs 1, 2 or 3 of the light chain may be characterized as having an amino acid sequence that shares at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the particular sets of CDRs listed in the corresponding SEQ ID NOs: 7, 8 and 9.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising an amino acid sequence selected from the group consisting of: QVQLVQSGAEVKKPGASVKVSCKAS (SEQ ID NO: 11) and QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 12).

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKAS (SEQ ID NO: 11). In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 12). In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 12.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising an amino acid sequence selected from the group consisting of:

WVRQAPGQX^{9}LEWMGX^{15} (SEQ ID NO: 13),

WVRQAPGQX^{9}LEWMGX^{15}WI (SEQ ID NO: 17),

HIQWVRQAPGQX^{12}LEWMGX^{18}WI (SEQ ID NO: 21), and

HIQWVRQAPGQX^{12}LEWMGX^{18} (SEQ ID NO: 25), wherein:

X^{9} is R or G and X^{15} is I or absent in SEQ ID NO: 13;

X^{9} is R or G, and X^{15} is I or absent in SEQ ID NO: 17;

X^{12} is R or G, and X^{18} is I or absent in SEQ ID NO: 21; and

X^{12} is R or G, and X^{18} is I or absent in SEQ ID NO: 25.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising the amino acid sequence WVRQAPGQX^{9}LEWMGX^{15} (SEQ ID NO: 13), wherein the amino acid at position 9 (X^{9}) is R or G, and the amino acid at position 15 (X^{15}) is I or absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 13, wherein the amino acid at position 9 is R or G, and the amino acid at position 15 is absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 14, 15, or 16. In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 14, 15, or 16.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising the amino acid sequence HIQWVRQAPGQX^{12}LEWMGX^{18} (SEQ ID NO: 25), wherein the amino acid at position 12 (X^{12}) is R or G, and the amino acid at position 18 (X^{18}) is I or absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 25, wherein the amino acid at position 12 is R or G, and the amino acid at position 18 is absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 26, 27, or 28. In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 26, 27, or 28.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising the amino acid sequence WVRQAPGQX^{9}LEWMGX^{15}WI (SEQ ID NO: 17), wherein the amino acid at position 9 (X^{9}) is R or G, and the amino acid at position 15 (X^{15}) is I or absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 17, wherein the amino acid at position 9 is R or G, and the amino acid at position 15 is absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 18, 19, or 20. In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 18, 19, or 20.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising the amino acid sequence HIQWVRQAPGQX^{12}LEWMGX^{18}WI (SEQ ID NO: 21), wherein the amino acid at position 12 (X^{12}) is R or G, and the amino acid at position 18 (X^{18}) is I or absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 21, wherein the amino acid at position 12 is R or G, and the amino acid at position 18 is absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 22, 23, or 24. In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 22, 23, or 24.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 3 (VH FR3) comprising an amino acid sequence selected from the group consisting of:

$X^1VTX^4TX^6DTSX^{10}STAYMX^{16}LSX^{19}LRSX^{23}DX^{25}AVYYCAR$ (SEQ ID NO: 29),

$TX^2YX^4X^5KFX^8GX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}$

AVYYCAR (SEQ ID NO: 35),

$TQYNEKFKGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}$

AVYYCAR (SEQ ID NO: 36), and

$TKYSQKFQGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}AVYYCAR$ (SEQ ID NO: 37), wherein:

X^1 is R or absent, X^4 is I or M, X^6 is R or A, X^{10} is A, T or I, X^{16} is E or L, X^{19} is S or R, X^{23} is E or D, and X^{25} is T or M in SEQ ID NO: 29;

X^2 is Q or K, X^4 is N or S, X^5 is E or Q, X^8 is K or Q, X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 35;

X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 36; and

X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 37.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 3 (VH FR3) comprising the amino acid

sequence $X^1VTX^4TX^6DTSX^{10}STAYMX^{16}LSX^{19}LRSX^{23}DX^{25}AVYYCAR$ (SEQ ID NO: 29), wherein the amino acid at position 1 (X^1) is R or absent, the amino acid at position 4 (X^4) is I or M, the amino acid at position 6 (X^6) is R or A, the amino acid at position 10 (X^{10}) is A, T or I, the amino acid at position 16 (X^{16}) is E or L, the amino acid at position 19 (X^{19}) is S or R, the amino acid at position 23 (X^{23}) is E or D, and the amino acid at position 25 (X^{25}) is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 29, wherein the amino acid at position 1 is R, the amino acid at position 4 is I or M, the amino acid at position 6 is R, the amino acid at position 10 is A or I, the amino acid at position 16 is E, the amino acid at position 19 is S or R, the amino acid at position 23 is E or D, and the amino acid at position 25 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth by SEQ ID NO: 29, wherein the amino acid at position 1 is R, the amino acid at position 4 is I or M, the amino acid at position 6 is R, the amino acid at position 10 is A or I, the amino acid at position 16 is E, the amino acid at position 19 is S or R, the amino acid at position 23 is E or D, and the amino acid at position 25 is T.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth by SEQ ID NO: 29, wherein the amino acid at position 1 is R, the amino acid at position 4 is I, the amino acid at position 6 is R, the amino acid at position 10 is A, the amino acid at position 16 is E, the amino acid at position 19 is S, the amino acid at position 23 is E, and the amino acid at position 25 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 30, 31, 32, 33, or 34. In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 30, 31, 32, 33, or 34.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 3 (VH FR3) comprising the amino acid sequence $TX^{2}YX^{4}X^{5}KFX^{8}GX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}$ AVYYCAR (SEQ ID NO: 35), wherein the amino acid at position 2 (X^{2}) is Q or K, the amino acid at position 4 (X^{4}) is N or S, the amino acid at position 5 (X^{5}) is E or Q, the amino acid at position 8 (X^{8}) is K or Q, the amino acid at position 10 (X^{10}) is R or absent, the amino acid at position 13 (X^{13}) is I or M, the amino acid at position 15 (X^{15}) is R or A, the amino acid at position 19 (X^{19}) is A, T or I, the amino acid at position 25 (X^{25}) is E or L, the amino acid at position 28 (X^{28}) is S or R, the amino acid at position 32 (X^{32}) is E or D, and the amino acid at position 34 (X^{34}) is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N or S, the amino acid at position 5 is E or Q, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N or S, the amino acid at position 5 is E or Q, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N or S, the amino acid at position 5 is E or Q, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I, the amino acid at position 15 is R, the amino acid at position 19 is A,

the amino acid at position 25 is E, the amino acid at position 28 is S, the amino acid at position 32 is E, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N, the amino acid at position 5 is E, the amino acid at position 8 is K, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q, the amino acid at position 4 is N or S, the amino acid at position 5 is E, the amino acid at position 8 is K, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q, the amino acid at position 4 is N, the amino acid at position 5 is E or Q, the amino acid at position 8 is K, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q, the amino acid at position 4 is N, the amino acid at position 5 is E, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N or S, the amino acid at position 5 is E, the amino acid at position 8 is K, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N, the amino acid at position 5 is E or Q, the amino acid at position 8 is K, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N, the amino acid at position 5 is E, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q, the amino acid at position 4 is N or S, the amino acid at position 5 is E or Q, the amino acid at position 8 is K, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino

acid at position 2 is Q, the amino acid at position 4 is N or S, the amino acid at position 5 is E, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q, the amino acid at position 4 is N, the amino acid at position 5 is E or Q, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N or S, the amino acid at position 5 is E or Q, the amino acid at position 8 is K, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N or S, the amino acid at position 5 is E, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q or K, the amino acid at position 4 is N, the amino acid at position 5 is E or Q, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid

at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 35, wherein the amino acid at position 2 is Q, the amino acid at position 4 is N or S, the amino acid at position 5 is E or Q, the amino acid at position 8 is K or Q, the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 3 (VH FR3) comprising the amino acid sequence TQYNEKFKGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}

AVYYCAR (SEQ ID NO: 36), wherein the amino acid at position 10 (X^{10}) is R or absent, the amino acid at position 13 (X^{13}) is I or M, the amino acid at position 15 (X^{15}) is R or A, the amino acid at position 19 (X^{19}) is A, T or I, the amino acid at position 25 (X^{25}) is E or L, the amino acid at position 28 (X^{28}) is S or R, the amino acid at position 32 (X^{32}) is E or D, and the amino acid at position 34 (X^{34}) is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 36, wherein the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 36, wherein the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at

position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 36, wherein the amino acid at position 10 is R, the amino acid at position 13 is I, the amino acid at position 15 is R, the amino acid at position 19 is A, the amino acid at position 25 is E, the amino acid at position 28 is S, the amino acid at position 32 is E, and the amino acid at position 34 is T or M.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 3 (VH FR3) comprising the amino acid sequence TKYSQKFQGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}AVYYCAR (SEQ ID NO: 37), wherein the amino acid at position 10 (X^{10}) is R or absent, the amino acid at position 13 (X^{13}) is I or M, the amino acid at position 15 (X^{15}) is R or A, the amino acid at position 19 (X^{19}) is A, T or I, the amino acid at position 25 (X^{25}) is E or L, the amino acid at position 28 (X^{28}) is S or R, the amino acid at position 32 (X^{32}) is E or D, and the amino acid at position 34 (X^{34}) is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 37, wherein the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 37, wherein the amino acid at position 10 is R, the amino acid at position 13 is I or M, the amino acid at position 15 is R, the amino acid at position 19 is A or I, the amino acid at position 25 is E, the amino acid at position 28 is S or R, the amino acid at position 32 is E or D, and the amino acid at position 34 is T.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 37, wherein the amino

acid at position 10 is R, the amino acid at position 13 is I, the amino acid at position 15 is R, the amino acid at position 19 is A, the amino acid at position 25 is E, the amino acid at position 28 is S, the amino acid at position 32 is E, and the amino acid at position 34 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 38, 39, 40, 41, 42, or 43. In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 38, 39, 40, 41, 42, or 43.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 4 (VH FR4) comprising the amino acid sequence WGQGTTVTVSS (SEQ ID NO: 44). In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 4 (VH FR4) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 44.

In one embodiment, the heavy chain variable region comprises:

a VH CDR1 set forth by SEQ ID NO: 1;

a VH CDR2 set forth by SEQ ID NO: 10, wherein the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q;

a VH CDR3 set forth by SEQ ID NO: 6;

a VH FR1 set forth by SEQ ID NO: 12;

a VH FR2 set forth by SEQ ID NO: 13, wherein the amino acid at position 9 (X^{9}) is R or G, and the amino acid at position 15 (X^{15}) is I or absent;

a VH FR3 set forth by SEQ ID NO: 29, wherein the amino acid at position 1 (X^{1}) is R or absent, the amino acid at position 4 (X^{4}) is I or M, the amino acid at position 6 (X^{6}) is R or A, the amino acid at position 10 (X^{10}) is A, T or I, the amino acid at position 16 (X^{16}) is E or L, the

amino acid at position 19 (X^{19}) is S or R, the amino acid at position 23 (X^{23}) is E or D, and the amino acid at position 25 (X^{25}) is T or M; and

a VH FR4 set forth by SEQ ID NO: 44.

In one embodiment, the heavy chain variable region comprises:

a VH CDR1 set forth by SEQ ID NO: 2;

a VH CDR2 set forth by SEQ ID NO: 10, wherein the amino acid at position 10 (X^{10}) is Q or K, the amino acid at position 12 (X^{12}) is N or S, the amino acid at position 13 (X^{13}) is E or Q, and the amino acid at position 16 (X^{16}) is K or Q;

a VH CDR3 set forth by SEQ ID NO: 6;

a VH FR1 set forth by SEQ ID NO: 11;

a VH FR2 set forth by SEQ ID NO: 25, wherein the amino acid at position 12 (X^{12}) is R or G, and the amino acid at position 18 (X^{18}) is I or absent;

a VH FR3 set forth by SEQ ID NO: 29, wherein the amino acid at position 1 (X^{1}) is R or absent, the amino acid at position 4 (X^{4}) is I or M, the amino acid at position 6 (X^{6}) is R or A, the amino acid at position 10 (X^{10}) is A, T or I, the amino acid at position 16 (X^{16}) is E or L, the amino acid at position 19 (X^{19}) is S or R, the amino acid at position 23 (X^{23}) is E or D, and the amino acid at position 25 (X^{25}) is T or M; and

a VH FR4 set forth by SEQ ID NO: 44.

In one embodiment, the heavy chain variable region comprises:

a VH CDR1 set forth by SEQ ID NO: 2;

a VH CDR2 set forth by SEQ ID NO:4;

a VH CDR3 set forth by SEQ ID NO: 6;

a VH FR1 set forth by SEQ ID NO: 11;

a VH FR2 set forth by SEQ ID NO: 21, wherein the amino acid at position 12 (X^{12}) is R or G, and the amino acid at position 18 (X^{18}) is I or absent;

a VH FR3 set forth by SEQ ID NO: 35, wherein the amino acid at position 2 (X^2) is Q or K, the amino acid at position 4 (X^4) is N or S, the amino acid at position 5 (X^5) is E or Q, the amino acid at position 8 (X^8) is K or Q, the amino acid at position 10 (X^{10}) is R or absent, the amino acid at position 13 (X^{13}) is I or M, the amino acid at position 15 (X^{15}) is R or A, the amino acid at position 19 (X^{19}) is A, T or I, the amino acid at position 25 (X^{25}) is E or L, the amino acid at position 28 (X^{28}) is S or R, the amino acid at position 32 (X^{32}) is E or D, and the amino acid at position 34 (X^{34}) is T or M; and

a VH FR4 set forth by SEQ ID NO: 44.

In one embodiment, the heavy chain variable region comprises:

a VH CDR1 set forth by SEQ ID NO: 1;

a VH CDR2 set forth by SEQ ID NO:4;

a VH CDR3 set forth by SEQ ID NO: 6;

a VH FR1 set forth by SEQ ID NO: 12;

a VH FR2 set forth by SEQ ID NO: 17, wherein the amino acid at position 9 (X^9) is R or G, and the amino acid at position 15 (X^{15}) is I or absent;

a VH FR3 set forth by SEQ ID NO: 35, wherein the amino acid at position 2 (X^2) is Q or K, the amino acid at position 4 (X^4) is N or S, the amino acid at position 5 (X^5) is E or Q, the amino acid at position 8 (X^8) is K or Q, the amino acid at position 10 (X^{10}) is R or absent, the amino acid at position 13 (X^{13}) is I or M, the amino acid at position 15 (X^{15}) is R or A, the amino acid at position 19 (X^{19}) is A, T or I, the amino acid at position 25 (X^{25}) is E or L, the amino acid at position 28 (X^{28}) is S or R, the amino acid at position 32 (X^{32}) is E or D, and the amino acid at position 34 (X^{34}) is T or M; and

a VH FR4 set forth by SEQ ID NO: 44.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising the amino acid sequence QVQLVQSGAEVKKPGAS VKVSCKASGYTFTSYHIQWVRQAPGQX^{44}LEWMGX^{50}WIYPGDGSTX^{60}Y$X^{62}$$X^{63}KFX^{66}$G X^{68}VTX^{71}TX^{73}DTSX^{77}STAYMX^{83}LSX^{86}LRSX^{90}DX^{92}AVYYCAREGTYYAMDYWGQGTT VTVSS (SEQ ID NO: 45), wherein the amino acid at position 44 (X^{44}) is R or G, the amino

acid at position 50 (X^{50}) is I or absent, the amino acid at position 60 (X^{60}) is Q or K, the amino acid at position 62 (X^{62}) is N or S, the amino acid at position 63 (X^{63}) is E or Q, the amino acid at position 66 (X^{66}) is K or Q, the amino acid at position 68 (X^{68}) is R or absent, the amino acid at position 71 (X^{71}) is I or M, the amino acid at position 73 (X^{73}) is R or A, the amino acid at position 77 (X^{77}) is A, T or I, the amino acid at position 83 (X^{83}) is E or L, the amino acid at position 86 (X^{86}) is S or R, the amino acid at position 90 (X^{90}) is E or D, and the amino acid at position 92 (X^{92}) is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G; the amino acid at position 50 is absent; the amino acid at position 60 is Q or K; the amino acid at position 62 is N or S; the amino acid at position 63 is E or Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R; the amino acid at position 71 is I or M; the amino acid at position 73 is R; the amino acid at position 77 is A or I; the amino acid at position 83 is E; the amino acid at position 86 is S or R; the amino acid at position 90 is E or D; and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is N or S; the amino acid at position 63 is E or Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is N or S; the amino acid at position 63 is E or Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I, the amino acid at position 73 is R, the amino acid at position 77 is A, the amino acid at position 83 is E, the amino acid at position 86 is S, the amino acid at position 90 is E, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q; the amino acid at position 62 is N; the amino acid at position 63 is E; the amino acid at position 66 is K; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q; the amino acid at position 62 is N; the amino acid at position 63 is E; the amino acid at position 66 is K; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R, the amino acid at position 50 is absent, the amino acid at position 60 is Q; the amino acid at position 62 is N; the amino acid at position 63 is E; the amino acid at position 66 is K; the amino acid at position 68 is R, the amino acid at position 71 is I, the amino acid at position 73 is R, the amino acid at position 77 is A, the amino acid at position 83 is E, the amino acid at position 86 is S, the amino acid at position 90 is E, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is S; the amino acid at position 63 is Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at

position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is S; the amino acid at position 63 is Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is S; the amino acid at position 63 is Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I, the amino acid at position 73 is R, the amino acid at position 77 is A, the amino acid at position 83 is E, the amino acid at position 86 is S, the amino acid at position 90 is E, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is S; the amino acid at position 63 is Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is N or S; the amino acid at position 63 is Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is S; the amino acid at position 63 is E or Q; the amino acid at position 66 is Q; the amino acid at

position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is S; the amino acid at position 63 is Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is N or S; the amino acid at position 63 is Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is S; the amino acid at position 63 is E or Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at

position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is S; the amino acid at position 63 is Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is N or S; the amino acid at position 63 is E or Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is N or S; the amino acid at position 63 is Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is S; the amino acid at position 63 is E or Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is N or S; the amino acid at position 63 is E or Q; the amino acid at position 66 is Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is N or S; the amino acid at position 63 is Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is Q or K; the amino acid at position 62 is S; the amino acid at position 63 is E or Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 45, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 60 is K; the amino acid at position 62 is N or S; the amino acid at position 63 is E or Q; the amino acid at position 66 is K or Q; the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at

position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 61, 62, 63, 64, 65, or 66. In one embodiment, the humanized anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 61, 62, 63, 64, 65, or 66.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a framework region 1 (VL FR1) comprising the amino acid sequence $DX^2VMTQX^7PLSX^{11}X^{12}VTX^{15}GQPASISX^{23}$ (SEQ ID NO: 46), wherein the amino acid at position 2 (X^2) is V or I, the amino acid at position 7 (X^7) is S or T, the amino acid at position 11 (X^{11}) is L or S; the amino acid at position 12 (X^{12}) is P or S; the amino acid at position 15 (X^{15}) is L or P, and the amino acid at position 23 (X^{23}) is C or F.

In one embodiment, the light chain variable region comprises a framework region 1 (VL FR1) comprising an amino acid sequence set forth in SEQ ID NO: 46, wherein the amino acid at position 2 is V or I; the amino acid at position 7 is S or T; the amino acid at position 11 is L or S; the amino acid at position 12 is P or S; the amino acid at position 15 is L or P; and the amino acid at position 23 is C.

In one embodiment, the light chain variable region comprises a framework region 1 (VL FR1) comprising an amino acid sequence set forth in SEQ ID NO: 46, wherein the amino acid at position 2 is I; the amino acid at position 7 is T; the amino acid at position 11 is L or S; the amino acid at position 12 is P or S; the amino acid at position 15 is L or P; and the amino acid at position 23 is C.

In one embodiment, the light chain variable region comprises a framework region 1 (VL FR1) comprising an amino acid sequence set forth in SEQ ID NO: 46, wherein the amino acid at position 2 is V or I; the amino acid at position 7 is S or T; the amino acid at position 11 is L; the amino acid at position 12 is P or S; the amino acid at position 15 is L or P; and the amino acid at position 23 is C.

In one embodiment, the light chain variable region comprises a framework region 1 (VL FR1) comprising an amino acid sequence set forth in SEQ ID NO: 46, wherein the amino acid at position 2 is V or I; the amino acid at position 7 is S or T; the amino acid at position 11 is L or S; the amino acid at position 12 is P; the amino acid at position 15 is L; and the amino acid at position 23 is C.

In one embodiment, the light chain variable region comprises a framework region 1 (VL FR1) comprising an amino acid sequence set forth in SEQ ID NO: 47, 48, 49, or 50. In one embodiment, the light chain variable region comprises a framework region 1 (VL FR1) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 47, 48, 49, or 50.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a framework region 2 (VL FR2) comprising the amino acid sequence $WX^2X^3QX^5PGQX^9PX^{11}X^{12}LIY$ (SEQ ID NO: 51), wherein the amino acid at position 2 (X^2) is F or Y, the amino acid at position 3 (X^3) is Q or L, the amino acid at position 5 (X^5) is R or K, the amino acid at position 9 (X^9) is S or P, the amino acid at position 11 (X^{11}) is R or Q, and the amino acid at position 12 (X^{12}) is R or L.

In one embodiment, the light chain variable region comprises a framework region 2 (VL FR2) comprising the amino acid sequence set forth in SEQ ID NO: 51, wherein the amino acid at position 2 is Y, the amino acid at position 3 is Q or L, the amino acid at position 5 is R or K, the amino acid at position 9 is S or P, the amino acid at position 11 is R or Q, and the amino acid at position 12 is L.

In one embodiment, the light chain variable region comprises a framework region 2 (VL FR2) comprising the amino acid sequence set forth in SEQ ID NO: 51, wherein the amino acid at position 2 is Y, the amino acid at position 3 is Q or L, the amino acid at position 5 is R or K, the amino acid at position 9 is S, the amino acid at position 11 is R or Q, and the amino acid at position 12 is L.

In one embodiment, the light chain variable region comprises a framework region 2 (VL FR2) comprising the amino acid sequence set forth in SEQ ID NO: 51, wherein the amino acid

at position 2 is Y, the amino acid at position 3 is Q, the amino acid at position 5 is R, the amino acid at position 9 is S or P, the amino acid at position 11 is R, and the amino acid at position 12 is L.

In one embodiment, the light chain variable region comprises a framework region 2 (VL FR2) comprising an amino acid sequence set forth in SEQ ID NO: 51, wherein the amino acid at position 2 is F or Y, the amino acid at position 3 is Q or L, the amino acid at position 5 is R or K, the amino acid at position 9 is S, the amino acid at position 11 is R or Q, and the amino acid at position 12 is R or L.

In one embodiment, the light chain variable region comprises a framework region 2 (VL FR2) comprising an amino acid sequence set forth in SEQ ID NO: 51, wherein the amino acid at position 2 is F or Y, the amino acid at position 3 is Q, the amino acid at position 5 is R, the amino acid at position 9 is S or P, the amino acid at position 11 is R, and the amino acid at position 12 is R or L.

In one embodiment, the light chain variable region comprises a framework region 2 (VL FR2) comprising the amino acid sequence set forth in SEQ ID NO: 52, 53, 54, or 55. In one embodiment, the light chain variable region comprises a framework region 2 (VL FR2) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 52, 53, 54, or 55.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a framework region 3 (VL FR3) comprising the amino acid sequence GVPDRFSGSGX^{11}GTDFTLKISRVEAEDVGVYYC (SEQ ID NO: 56), wherein the amino acid at position 11 (X^{11}) is S or A.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a framework region 3 (VL FR3) comprising an amino acid sequence set forth in SEQ ID NO: 57 or 58. In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a framework region 3 (VL FR3) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%,

88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 57 or 58.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a framework region 4 (VL FR4) comprising the amino acid sequence FGGGTKVEIK (SEQ ID NO: 59). In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising a framework region 4 (VL FR4) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 59.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising an amino acid sequence $DX^{2}VMTQX^{7}PLSX^{11}X^{12}VTX^{15}GQPASISX^{23}RSSQSIVHSNGNTYLEWX^{41}X^{42}QX^{44}PGQX^{48}PX^{50}X^{51}LIYKVSNRFSGVPDRFSGSGX^{72}GTDFTLKISRVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK$ (SEQ ID NO: 60), wherein the amino acid at position 2 (X^{2}) is V or I, the amino acid at position 7 (X^{7}) is S or T, the amino acid at position 11 (X^{11}) is L or S, the amino acid at position 12 (X^{12}) is P or S, the amino acid at position 15 (X^{15}) is L or P, the amino acid at position 23 (X^{23}) is C or F, the amino acid at position 41 (X^{41}) is F or Y, the amino acid at position 42 (X^{42}) is Q or L, the amino acid at position 44 (X^{44}) is R or K, the amino acid at position 48 (X^{48}) is S or P, the amino acid at position 50 (X^{50}) is R or Q, the amino acid at position 51 (X^{51}) is R or L, and the amino acid at position 72 (X^{72}) is S or A.

In one embodiment, the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 60, wherein the amino acid at position 2 is V or I, the amino acid at position 7 is S or T, the amino acid at position 11 is L or S, the amino acid at position 12 is P or S, the amino acid at position 15 is L or P, the amino acid at position 23 is C, the amino acid at position 41 is Y, the amino acid at position 42 is Q or L, the amino acid at position 44 is R or K, the amino acid at position 48 is S or P, the amino acid at position 50 is R or Q, the amino acid at position 51 is L, and the amino acid at position 72 is S or A.

In one embodiment, the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 60, wherein the amino acid at position 2 is I, the amino acid at position 7 is T, the amino acid at position 11 is L or S, the amino acid at position 12 is P or S, the amino

acid at position 15 is L or P, the amino acid at position 23 is C, the amino acid at position 41 is Y, the amino acid at position 42 is Q or L, the amino acid at position 44 is R or K, the amino acid at position 48 is S or P, the amino acid at position 50 is R or Q, the amino acid at position 51 is L, and the amino acid at position 72 is S or A.

In one embodiment, the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 60, wherein the amino acid at position 2 is V or I, the amino acid at position 7 is S or T, the amino acid at position 11 is L, the amino acid at position 12 is P or S, the amino acid at position 15 is L or P, the amino acid at position 23 is C, the amino acid at position 41 is Y, the amino acid at position 42 is Q or L, the amino acid at position 44 is R or K, the amino acid at position 48 is S, the amino acid at position 50 is R or Q, the amino acid at position 51 is L, and the amino acid at position 72 is S.

In one embodiment, the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 60, wherein the amino acid at position 2 is V or I, the amino acid at position 7 is S or T, the amino acid at position 11 is L or S, the amino acid at position 12 is P, the amino acid at position 15 is L, the amino acid at position 23 is C, the amino acid at position 41 is Y, the amino acid at position 42 is Q, the amino acid at position 44 is R, the amino acid at position 48 is S or P, the amino acid at position 50 is R, the amino acid at position 51 is L, and the amino acid at position 72 is S or A.

In one embodiment, the light chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 60, wherein the amino acid at position 2 is V or I, the amino acid at position 7 is S or T, the amino acid at position 11 is L or S, the amino acid at position 12 is P or S, the amino acid at position 15 is L or P, the amino acid at position 23 is C, the amino acid at position 41 is F or Y, the amino acid at position 42 is Q or L, the amino acid at position 44 is R or K, the amino acid at position 48 is S or P, the amino acid at position 50 is R or Q, the amino acid at position 51 is R or L, and the amino acid at position 72 is S or A.

In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 67, 68, 69, 70, or 71. In one embodiment, the humanized anti-HLA-A2 antibody comprises a light chain variable region comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 67, 68, 69, 70, or 71.

In one embodiment, the humanized anti-HLA-A2 antibody is an scFv, scFab or sdAb. In one embodiment, the humanized anti-HLA-A2 antibody is an scFv or scFab. In one embodiment, the humanized anti-HLA-A2 antibody is an sdAb. In one embodiment, the humanized anti-HLA-A2 antibody is an scFab. In one embodiment, the humanized anti-HLA-A2 antibody is an scFv. In one embodiment, the humanized anti-HLA-A2 antibody is an scFv comprising an amino acid sequence set forth in SEQ ID NO: 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, or 91. In one embodiment, the humanized anti-HLA-A2 antibody is an scFv comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, or 91.

In one embodiment, the humanized anti-HLA-A2 antibody comprises an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the SEQ ID NOs recited above. In one embodiment, the humanized anti-HLA-A2 antibody is an scFv or scFab comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the SEQ ID NOs recited above.

In one aspect, the invention provides an anti-HLA antibody comprising a heavy chain variable region comprising the amino acid sequence WIYPGDGSTKYSQKFQG (SEQ ID NO: 5).

In one embodiment, said antibody is an antibody mimetic selected from the group consisting of an affibody, an alphabody, an armadillo repeat protein-based scaffold, a knottin, a kunitz domain peptide, an affilin, an affitin, an adnectin, an atrimer, an evasin, a DARPin, an anticalin, an avimer, a fynomer, a versabody and a duocalin.

The anti-HLA-A2 antibodies provided herein bind specifically to HLA-A2. In one embodiment, the anti-HLA-A2 antibodies provided herein bind specifically to HLA-A*02:01.

As would be appreciated by one skilled in the art, the ability of an antibody to bind to HLA-A2 may be detected through the use of techniques known in the art. For example, binding of an antibody to HLA-A2 may be detected through the use of an HLA-A2 tetramer as exemplified herein. In one embodiment, the anti-HLA-A2 antibodies provided herein compete for binding to HLA-A2 with an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the anti-HLA-A2 antibodies provided herein bind to the same HLA-A2 epitope as an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the anti-HLA-A2 antibodies provided herein compete for binding to HLA-A2 with a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein bind to the same HLA-A2 epitope as a BB7.2 antibody. In one embodiment, the anti-HLA antibody comprises a heavy chain variable region comprising a complementarity determining region 2 (VH CDR2) set forth by SEQ ID NO: 5. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from two or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-

A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from three or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from four or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from five or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from six or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from seven or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from each of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from two or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from each of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least two of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01,

HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least three of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least four of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least five of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least six of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least seven of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least two of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*25:01 as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*29:02 as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*30:01 as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*03:01 as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-

A*31:01 as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*33:01 as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*36:01 as compared to a BB7.2 antibody. In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to HLA-A*68:01 as compared to a BB7.2 antibody. The BB7.2 antibody may be isolated from the BB7.2 hybridoma (ATCC Deposit No. HB-82). Techniques for determining the reactivity of the anti-HLA-A2 antibodies to HLA-A subtypes would be known to those of ordinary skill in the art. For example, the reactivity of the anti-HLA-A2 antibodies to HLA-A subtypes may be determined by a single antigen bead assay. Such single antigen bead assays are commercially available (e.g., FlowPRA Single Antigen Antibody; ONE LAMBDA).

In one embodiment, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, and any combination thereof, as compared to a BB7.2 antibody when measured in the conditions of Test A. For example, in some embodiments, the anti-HLA-A2 antibodies provided herein have less reactivity to at least one HLA-A subtype selected from the group comprising HLA-A* 25, HLA-A* 29, HLA-A* 30 and any combination thereof, as compared to a BB7.2 antibody when measured in the conditions of Test A.

Test A:

$0.25.10^6$ T cells expressing a CAR comprising the anti-HLA-A2 antibody or a BB7.2 antibody (e.g., a BB7.2 scFv (mA2 CAR)) are incubated with FlowPRA single antigen antibody beads panel (FL1HD01, FL1HD02, FL1HD03, FL1HD04, FL1HD06 and FL1HD08, One Lambda) and fixable viability dye (FVD, ThermoFisher, 65-0865-14, eBioscience) for 30 minutes at room temperature. Samples are washed, fixed with 0.5% formaldehyde and analyzed via flow cytometry. Two hundred negative control beads are acquired per sample. Beads alone were used as a negative control. For analysis, dead cells are first eliminated using the fixable viability dye. Single antigen beads are then gated after exclusion of dead cells and doublets. Then, the number of beads per HLA is determined by their respective PE intensity peak. Data are normalized by multiplying the number of beads of interest in each HLA-peak by 200,

divided by the number of negative beads in the sample. For each HLA-peak the percent relative binding of CAR Tregs compared to control (non-CAR-expressing cells) is determined by subtracting the number of beads in the CAR-Treg from the number of beads in the control sample then dividing the average number of beads in the non-CAR-expressing control, times 100. In one embodiment, the anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 statistically inferior to a BB7.2 antibody, e.g., when measured in the conditions of Test A.

In one embodiment, the anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 statistically inferior to a BB7.2 antibody, e.g., when measured in the conditions of Test A.

In one embodiment, the term "statistically inferior" means that the reactivity (*i.e.*, for example, the relative binding in the conditions of Test A) measured for the anti-HLA-A2 antibody of the invention is inferior to the reactivity measured for a BB7.2 antibody with a p value of at most about 0.05, preferably of at most about 0.01, more preferably of at most about 0.005, and even more preferably of at most about 0.001, in particular when analyzed by 2-way ANOVA, Dunnett post-test.

In one embodiment, the anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 inferior to a BB7.2 antibody In some embodiments, such an anti-HLA-A2 antibody has a relative binding for at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 inferior to a BB7.2 antibody when measured in the conditions of Test A. In certain aspects, the relative binding measured for such an anti-HLA-A2 antibody is at most about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less of the relative binding measured for a BB7.2 antibody.

In one embodiment, the anti-HLA-A2 antibody of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 inferior to a BB7.2 antibody In some embodiments, such an anti-HLA-A2 antibody has a

relative binding for at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 inferior to a BB7.2 antibody when measured in the conditions of Test A. In certain aspects, the relative binding measured for such an anti-HLA-A2 antibody is at most about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less of the relative binding measured for a BB7.2 antibody.

Further, the anti-HLA-A2 antibodies provided herein with antigen binding activity, are capable of constituting antigen binding domains of CARs, wherein such CARs are capable of being expressed in human cells such that the CARs specifically bind to HLA-A2. In one embodiment, the CARs specifically bind to HLA-A*02:01. As would be appreciated by one skilled in the art, the ability of a CAR to bind to HLA-A2 may be detected through the use of techniques known in the art. For example, binding of a CAR to HLA-A2 may be detected through the use of an HLA-A2 tetramer as exemplified herein. In one embodiment, the human cell is an immune cell. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg.

Further, anti-HLA-A2 antibodies provided herein with antigen binding activity, are capable of constituting antigen binding domains of chimeric antigen receptors (CARs), wherein such CARs are capable of being expressed in a T regulatory cell (Treg) such that the CARs specifically bind to HLA-A2. In one embodiment, the CARs specifically bind to HLA-A*02:01. In one embodiment, the Treg is a human Treg.

In one embodiment, the anti-HLA-A2 antibody is capable of constituting an antigen binding domain of a CAR, wherein such CAR is capable of being expressed in an immune cell such that the immune cell is activated by HLA-A2. In one embodiment, the immune cell is activated by HLA-A*02:01. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg. In one embodiment, the immune cell is a human immune cell. In one embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the T cell is a human T cell. In one embodiment, the Treg is a human Treg.

In one embodiment, such anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: SYHIQ (SEQ ID NO: 1) and GYTFTSY (SEQ ID NO: 2).

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 1 (VH CDR1) selected from SEQ ID NOs: 1-2. In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a VH CDR1 set forth by SEQ ID NO: 1. In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a VH CDR1 set forth by SEQ ID NO: 2.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising the amino acid sequence EGTYYAMDY (SEQ ID NO: 6).

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a complementarity determining region 3 (VH CDR3) set forth by SEQ ID NO: 6.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR2 set forth by SEQ ID NO: 5 and at least one of the following CDRs:

a VH CDR1 set forth by SEQ ID NO: 1; or

a VH CDR3 set forth by SEQ ID NO: 6.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR1 set forth by SEQ ID NO: 1; one VH CDR2 set forth by SEQ ID NO: 5; and one VH CDR3 set forth by SEQ ID NO: 6.

According to the present invention, any of the CDRs 1, 2 or 3 of the heavy chain may be characterized as having an amino acid sequence that shares at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the particular sets of CDRs listed in the corresponding SEQ ID NOs: 1, 5 and 6.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR2 set forth by SEQ ID NO: 5 and at least one of the following CDRs:

a VH CDR1 set forth by SEQ ID NO: 2; or

a VH CDR3 set forth by SEQ ID NO: 6.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising one VH CDR1 set forth by SEQ ID NO: 2; one VH CDR2 set forth by SEQ ID NO: 5; and one VH CDR3 set forth by SEQ ID NO: 6.

According to the present invention, any of the CDRs 1, 2 or 3 of the heavy chain may be characterized as having an amino acid sequence that shares at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the particular sets of CDRs listed in the corresponding SEQ ID NOs: 2, 5 and 6.

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising the amino acid sequence RSSQSIVHSNGNTYLE (SEQ ID NO: 7).

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising a complementarity determining region 1 (VL CDR1) set forth by SEQ ID NO: 7.

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising the amino acid sequence KVSNRFS (SEQ ID NO: 8).

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising a complementarity determining region 2 (VL CDR2) set forth by SEQ ID NO: 8.

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising the amino acid sequence FQGSHVPRT (SEQ ID NO: 9).

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising a complementarity determining region 3 (VL CDR3) set forth by SEQ ID NO: 9.

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising at least one of the following CDRs:

a VL CDR1 set forth by SEQ ID NO: 7; or

a VL CDR2 set forth by SEQ ID NO: 8; or

a VL CDR3 set forth by SEQ ID NO: 9.

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region comprising one VL CDR1 set forth by SEQ ID NO: 7; one VL CDR2 set forth by SEQ ID NO: 8; and one VL CDR3 set forth by SEQ ID NO: 9.

According to the present invention, any of the CDRs 1, 2 or 3 of the light chain may be characterized as having an amino acid sequence that shares at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity with the particular sets of CDRs listed in the corresponding SEQ ID NOs: 7, 8 and 9.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising an amino acid sequence selected from the group consisting of: QVQLVQSGAEVKKPGASVKVSCKAS (SEQ ID NO: 11) and QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 12).

In one embodiment, the anti-HLA-A2 antibody thereof comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKAS (SEQ ID NO: 11). In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising the amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 12). In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 12.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising an amino acid sequence selected from the group consisting of: WVRQAPGQX^{9}LEWMGX^{15} (SEQ ID NO: 13), and HIQWVRQAPGQX^{12}LEWMGX^{18} (SEQ ID NO: 25), wherein: X^{9} is R or G and X^{15} is I or absent in SEQ ID NO: 13; and X^{12} is R or G, and X^{18} is I or absent in SEQ ID NO: 25.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising the amino acid sequence

WVRQAPGQX^{9}LEWMGX^{15} (SEQ ID NO: 13), wherein the amino acid at position 9 (X^{9}) is R or G, and the amino acid at position 15 (X^{15}) is I or absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 13, wherein the amino acid at position 9 is R or G, and the amino acid at position 15 is absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 14, 15, or 16. In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 14, 15, or 16.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising the amino acid sequence HIQWVRQAPGQX^{12}LEWMGX^{18} (SEQ ID NO: 25), wherein the amino acid at position 12 (X^{12}) is R or G, and the amino acid at position 18 (X^{18}) is I or absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 25, wherein the amino acid at position 12 is R or G, and the amino acid at position 18 is absent.

In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence set forth in SEQ ID NO: 26, 27, or 28. In one embodiment, the heavy chain variable region comprises a framework region 2 (VH FR2) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 26, 27, or 28.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 3 (VH FR3) comprising the amino acid sequence X^{1}VTX^{4}TX^{6}DTSX^{10}STAYMX^{16}LSX^{19}LRSX^{23}DX^{25}AVYYCAR (SEQ ID NO: 29), wherein the amino acid at position 1 (X^{1}) is R or absent, the amino acid at position 4 (X^{4}) is I or M, the amino acid at position 6 (X^{6}) is R or A, the amino acid at position 10 (X^{10}) is A, T or I, the

amino acid at position 16 (X^{16}) is E or L, the amino acid at position 19 (X^{19}) is S or R, the amino acid at position 23 (X^{23}) is E or D, and the amino acid at position 25 (X^{25}) is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 29, wherein the amino acid at position 1 is R, the amino acid at position 4 is I or M, the amino acid at position 6 is R, the amino acid at position 10 is A or I, the amino acid at position 16 is E, the amino acid at position 19 is S or R, the amino acid at position 23 is E or D, and the amino acid at position 25 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth by SEQ ID NO: 29, wherein the amino acid at position 1 is R, the amino acid at position 4 is I or M, the amino acid at position 6 is R, the amino acid at position 10 is A or I, the amino acid at position 16 is E, the amino acid at position 19 is S or R, the amino acid at position 23 is E or D, and the amino acid at position 25 is T.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth by SEQ ID NO: 29, wherein the amino acid at position 1 is R, the amino acid at position 4 is I, the amino acid at position 6 is R, the amino acid at position 10 is A, the amino acid at position 16 is E, the amino acid at position 19 is S, the amino acid at position 23 is E, and the amino acid at position 25 is T or M.

In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence set forth in SEQ ID NO: 30, 31, 32, 33, or 34. In one embodiment, the heavy chain variable region comprises a framework region 3 (VH FR3) comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 30, 31, 32, 33, or 34.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 4 (VH FR4) comprising the amino acid sequence WGQGTTVTVSS (SEQ ID NO: 44). In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising a framework region 4 (VH FR4)

comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 44.

In one embodiment, the heavy chain variable region comprises:

a VH CDR1 set forth by SEQ ID NO: 1;

a VH CDR2 set forth by SEQ ID NO: 5;

a VH CDR3 set forth by SEQ ID NO: 6;

a VH FR1 set forth by SEQ ID NO: 12;

a VH FR2 set forth by SEQ ID NO: 13, wherein the amino acid at position 9 (X^9) is R or G, and the amino acid at position 15 (X^{15}) is I or absent;

a VH FR3 set forth by SEQ ID NO: 29, wherein the amino acid at position 1 (X^1) is R or absent, the amino acid at position 4 (X^4) is I or M, the amino acid at position 6 (X^6) is R or A, the amino acid at position 10 (X^{10}) is A, T or I, the amino acid at position 16 (X^{16}) is E or L, the amino acid at position 19 (X^{19}) is S or R, the amino acid at position 23 (X^{23}) is E or D, and the amino acid at position 25 (X^{25}) is T or M; and

a VH FR4 set forth by SEQ ID NO: 44.

In one embodiment, the heavy chain variable region comprises:

a VH CDR1 set forth by SEQ ID NO: 2;

a VH CDR2 set forth by SEQ ID NO: 5;

a VH CDR3 set forth by SEQ ID NO: 6;

a VH FR1 set forth by SEQ ID NO: 11;

a VH FR2 set forth by SEQ ID NO: 25, wherein the amino acid at position 12 (X^{12}) is R or G, and the amino acid at position 18 (X^{18}) is I or absent;

a VH FR3 set forth by SEQ ID NO: 29, wherein the amino acid at position 1 (X^1) is R or absent, the amino acid at position 4 (X^4) is I or M, the amino acid at position 6 (X^6) is R or A, the amino acid at position 10 (X^{10}) is A, T or I, the amino acid at position 16 (X^{16}) is E or L, the

amino acid at position 19 (X^{19}) is S or R, the amino acid at position 23 (X^{23}) is E or D, and the amino acid at position 25 (X^{25}) is T or M; and

a VH FR4 set forth by SEQ ID NO: 44.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence QVQLVQSGAEVKKPGASVKVSC KASGYTFTSYHIQWVRQAPGQX^{44}LEWMGX^{50}WIYPGDGSTKYSQKFQGX^{68}VTX^{71}TX^{73} DTSX^{77}STAYMX^{83}LSX^{86}LRSX^{90}DX^{92}AVYYCAREGTYYAMDYWGQGTTVTVSS (SEQ ID NO: 92), wherein the amino acid at position 44 is R or G, the amino acid at position 50 is I or absent, the amino acid at position 68 is R or absent, the amino acid at position 71 is I or M, the amino acid at position 73 is R or A, the amino acid at position 77 is A, T or I, the amino acid at position 83 is E or L, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 92, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T or M.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 92, wherein the amino acid at position 44 is R or G, the amino acid at position 50 is absent, the amino acid at position 68 is R, the amino acid at position 71 is I or M, the amino acid at position 73 is R, the amino acid at position 77 is A or I, the amino acid at position 83 is E, the amino acid at position 86 is S or R, the amino acid at position 90 is E or D, and the amino acid at position 92 is T.

In one embodiment, the heavy chain variable region comprises an amino acid sequence set forth in SEQ ID NO: 92, wherein the amino acid at position 44 is R, the amino acid at position 50 is absent, the amino acid at position 68 is R, the amino acid at position 71 is I, the amino acid at position 73 is R, the amino acid at position 77 is A, the amino acid at position 83

is E, the amino acid at position 86 is S, the amino acid at position 90 is E, and the amino acid at position 92 is T or M.

In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 66. In one embodiment, the anti-HLA-A2 antibody comprises a heavy chain variable region comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 66.

In one embodiment, the anti-HLA-A2 antibody comprises a light chain variable region as defined anywhere herein. For example, in one embodiment, the anti-HLA-A2 antibody may comprise a light chain variable region as defined anywhere herein for the humanized anti-HLA-A2 antibody.

In one embodiment, the anti-HLA-A2 antibody is an scFv, scFab or sdAb. In one embodiment, the anti-HLA-A2 antibody is an scFv or scFab. In one embodiment, the anti-HLA-A2 antibody is an sdAb. In one embodiment, the anti-HLA-A2 antibody is an scFab. In one embodiment, the anti-HLA-A2 antibody is an scFv. In one embodiment, the anti-HLA-A2 antibody is an scFv comprising an amino acid sequence set forth in SEQ ID NO: 91. In one embodiment, the anti-HLA-A2 antibody is an scFv comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 91.

In one embodiment, the anti-HLA-A2 antibody is a humanized anti-HLA-A2 antibody. In one embodiment, the anti-HLA-A2 antibody is a human antibody. In one embodiment, the anti-HLA-A2 antibody is a non-humanized antibody. In one embodiment, the anti-HLA-A2 antibody is a non-human antibody.

In one embodiment, the anti-HLA-A2 antibody comprises an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the SEQ ID NOs recited above. In one embodiment, the anti-HLA-A2 antibody is an scFv or scFab comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the SEQ ID NOs recited above.

Also provided is a composition comprising, consisting essentially of, or consisting of, at least one anti-HLA-A2 antibody of the invention.

As used herein, "consisting essentially of", with reference to a composition, means that at least one anti-HLA-A2 antibody of the invention as described here above is the only one therapeutic agent or agent with a biologic activity within said composition.

In another embodiment, there is provided a pharmaceutical composition comprising at least one anti-HLA-A2 antibody of the invention, and a pharmaceutically acceptable carrier.

In one embodiment, the anti-HLA-A2 antibody is a humanized anti-HLA-A2 antibody.

Examples of pharmaceutically acceptable carriers include, but are not limited to, media, solvents, coatings, isotonic and absorption delaying agents, additives, stabilizers, preservatives, surfactants, substances which inhibit enzymatic degradation, alcohols, pH controlling agents, antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); preservatives and propellants.

Examples of pharmaceutically acceptable media include, but are not limited to, water, neutral buffered saline, phosphate buffered saline, normal saline or other physiologically buffered saline, or other solvent such as glycol, glycerol, and oil such as olive oil or an injectable organic ester. A pharmaceutically acceptable medium can also contain liposomes or micelles.

Examples of coating materials include, but are not limited to, lecithin.

Examples of isotonic agents include, but are not limited to, sugars, sodium chloride, and the like.

Examples of agents that delay absorption include, but are not limited to, aluminum monostearate and gelatin.

Examples of additives include, but are not limited to, mannitol, dextran, carbohydrates (such as, for example, glucose, mannose, sucrose or dextrans); glycine, lactose or polyvinylpyrrolidone or other additives such as antioxidants or inert gas, stabilizers or recombinant proteins (e. g. human serum albumin) suitable for in vivo administration.

Examples of suitable stabilizers include, but are not limited to, sucrose, gelatin, peptone, digested protein extracts such as NZ- Amine or NZ-Amine AS.

Pharmaceutically acceptable carriers that may be used in these compositions further include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylenepolyoxypropylene-block polymers, polyethylene glycol and wool fat.

Also provided is a medicament comprising, consisting or consisting essentially of at least one anti-HLA-A2 antibody of the invention, as described hereinabove. In one embodiment, the anti-HLA-A2 antibody is a humanized anti-HLA-A2 antibody.

IV. CHIMERIC ANTIGEN RECEPTORS (CARs)

In one aspect, the present invention provides chimeric antigen receptors (CARs). CARs are chimeric protein molecules that combine antibody-based specificity for a target antigen with an immune cell receptor-activating intracellular domain.

The CARs of the invention comprise an extracellular domain that specifically binds to HLA-A2. The extracellular domain comprises an anti-HLA-A2 antibody of the invention. In one embodiment, the anti-HLA-A2 antibody is a humanized anti-HLA-A2 antibody. The CARs of the invention further comprise a transmembrane domain and a cytoplasmic domain comprising an intracellular signaling domain. In one embodiment, the CARs of the invention are capable of being expressed in a human cell such that the CARs specifically bind to HLA-A2. In other embodiments, the CARs of the invention are capable of being expressed in an immune cell such that the CARs specifically bind to HLA-A2. In one embodiment, the CARs specifically bind to HLA-A*02:01. As would be appreciated by one skilled in the art, the ability of a CAR to bind to HLA-A2 may be detected through the use of techniques known in the art. For example, binding of a CAR to HLA-A2 may be detected through the use of an HLA-A2 tetramer as exemplified herein. In one embodiment, the CARs provided herein have

less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from two or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from three or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from four or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from five or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from six or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from seven or more of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from each of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from two or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one HLA-A subtype selected from each of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs

provided herein have less reactivity to at least one of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least two of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least three of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least four of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least five of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least six of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least seven of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least one of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to at least two of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01, as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*25:01 as compared to a CAR comprising a BB7.2 antibody. In one

embodiment, the CARs provided herein have less reactivity to HLA-A*29:02 as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*30:01 as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*03:01 as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*31:01 as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*33:01 as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*36:01 as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the CARs provided herein have less reactivity to HLA-A*68:01 as compared to a CAR comprising a BB7.2 antibody. In one embodiment, the human cell is an immune cell. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg.

Techniques for determining the reactivity of the CAR of the invention to HLA-A subtypes would be known to those of ordinary skill in the art. For example, the reactivity of the CAR of the invention to HLA-A subtypes may be determined by a single antigen bead assay. Such single antigen bead assays are commercially available (e.g., FlowPRA Single Antigen Antibody; ONE LAMBDA).

In one embodiment, the CAR of the invention has less reactivity to an HLA-A subtype selected from the group comprising of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68, and any combination thereof, as compared to a CAR comprising a BB7.2 antibody, when measured in the conditions of Test A, as described hereinabove.

In one embodiment, the CAR of the invention has less reactivity to an HLA-A subtype selected from the group comprising of HLA-A*25, HLA-A*29, HLA-A*30, and any combination thereof, as compared to a CAR comprising a BB7.2 antibody, when measured in the conditions of Test A, as described hereinabove.

In one embodiment, the CAR of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30,

HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 statistically inferior to the one of a CAR comprising a BB7.2 antibody, e.g., when measured in the conditions of Test A.

In one embodiment, the CAR of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 statistically inferior to the one of a CAR comprising a BB7.2 antibody, e.g., when measured in the conditions of Test A.

In one embodiment, the term "statistically inferior" means that the reactivity (*i.e.*, for example, the relative binding in the conditions of Test A) measured for the CAR of the invention is inferior to the reactivity measured for a CAR comprising a BB7.2 antibody, with a p value of at most about 0.05, preferably of at most about 0.01, more preferably of at most about 0.005, and more preferably of at most about 0.001, in particular when analyzed by 2-way ANOVA, Dunnett post-test.

In one embodiment, the CAR of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 inferior to a CAR comprising a BB7.2 antibody In some embodiments, such a CAR has a relative binding for at least one HLA-A subtype selected from the group comprising HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68 inferior to a CAR comprising a BB7.2 antibody when measured in the conditions of Test A. In certain aspects, the relative binding for such a CAR is at most about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less of the relative binding measured for a CAR comprising a BB7.2 antibody.

In one embodiment, the CAR of the invention has a reactivity to at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 inferior to a CAR comprising a BB7.2 antibody In some embodiments, such a CAR has a relative binding for at least one HLA-A subtype selected from the group comprising HLA-A*25, HLA-A*29, HLA-A*30 inferior to a CAR comprising a BB7.2 antibody when measured in the conditions of Test A. In certain aspects, the relative binding measured for such a CAR is at most about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less of the binding of a CAR comprising a BB7.2 antibody.

In one embodiment, the CAR is capable of being expressed in an immune cell such that the immune cell is activated by HLA-A2. In one embodiment, the immune cell is activated by HLA-A*02:01. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg. In one embodiment, the immune cell is a human immune cell. In one embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the T cell is a human T cell. In one embodiment, the Treg is a human Treg.

In one embodiment, the CAR competes for binding to HLA-A2 with an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the CAR binds to the same HLA-A2 epitope as an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the CAR competes for binding to HLA-A2 with the BB7.2 antibody. In one embodiment, the CAR binds to the same HLA-A2 epitope as a BB7.2 antibody. The BB7.2 antibody may be isolated from the BB7.2 hybridoma (ATCC Deposit No. HB-82).

In one embodiment, the CAR of the invention comprises: an extracellular domain, comprising an anti-HLA-A2 antibody; a transmembrane domain; and a cytoplasmic domain comprising an intracellular signaling domain. In one embodiment, the anti-HLA-A2 antibody is a humanized anti-HLA-A2 antibody.

In one embodiment, the chimeric receptor further comprises a Tag and/or a leader sequence.

In one embodiment, the chimeric receptor further comprises a tag, such as, for example, a tag for quality control, enrichment, tracking in vivo and the like. Said Tag may be localized N-terminally, C-terminally and/or internally. Examples of tags that may be used in the chimeric receptor of the invention are well known by the skilled artisan. For example, but without limitation, a tag used in the invention can be a tag selected from the group comprising or consisting of Hemagglutinin Tag, Poly Arginine Tag, Poly Histidine Tag, Myc Tag, Strep Tag, S-Tag, HAT Tag, 3x Flag Tag, Calmodulin-binding peptide Tag, SBP Tag, Chitin binding domain Tag, GST Tag, Maltose-Binding protein Tag, Fluorescent Protein Tag (*e.g.*, eGFP), T7 Tag, V5 Tag and Xpress Tag.

The extracellular domain is a target-specific binding element also sometimes referred to as a targeting arm of the CAR. The extracellular domain is chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. A CAR of the present invention is engineered to target a cell displaying HLA-A2 by engineering an appropriate extracellular domain that specifically binds to an HLA-A2 epitope. The target-specific binding element or antigen binding domain of the CAR of the present invention may be referred to herein as an anti-HLA-A2 binding domain. In some embodiments, the anti-HLA-A2 binding domain may be a humanized anti-HLA-A2 binding domain.

The transmembrane domain is attached to the extracellular domain and the cytoplasmic domain of the CAR. The transmembrane domain is capable of signaling to the intracellular signaling domain(s) of the cytoplasmic domain whenever the extracellular domain of the CAR is bound to a target.

The cytoplasmic domain which includes the intracellular signaling domain of the CAR is responsible for activation of at least one of the physiological effector functions of the immune cell (e.g., regulatory T cell) in which the CAR has been placed in. The term "effector function" refers to a specialized function of an immune cell. For example, an effector function

of a regulatory T cell may include suppressing or downregulating the induction and/or proliferation of other immune cells. In addition, the effector function of Tregs may include effects on non-immune cells that result in an improved clinical state such as promoting tissue repair or regeneration. Thus, the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the immune cell to perform its specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

In one embodiment, there may be a spacer domain (or linker or hinge) incorporated between the antigen binding domain and the transmembrane domain of the CAR or between the intracellular signaling domain and the transmembrane domain of the CAR. As defined hereinabove, spacer domain, linker and hinge are oligo- or polypeptides that function to link the transmembrane domain to, either the antigen binding domain or, the intracellular signaling domain in the polypeptide chain. A spacer domain may comprise, e.g., up to 300 amino acids, 10 to 100 amino acids, 25 to 75 amino acids, or 25 to 50 amino acids, or amino acids of any subranges or individual numerical values within these ranges.

Extracellular Domain

The extracellular domain comprises an anti-HLA-A2 antibody of the present invention. In one embodiment, the extracellular domain comprises a humanized anti-HLA-A2 antibody of the present invention.

In one embodiment, the extracellular domain comprises an anti-HLA-A2 antibody of the present invention which is an scFv, scFab or sdAb. In one embodiment, the extracellular domain comprises an anti-HLA-A2 antibody of the present invention which is an scFv or scFab. In one embodiment, the extracellular domain comprises an anti-HLA-A2 antibody of the present invention which is an sdAb. In one embodiment, the extracellular domain comprises an anti-HLA-A2 antibody of the present invention which is an scFv. In one embodiment, the extracellular domain comprises an anti-HLA-A2 antibody of the present invention which is an

scFab. In one embodiment, the extracellular domain comprises any humanized anti-HLA-A2 antibody of the present invention wherein the humanized anti-HLA-A2 antibody is an scFv, scFab or sdAb. In one embodiment, the extracellular domain comprises any humanized anti-HLA-A2 antibody of the present invention wherein the humanized anti-HLA-A2 antibody is an scFv or scFab. In one embodiment, the extracellular domain comprises a humanized anti-HLA-A2 antibody of the present invention which is an sdAb. In one embodiment, the extracellular domain comprises a humanized anti-HLA-A2 antibody of the present invention which is an scFv. In one embodiment, the extracellular domain comprises a humanized anti-HLA-A2 antibody of the present invention which is an scFab.

In some embodiments, the extracellular domain may comprise a hinge, where the transmembrane domain is attached to the extracellular region of the CAR, e.g., the antigen binding domain of the CAR, via the hinge. In one embodiment, the hinge may be from a human protein. For example, in one embodiment, the hinge may be a human Ig (immunoglobulin) hinge, e.g., an IgG4 hinge, or a CD8α hinge. In some instances, the extracellular domain of the CAR of the invention may comprise a CD8α hinge. In one embodiment, the hinge region comprises a stalk region of CD8α. In one embodiment, the CD8 hinge may be encoded by the nucleic acid sequence of SEQ ID NO: 15 of US Patent No. 9,102,760. In one embodiment, the CD8 hinge may comprise the amino acid sequence of SEQ ID NO: 21 of US Patent No. 9,102,760. In another embodiment, the CD8 hinge may comprise the amino acid sequence of SEQ ID NO: 21 of US Patent No. 9,102,760. In one embodiment, the hinge or spacer may comprise the amino acid sequence of SEQ ID NO: 115 or 219 in Table 3. In one embodiment, the hinge or spacer may be encoded by a nucleic acid sequence of SEQ ID NO: 159 or 220 in Table 4.

Transmembrane Domain

The transmembrane domain may be derived either from a natural source or a synthetic source. In one embodiment, the transmembrane domain may be derived from a natural source, for example, from any membrane-bound or transmembrane protein.

In one embodiment, the transmembrane domain of the CAR may be derived from a transmembrane domain that is naturally associated with one of the domains of the CAR. In other embodiments, the transmembrane domain may be selected or modified by amino acid

substitution to avoid binding of such domains to the transmembrane domain of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

In one embodiment, the transmembrane domain may include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid associated with the extracellular region of the protein from which the transmembrane is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the cytoplasmic region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids of the cytoplasmic region).

In one embodiment, the transmembrane domain may comprise a transmembrane domain of a protein selected from the group consisting of CD3 gamma, CD3 delta, CD3 epsilon, CD3 zeta, the alpha chain of the T-cell receptor, the beta chain of the T-cell receptor, the gamma chain of the T-cell receptor, the delta chain of the T-cell receptor, CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, KIRDS2, OX40, CD2, CD27, LFA-1 (CDl la, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFl), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, PD1, ITGAX, CDl1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CDIOO (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C and CD154, and any combination thereof. In one embodiment, the transmembrane domain may comprise a transmembrane domain of CD28. In one embodiment, the CD28 transmembrane domain is encoded by the nucleic acid sequence of SEQ ID NO: 160 in Table 4. In one embodiment, the CD28 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 116 in Table 3. In another embodiment, the CD28 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 116 in Table 3.

In one embodiment, the transmembrane domain may comprise a transmembrane domain of CD8. In one embodiment, the CD8 transmembrane domain is encoded by the nucleic acid sequence of SEQ ID NO: 16 of US Patent No. 9,102,760. In one embodiment, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 22 of US Patent No. 9,102,760. In another embodiment, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 22 of US Patent No. 9,102,760.

In one embodiment, the CD8 transmembrane domain is encoded by the nucleic acid sequence of SEQ ID NO: 224 in Table 4. In one embodiment, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 223 in Table 3.

In other embodiments, the transmembrane domain may be synthetic, in which case it may comprise predominantly hydrophobic residues including leucine and valine. In one embodiment, a triplet of phenylalanine, tryptophan and valine may be found at each end of a synthetic transmembrane domain.

In one embodiment, a short oligo- or polypeptide linker may form a linkage between the transmembrane domain and the cytoplasmic domain of the CAR. In one embodiment, the linker may comprise between 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids in length. In one embodiment, the linker may comprise a glycine-serine doublet.

Cytoplasmic Domain

In one embodiment, intracellular signaling domains for use in the CAR of the invention may include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the invention include those derived from CD3 zeta, FcR gamma, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22,

CD79a, CD79b, and CD66d. In one embodiment, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In an embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

In one embodiment, the intracellular signaling domain comprises a functional signaling domain of a protein selected from the group consisting of CD3 gamma, CD3 delta, CD3 epsilon, CD3 zeta, FcR gamma (e.g., FCγRI, RCγRIIA, FcγRIIB1, FcγRIIB2, FcγRIIIA, or FcγRIIIB), FcR alpha (e.g., FcαRI), FcR epsilon (e.g., FcεRI or FcεRII), CD5, CD22, CD79a, CD79b, DAP10, DAP12 and CD66d, and any combination thereof. In one embodiment, the intracellular signaling domain comprises or consists of a primary signaling domain of CD3-zeta.

It is known that signals generated through the TCR alone may be insufficient for full activation of the T cell and that a secondary or co-stimulatory signal may also be required. Thus, in certain embodiments, T cell activation may be mediated by two classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences). In one embodiment, the intracellular signaling domain may further comprise a costimulatory domain.

In one embodiment, the intracellular signaling domain of the CAR can comprise the CD3-zeta signaling domain by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the invention. For example, the intracellular signaling domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include

OX40, CD27, CD28, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18), TNFR1 (CD120a/TNFRSF1A), TNFR2 (CD120b/TNFRSF1B), CTLA-4 (CD152), CD95, ICOS (CD278), 4-1BB (CD137), CD2, CD30, CD40, PD-1, CD7, LIGHT, NKG2C, B7-H3, ICAM-1, a ligand that specifically binds with CD83, IL2ra (CD25), IL6Ra (CD126), IL-7Ra (CD127), IL-13RA1, IL-13RA2, IL-33R(IL1RL1), IL-10RA, IL-10RB, IL-4R, IL-5R (CSF2RB), ARHR, BAFF receptor, IL-21R, TGFbR1, TGFbR2, TGFbR3, common gamma chain, an MHC class I molecule, BTLA and a Toll ligand receptor, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160 (BY55), CD19, CD19a, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CitgbD18, ITGB7, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKG2D and the like.

The intracellular signaling sequences within the cytoplasmic portion of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, for example, between 1 and 10 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) in length may form the linkage between intracellular signaling sequences. In one embodiment, a glycine-serine doublet can be used as a suitable linker. In one embodiment, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable linker.

In one embodiment, the intracellular signaling domain is designed to comprise two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains. In an embodiment, the two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, e.g., a linker molecule described herein. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

In one embodiment, the cytoplasmic domain may comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the cytoplasmic domain may comprise the signaling domain of CD3-zeta and the signaling domain of 4-IBB. In yet

another embodiment, the cytoplasmic domain may comprise the signaling domain of CD3-zeta and the signaling domains of CD28 and 4-1BB.

In one embodiment, the cytoplasmic domain may comprise the signaling domain of CD28 and the signaling domain of CD3-zeta, wherein the signaling domain of CD28 is encoded by the nucleic acid sequence set forth in SEQ ID NO: 161 in Table 4 and the signaling domain of CD3-zeta is encoded by the nucleic acid sequence set forth in SEQ ID NO: 162 in Table 4.

In one embodiment, the cytoplasmic domain may comprise the signaling domain of CD28 and the signaling domain of CD3-zeta, wherein the signaling domain of CD28 comprises the amino acid sequence of SEQ ID NO: 117 in Table 3 and the signaling domain of CD3-zeta comprises the amino acid sequence of SEQ ID NO: 118 in Table 3.

In one embodiment, the cytoplasmic domain may comprise the signaling domain of CD28 and the signaling domain of CD3-zeta, wherein the signaling domain of CD28 comprises the amino acid sequence set forth in SEQ ID NO: 117 in Table 3 and the signaling domain of CD3-zeta comprises the amino acid sequence set forth in SEQ ID NO: 118 in Table 3.

In one embodiment, the cytoplasmic domain may comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB is encoded by the nucleic acid sequence set forth in SEQ ID NO: 17 of US Patent No. 9,102,760 and the signaling domain of CD3-zeta is encoded by the nucleic acid sequence set forth in SEQ ID NO: 162 in Table 4.

In one embodiment, the cytoplasmic domain may comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the amino acid sequence of SEQ ID NO: 23 of US Patent No. 9,102,760 and the signaling domain of CD3-zeta comprises the amino acid sequence of SEQ ID NO: 118 in Table 3.

In one embodiment, the cytoplasmic domain may comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the amino acid sequence set forth in SEQ ID NO: 23 of US Patent No. 9,102,760 and the signaling domain of CD3-zeta comprises the amino acid sequence set forth in SEQ ID NO: 118 in Table 3.

In one embodiment, the CAR comprises an amino acid sequence set forth in SEQ ID NO: 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138 or 213. In one embodiment, the CAR comprises an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138 or 213.

V. NUCLEIC ACIDS AND VECTORS

In some embodiments, the invention provides a nucleic acid encoding an anti-HLA-A2 antibody of the present invention. In some embodiments, the invention provides a nucleic acid encoding a humanized anti-HLA-A2 antibody of the present invention. In some embodiments, the invention provides a nucleic acid encoding a protein comprising the amino acid sequence set forth in SEQ ID NO: 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, or 91. In some embodiments, the invention provides a nucleic acid encoding a protein comprising the amino acid sequence set forth in SEQ ID NO: 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, or 112. In some embodiments, the invention provides a nucleic acid encoding a protein comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, or 91. In some embodiments, the invention provides a nucleic acid encoding a protein comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, or 112. In some embodiments, the invention provides a nucleic acid comprising the nucleic acid sequence set forth in SEQ ID NO: 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, or 158. In some embodiments, the invention provides a nucleic acid comprising a nucleic acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, or 158. In some embodiments, the invention provides a protein encoded by a nucleic acid comprising the nucleic acid sequence set

forth in SEQ ID NO: 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, or 158. In some embodiments, the invention provides a protein encoded by a nucleic acid comprising a nucleic acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, or 158. In some embodiments, the invention provides a nucleic acid encoding a protein comprising the amino acid sequence set forth in SEQ ID NO: 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, or 71. In some embodiments, the invention provides a nucleic acid encoding a protein comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, or 71.

In some embodiments, the invention provides a nucleic acid encoding a chimeric antigen receptor (CAR) of the present invention. The CAR-encoding nucleic acid sequences of the invention may encode CARs which comprise an extracellular domain as described anywhere herein.

The extracellular domain may comprise an anti-HLA-A2 antibody of the present invention. The extracellular domain may comprise a humanized anti-HLA-A2 antibody of the present invention. In one embodiment, the extracellular domain may further comprise a leader sequence. In one embodiment, the leader sequence comprises an amino acid sequence set forth in SEQ ID NO: 113. In one embodiment, the extracellular domain comprises a hinge region, wherein the anti-HLA-A2 binding domain is connected to the transmembrane domain by the hinge region. In one embodiment, the hinge region comprises a stalk region of CD8α.

The nucleic acid sequences of the invention may encode CARs which comprise a transmembrane domain as described anywhere herein. For example, in one embodiment, the transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of CD3 gamma, CD3 delta, CD3 epsilon, CD3 zeta, the alpha chain of the T-cell receptor, the beta chain of the T-cell receptor, the gamma chain of the T-cell receptor, the delta chain of the T-cell receptor, CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, KIRDS2, OX40, CD2, CD27, LFA-1 (CDl la, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7,

NKp80 (KLRFl), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, PD1, ITGAX, CDl1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CDIOO (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C and CD154, and any combination thereof. In one embodiment, the transmembrane domain comprises a transmembrane domain of CD28.

The nucleic acid sequences of the invention may encode CARs which comprise a cytoplasmic domain as described anywhere herein. Furthermore, the cytoplasmic domain may comprise an intracellular signaling domain as described anywhere herein. For example, in one embodiment, the intracellular signaling domain comprises a functional signaling domain of a protein selected from the group consisting of CD3 gamma, CD3 delta, CD3 epsilon, CD3 zeta, FcR gamma (e.g., FCγRI, RCγRIIA, FcγRIIB1, FcγRIIB2, FcγRIIIA, or FcγRIIIB), FcR alpha (e.g., FcαRI), FcR epsilon (e.g., FcεRI or FcεRII), CD5, CD22, CD79a, CD79b, DAP10, DAP12 and CD66d, and any combination thereof. In one embodiment, the intracellular signaling domain comprises a functional signaling domain of CD3 zeta. In one embodiment, the intracellular signaling domain further comprises a costimulatory domain. The costimulatory domain of the CARs encoded by the nucleic acid sequences of the invention may be a costimulatory domain as described anywhere herein. For example, in one embodiment, the costimulatory domain comprises a functional signaling domain of a protein selected from the group consisting of OX40, CD27, CD28, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18), TNFR1 (CD120a/TNFRSF1A), TNFR2 (CD120b/TNFRSF1B), CTLA-4 (CD152), CD95, ICOS (CD278), 4-1BB (CD137), CD2, CD30, CD40, PD-1, CD7, LIGHT, NKG2C, B7-H3, ICAM-1, a ligand that specifically binds with CD83, IL2ra (CD25), IL6Ra (CD126), IL-7Ra (CD127), IL-13RA1, IL-13RA2, IL-33R(IL1RL1), IL-10RA, IL-10RB, IL-4R, IL-5R (CSF2RB), ARHR, BAFF receptor, IL-21R, TGFbR1, TGFbR2, TGFbR3, common gamma chain, an MHC class I molecule, BTLA and a Toll ligand receptor, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160 (BY55), CD19, CD19a, CD4, CD8alpha, CD8beta, IL2R

beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CitgbD18, ITGB7, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKG2D and any combination thereof. In one embodiment, the costimulatory domain comprises a functional signaling domain of a protein selected from the group consisting of CD28, 4-1BB and a combination thereof. In one embodiment, the costimulatory domain comprises a functional signaling domain of CD28. In one embodiment, the costimulatory domain comprises a functional signaling domain of 4-1BB. In one embodiment, the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

The nucleic acid sequences of the present invention may be isolated nucleic acid sequences.

In one embodiment, the nucleic acid is provided as a messenger RNA transcript. In one embodiment, the nucleic acid is provided as a DNA construct.

In one embodiment, there is provided a recombinant DNA construct comprising sequences encoding a CAR, wherein the CAR comprises: (i) an extracellular domain comprising an anti-HLA-A2 antibody; (ii) a transmembrane domain; and (iii) a cytoplasmic domain comprising an intracellular signaling domain, wherein the encoded CAR is capable of being expressed in a human cell such that the CAR is capable of specifically binding to HLA-A2. In one embodiment, the CAR is capable of specifically binding to HLA-A*02:01. In one embodiment, the anti-HLA-A2 antibody is a humanized anti-HLA-A2 antibody. In one embodiment, the human cell is an immune cell. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg.

In one embodiment, there is provided a recombinant DNA construct comprising sequences encoding a CAR, wherein the CAR comprises: (i) an extracellular domain comprising an anti-HLA-A2 antibody; (ii) a transmembrane domain; and (iii) a cytoplasmic

domain comprising an intracellular signaling domain, wherein the encoded CAR is capable of being expressed in a T regulatory cell (Treg) such that the CAR is capable of specifically binding to HLA-A2. In one embodiment, the CAR is capable of specifically binding to HLA-A*02:01. In one embodiment, binding of a CAR to HLA-A2 may be detected through the use of an HLA-A2 tetramer as exemplified herein. In one embodiment, the anti-HLA-A2 antibody is a humanized anti-HLA-A2 antibody. In one embodiment, the Treg is a human Treg.

In one embodiment, the encoded CAR is capable of being expressed in an immune cell such that the immune cell is activated by HLA-A2. In one embodiment, the immune cell is activated by HLA-A*02:01. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is a Treg. In one embodiment, the immune cell is a human immune cell. In one embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the T cell is a human T cell. In one embodiment, the Treg is a human Treg.

In one embodiment, the encoded CAR competes for binding to HLA-A2 with an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the encoded CAR binds to the same HLA-A2 epitope as an antibody comprising: a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183; a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185; a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187; a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188; a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ

ID NO: 189; and a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190. In one embodiment, the encoded CAR competes for binding to HLA-A2 with a BB7.2 antibody. In one embodiment, the encoded CAR binds to the same HLA-A2 epitope as a BB7.2 antibody.

In some embodiments, the invention provides a nucleic acid encoding a protein comprising the amino acid sequence set forth in SEQ ID NO: 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138 or 213. In some embodiments, the invention provides a nucleic acid encoding a protein comprising an amino acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138 or 213. In some embodiments, the invention provides a nucleic acid comprising the nucleic acid sequence set forth in SEQ ID NO: 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 or 214. In some embodiments, the invention provides a nucleic acid comprising a nucleic acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 or 214. In some embodiments, the invention provides a protein encoded by a nucleic acid comprising the nucleic acid sequence set forth in SEQ ID NO: 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 or 214. In some embodiments, the invention provides a protein encoded by a nucleic acid comprising a nucleic acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 or 214.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the nucleic acid of interest can be produced synthetically, rather than cloned.

The present invention further provides a vector comprising an anti-HLA-A2 antibody-encoding nucleic acid molecule or a CAR-encoding nucleic acid molecule. In one embodiment, the invention provides a vector comprising a humanized anti-HLA-A2 antibody-encoding nucleic acid molecule. In one embodiment, the invention provides vectors in which such a nucleic acid molecule is inserted.

In one embodiment, the present invention provides a vector comprising a nucleic acid sequence as described anywhere herein.

In some embodiments, the present invention provides a vector comprising a nucleic acid that encodes any of the anti-HLA-A2 antibodies described herein. In other embodiments, the present invention provides a vector comprising a nucleic acid that encodes any of the humanized anti-HLA-A2 antibodies described herein. In some embodiments, the present invention provides a vector comprising a nucleic acid comprising the nucleic acid sequence set forth in SEQ ID NO: 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, or 158. In some embodiments, the invention provides a vector comprising a nucleic acid comprising a nucleic acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, or 158. In certain aspects, provided is a host cell that includes such a vector.

In one embodiment, the present invention provides a vector comprising a CAR-encoding nucleic acid of the invention. In some embodiments, the present invention provides a vector comprising a nucleic acid sequence set forth in SEQ ID NO: 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 or 214. In some embodiments, the invention provides a vector comprising a nucleic acid comprising a nucleic acid sequence having about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182 or 214.

In one embodiment, the vectors of the invention may be transduced into a cell. In one embodiment, the vectors of the invention may be transduced or engineered via non-viral vector methods into a human cell. In one embodiment, the vectors of the invention can be transduced

into an immune cell. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the immune cell is a T regulatory cell (Treg). In one embodiment, the immune cell is a T cell. In one embodiment, the T cell may be a Treg. In one embodiment, the vector is capable of expressing the CAR in mammalian immune cells. In one embodiment, the mammalian immune cell is a human immune cell. In one embodiment, the vector is capable of expressing the CAR in mammalian regulatory immune cells. In one embodiment, the mammalian regulatory immune cell is a human regulatory immune cell. In one embodiment, the vector is capable of expressing the CAR in mammalian T cells. In one embodiment, the mammalian T cells are mammalian T regulatory cells. In one embodiment, the mammalian T cell is a human T cell. In one embodiment, the mammalian T cell is a human T regulatory cell.

In one embodiment, the vector is a cloning or expression vector, e.g., a vector including, but not limited to, one or more plasmids (e.g., expression plasmids, cloning vectors, minicircles, minivectors, double minute chromosomes), retroviral and lentiviral vector constructs.

The present invention includes retroviral and lentiviral vector constructs expressing a CAR that can be directly transduced into a cell. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

In brief summary, the expression of natural or synthetic nucleic acids encoding CARs is typically achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

In addition to the methods described above, the following methods may be used.

The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector. In another embodiment, genome editing techniques such as CRISPR-Cas9 or TALEN-based techniques may be used to introduce nucleic acids encoding CARs of the present invention into the genome of recipient immune cells (Delhove, J. M. K. M., et al., 2017; Eyquem, J. et al., 2017).

The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, poxviruses, and lentiviruses (see, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362). In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

Additional transcriptionally active elements, *e.g.*, promoters and enhancers, may regulate the frequency of transcriptional initiation. Typically, regarding core promoter, these

are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well, and enhancers elements are generally located 500-2000bp upstream of the start site. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1α (EF-1α). Another example of a suitable promoter is phosphoglycerate kinase (PGK) promoter. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate

regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art (see, for example, Sambrook et al., 2001). For example, a polynucleotide may be introduced into a host cell by calcium phosphate transfection. Alternatively, a host cell may be transfected with a polynucleotide such as RNA by electroporation (see, for example, WO 2007/065957).

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle). Other methods of state-of-the-art targeted delivery of

nucleic acids are available, such as delivery of polynucleotides with targeted nanoparticles or other suitable sub-micron sized delivery system.

In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, Mo.; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, N.Y.); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous

solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

VI. CAR-MODIFIED IMMUNE CELLS

CAR-encoding nucleic acids of the invention may be introduced into immune cells to generate CAR-modified immune cells expressing CARs of the present invention, which modified immune cells find use as disclosed herein. CAR-modified immune cells may also be referred to herein as “CAR-engineered immune cells”. In an embodiment, there is provided a modified immune cell comprising a CAR as described anywhere herein. In another embodiment, there is provided an immune cell comprising an expression vector as described anywhere herein. The immune cell may be any immune cell suitable for use in cellular therapy. In one embodiment, the immune cell may be a human immune cell.

In one embodiment, the immune cell is selected from the group consisting of a lymphocyte, a myeloid-derived cell, and any combination thereof. In one embodiment, the lymphocyte is selected from the group consisting of a T cell, a B cell, a natural killer (NK) cell, and any combination thereof. In one embodiment, the immune cell is a T cell. In one embodiment, the T cell is selected from the group consisting of a $CD4^+$ T cell, a $CD8^+$ T cell, a γδ T cell, a double negative (DN) T cell, and any combination thereof. In one embodiment, the $CD4^+$ T cell is selected from the group consisting of a T helper cell, a regulatory T cell, and any combination thereof. In one embodiment, the immune cell is a regulatory T cell. In one

embodiment, the Treg is a thymus derived Treg or an adaptive or induced Treg. In one embodiment, the Treg is a $CD4^+FOXP3^+$ regulatory T cell or a $CD4^+FOXP3^-$ regulatory T cell (T_R1 cell). In one embodiment, the Treg is a $CD4^+FOXP3^+$ regulatory T cell. In one embodiment, the Treg is a $CD4^+FOXP3^-$ regulatory T cell (T_R1 cell). In one embodiment, the $CD8^+$ T cell is a cytotoxic $CD8^+$ T cell or a $CD8^+$ regulatory T cell. In one embodiment, the $CD8^+$ T cell is a $CD8^+$ regulatory T cell. In one embodiment, the $CD8^+$ regulatory T cell is selected from the group consisting of a $CD8^+CD28^-$ regulatory T cell, a $CD8^+CD103^+$ regulatory T cell, a $CD8^+FoxP3^+$ regulatory T cell, a $CD8^+CD122^+$ regulatory T cell and any combination thereof. In one embodiment, the $CD8^+$ regulatory T cell is an $INF\gamma^+IL10^+IL34^+$ $CD8^+CD45RC^{low}$ regulatory T cell. In one embodiment, the γδ T cell is a regulatory γδ T cell. In one embodiment, the DN T cell is a regulatory DN T cell. In one embodiment, the immune cell is a B cell. In one embodiment, the B cell is a regulatory B cell. In one embodiment, the regulatory B cell is a $CD19^+CD24^{hi}CD38^{hi}$ B cell. In one embodiment, the NK cell is a regulatory NK cell. In one embodiment, the myeloid-derived cell is selected from the group consisting of a neutrophil, an eosinophil, a basophil, a monocyte, a macrophage, a dendritic cell, or any combination thereof. In one embodiment, the macrophage is a regulatory macrophage. In one embodiment, the dendritic cell is a regulatory dendritic cell.

In one embodiment, the immune cell is a regulatory immune cell. The immune cell may be any regulatory immune cell suitable for use in cellular therapy (see, e.g., Wood, K.J. et al., 2012; Papp, G. et al., 2017). In one embodiment, the regulatory immune cell may be a human regulatory immune cell. In one embodiment, the regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In one embodiment, the regulatory immune cell is a $CD8^+$ regulatory T cell (see, e.g., Guillonneau, C. et al., 2010). In one embodiment, the $CD8^+$ regulatory T cell is selected from the group consisting of a $CD8^+CD28^-$ regulatory T cell, a $CD8^+CD103^+$ regulatory T cell, a $CD8^+FoxP3^+$ regulatory T cell, a $CD8^+CD122^+$ regulatory T cell, and any combination thereof. In one embodiment, the $CD8^+$ regulatory T cell is an $INF\gamma^+IL10^+IL34^+$ $CD8^+CD45RC^{low}$ regulatory T cell. In one embodiment, the regulatory immune cell is a regulatory γδ T cell (see, e.g., Wesch,

D., et al., 2014). In one embodiment, the immune cell is a regulatory DN T cell (see, e.g., Juvet, S. C., et al., 2012). In one embodiment, the regulatory immune cell is a regulatory B cell (see, e.g., Chong, A.S., et al., 2017). In one embodiment, the regulatory B cell is a $CD19^{+}CD24^{hi}CD38^{hi}$ B cell. In one embodiment, the regulatory immune cell is a regulatory NK cell (see, e.g., Fu, B. et al., 2013; Crome, S. Q., et al., 2013; Crome, S. Q., et al., 2017). In one embodiment, the regulatory immune cell is a regulatory macrophage (see, e.g., Hutchinson, J. A., et al., 2017). In one embodiment, the regulatory immune cell is a regulatory dendritic cell. In one embodiment, the regulatory immune cell is a regulatory T cell. In one embodiment, the Treg is a thymus derived Treg or an adaptive or induced Treg. In one embodiment, the Treg is a $CD4^{+}FOXP3^{+}$ regulatory T cell or a $CD4^{+}FOXP3^{-}$ regulatory T cell (T_R1 cell). In one embodiment, the Treg is a $CD4^{+}FOXP3^{+}$ regulatory T cell. In one embodiment, the Treg is a $CD4^{+}FOXP3^{-}$ regulatory T cell (T_R1 cell).

In one embodiment, the regulatory immune cell has the following phenotype: $CD4^{+}CD25^{+}$, such as, for example, $CD4^{+}CD25^{+}CD127^{-}$, such as, for example, $CD4^{+}CD25^{+}CD127^{-}CD45RA^{+}$. In further embodiments, the regulatory immune cell has the following phenotype: $FoxP3^{+}CD4^{+}CD25^{+}$, such as, for example, $FoxP3^{+}CD4^{+}CD25^{+}CD127^{-}$, such as, for example, $FoxP3^{+}CD4^{+}CD25^{+}CD127^{-}CD45RA^{+}$.

In one embodiment, the immune regulatory cell presents at least one of the following phenotypes: $CD4^{+}CD25^{+}$, FoxP3+, $CD127^{lo/-}$, CTLA-4^{+}, $CD39^{+}$, $Helios^{+}$, $CD62L^{+/hi}$, $VLA4^{+}$, $LFA1^{+}$, $CD49^{bint}$, $ITGb^{7int}$, PSGL-1^{+}, $ICOS^{+}$, $GITR^{+}$, PD-1^{int}, $Perf^{lo/-}$, $CCR7^{+}$. In one embodiment, the immune regulatory cell does not express Granzyme A and/or Granzyme B.

In one embodiment, the determination of the expression level of molecules is conducted by flow cytometry, immunofluorescence or image analysis, for example high content analysis. Preferably, the determination of the expression level of molecules is conducted by flow cytometry. In one embodiment, before conducting flow cytometry analysis, cells are fixed and permeabilized, thereby allowing detecting intracellular proteins.

In one embodiment, the determination of the expression level of a molecule in a cell population comprises determining the percentage of cells of the cell population expressing the molecule (*i.e.* cells "+" for the molecule). Preferably, said percentage of cells expressing the molecule is measured by FACS.

The terms "expressing (or +)" and "not expressing (or -)" are well known in the art and refer to the expression level of the cell marker of interest, in that the expression level of the cell marker corresponding to "+" is high or intermediate, also referred as "+/-", and the expression level of the cell marker corresponding to "-" is null.

The term "low" or "lo" or "lo/-" is well known in the art and refers to the expression level of the cell marker of interest, in that the expression level of the cell marker is low by comparison with the expression level of that cell marker in the population of cells being analyzed as a whole. More particularly, the term “lo” refers to a distinct population of cells that express the cell marker at a lower level than one or more other distinct population of cells.

The term "high" or "hi" or "bright" is well known in the art and refers to the expression level of the cell marker of interest, in that the expression level of the cell marker is high by comparison with the expression level of that cell marker in the population of cells being analyzed as a whole.

Generally, cells in the top 2, 3, 4, or 5% of staining intensity are designated "hi", with those falling in the top half of the population categorized as being "+". Those cells falling below 50%, of fluorescence intensity are designated as "lo" cells and below 5% as "-" cells.

The expression level of the cell marker of interest is determined by comparing the Median Fluorescence Intensity (MFI) of the cells from the cell population stained with fluorescently labeled antibody specific for this marker to the fluorescence intensity (FI) of the cells from the same cell population stained with fluorescently labeled antibody with an irrelevant specificity but with the same isotype, the same fluorescent probe and originated from the same specie (referred as Isotype control). The cells from the population stained with fluorescently labeled antibody specific for this marker and that show equivalent MFI or a lower MFI than the cells stained with the isotype controls are not expressing this marker and then are designated (-) or negative. The cells from the population stained with fluorescently labeled antibody specific for this marker and that show a MFI value superior to the cells stained with the isotype controls are expressing this marker and then are designated (+) or positive.

In one embodiment, the immune cell of the invention expresses at its cell surface a CAR of the invention, and another receptor (herein referred to as “second receptor”), that binds to

another ligand than HLA-A2. According to the invention, this second receptor comprises an extracellular ligand binding domain, optionally a hinge, optionally a transmembrane domain, and an intracellular signaling domain, as previously described.

In one embodiment, the second receptor is endogenous (such as, for example, the endogenous TCR). In another embodiment, the second receptor is exogenous, and its expression is induced in the immune cell of the invention by transformation or transduction of a nucleic acid encoding it. Said exogenous receptor may be an exogenous TCR or a chimeric antigen receptor. Therefore, in one embodiment, the immune cell of the invention expresses two chimeric antigen receptors, wherein the first one recognizes HLA-A2, and the second one recognizes a distinct ligand.

In another embodiment, the immune cell of the invention expresses at its cell surface a CAR of the invention, and another receptor (herein referred to as "second receptor"), that binds to another epitope in HLA-A2. According to the invention, this second receptor comprises an extracellular ligand binding domain, optionally a hinge, optionally a transmembrane domain, and an intracellular signaling domain, as previously described.

In another embodiment, the immune cell of the invention expresses two CARs, wherein the first one recognizes a first epitope of HLA-A2, and the second one recognizes a distinct epitope on HLA-A2.

In one embodiment, the CAR of the invention comprises a first intracellular signaling domain, and the second receptor comprises a distinct second intracellular signaling domain. In a first embodiment, the CAR of the invention comprises a T cell primary signaling domain (such as, for example, CD3zeta), and the second receptor comprises a costimulatory signaling domain (such as, for example, of 4-1BB, CD28 or a combination of costimulatory signaling domain of 4-1BB and CD28). In a second embodiment, the CAR of the invention comprises a costimulatory signaling domain (such as, for example, of 4-1BB, CD28 or a combination of costimulatory signaling domain of 4-1BB and CD28), and the second receptor comprises a T cell primary signaling domain (such as, for example, CD3zeta).

Consequently, according to these embodiments, the complete activation of the immune cell of the invention requires both the binding of the CAR of the invention to HLA-A2, and the binding of the second receptor to the ligand to which it is directed.

In one embodiment, the ligand recognized by the second receptor is expressed or present at a diseased tissue or organ, or at a site of an autoimmune response.

Examples of ligands that may be recognized by the second receptor include, but are not limited to, food antigens from the common human diet, autoantigens, inhaled allergens, ingested allergens or contact allergens.

The term "food antigen from common human diet" refers to an immunogenic peptide, which comes from foodstuffs common for humans, such as food antigens of the following non-limiting list: bovine antigens such as lipocalin, Ca-binding S100, alpha-lactalbumin, lactoglobulins such as beta-lactoglobulin, bovine serum albumin, caseins. Food-antigens may also be atlantic salmon antigens such as parvalbumin; chicken antigens such as, for example, ovomucoid, ovalbumin, Ag22, conalbumin, lysozyme or chicken serum albumin; peanut antigens; shrimp antigens such as tropomyosin; wheat antigens such as agglutinin or gliadin; celery antigens such as celery profilin; carrot antigens such as carrot profilin; apple antigens such as thaumatin, apple lipid transfer protein, or apple profilin; pear antigens such as pear profilin, or isoflavone reductase; avocado antigens such as endochitinase; apricot antigens such as apricot lipid transfer protein; peach antigens such as peach lipid transfer protein or peach profilin; soybean antigens such as HPS, soybean profilin or (SAM22) PR-I0 prot; fragments, variants and mixtures thereof.

In one embodiment, said autoantigen is a multiple sclerosis-associated antigen, a joint-associated antigen, an eye-associated antigen, a human HSP antigen, a skin-associated antigen or an antigen involved in graft rejection or GVHD

The term "multiple sclerosis-associated antigen" refers to myelin basic protein (MBP). myelin associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), oligodendrocyte myelin oligoprotein (OMGP), myelin associated oligodendrocyte basic protein (MOBP), oligodendrocyte specific protein (OSP/Claudinl 1), heat shock proteins, oligodendrocyte specific proteins (OSP), NOGO A, glycoprotein Po,

peripheral myelin protein 22 (PMP22), 2'3'-cyclic nucleotide 3'-phosphodiesterase (CNPase), fragments, variants and mixtures thereof.

The term "joint-associated antigen" refers to citrulline-substituted cyclic and linear filaggrin peptides, type II collagen peptides, human cartilage glycoprotein 39 (HCgp39) peptides, HSP, heterogeneous nuclear ribonucleoprotein (hnRNP) A2 peptides, hnRNP B1, hnRNP D, Ro60/52, HSP60, HSP65, HSP70 and HSP90, BiP, keratin, vimentin, fibrinogen, type I, III, IV and V collagen peptides, annexin V, Glucose 6 phosphate isomerase (GPI), acetyl-calpastatin, pyruvate dehydrogenase (PDH), aldolase, topoisomerase I, snRNP, PARP, Scl-70, Scl-100, phospholipid antigens including anionic cardiolipin and phosphatidylserine, neutrally charged phosphatidylethanolamine and phosphatidylcholine, matrix metalloproteinase, fibrillin, aggreccan, fragments, variants and mixtures thereof.

The term "eye-associated antigen" refers to type II collagen, retinal arrestin, S-arrestin, interphotoreceptor retinoid-binding proteins (IRBP1), beta-crystallin B1, retinal proteins, choroid proteins and fragments, variants and mixtures thereof.

The term "human HSP antigen" refers to human HSP60, HSP70, HSP90, fragments, variants and mixtures thereof.

Examples of skin-associated antigens include, but are not limited to, keratinocytes antigens, an antigen present in the dermis or epidermis, a melanocyte antigen (such as, for example, melanin or tyrosinase), desmoglein (*e.g.*, desmoglein 1 or 3, that may also be referred to as Dsg1/3), BP180, BP230, plectin, integrins (*e.g.*, integrin α4β6), collagens (*e.g.*, collagen type VII), laminins (*e.g.*, laminin 332 or laminin γ1), plakins (*e.g.*, envoplakin, periplakin, or desmoplakins), keratins (*e.g.*, KRT5, KRT8, KRT15, KRT17 and KRT31), keratin filament-associated proteins, filaggrin, corneodesmosin, and elastin.

In one embodiment, the ligand is an antigen involved in graft rejection or GVHD. Examples of such antigens include, but are not limited to, the MHC specific to the transplanted tissue or to the host, β2-microglobulin, antigens from ABO system, antigens from rhesus system (in particular antigens from the C, c, E et e and D system) and isohaemagglutinins. Other examples of antigens that may be involved in graft rejection or GVHD include, but are not limited to HLA-DR (in particular during the first six months following grafting), HLA-B

(in particular during the first two years following grafting), minor histocompatibility antigens (miHA, *e.g.*, HLA-E, HLA-F and HLA-G), HLAs corresponding to MHC class I (B, and C), HLAs corresponding to MHC class II (DP, DM, DOA, DOB, DQ, and DR) and HLAs corresponding to MHC class III (*e.g.*, components of the complement system).

Other examples of autoantigens include, without limitation, aquaporin water channels (such as, for example, aquaporin-4 water channel (AQP4)), Hu, Ma2, collapsin response-mediator protein 5 (CRMP5), and amphiphysin, voltage-gated potassium channel (VGKC), N-methyl-d-aspartate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPAR), thyroid peroxidase, thyroglobulin, anti–N-methyl-D-aspartate receptor (NR1 subunit), Rh blood group antigens, I antigen, desmoglein 1 or 3 (Dsg1/3), BP180, BP230, Acetylcholine nicotinic postsynaptic receptors, thyrotropin receptors, platelet integrin, GpIIb:IIIa, Collagen (such as, for example, Collagen alpha-3(IV) chain), rheumatoid factor, calpastatin, citrullinated proteins, Myelin basic protein (MBP), Myelin oligodendrocyte glycoprotein (MOG) peptides, alpha-beta-crystallin, DNA, histone, ribosomes, RNP, tissue transglutaminase (TG2), intrinsic factor, 65-kDa antigen, phosphatidylserine, ribosomal phosphoproteins, anti-neutrophil cytoplasmic antibody, Scl-70, U1-RNP, ANA, SSA, anti–SSB, antinuclear antibodies (ANA), antineutrophil cytoplasm antibodies (ANCA), Jo-1, antimitochondrial antibodies, gp210, p62, sp100, antiphospholipid antibodies, U1-70 kd snRNP, GQ1b ganglioside, GM1, asialo GM1, GD1b, anti-smooth muscle antibodies (ASMA), anti-liver-kidney microsome-1 antibodies (ALKM-1), anti-liver cytosol antibody-1 (ALC-1), IgA antiendomysial antibodies, neutrophil granule proteins, streptococcal cell wall antigen, intrinsic factor of gastric parietal cells, insulin (IAA), glutamic acid decarboxylase (GAA or GAD) and protein tyrosine phosphatase (such as, for example, IA2 or ICA512), PLA2R1 and THSD7A1.

In one embodiment, said ligand is selected from the group comprising ovalbumin, MOG, type II collagen fragments, variants and mixtures thereof.

In one embodiment, said ligand is ovalbumin, fragments, variants and mixtures thereof.

In another embodiment, said ligand is MOG, fragments, variants and mixtures thereof.

In another embodiment, said ligand is type II collagen, fragments, variants and mixtures thereof.

In another embodiment, said ligand is IL23R, fragments, variants and mixtures thereof.

In one embodiment, the CAR of the invention further comprises an extracellular ligand binding domain recognizing a ligand distinct from HLA-A2. In one embodiment, said ligand binding domain is an antibody or an antigen binding fragment thereof.

In one embodiment, the ligand binding domain of the CAR of the invention is a multifunctional antibody recognizing multiple distinct epitopes on HLA-A2. In one embodiment, the ligand binding domain of the CAR of the invention is a bifunctional antibody recognizing two distinct epitopes on HLA-A2.

In one embodiment, the CAR of the invention comprises an extracellular ligand binding domain comprising a HLA-A2 binding domain and another ligand binding domain recognizing a ligand distinct from HLA-A2. In one embodiment, said ligand binding domain is a bifunctional antibody recognizing both HLA-A2 and the distinct ligand.

Examples of ligands distinct from HLA-A2 that may be recognized by the CAR of the invention are listed hereinabove.

In one embodiment, CAR-modified immune cells of the invention may be generated by introducing a lentiviral vector comprising a desired CAR into the cells. In one embodiment, there is provided a method of making an immune cell modified to express a CAR, wherein the method comprises transducing an immune cell with a vector as described anywhere herein, thereby generating said modified immune cell.

In one embodiment, the genetically modified immune cells of the invention are modified through the introduction of RNA. In one embodiment, an *in vitro* transcribed RNA CAR can be introduced in a cell as a form of transient transfection. The RNA may be produced by *in vitro* transcription using a polymerase chain reaction (PCR)-generated template. DNA of interest from any source can be directly converted by PCR into a template for *in vitro* mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA can be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA, synthetic DNA sequence or any

other appropriate source of DNA. The desired template for *in vitro* transcription is the CAR of the present invention.

In one embodiment, the DNA to be used for PCR contains an open reading frame. The DNA can be from a naturally occurring DNA sequence from the genome of an organism. In one embodiment, the DNA is a full-length gene of interest or a portion of a gene. The gene can include some or all of the 5' and/or 3' untranslated regions (UTRs). The gene can include exons and introns. In one embodiment, the DNA to be used for PCR is a human gene. In another embodiment, the DNA to be used for PCR is a human gene including the 5' and 3' UTRs. The DNA can alternatively be an artificial DNA sequence that is not normally expressed in a naturally occurring organism. An exemplary artificial DNA sequence is one that contains portions of genes that are ligated together to form an open reading frame that encodes a fusion protein. The portions of DNA that are ligated together can be from a single organism or from more than one organism.

PCR may be used to generate a template for *in vitro* transcription of mRNA which is used for transfection. Methods for performing PCR are well known in the art. Primers for use in PCR are designed to have regions that are substantially complementary to regions of the DNA to be used as a template for the PCR. "Substantially complementary", as used herein, refers to sequences of nucleotides where a majority or all of the bases in the primer sequence are complementary, or one or more bases are non-complementary, or mismatched. Substantially complementary sequences are able to anneal or hybridize with the intended DNA target under annealing conditions used for PCR. The primers can be designed to be substantially complementary to any portion of the DNA template. For example, the primers can be designed to amplify the portion of a gene that is normally transcribed in cells (the open reading frame), including 5' and 3' UTRs. The primers can also be designed to amplify a portion of a gene that encodes a particular domain of interest. In one embodiment, the primers are designed to amplify the coding region of a human cDNA, including all or portions of the 5' and 3' UTRs. Primers useful for PCR are generated by synthetic methods that are well known in the art.

"Forward primers" are primers that contain a region of nucleotides that are substantially complementary to nucleotides on the DNA template that are upstream of the DNA sequence that is to be amplified. "Upstream" is used herein to refer to a location 5', to the DNA sequence

to be amplified relative to the coding strand. "Reverse primers" are primers that contain a region of nucleotides that are substantially complementary to a double-stranded DNA template that are downstream of the DNA sequence that is to be amplified. "Downstream" is used herein to refer to a location 3' to the DNA sequence to be amplified relative to the coding strand.

Any DNA polymerase useful for PCR can be used in the methods disclosed herein. The reagents and polymerase are commercially available from a number of sources.

Chemical structures with the ability to promote stability and/or translation efficiency may also be used. The RNA preferably has 5' and 3' UTRs. In one embodiment, the 5' UTR is between zero and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA. The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the gene of interest. Alternatively, UTR sequences that are not endogenous to the gene of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the gene of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of mRNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous gene. Alternatively, when a 5' UTR that is not endogenous to the gene of interest is being added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many mRNAs is known in the art. In other embodiments, the 5' UTR can be derived from an RNA virus whose RNA genome is stable in cells. In other embodiments, various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the mRNA.

To enable synthesis of RNA from a DNA template without the need for gene cloning, a promoter of transcription should be attached to the DNA template upstream of the sequence to be transcribed. When a sequence that functions as a promoter for an RNA polymerase is added to the 5' end of the forward primer, the RNA polymerase promoter becomes incorporated into the PCR product upstream of the open reading frame that is to be transcribed. In one preferred embodiment, the promoter is a T7 polymerase promoter, as described elsewhere herein. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

In a preferred embodiment, the mRNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability mRNA in the cell. On a circular DNA template, for instance, plasmid DNA, RNA polymerase produces a long concatemeric product which is not suitable for expression in eukaryotic cells. The transcription of plasmid DNA linearized at the end of the 3' UTR results in normal sized mRNA which is not effective in eukaryotic transfection even if it is polyadenylated after transcription.

On a linear DNA template, phage T7 RNA polymerase can extend the 3' end of the transcript beyond the last base of the template (Schenborn and Mierendorf, Nuc Acids Res., 13:6223-36 (1985); Nacheva and Berzal-Herranz, Eur. J. Biochem., 270: 1485-65 (2003).

The conventional method of integration of polyA/T stretches into a DNA template is molecular cloning. However, polyA/T sequence integrated into plasmid DNA can cause plasmid instability, which is why plasmid DNA templates obtained from bacterial cells are often highly contaminated with deletions and other aberrations. This makes cloning procedures not only laborious and time consuming but often not reliable. That is why a method which allows construction of DNA templates with polyA/T 3' stretch without cloning is highly desirable.

The polyA/T segment of the transcriptional DNA template can be produced during PCR by using a reverse primer containing a polyT tail, such as 100T tail (size can be 50-5000 T), or after PCR by any other method, including, but not limited to, DNA ligation or *in vitro* recombination. Poly(A) tails also provide stability to RNAs and reduce their degradation. Generally, the length of a poly(A) tail positively correlates with the stability of the transcribed RNA. In one embodiment, the poly(A) tail is between 100 and 5000 adenosines.

Poly(A) tails of RNAs can be further extended following *in vitro* transcription with the use of a poly(A) polymerase, such as *E. coli* polyA polymerase (E-PAP). In one embodiment, increasing the length of a poly(A) tail from 100 nucleotides to between 300 and 400 nucleotides results in about a two-fold increase in the translation efficiency of the RNA. Additionally, the attachment of different chemical groups to the 3' end can increase mRNA stability. Such attachment can contain modified/artificial nucleotides, aptamers and other compounds. For example, ATP analogs can be incorporated into the poly(A) tail using poly(A) polymerase. ATP analogs can further increase the stability of the RNA.

5' caps on RNAs also provide stability to RNA molecules. In a preferred embodiment, RNAs produced by the methods disclosed herein include a 5' cap. The 5' cap is provided using techniques known in the art and described herein (Cougot, et al., Trends in Biochem. Sci., 29:436-444 (2001); Stepinski, et al., RNA, 7: 1468-95 (2001); Elango, et al., Biochim. Biophys. Res. Commun., 330:958-966 (2005)).

The RNAs produced by the methods disclosed herein can also contain an internal ribosome entry site (IRES) sequence. The IRES sequence may be any viral, chromosomal or artificially designed sequence which initiates cap-independent ribosome binding to mRNA and facilitates the initiation of translation. Any solutes suitable for cell electroporation, which can contain factors facilitating cellular permeability and viability such as sugars, peptides, lipids, proteins, antioxidants, and surfactants can be included.

RNA can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaxa Nucleofector-II (Amaxa Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendort, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, or biolistic particle delivery systems such as "gene guns" (see, for example, Nishikawa, et al. Hum Gene Ther., 12(8):861-70 (2001).

In one embodiment, the CAR sequences are delivered into cells using a retroviral or lentiviral vector. CAR-expressing retroviral and lentiviral vectors can be delivered into different types of eukaryotic cells as well as into tissues and whole organisms using transduced

cells as carriers or cell-free local or systemic delivery of encapsulated, bound or naked vectors. The method used can be for any purpose where stable expression is required or sufficient.

In another embodiment, the CAR sequences are delivered into cells using *in vitro* transcribed mRNA. *In vitro* transcribed mRNA CAR can be delivered into different types of eukaryotic cells as well as into tissues and whole organisms using transfected cells as carriers or cell-free local or systemic delivery of encapsulated, bound or naked mRNA. The method used can be for any purpose where transient expression is required or sufficient.

In another embodiment, the desired CAR can be expressed in the cells by way of transposons.

In one embodiment, the immune cell of the invention is a T cell. Prior to expansion and genetic modification of the T cells of the invention, a source of T cells is obtained from a subject. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available in the art, may be used. In certain embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation or Sepax separation system. In one preferred embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca^{2+}-free, Mg^{2+}-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

Sources, Activation and Expansion of T Cells

In one embodiment, the immune cell may be a T cell. Prior to expansion and genetic modification of the T cells of the invention, a source of T cells or progenitor cells from which T cells can be made, is obtained from a subject. T cells can be obtained from any source where these cells reside including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, germinal centres, tissue from a site of infection, ascites, pleural effusion, spleen, tumors, and transplanted organs/tissue. In certain embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one embodiment, cells from the circulating blood of a subject are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, Tregs, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment of the invention, the cells may be washed with a wash solution (e.g., phosphate buffered saline (PBS)). As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca^{2+}-free, Mg^{2+}-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, $CD25^+$, CD127neg, HLA-DR^+, $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and/or $CD45RO^+$T cells, can be further isolated by positive or negative selection techniques. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain embodiments, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected"

cells can also be subjected to further rounds of selection.

Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for $CD4^+$ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, $CD62L^{hi}$, $GITR^+$, and $FoxP3^+$.

For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, or from samples where there are many tumor cells present (i.e., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain.

In a related embodiment, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, $CD4^+$ T cells express higher levels of CD28 and are more efficiently captured than $CD8^+$ T cells in dilute concentrations. In one embodiment, the concentration of cells used is $5x10^6$/ml. In other

embodiments, the concentration used can be from about $1x10^5$/ml to $1x10^6$/ml, and any integer value in between.

In other embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10°C or at room temperature.

T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20°C or in liquid nitrogen.

In certain embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

Also contemplated in the context of the invention is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as those described herein. In certain embodiments, a blood sample or an apheresis is taken from a subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the T cells may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a subject shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further embodiment, the cells are isolated from a blood sample or

an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun 73:316-321, 1991; Bierer et al., Curr. Opin. Immun 5:763-773, 1993). In a further embodiment, the cells are isolated for a patient and frozen for later use in conjunction with (e.g., before, simultaneously or following) bone marrow or stem cell transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cells are isolated prior to and can be frozen for later use for treatment following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan.

In a further embodiment of the present invention, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain immunosuppressive treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand ex vivo. Likewise, following ex vivo manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and in vivo expansion. Thus, it is contemplated within the context of the present invention to collect immune cells during this recovery phase. Further, in certain embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy.

Whether prior to or after genetic modification of the T cells to express a desirable CAR, the T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681;

7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

Generally, the T cells of the invention are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For costimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of $CD4^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besancon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):13191328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

In certain embodiments, the primary stimulatory signal and the co-stimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in "cis" formation) or to separate surfaces (i.e., in "trans" formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the co-stimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

In one embodiment, the two agents are immobilized on beads, either on the same bead, i.e., "cis," or to separate beads, *i.e.*, "trans." By way of example, the agent providing the

primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the co-stimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one embodiment, a 1:1 ratio of each antibody bound to the beads for $CD4^+$ T cell expansion and T cell growth is used. In certain aspects of the present invention, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular embodiment an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one embodiment, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect of the present invention, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, i.e., the ratio of CD3:CD28 is less than one. In certain embodiments of the invention, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular embodiment, a 1:100 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further embodiment, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one preferred embodiment, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet another embodiment, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain embodiments the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further embodiments the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above. For example, such values may include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, and 1:1 particles per T cell. In one embodiment, a ratio of particles to cells of 1:1 or less is used. In one particular

embodiment, a particle: cell ratio may be 1:5. In further embodiments, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one embodiment, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular embodiment, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In another embodiment, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type.

In further embodiments of the present invention, the cells, such as T cells (e.g., Treg cells), are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one embodiment the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, preferably PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used.

For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (i.e., 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present invention. In certain

embodiments, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one embodiment, a concentration of about 2 billion cells/ml is used. In another embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest. Such populations of cells may have therapeutic value and would be desirable to obtain in certain embodiments.

In one embodiment of the present invention, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In another embodiment, the mixture may be cultured for 21 days. In one embodiment of the invention the beads and the T cells are cultured together for about eight days. In another embodiment, the beads and T cells are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of T cells can be 60 days or more. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGFβ, and TNF-α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, Immunocult (StemCell), AIM-V, DMEM, MEM, α-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37˚C) and atmosphere (e.g., air plus 5% CO_2).

T cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (T_H, $CD4^+$) that is greater than the cytotoxic or suppressor T cell population (T_C, $CD8^+$). Ex vivo expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of T_H cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of T_C cells.

Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

In one embodiment, regulatory T cell may be obtained by isolating $CD4^+$ T cells from HLA-$A2^-$ donors via RosetteSep (Stemcell) and enriched for $CD25^+$ cells (Miltenyi) prior to sorting live $CD4^+CD127^{lo}CD25^{hi}$ Tregs using a FACSAria II (BD Biosciences). Sorted Tregs may be stimulated with L cells and αCD3 mAb (OKT3; 200ng/mL) in 1000U/ml of IL-2.

In one embodiment of the invention, the T cells may be cultured in the presence of rapamycin in order to obtain regulatory T cells, as described for example in WO2007110785 incorporated herein by reference. Another method to generate regulatory T cells is described in US2016024470 incorporated herein by reference, where T cells are cultured with a T cell receptor (TCR)/CD3 activator such as for example TCR/CD3 antibodies, a TCR co-stimulator activator such as for example CD28, CD137 (4-1 BB), GITR, B7-1/2, CD5, ICOS, OX40, CD40 or CD137 antibodies, and rapamycin.

In one embodiment of the invention, the T cells genetically modified by expression of the CAR may also have been genetically modified by expression of at least one intracellular factor such as ROR-C, Foxo1, T-bet, or Gata 3, c-Maf, AhR. In one embodiment, the genetically modified immune cell of the invention expresses Foxo1.

In one embodiment, the genetically modified cells of the present invention can be an allogeneic immune cell, such as, for example, an allogenic T or Treg cell. For example, the allogeneic immune cell can be an immune cell lacking expression of a functional human

leukocyte antigen (HLA), *e.g.*, HLA class I and/or HLA class II, or lacking expression of a functional T cell receptor (TCR).

In one embodiment, a T cell lacking a functional TCR can be engineered such that it does not express any functional TCR on its surface, engineered such that it does not express one or more subunits that comprise a functional TCR or engineered such that it produces very little functional TCR on its surface. Alternatively, the T cell can express a substantially impaired TCR, *e.g.*, by expression of mutated or truncated forms of one or more of the subunits of the TCR. The term "substantially impaired TCR" means that this TCR will not elicit an adverse immune reaction in a host.

In another embodiment, the genetically modified cells described herein can be engineered such that it does not express a functional HLA on its surface. For example, an immune cell described herein, can be engineered such that cell surface expression HLA, *e.g.*, HLA class 1 and/or HLA class II or non-classical HLA molecules is downregulated.

In another embodiment, the T cell can lack a functional TCR and a functional HLA such as HLA class I and/or HLA class II.

Modified immune cells (such as, for example, modified T or Treg cells) that lack expression of a functional TCR and/HLA can be obtained by any suitable means, including a knock out or knock down of one or more subunit of TCR and/or HLA. For example, the immune cell can include a knock down of TCR and/or HLA using siRNA, shRNA, clustered regularly interspaced short palindromic repeats (CRISPR) transcription-activator like effector nuclease (TALEN), zinc finger endonuclease (ZFN), meganuclease (mn, also known as homing endonuclease), or megaTAL (combining a TAL effector with a mn cleavage domain) (Osborn et al, "Evaluation of TCR Gene Editing Achieved by TALENs, CRISPR/Cas9, and megaTAL Nucleases" Mol Ther. 2016 Mar;24(3):570-81).

In one embodiment, TCR expression and/or HLA expression can be inhibited using siRNA or shRNA that targets a nucleic acid encoding a TCR and/or HLA in a T cell. Expression of siRNA and shRNAs in T cells can be achieved using any conventional expression system, *e.g.*, such as a lentiviral expression system. Exemplary siRNA and shRNA that downregulate expression of HLA class I and/or HLA class II genes are described, e.g., in

US2007/0036773. Exemplary shRNAs that downregulate expression of components of the TCR are described, e.g., in US2012/0321667.

"CRISPR" or "CRISPR to TCR and/or HLA" or "CRISPR to inhibit TCR and/or HLA" as used herein refers to a set of clustered regularly interspaced short palindromic repeats, or a system comprising such a set of repeats. "Cas", as used herein, refers to a CRISPR-associated protein. A "CRISPR/Cas" system refers to a system derived from CRISPR and Cas which can be used to silence or mutate a TCR and/or HLA gene.

Naturally-occurring CRISPR/Cas systems are found in approximately 40% of sequenced eubacteria genomes and 90% of sequenced archaea. Grissa et al. (2007) BMC Bioinformatics 8: 172. This system is a type of prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. Barrangou et al. (2007) Science 315: 1709-1712; Marragini et al. (2008) Science 322: 1843-1845. The CRISPR/Cas system has been modified for use in gene editing (silencing, enhancing or changing specific genes) in eukaryotes such as mice or primates. Wiedenheft et al. (2012) Nature 482: 331-8. This is accomplished by introducing into the eukaryotic cell a plasmid containing a specifically designed CRISPR and one or more appropriate Cas. The CRISPR sequence, sometimes called a CRISPR locus, comprises alternating repeats and spacers. In a naturally-occurring CRISPR, the spacers usually comprise sequences foreign to the bacterium such as a plasmid or phage sequence; in the TCR and/or HLA CRISPR/Cas system, the spacers are derived from the TCR or HLA gene sequence. RNA from the CRISPR locus is constitutively expressed and processed by Cas proteins into small RNAs. These comprise a spacer flanked by a repeat sequence. The RNAs guide other Cas proteins to silence exogenous genetic elements at the RNA or DNA level. Horvath et al. (2010) Science 327: 167-170; Makarova et al. (2006) Biology Direct 1: 7. The spacers thus serve as templates for RNA molecules, analogously to siRNAs. Pennisi (2013) Science 341: 833-836. The CRISPR/Cas system can thus be used to edit a TCR and/or HLA gene (adding or deleting a base pair), or introducing a premature stop which thus decreases expression of a TCR and/or HLA. The CRISPR/Cas system can alternatively be used like RNA interference, turning off HLA gene in a reversible fashion. In a mammalian cell, for example, the RNA can guide the Cas protein to a TCR and/or HLA promoter, sterically blocking RNA polymerases.

Artificial CRISPR/Cas systems can be generated which inhibit TCR and/or HLA, using technology known in the art, *e.g.*, that described in US20140068797, and Cong (2013) Science 339: 819-823. Other artificial CRISPR/Cas systems that are known in the art may also be generated which inhibit TCR and/or HLA, *e.g.*, that described in Tsai (2014) Nature Biotechnol., 32:6 569-576, US8,871,445; 8,865,406; 8,795,965; 8,771,945; and 8,697,359.

"TALEN" or "TALEN to TCR and/or HLA" or "TALEN to inhibit TCR and/or HLA" refers to a transcription activator-like effector nuclease, an artificial nuclease which can be used to edit the TCR and/or HLA. TALENs are produced artificially by fusing a TAL effector DNA binding domain to a DNA cleavage domain. Transcription activator-like effectors (TALEs) can be engineered to bind any desired DNA sequence, including a portion of the TCR and/or HLA gene. By combining an engineered TALE with a DNA cleavage domain, a restriction enzyme can be produced which is specific to any desired DNA sequence, including a TCR and/or HLA sequence. These can then be introduced into a cell, wherein they can be used for genome editing. Boch (2011) Nature Biotech. 29: 135-6; and Boch et al. (2009) Science 326: 1509-12; Moscou et al. (2009) Science 326: 3501.

TALEs are proteins secreted by Xanthomonas bacteria. The DNA binding domain contains a repeated, highly conserved 33-34 amino acid sequence, with the exception of the 12^{th} and 13^{th} amino acids. These two positions are highly variable, showing a strong correlation with specific nucleotide recognition. They can thus be engineered to bind to a desired DNA sequence. To produce a TALEN, a TALE protein is fused to a nuclease (N), which is a wild-type or mutated FokI endonuclease. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. Cermak et al. (2011) Nucl. Acids Res. 39: e82; Miller et al. (2011) Nature Biotech. 29: 143-8; Hockemeyer et al. (2011) Nature Biotech. 29: 731-734; Wood et al. (2011) Science 333: 307; Doyon et al. (2010) Nature Methods 8: 74-79; Szczepek et al. (2007) Nature Biotech. 25: 786-793; and Guo et al. (2010) /. Mol. Biol. 200: 96. The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al. (2011)

Nature Biotech. 29: 143-8. A TCR and/or HLA TALEN can be used inside a cell to produce a double- stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break via non-homologous end joining. For example, improper repair may introduce a frame shift mutation. Alternatively, foreign DNA can be introduced into the cell along with the TALEN; depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to correct a defect in the TCR and/or HLA gene or introduce such a defect into a wt TCR and/or HLA gene, thus decreasing expression of TCR and/or HLA. TALENs specific to sequences in TCR and/or HLA can be constructed using any method known in the art, including various schemes using modular components. Zhang et al. (2011) Nature Biotech. 29: 149-53; Geibler et al. (2011) PLoS ONE 6: el9509.

"ZFN" or "Zinc Finger Nuclease" or "ZFN to TCR and/or HLA" or "ZFN to inhibit TCR and/or HLA" refer to a zinc finger nuclease, an artificial nuclease which can be used to edit the TCR and/or HLA gene. Like a TALEN, a ZFN comprises a Fokl nuclease domain (or derivative thereof) fused to a DNA-binding domain. In the case of a ZFN, the DNA-binding domain comprises one or more zinc fingers. Carroll et al. (2011) Genetics Society of America 188: 773-782; and Kim et al. (1996) Proc. Natl. Acad. Sci. USA 93: 1156-1160. A zinc finger is a small protein structural motif stabilized by one or more zinc ions. A zinc finger can comprise, for example, Cys_2His_2, and can recognize an approximately 3-bp sequence. Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15 or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one -hybrid systems, bacterial one -hybrid and two-hybrid systems, and mammalian cells.

Like a TALEN, a ZFN must dimerize to cleave DNA. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. Bitinaite et al. (1998) Proc. Natl. Acad. Sci. USA 95: 10570-5. Also like a TALEN, a ZFN can create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression and amount of TCR and/or HLA in a cell. ZFNs can also be used with

homologous recombination to mutate in the TCR and/or HLA gene. ZFNs specific to sequences in TCR and/or HLA can be constructed using any method known in the art. See, e.g., Provasi (2011) Nature Med. 18: 807-815; Torikai (2013) Blood 122: 1341-1349; Cathomen et al. (2008) Mol. Ther. 16: 1200-7; Quo et al. (2010) /. Mol. Biol. 400: 96; US2011/0158957; and US2012/0060230.

"Meganuclease" or "meganuclease to TCR and/or HLA" or "meganuclease to inhibit TCR and/or HLA" refers to a monomeric endonuclease with large (>14 base pairs) recognition sites, which can be used to edit the TCR and/or HLA gene. Meganucleases (mn) are monomeric proteins with innate nuclease activity that are derived from bacterial homing endonucleases and engineered for a unique target site. Homing endonucleases are DNA-cleaving enzymes that can generate double strand breaks at individual loci in their host genomes, and thereby drive site-specific gene conversion events. (Stoddard, Structure. 2011 Jan 12;19(1):7-15). Despite their small size, homing endonucleases recognize long DNA sequences (typically 20 to 30 base pairs). Homing endonucleases are extremely widespread and are found in microbes, as well as in phages and viruses. The LAGLIDADG and His-Cys box enzymes (which are the most sequence-specific of these enzymes) rely upon antiparallel β-sheets that dock into the major grooves of their DNA target sites (Flick et al., 1998; Jurica et al., 1998). There they establish a collection of sequence-specific and non-specific contacts that are distributed nonuniformly across multiple consecutive basepairs (Chevalier et al., 2003; Scalley-Kim et al., 2007).

The LAGLIDADG homing endonuclease (LHE) family is the primary source of the engineered enzymes used for gene targeting applications. The LHE family is primarily encoded within archaea and in the chloroplast and mitochondrial genomes of algae and fungi (Chevalier et al., 2005; Dalgaard et al., 1997; Sethuraman et al., 2009). Meganucleases that possess a single conserved LAGLIDADG motif (SEQ ID NO: 85) per protein chain form homodimeric proteins that cleave palindromic and nearly palindromic DNA target sequences, while those that contain two such motifs per protein chain form larger, pseudo-symmetric monomers that can target completely asymmetric DNA sequences.

Meganucleases can be engineered to target TCR and/or HLA and thus create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression and amount of TCR and/or HLA in a cell.

"MegaTAL" or "megaTAL to TCR and/or HLA" or " megaTAL to inhibit TCR and/or HLA" refers to an artificial nuclease, which can be used to edit the TCR and/or HLA gene. MegaTALs are hybrid monomeric nucleases obtained through the fusion of minimal TAL (transcription activator-like) effector domains to the N-terminus of meganucleases derived from the LAGLIDADG homing endonuclease family (Nucleic Acids Res. 2014 Feb;42(4):2591-601; Takeuchi et al, Methods Mol Biol. 2015;1239:105-32. doi: 10.1007/978-1-4939-1862-1_6).

MegaTALs thus consist of a site-specific meganuclease cleavage head with additional affinity and specificity provided by a TAL effector DNA binding domain. MegaTALs can be engineered to target TCR and/or HLA and thus create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression and amount of HLA in a cell. A variant of the I-OnuI meganuclease (mn) was used to design a TCRα-megaTAL to knockout the T-cell receptor alpha (TCRα) gene. The TCRα mn was fused to a 10.5 repeat TALE array designed to bind a DNA sequence upstream of the TCRα mn binding site. It was found that the megaTAL targeting TCRα achieved extremely high gene disruption with no detectable off-target cleavage in human primary T-cells (Boissel et al, Nucleic Acids Res. 2014 Feb;42(4):2591-601).

While not wishing to be bound by any particular theory, in some embodiments, a therapeutic T cell has short term persistence in a patient, due to shortened telomeres in the T cell; accordingly, transfection with a telomerase gene can lengthen the telomeres of the T cell and improve persistence of the T cell in the patient. See Carl June, "Adoptive T cell therapy for cancer in the clinic", Journal of Clinical Investigation, 117: 1466-1476 (2007). Thus, in an embodiment, the genetically modified immune cell of the invention ectopically expresses a telomerase subunit, *e.g.*, the catalytic subunit of telomerase, *e.g.*, TERT, *e.g.*, hTERT. In some aspects, this disclosure provides a method of producing a CAR-expressing immune cell, comprising contacting an immune cell with a nucleic acid encoding a telomerase subunit, *e.g.*, the catalytic subunit of telomerase, *e.g.*, TERT, *e.g.*, hTERT. The cell may be contacted with

the nucleic acid before, simultaneous with, or after being contacted with a construct encoding a CAR.

VII. THERAPEUTIC APPLICATION

The invention provides immune cells modified to express a CAR targeting an HLA-A2 antigen as a means to recruit said immune cells to sites of specific immune or inflammatory response, and to activate the immune cells to suppress the immunological activity of immune effector cells at these sites. The CAR-mediated recruitment and activation of immune cells provide a method of preventing or treating a number of immunological diseases or disorders for which such immune suppression activity is beneficial.

In one embodiment, the immune cell is a regulatory immune cell. The immune cell may be any regulatory immune cell suitable for use in cellular therapy (see, e.g., Wood, K.J. et al., 2012; Papp, G. et al., 2017). In one embodiment, the regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In one embodiment, the immune cell is a human immune cell. In another embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the human regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof.

In one embodiment, the immune cell may be a regulatory T cell. In another embodiment, the regulatory T cell may be a human regulatory T cell. Although activation of regulatory T cells is antigen-dependent, the suppressive action of these cells is antigen-, TCR-, and MHC-independent. Accordingly, expression of CARs in regulatory T cells redirects these cells and their activation to the appropriate target tissue so that they are activated in an antigen-specific manner; however, their suppressive effects take place without a need for further recognition of disease-associated-antigens. Therefore, as long as the regulatory T cells are in the correct vicinity of where immune effector cells are located and mediating their undesired

effects, the redirected regulatory T cells can be triggered or activated at that location to provide their suppressive effects.

In one embodiment, the target HLA-A2 antigen may be present or expressed at a site or target tissue of an undesirable immune or inflammatory response mediated by immune effector cells.

In one embodiment, the CAR-modified immune cells of the invention may be used in the prevention or treatment of one or more diseases, disorders, symptoms, or conditions associated with organ or tissue transplant (e.g., organ or tissue rejection/dysfunction, GVHD, and/or conditions associated therewith). Transplant rejection involves the destruction of the donor's transplanted tissue by the recipient's immune cells through an immune response. An immune response is also involved in GVHD; however, in this case, the recipient's tissues are destroyed by the donor's immune cells transferred to the recipient via the transplant. Accordingly, CAR-mediated redirection and activation of immune cells provide a method of suppressing rejection of mismatched cells and/or tissues by immune effector cells in transplant recipients or inhibiting the pathogenic action of transplanted immunocompetent cells in the case of GVHD. In one embodiment, the mismatched cells and/or tissues comprise HLA-A2 mismatched cells and/or tissues.

Another embodiment of the present invention is thus a method for treating one or more diseases, disorders, symptoms, or conditions associated with organ or tissue transplant (*e.g.*, organ or tissue rejection/dysfunction, GVHD, and/or conditions associated therewith) in a subject, wherein said method comprises administering to the subject a CAR-engineered immune cell or a composition as described herein.

In one embodiment, the method is a cell therapy method. In one embodiment, the cell therapy is autologous. In one embodiment, the cell therapy is heterologous. In one embodiment, the cell therapy is allogenic. In one embodiment, the method is a gene therapy method.

Another embodiment of the present invention is thus a CAR-engineered immune cell or a composition as described herein for use in treating one or more diseases, disorders, symptoms, or conditions associated with organ or tissue transplant (*e.g.*, organ or tissue rejection/dysfunction, GVHD, and/or conditions associated therewith) in a subject.

The CAR-modified immune cells of the invention may be used to promote immune tolerance, operational tolerance, and/or immune accommodation in a subject. The CAR-modified immune cells of the invention may be used to promote immune tolerance, operational tolerance, and/or immune accommodation in a subject following organ or tissue transplantation. In one embodiment, there is provided a method of promoting immune tolerance, operational tolerance, and/or immune accommodation in a subject, the method comprising administering to the subject a CAR-modified immune cell as described anywhere herein. In another embodiment, there is provided a method of promoting immune tolerance, operational tolerance, and/or immune accommodation in a subject, the method comprising administering to the subject a pharmaceutical composition as described anywhere herein. In one embodiment, the use may be for promoting immune tolerance, operational tolerance, and/or immune accommodation to a transplanted organ or tissue in a subject. In one embodiment, the CAR-modified immune cell is administered at the same time as, before, or after the transplantation of the organ or tissue. In one embodiment, the CAR-modified immune cell is administered at the same time as the transplantation of the organ or tissue. In another embodiment, the CAR-modified immune cell is administered before the transplantation of the organ or tissue. In one embodiment, the CAR-modified immune cell is administered after the transplantation of the organ or tissue. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In one embodiment, the immune cell is a regulatory T cell. In one embodiment, the immune cell is a $CD4^+$ regulatory T cell. In one embodiment, the immune cell is a human immune cell. In another embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the human regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In another embodiment, the immune cell is a human regulatory T cell. In one embodiment, the immune cell is a human $CD4^+$ regulatory T cell.

The CAR-modified immune cells of the present invention may be used to prevent or treat rejection of a transplanted organ or tissue. In one embodiment, the CAR-modified immune (e.g., the CAR-engineered Treg cells) cells of the present invention may be used to prevent or treat hyperacute rejection of a transplanted organ or tissue. In one embodiment, the CAR-modified immune cells (e.g., the CAR-engineered Treg cells) of the present invention may be used to prevent or treat antibody-mediated rejection of a transplanted organ or tissue. In one embodiment, the method comprises administering CAR-modified immune cells (e.g., the CAR-engineered Treg cells) of the present invention to a subject exposed to a transplanted organ or tissue. In one embodiment, the transplanted organ or tissue may encompass a bone marrow transplant, an organ transplant, a blood transfusion or any other foreign tissue or cell that is purposefully introduced into a subject. In one embodiment, the CAR-modified immune cells (e.g., the CAR-engineered Treg cells) of the present invention may be used as a therapy to inhibit graft rejection following transplantation. In one embodiment, the graft rejection may be allograft rejection. In one embodiment, the graft rejection may be xenograft rejection. In one embodiment, there is provided a method of preventing or treating organ or tissue transplant rejection in a subject, the method comprising administering to the subject a CAR-modified immune cell as described anywhere herein. In one embodiment, there is provided a method of preventing or treating organ or tissue transplant rejection in a subject, the method comprising administering to the subject a pharmaceutical composition as described anywhere herein. Another embodiment of the present invention is thus a CAR-engineered immune cell (e.g., the CAR-engineered Treg cells) of the invention, or a pharmaceutical composition comprising said immune cells, for use in preventing or treating organ or tissue transplant rejection in a subject. In one embodiment, the present invention provides a method of increasing the time period of graft survival in a subject, the method comprising administering to the subject a CAR-modified immune cell (e.g., the CAR-engineered Treg cells) as described anywhere herein. In one embodiment, the present invention provides a method of increasing the time period of graft survival in a subject, the method comprising administering to the subject a pharmaceutical composition as described anywhere herein. In one embodiment, the method provides a time period of graft survival of 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 20 years, 30 years, 40 years,

50 years, 60 years, 70 years, 80 years, 90 years, 100 years, or the lifetime of the subject. In one embodiment, the subject is not undergoing any immunosuppressant agent therapies. In one embodiment, the administration of an immune cell or composition of the invention allows reducing the amount of an immunosuppressant agent therapy received by the subject. In one embodiment, the graft may be an allograft. In one embodiment, the transplant may be exposed to the CAR-modified immune cells of the present invention at the same time as, before, or after the transplantation of the transplant into the recipient. In one embodiment, the organ or tissue transplant may be a heart, heart valve, lung, kidney, liver, pancreas, intestine, skin, blood vessels, bone marrow, stem cells, bone, or, islet cells. However, the invention is not limited to a specific type of transplantation. In one embodiment, the donor transplant may be "preconditioned" or "pretreated" by treating the organ or tissue transplant prior to transplantation into the recipient with the CAR-modified immune cells of the invention in order to reduce the immunogenicity of the transplant against the recipient, thereby reducing or preventing graft rejection. In one embodiment, the transplant host or recipient is HLA-A2 negative. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two different HLA-A subtypes selected from one or two of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one HLA-A subtype selected from one of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one or two of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from two of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two different HLA-A subtypes selected from one or two of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one HLA-A subtype

selected from one of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one or two of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from two of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*03. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*25. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*29. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*31. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*33. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*36. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is

positive for HLA-A*25:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*29:02. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*30:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*03:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*31:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*33:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*36:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*68:01. In one embodiment, the transplant is HLA-A2 positive.

In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In one embodiment, the immune cell is a regulatory T cell. In one embodiment, the immune cell is a $CD4^+$ regulatory T cell. In one embodiment, the immune cell is a human immune cell. In another embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the human regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In another embodiment, the immune cell is a human regulatory T cell. In one embodiment, the immune cell is a human $CD4^+$ regulatory T cell.

The CAR-modified immune cells (e.g., the CAR-engineered Treg cells) of the present invention may be used to prevent or treat graft versus host disease (GVHD). In one embodiment, the method comprises administering a CAR-modified immune cell (e.g., the CAR-engineered Treg cells) of the present invention to a subject exposed to a transplanted organ or tissue. In one embodiment, the transplanted organ or tissue may encompass a bone marrow transplant, an organ transplant, a blood transfusion, or any other foreign tissue or cell that is purposefully introduced into a subject. For example, GVHD may occur after heart, heart

valve, lung, kidney, liver, pancreas, intestine, skin, blood vessel, bone marrow, stem cell, bone or islet cell transplantation. However, the invention is not limited to a specific type of transplantation. In one embodiment, the GVHD may occur after hematopoietic stem cell transplantation. In one embodiment, there is provided a method of preventing or treating graft versus host disease (GVHD) in a subject, the method comprising administering to the subject a CAR-modified immune cell as described anywhere herein. In one embodiment, there is provided a method of preventing or treating graft versus host disease (GVHD) in a subject, the method comprising administering to the subject a pharmaceutical composition as described anywhere herein. In one embodiment, the invention provides a method of contacting a donor transplant, for example, a biocompatible lattice or a donor tissue, organ or cell, with CAR-modified immune cells of the present invention at the same time as, before, or after the transplantation of the transplant into a recipient. In one embodiment, the CAR-modified immune cells of the present invention may be used to ameliorate, inhibit or reduce an adverse response by the donor transplant against the recipient, thereby preventing or treating GVHD. In one embodiment, the present invention provides a method of preventing or delaying onset of GVHD in a subject, the method comprising administering to the subject a CAR-modified immune cell as described anywhere herein. In one embodiment, the present invention provides a method of preventing or delaying onset of GVHD in a subject, the method comprising administering to the subject a pharmaceutical composition as described anywhere herein. In one embodiment, the onset of GVHD is delayed for 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 20 years, 30 years, 40 years, 50 years, 60 years, 70 years, 80 years, 90 years, 100 years, or the lifetime of the subject. In one embodiment, the subject is not undergoing any immunosuppressant agent therapies. In one embodiment, the subject is undergoing an immunosuppressant therapy. In one embodiment, the immune cells of the invention are administered to the subject with the aim to decrease the therapeutically effective amount of said immunosuppressant therapy. In one embodiment, GVHD may be acute GVHD or chronic GVHD. In one embodiment, the donor transplant may be “preconditioned” or “pretreated” by treating the transplant prior to transplantation into the recipient with CAR-modified immune cells (e.g., the CAR-engineered Treg cells) of the invention in order to reduce the

immunogenicity of the transplant against the recipient, thereby reducing or preventing GVHD. In one embodiment, the transplant may be contacted with cells or a tissue from the recipient prior to transplantation in order to activate T cells that may be associated with the transplant. Following the treatment of the transplant with cells or a tissue from the recipient, the cells or tissue may be removed from the transplant. The treated transplant may be then further contacted with CAR-modified immune cells (e.g., the CAR-engineered Treg cells) of the present invention to reduce, inhibit or eliminate the activity of the immune effector cells that were activated by the treatment of the cells or tissue from the recipient. Following this treatment of the transplant with CAR-modified immune cells of the present invention, the CAR-modified immune cells may be removed from the transplant prior to transplantation into the recipient. However, some CAR-modified immune cells may adhere to the transplant, and therefore, may be introduced to the recipient with the transplant. In this situation, the CAR-modified immune cells introduced into the recipient may suppress an immune response against the recipient caused by a cell associated with the transplant. In one embodiment, the transplant host or recipient is HLA-A2 negative. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two different HLA-A subtypes selected from one or two of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one HLA-A subtype selected from one of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one or two of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from two of HLA-A*03, HLA-A*25, HLA-A*29, HLA-A*30, HLA-A*31, HLA-A*33, HLA-A*36, HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two different HLA-A subtypes selected from one or two of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one

HLA-A subtype selected from one of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one or two of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from one of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two different HLA-A subtypes selected from two of HLA-A*25, HLA-A*29, HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*03. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*25. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*29. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*30. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*31. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*33. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*36. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for an HLA-A subtype of HLA-A*68. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two of HLA-A*03:01, HLA-A*25:01, HLA-A*29:02, HLA-A*30:01, HLA-A*31:01, HLA-A*33:01, HLA-A*36:01, and HLA-A*68:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one or two of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for one of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for two of HLA-A*25:01, HLA-A*29:02, and HLA-A*30:01. In one embodiment, the transplant host or

recipient is HLA-A2 negative and is positive for HLA-A*25:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*29:02. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*30:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*03:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*31:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*33:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*36:01. In one embodiment, the transplant host or recipient is HLA-A2 negative and is positive for HLA-A*68:01. In one embodiment, the transplant is HLA-A2 positive. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In one embodiment, the immune cell is a regulatory T cell. In one embodiment, the immune cell is a $CD4^+$ regulatory T cell. In one embodiment, the immune cell is a human immune cell. In another embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the human regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In another embodiment, the immune cell is a human regulatory T cell. In one embodiment, the immune cell is a human $CD4^+$ regulatory T cell.

In one embodiment, the present invention provides a method of expanding a population of immune cells in a subject wherein the immune cells are modified to express a chimeric antigen receptor (CAR), the method comprising administering to the subject an immune cell as described anywhere herein, wherein the administered modified immune cell produces a population of progeny immune cells in the subject. In one embodiment, the immune cell is a regulatory immune cell. In one embodiment, the regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a

regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In one embodiment, the immune cells may be regulatory T cells. In one embodiment, the immune cells may be $CD4^+$ regulatory T cells. In one embodiment, the immune cell is a human immune cell. In another embodiment, the regulatory immune cell is a human regulatory immune cell. In one embodiment, the human regulatory immune cell is selected from the group consisting of a regulatory T cell, a $CD4^+$ regulatory T cell, a $CD8^+$ regulatory T cell, a regulatory γδ T cell, a regulatory DN T cell, a regulatory B cell, a regulatory NK cell, a regulatory macrophage, a regulatory dendritic cell, and any combination thereof. In another embodiment, the immune cell is a human regulatory T cell. In one embodiment, the immune cell is a human $CD4^+$ regulatory T cell. In one embodiment, the CAR-modified Treg cells of the present invention are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained suppression of an immune response of a targeted cell and immune tolerance. In one embodiment, there is provided a method of generating a persisting population of regulatory T cells in a subject wherein the regulatory T cells are modified to express a chimeric antigen receptor (CAR), the method comprising administering to the subject a regulatory T cell as described anywhere herein, wherein the persisting population of modified regulatory T cells persists in the subject for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 30 days, 40 days, 50 days, 60 days, 70 days, 80 days, 90 days, 100 days, 200 days, 300 days, 400 days, 500 days, 600 days, 700 days, 800 days, 900 days, or 1000 days after administration. In one embodiment, the CAR-Treg cells may be capable of self-renewing and being re-activated *in vivo* to suppress an immune response of a targeted cell. In one embodiment, the CAR-Treg cells may be memory CAR-Treg cells that can be re-activated to suppress an immune response of a targeted cell.

The immune cells may be obtained from any source. For example, in one embodiment, immune cells may be obtained from the tissue donor, the transplant recipient or an otherwise unrelated source (a different subject or species altogether) for generation of CAR-modified immune cells of the present invention. Accordingly, CAR-modified immune cells of the present invention may be autologous, allogeneic or xenogeneic to the transplant recipient or an otherwise unrelated source. In one embodiment, the CAR-Treg cells of the present invention

may be autologous, allogeneic or xenogeneic to the transplant recipient. In one embodiment, the CAR-Treg cells of the present invention may be autologous to the transplant recipient.

In one embodiment, the subject may be a mammal. In one embodiment, the subject may be a human.

In one embodiment, it may be desirable to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom according to the present invention, and reinfuse the subject with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain embodiments, T cells can be activated from blood draws of from 10 cc to 400 cc. In certain embodiments, 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. Not to be bound by theory, using this multiple blood draw/multiple reinfusion protocol may serve to select out certain populations of T cells.

The CAR-modified immune cells of the present invention may be administered either alone or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. In one embodiment, the pharmaceutical composition may comprise a plurality of the modified immune cells as described anywhere herein. In one embodiment, the pharmaceutical compositions of the present invention may comprise a CAR-modified immune cell or cell population as described anywhere herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. The pharmaceutical compositions of the present invention may be administered to a subject in any suitable manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The pharmaceutical compositions described anywhere herein may be administered to a subject by parenteral administration. The pharmaceutical compositions described anywhere herein may be administered to a subject subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, intrasternally, by intravenous (i.v.) injection, by infusion techniques or intraperitoneally. In one

embodiment, the CAR-modified immune cell compositions of the present invention may be administered to a subject by intradermal or subcutaneous injection. In another embodiment, the CAR-modified immune cell compositions of the present invention may be administered by i.v. injection. In one embodiment, the compositions of CAR-modified immune cells may be injected directly into a lymph node, site of infection, site of inflammation or site of tissue or organ ejection.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be prevented or treated. The quantity and frequency of administration will be determined by such factors as the condition of the subject, and the type and severity of the subject's disease, although appropriate dosages may be determined by clinical trials.

When an "effective amount" or "therapeutic amount" is indicated, the amount of the compositions of the present invention to be administered may be determined with consideration of individual differences in age, weight, antibody titer, and condition of the subject. It can generally be stated that a pharmaceutical composition comprising the CAR-modified immune cells as described anywhere herein may be administered at a dosage of 1×10^4 to 1×10^9 cells/kg body weight. In one embodiment, the CAR-modified immune cells may be administered at a dosage of 1×10^5 to 100×10^5 cells/kg body weight, including all integer values within those ranges. CAR-modified immune cell compositions may also be administered multiple times at these dosages. The CAR-modified immune cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al, 1988). The optimal dosage and treatment regime for a particular subject can readily be determined by monitoring the subject for signs of disease and adjusting the treatment accordingly.

In one embodiment, the at least one CAR-engineered immune cell of the invention is administered to the subject in need thereof in combination with another active agent. According to one embodiment, the at least one immune cell population is administered before, at the same time or after the administration of another active agent.

In one embodiment, the CAR-modified immune cells of the present invention may be administered to a subject in conjunction with (e.g., before, simultaneously or following) any

number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, chemotherapy, radiation, immunosuppressive agents, antibodies, immunoablative agents, cytokines, and irradiation. In one embodiment, the CAR-modified immune cells of the present invention may be administered in conjunction with an immunosuppressant agent. Any immunosuppressant agent known in the art may be used. For example, the immunosuppressant agent may be calcineurin inhibitors such as cyclosporine, tacrolimus, azathioprine, methotrexate, methoxsalen, rapamycin, mycophenolate mofetil, mycophenolic acid, mycophenolate sodium, 6-mercaptopurine, 6-thioguanine, rituximab, mTOR inhibitors such as sirolimus, everolimus, basiliximab, daclizumab, belatacept, alemtuzumab, muromonab-CD3, anti-thymocyte globulin, glucorticosteroids, or adrenocortical steroids such as prednisone and prednisolone, or any combination thereof. The CAR-modified immune cells of the present invention may be administered to the subject before, after, or concomitant with the immunosuppressant agent. For example, the CAR-modified immune cells of the present invention may be administered after the immunosuppressant agent is administered to the subject or the CAR-modified immune cells of the invention may be administered before the immunosuppressant agent is administered to the subject. Alternatively, or in addition, the CAR-modified immune cells of the invention may be administered at the same time the immunosuppressant agent is administered to the subject. The CAR-modified immune cells of the present invention and/or the immunosuppressant agent may be administered to the subject after transplantation. Alternatively, or in addition, the CAR-modified immune cells of the present invention and/or the immunosuppressant agent may be administered to the subject before transplantation. The CAR-modified immune cells of the present invention and/or the immunosuppressant agent may be administered to the subject during transplantation surgery. In one embodiment, the method of the invention of administering CAR-modified immune cells to the subject is carried out once immunosuppressive therapy has been initiated. In some embodiments, the method is carried out more than once, e.g., to monitor the transplant recipient over time, and, if applicable, in different immunosuppressive therapy regimes. In some embodiments, immunosuppressive therapy is reduced if the transplant recipient is predicted to be tolerant of the transplant. In some embodiments, no immunosuppressive therapy is prescribed, e.g., immunosuppressive therapy is ceased, if the transplant recipient is predicted to be tolerant of the transplant.

Also provided is a method of preventing or treating organ or tissue transplant rejection in a subject, the method comprising administering to the subject (i) at least one immunosuppressive agent and (ii) CAR-engineered immune cells (e.g., the CAR-engineered Treg cells) of the invention, or a pharmaceutical composition comprising said immune cells.

Another embodiment of the present invention is thus a combination of a CAR-engineered immune cell (e.g., the CAR-engineered Treg cells) of the invention, or a pharmaceutical composition comprising said immune cells, and of at least one immunosuppressive agent, for use in preventing or treating organ or tissue transplant rejection in a subject.

Also provided is a method of increasing the time period of graft survival in a subject, the method comprising administering to the subject at least one immunosuppressive agent and CAR-engineered immune cells (e.g., the CAR-engineered Treg cells) of the present invention, or a pharmaceutical composition comprising the same.

In one embodiment, a combination of at least one immunosuppressive agent with CAR-engineered immune cells (e.g., the CAR-engineered Treg cells) of the present invention is used to prevent or treat graft versus host disease (GVHD). In one embodiment, the GVHD may occur after hematopoietic stem cell transplantation.

Another embodiment of the invention is thus a method of preventing or treating graft versus host disease (GVHD) in a subject, the method comprising administering to the subject at least one immunosuppressive agent and CAR-engineered immune cells (e.g., the CAR-engineered Treg cells) or a pharmaceutical composition as described herein.

In one embodiment, the invention provides a method of contacting a donor transplant, for example, a biocompatible lattice or a donor tissue, organ or cell, with at least one immunosuppressive agent and CAR-engineered immune cells (e.g., the CAR-engineered Treg cells) of the present invention at the same time as, before, or after the transplantation of the transplant into a recipient.

In one embodiment, the combination of at least one immunosuppressive agent with CAR-engineered immune cells (e.g., the CAR-engineered Treg cells) of the present invention

may be used to ameliorate, inhibit or reduce an adverse response by the donor transplant against the recipient, thereby preventing or treating GVHD.

Another embodiment of the present invention is thus a method of preventing or delaying onset of GVHD in a subject, the method comprising administering to the subject at least one immunosuppressive agent and CAR-engineered immune cells (e.g., the CAR-engineered Treg cells) or a pharmaceutical composition as described herein.

In one embodiment, the CAR-engineered immune cells of the invention and the at least one immunosuppressive agent are administered simultaneously or sequentially.

In one embodiment, the CAR-engineered immune cells of the present invention, and optionally the at least one other active agent, e.g., immunosuppressive agent, is administered in conjunction with (*e.g.*, before, simultaneously or following) the transplant.

The CAR-modified immune cells of the present invention may be administered following a diagnosis of transplant organ or tissue rejection followed by doses of both the CAR-modified immune cells of the invention and an immunosuppressant agent until symptoms of organ or tissue rejection subside. In a further embodiment, the CAR-modified immune cell compositions of the present invention may be administered to a subject in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation. In another embodiment, the CAR-modified immune cells of the present invention may be administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituximab. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects may receive an infusion of the expanded CAR-modified immune cells of the present invention. In an additional embodiment, expanded CAR-modified immune cells may be administered before or following surgery.

In one embodiment, the subject (*e.g.*, human) receives an initial administration of the at least one immune cell or population of the invention, and one or more subsequent administrations, 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, a therapeutically effective amount of immune cells of the invention is

administered or is to be administered to the subject. In one embodiment, the amount of immune cells of the at least one immune cell population of the invention administered to the subject is at least of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8 or 10^9 cells. In one embodiment, the amount of immune cells of the invention administered to the subject ranges from about 10^2 to about 10^9, from about 10^3 to about 10^8, from about 10^4 to about 10^7, or from about 10^5 to about 10^6 cells. In another embodiment, the amount of immune cells of the invention administrated to the subject ranges from about 10^6 to about 10^9, from about 10^6 to 10^7, from about 10^6 to 10^8, from about 10^7 to 10^9, from about 10^7 to 10^8, from about 10^8 to 10^9. In another embodiment the amount of immune cells of the invention administrated to the subject is about 10^6, about 10^7, about 10^8, or is about 10^9. In one embodiment, the amount of immune cells of the at least one immune cell population of the invention administered to the subject is at least of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8 or 10^9 cells/kg body. In one embodiment, the amount of immune cells of the invention administered to the subject ranges from about 10^4 to 10^9 cells/kg body weight or 10^5 to 10^8 cells/kg body weight, including all integer values within those ranges. In one embodiment, more than one administration of the at least one immune cell or population of the invention are administered to the subject (*e.g.*, human) per week, *e.g.*, 2, 3, or 4 administrations of the genetically modified immune cell or population of the invention are administered per week.

In some embodiments, it may be desirable to incorporate a suicide gene into the CAR-modified immune cells of the present invention in order to enable selective destruction of CAR-modified immune cells *in vivo*. In one embodiment, the CAR-modified immune cells of the present invention may further comprise a suicide gene system. In one embodiment, the CAR-modified immune cells of the present invention may further comprise a nucleic acid encoding a suicide gene system. "Suicide gene therapy," "suicide gene" and "suicide gene system" as described herein refer to methods to selectively destroy a cell through apoptosis. Such methods employ a suicide gene that will cause a cell to kill itself by apoptosis. The suicide gene encodes for a product which causes cell death by itself or in the presence of other compounds. The suicide gene may be from any suicide gene system suitable for use in cellular therapy (see, for example, Jones et al., 2014; Wang et al., 2017; Zhang et al., 2017; Casucci et al., 2011; Gargett et al., 2014; Khaleghi, 2012; Philip et al., 2014).

In one embodiment, the suicide gene system may involve a gene-directed enzyme prodrug therapy (GDEPT) where a nontoxic drug (i.e., suicide gene prodrug) is converted to a toxic compound in suicide-gene modified cells. For example, in one embodiment, the suicide gene system may be a herpes simplex virus thymidine kinase (HSV-TK)/ganciclovir (GCV) suicide gene system which uses the thymidine kinase gene from the herpes simplex virus in combination with the prodrug ganciclovir. In other embodiments, the suicide gene system may be a cytosine deaminase/ 5-fluorocytosine suicide gene system. In some alternatives of the methods provided herein wherein the CAR-modified immune cells comprise a suicide gene system, the methods further comprise administering to the subject a suicide gene prodrug.

In other embodiments, the suicide gene system may involve a dimerization inducing mechanism where apoptotic genes such as caspases destroy cells by inducing apoptosis. Dimerization inducing suicide gene systems may involve chimeric proteins composed of a drug binding domain linked with components of the apoptotic pathway allowing conditional dimerization and apoptosis of the modified immune cells after administration of a chemical inducer of dimerization (CID). For example, in one embodiment, the suicide gene system may be a caspase based suicide gene system. Non-limiting examples of inducible caspase suicide gene systems may include the inducible caspase-9 suicide gene system (iCasp9) and the caspase-8 suicide gene system (iCasp8) where the caspase is activated by the CID, FK506 or an analogue thereof. In some alternatives of the methods provided herein wherein the CAR-modified immune cells comprise a suicide gene system, the methods further comprise administering to the subject a chemical inducer of dimerization.

In other embodiments, the suicide gene system may be mediated by a therapeutic monoclonal antibody. Therapeutic mAb-mediated suicide gene systems involve the expression of proteins at the cell surface which make the modified immune cells sensitive to the therapeutic mAbs. Modified immune cells may be selectively destroyed after administration of the specific therapeutic mAb. Non-limiting examples of therapeutic mAb-mediated suicide gene systems may include the CD20 suicide gene system and the RQR8 suicide gene system where the therapeutic mAb is an anti-CD20 mAb. In some embodiments, the anti-CD20 mAb may be rituximab. In some alternatives of the methods provided herein wherein the CAR-

modified immune cells comprise a suicide gene system, the methods further comprise administering to the subject a therapeutic monoclonal antibody.

VIII. ARTICLES OF MANUFACTURE AND KITS

In another embodiment, the invention provides an article of manufacture containing materials useful for the prevention and/or treatment of transplant rejection or GVHD. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for preventing and/or treating the immunological condition, such as transplant rejection or GVHD, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a CAR-modified immune cell of the present invention. In one embodiment, the CAR-modified immune cell is a CAR-modified Treg. In one embodiment, the CAR-modified Treg is a CAR-modified human Treg. The label or package insert indicates that the composition is used for preventing or treating transplant rejection or GVHD. The article of manufacture, label or package insert may further comprise instructional material for administering the CAR-modified immune cell composition to the patient. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Kits are also provided that are useful for various purposes (e.g., for preventing or treating transplant rejection or GVHD). Kits can be provided which contain the CAR-modified immune cells. As with the article of manufacture, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition comprising at least one CAR-modified immune cell of the present invention. Additional containers may be included that contain, e.g., diluents and buffers. The label or package insert may provide a description of the composition as well as instructions for the intended use. In

one embodiment, the CAR-modified immune cells are CAR-modified Tregs. In one embodiment, the CAR-modified Tregs are CAR-modified human Tregs.

The present invention thus further relates to a kit of parts comprising, in a first part, an immune cell or an immune cell population of the present invention, and in a second part, another active agent, including without limitations, antiviral therapy, chemotherapy, radiation, immunosuppressive agents, antibodies, immunoablative agents, cytokines, and irradiation. In some embodiments, the kit of parts of the present invention comprises in a first part, an immune cell or an immune cell population of the present invention, and in a second part, one or more immunosuppressive agents. Examples of immunosuppressive agents that may be present in the kit of the invention include, but are not limited to, calcineurin inhibitors such as cyclosporine, tacrolimus, azathioprine, methotrexate, methoxsalen, rapamycin, mycophenolate mofetil, mycophenolic acid, mycophenolate sodium, 6-mercaptopurine, 6-thioguanine, rituximab, mTOR inhibitors such as sirolimus, everolimus, basiliximab, daclizumab, belatacept, alemtuzumab, muromonab-CD3, anti-thymocyte globulin, glucorticosteroids, or adrenocortical steroids such as prednisone and prednisolone, or any combination thereof.

Table 1

	Kabat	Chothia
VH CDR1	SYHIQ (SEQ ID NO: 1)	GYTFTSY (SEQ ID NO: 2)
VH CDR2	WIYPGDGSTQYNEKFKG (SEQ ID NO: 3)	YPGDGS (SEQ ID NO: 4)
VH CDR2	WIYPGDGSTKYSQKFQG (SEQ ID NO: 5)	YPGDGS (SEQ ID NO: 4)
VH CDR3	EGTYYAMDY (SEQ ID NO: 6)	EGTYYAMDY (SEQ ID NO: 6)

Table 2

	Kabat	Chothia
VL CDR1	RSSQSIVHSNGNTYLE (SEQ ID NO: 7)	RSSQSIVHSNGNTYLE (SEQ ID NO: 7)
VL CDR2	KVSNRFS (SEQ ID NO: 8)	KVSNRFS (SEQ ID NO: 8)
VL CDR3	FQGSHVPRT (SEQ ID NO: 9)	FQGSHVPRT (SEQ ID NO: 9)

Table 3

SEQ Name	SEQ ID	AA
VH CDR2 (Kabat)	10	WIYPGDGSTX[10]YX[12]X[13]KFX[16]G
VH FR1 (chothia)	11	QVQLVQSGAEVKKPGASVKVSCKAS
VH FR1 (kabat)	12	QVQLVQSGAEVKKPGASVKVSCKASGYTFT
VH FR2 (kabat/kabat variable)	13	WVRQAPGQX[9]LEWMGX[15]
VH1/2/5/6 FR2 (kabat/kabat)	14	WVRQAPGQRLEWMG
VH3 FR2 (kabat/kabat)	15	WVRQAPGQGLEWMGI
VH4 FR2 (kabat/kabat)	16	WVRQAPGQGLEWMG
VH FR2 (kabat/chothia variable)	17	WVRQAPGQX[9]LEWMGX[15]WI
VH1/2/5/6 FR2 (kabat/chothia)	18	WVRQAPGQRLEWMGWI
VH3 FR2 (kabat/chothia)	19	WVRQAPGQGLEWMGIWI
VH4 FR2 (kabat/chothia)	20	WVRQAPGQGLEWMGWI
VH FR2 (chothia/chothia variable)	21	HIQWVRQAPGQX[12]LEWMGX[18]WI
VH1/2/5/6 FR2	22	HIQWVRQAPGQRLEWMGWI

(chothia/chothia)		
VH3 FR2 (chothia/chothia)	23	HIQWVRQAPGQGLEWMGIWI
VH4 FR2 (chothia/chothia)	24	HIQWVRQAPGQGLEWMGWI
VH FR2 (chothia/kabat variable)	25	HIQWVRQAPGQX[12]LEWMGX[18]
VH1/2/5/6 FR2 (chothia/kabat)	26	HIQWVRQAPGQRLEWMG
VH3 FR2 (chothia/kabat)	27	HIQWVRQAPGQGLEWMGI
VH4 FR2 (chothia/kabat)	28	HIQWVRQAPGQGLEWMG
VH FR3 (kabat variable)	29	X[1]VTX[4]TX[6]DTSX[10]STAYMX[16]LSX[19]LRSX[23]DX[25]AVYYCAR
VH1/6 FR3 (kabat)	30	RVTITRDTSASTAYMELSSLRSEDTAVYYCAR
VH2 FR3 (kabat)	31	RVTITADTSASTAYMLLSSLRSEDTAVYYCAR
VH3 FR3 (kabat)	32	VTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
VH4 FR3 (kabat)	33	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR
VH5 FR3 (kabat)	34	RVTITRDTSASTAYMELSSLRSEDMAVYYCAR
VH FR3 (chothia variable)	35	TX[2]YX[4]X[5]KFX[8]GX[10]VTX[13]TX[15]DTSX[19]STAYMX[25]LSX[28]LRSX[32]DX[34]AVYYCAR
VH1/2/3/4/5 FR3 (chothia	36	TQYNEKFKGX[10]VTX[13]TX[15]DTSX[19]STAYMX[25]LSX[28]LRSX[32]DX[34]AVYYCAR

variable)		
VH6 FR3 (chothia variable)	37	TKYSQKFQGX[10]VTX[13]TX[15]DTSX[19]STAYMX[25]LSX[28]LRSX[32]DX[34]AVYYCAR
VH 1 FR3 (chothia)	38	TQYNEKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCAR
VH 2 FR3 (chothia)	39	TQYNEKFKGRVTITADTSASTAYMLLSSLRSEDTAVYYCAR
VH 3 FR3 (chothia)	40	TQYNEKFKGVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
VH 4 FR3 (chothia)	41	TQYNEKFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAR
VH 5 FR3 (chothia)	42	TQYNEKFKGRVTITRDTSASTAYMELSSLRSEDMAVYYCAR
VH 6 FR3 (chothia)	43	TKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCAR
VH FR4	44	WGQGTTVTVSS
VH	45	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQX[44]LEWMGX[50]WIYPGDGSTX[60]YX[62]X[63]KFX[66]GX[68]VTX[71]TX[73]DTSX[77]STAYMX[83]LSX[86]LRSX[90]DX[92]AVYYCAREGTYYAMDYWGQGTTVTVSS
VL FR1	46	DX[2]VMTQX[7]PLSX[11]X[12]VTX[15]GQPASISX[23]
VL1/4	47	DVVMTQSPLSLPVTLGQPASISC
VL2 FR1	48	DIVMTQTPLSLSVTPGQPASISC
VL3 FR1	49	DIVMTQTPLSSPVTLGQPASISC
VL5 FR1	50	DIVMTQTPLSSPVTLGQPASISF
VL FR2	51	WX[2]X[3]QX[5]PGQX[9]PX[11]X[12]LIY
VL1 FR2	52	WFQQRPGQSPRRLIY
VL2 FR2	53	WYLQKPGQSPQLLIY
VL3/5 FR2	54	WYQQRPGQPPRLLIY
VL4 FR2	55	WYQQRPGQSPRLLIY
VL FR3	56	GVPDRFSGSGX[11]GTDFTLKISRVEAEDVGVYYC
VL1/2/4 FR3	57	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
VL3/5 FR3	58	GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC
VL FR4	59	FGGGTKVEIK

VL	60	DX[2]VMTQX[7]PLSX[11]X[12]VTX[15]GQPASISX[23]RSSQSIVHSNGNTYL EWX[41]X[42]QX[44]PGQX[48]PX[50]X[51]LIYKVSNRFSGVPDRFSGSGX[72]G TDFTLKISRVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H1 (without leader)	61	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSS
H2 (without leader)	62	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITADTSASTAYMLLSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSS
H3 (without leader)	63	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSS
H4 (without leader)	64	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSS
H5 (without leader)	65	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSS
H6 (without leader)	66	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTKYSQKFQGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSS
K1	67	DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTYLEWFQQR PGQSPRRLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCFQGSHVPRTFGGGTKVEIK
K2	68	DIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTYLEWYLQK PGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCFQGSHVPRTFGGGTKVEIK
K3	69	DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTYLEWYQQR PGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKISRVEAEDV GVYYCFQGSHVPRTFGGGTKVEIK
K4	70	DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTYLEWYQQR PGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV GVYYCFQGSHVPRTFGGGTKVEIK
K5	71	DIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTYLEWYQQR PGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKISRVEAEDV GVYYCFQGSHVPRTFGGGTKVEIK
H1_k1_AA (without leader)	72	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY

		LEWFQQRPGQSPRRLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H1_k2_AA (without leader)	73	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H1_k3_AA (without leader)	74	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H1_k4_AA (without leader)	75	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H1_k5_AA (without leader)	76	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H2_k1_AA (without leader)	77	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITADTSASTAYMLLSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWFQQRPGQSPRRLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H2_k2_AA (without leader)	78	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITADTSASTAYMLLSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H3_k2_AA (without leader)	79	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS

		RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H3_k3_AA (without leader)	80	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H3_k4_AA (without leader)	81	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H3_k5_AA (without leader)	82	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H4_k2_AA (without leader)	83	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H4_k3_AA (without leader)	84	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H4_k4_AA (without leader)	85	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H4_k5_AA (without leader)	86	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK

H5_k2_AA (without leader)	87	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H5_k3_AA (without leader)	88	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H5_k4_AA (without leader)	89	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H5_k5_AA (without leader)	90	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H6_k2_AA (without leader)	91	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTKYSQKFQGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIK
H6	92	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQX^{4} ^{4}LEWMGX^{50}WIYPGDGSTKYSQKFQGX^{68}VTX^{71}TX^{73}DTSX^{77}STAY MX^{83}LSX^{86}LRSX^{90}DX^{92}AVYYCAREGTYYAMDYWGQGTTVTVSS
H1_k1_AA (with leader)	93	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDVVMTQSPLSLPVT LGQPASISCRSSQSIVHSNGNTYLEWFQQRPGQSPRRLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H1_k2_AA (with	94	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDTAVYYCAREGTYYAMDYWG

leader)		QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSLSVT PGQPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H1_k3_AA (with leader)	95	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H1_k4_AA (with leader)	96	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDVVMTQSPLSLPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQSPRLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H1_k5_AA (with leader)	97	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISFRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H2_k1_AA (with leader)	98	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITADTSASTAYMLLSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDVVMTQSPLSLPVT LGQPASISCRSSQSIVHSNGNTYLEWFQQRPGQSPRRLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H2_k2_AA (with leader)	99	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITADTSASTAYMLLSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSLSVT PGQPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H3_k2_AA (with leader)	100	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGIWIYPGDGSTQYNEKF KGVTMTRDTSTSTVYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSLSVT PGQPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSN

		RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H3_k3_AA (with leader)	101	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGIWIYPGDGSTQYNEKF KGVTMTRDTSTSTVYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H3_k4_AA (with leader)	102	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGIWIYPGDGSTQYNEKF KGVTMTRDTSTSTVYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDVVMTQSPLSLPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQSPRLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H3_k5_AA (with leader)	103	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGIWIYPGDGSTQYNEKF KGVTMTRDTSTSTVYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISFRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H4_k2_AA (with leader)	104	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGWIYPGDGSTQYNEKFK GRVTMTRDTSISTAYMELSRLRSDDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSLSVT PGQPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H4_k3_AA (with leader)	105	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGWIYPGDGSTQYNEKFK GRVTMTRDTSISTAYMELSRLRSDDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H4_k4_AA (with leader)	106	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGWIYPGDGSTQYNEKFK GRVTMTRDTSISTAYMELSRLRSDDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDVVMTQSPLSLPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQSPRLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT

		FGGGTKVEIK
H4_k5_AA (with leader)	107	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQGLEWMGWIYPGDGSTQYNEKFK GRVTMTRDTSISTAYMELSRLRSDDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISFRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H5_k2_AA (with leader)	108	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDMAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSLSVT PGQPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H5_k3_AA (with leader)	109	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDMAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H5_k4_AA (with leader)	110	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDMAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDVVMTQSPLSLPVT LGQPASISCRSSQSIVHSNGNTYLEWYQQRPGQSPRLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H5_k5_AA (with leader)	111	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTQYNEKFK GRVTITRDTSASTAYMELSSLRSEDMAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSSPVT LGQPASISFRSSQSIVHSNGNTYLEWYQQRPGQPPRLLIYKVSN RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK
H6_k2_AA (with leader)	112	MDFQVQIFSFLLISASVIMSRASQVQLVQSGAEVKKPGASVKVS CKASGYTFTSYHIQWVRQAPGQRLEWMGWIYPGDGSTKYSQKFQ GRVTITRDTSASTAYMELSSLRSEDTAVYYCAREGTYYAMDYWG QGTTVTVSSVDSSGGGGSGGGGSGGGGSTSDIVMTQTPLSLSVT PGQPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSN RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPRT FGGGTKVEIK

leader	113	MDFQVQIFSFLLISASVIMSRAS
linker	114	VDSSGGGGSGGGGSGGGGSTS
CD8 hinge	115	SALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSL RPEACRPAAGGAVHTRGLD
CD28 TM	116	FWVLVVVGGVLACYSLLVTVAFIIFWV
CD28 IC	117	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
CD3z	118	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR
CAR_H1_k1_AA (without leader)	119	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWFQQRPGQSPRRLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H1_k2_AA (without leader)	120	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H1_k3_AA (without leader)	121	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD

		PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H1_k4_AA (without leader)	122	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H1_k5_AA (without leader)	123	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H2_k1_AA (without leader)	124	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITADTSASTAYMLLSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWFQQRPGQSPRRLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H2_k2_AA (without leader)	125	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITADTSASTAYMLLSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA

		CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H3_k2_AA (without leader)	126	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H3_k3_AA (without leader)	127	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H3_k4_AA (without leader)	128	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H3_k5_AA (without leader)	129	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGIWIYPGDGSTQYNEKFKGVTMTRDTSTSTVYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY

		LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H4_k2_AA (without leader)	130	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H4_k3_AA (without leader)	131	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H4_k4_AA (without leader)	132	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR

CAR_H4_k5_AA (without leader)	133	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQG LEWMGWIYPGDGSTQYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H5_k2_AA (without leader)	134	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H5_k3_AA (without leader)	135	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H5_k4_AA (without leader)	136	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTY LEWYQQRPGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE

		RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H5_k5_AA (without leader)	137	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDMAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSSPVTLGQPASISFRSSQSIVHSNGNTY LEWYQQRPGQPPRLLIYKVSNRFSGVPDRFSGSGAGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H6_k2_AA (without leader)	138	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTKYSQKFQGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSVDSSGGGGSGGGGS GGGGSTSDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCFQGSHVPRTFGGGTKVEIKNRIRGVTVSSALS NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEA CRPAAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLE RVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
CAR_H1_k2_AA	213	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYHIQWVRQAPGQR LEWMGWIYPGDGSTQYNEKFKGRVTITRDTSASTAYMELSSLRS EDTAVYYCAREGTYYAMDYWGQGTTVTVSSGGGGSGGGGSGGGG SDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTYLEWYLQ KPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCFQGSHVPRTFGGGTKVEIKRTTTPAPRPPTPAPTIASQ PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLYCRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFA AYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
4-1BB_AA (SEQ ID NO: 23 of US 9,102,760)	216	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
CD8 hinge AA (SEQ ID	218	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIY

NO:21 of US 9,102,760)		
CD8 hinge AA	219	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC D
CD8 transmembrane _AA (SEQ ID NO:22 of US 9,102,760)	221	IWAPLAGTCGVLLLSLVITLYC
CD8_transmembrane AA	223	IYIWAPLAGTCGVLLLSLVITLYC

Table 4

SEQ Name	SEQ ID	NT
H1_k1_NT	139	ATGGATTTCCAAGTTCAAATCTTCAGTTTCTTGCTTATCAGTGC TTCTGTTATTATGTCACGAGCAAGTCAAGTTCAACTCGTACAGT CTGGAGCCGAGGTGAAAAAACCGGGAGCGTCCGTGAAAGTGAGT TGCAAGGCGAGTGGATACACCTTCACTTCATACCATATACAATG GGTTCGGCAGGCGCCTGGTCAACGGCTGGAATGGATGGGCTGGA TTTATCCCGGAGATGGTTCCACGCAGTACAATGAGAAATTCAAA GGGAGAGTGACAATCACCCGAGATACCAGTGCCTCTACGGCATA TATGGAACTGAGTAGTCTGCGGTCTGAAGATACGGCGGTGTATT ATTGTGCGAGAGAAGGGACGTACTACGCCATGGATTATTGGGGA CAAGGAACAACAGTCACAGTCTCCAGCGTTGATTCCTCAGGCGG GGGCGGAAGTGGGGGCGGCGGATCTGGCGGCGGCGGGTCTACGT CTGACGTGGTCATGACCCAATCTCCATTGTCTTTGCCAGTTACT CTGGGACAGCCTGCAAGTATCAGTTGCCGATCCTCCCAATCTAT CGTCCATTCAAACGGGAACACTTATTTGGAATGGTTTCAACAGA GACCTGGGCAAAGTCCGCGCCGACTGATATATAAGGTCAGTAAC CGCTTTTCAGGCGTCCCCGATCGATTCAGTGGATCTGGGTCAGG GACTGACTTCACTCTGAAAATATCAAGAGTCGAAGCTGAAGATG TCGGAGTATATTACTGTTTCCAGGGGTCTCACGTCCCTCGGACG TTTGGAGGCGGAACTAAGGTTGAGATAAAA
H1_k2_NT	140	ATGGATTTTCAGGTTCAAATCTTTAGCTTTCTCTTGATTTCCGC CTCCGTAATAATGAGTCGGGCCAGTCAGGTACAGCTCGTTCAAT CTGGGGCTGAAGTAAAAAAGCCTGGAGCGTCTGTAAAGGTATCT TGCAAAGCGAGCGGCTACACATTCACAAGTTATCACATCCAATG GGTGAGACAGGCCCCAGGACAACGCTTGGAGTGGATGGGGTGGA TTTACCCTGGCGACGGCAGCACACAGTACAATGAGAAATTTAAA GGCCGGGTGACTATCACTCGGGACACCTCCGCCAGCACGGCTTA TATGGAGCTTAGCAGTTTGAGATCCGAAGATACAGCGGTATATT ACTGCGCGAGAGAAGGAACGTACTACGCTATGGACTATTGGGGT

		CAGGGCACAACCGTTACAGTCTCCTCTGTGGACAGCTCCGGAGG TGGGGGTTCAGGAGGGGGTGGAAGCGGTGGTGGTGGCAGTACAA GCGATATAGTAATGACCCAAACCCCGCTCTCTCTGAGCGTCACG CCAGGACAACCAGCATCAATCTCTTGCCGCAGTAGTCAATCCAT CGTTCACTCTAATGGAAACACATACCTTGAGTGGTATCTTCAGA AACCAGGTCAGAGCCCTCAGCTCCTCATCTATAAAGTCTCTAAC CGGTTCTCAGGTGTTCCGGACCGGTTCAGTGGTTCCGGCTCAGG AACAGACTTCACCTTGAAGATCAGTCGAGTAGAAGCCGAAGACG TGGGTGTATATTATTGCTTTCAGGGTTCCCACGTTCCGCGCACC TTCGGCGGCGGGACCAAAGTTGAGATCAAA
H2_k1_NT	141	ATGGACTTTCAAGTCCAAATCTTCTCATTCCTCCTGATCTCTGC GTCAGTAATCATGTCCAGAGCGTCACAAGTGCAACTCGTTCAAT CCGGAGCTGAGGTAAAGAAGCCCGGCGCCAGCGTGAAAGTCTCC TGCAAAGCGAGCGGCTACACGTTCACCTCATATCACATTCAATG GGTAAGACAAGCACCTGGGCAACGACTCGAGTGGATGGGGTGGA TCTACCCTGGGGACGGGAGCACGCAGTATAATGAGAAATTCAAA GGCAGGGTTACAATTACAGCCGATACCAGTGCATCTACGGCTTA TATGCTCCTCTCCTCACTCCGGTCTGAGGACACAGCGGTTTATT ATTGCGCACGGGAGGGAACGTACTACGCGATGGACTATTGGGGG CAAGGCACCACAGTTACAGTGAGCTCAGTTGACTCATCAGGAGG CGGAGGATCAGGGGGAGGTGGTAGTGGGGGCGGTGGGAGCACAT CAGATGTTGTCATGACTCAGAGCCCACTTTCTTTGCCGGTGACG CTGGGGCAGCCCGCTTCAATCTCTTGCCGCTCATCACAGTCTAT CGTTCATAGCAATGGTAACACTTACTTGGAATGGTTCCAACAAA GACCGGGTCAAAGTCCACGGCGCTTGATATATAAAGTATCAAAT AGATTTTCAGGGGTGCCTGATCGGTTCAGCGGTTCTGGATCTGG CACCGACTTCACGCTTAAAATAAGTAGGGTAGAAGCCGAAGACG TGGGAGTGTATTATTGTTTCCAGGGGTCACACGTCCCTCGCACG TTCGGCGGAGGCACTAAAGTGGAGATCAAA
H2_k2_NT	142	ATGGACTTTCAAGTCCAAATCTTCAGCTTTCTCCTTATATCTGC GTCTGTCATTATGAGTAGAGCAAGTCAAGTCCAGCTCGTACAAA GTGGAGCTGAGGTGAAAAAGCCGGGCGCGAGTGTGAAAGTCTCA TGCAAGGCGAGTGGATACACCTTTACCTCTTACCACATTCAATG GGTGCGGCAGGCGCCTGGGCAGCGCTTGGAATGGATGGGCTGGA TATATCCTGGCGACGGAAGTACCCAGTACAACGAAAAATTCAAA GGTAGGGTTACCATCACTGCTGATACCTCCGCGTCCACTGCTTA TATGCTTCTTAGCTCCTTGCGAAGCGAGGATACAGCCGTGTATT ATTGTGCCAGAGAGGGGACTTATTATGCCATGGACTATTGGGGT CAGGGTACAACGGTCACTGTCTCATCTGTTGACAGTAGCGGGGG AGGGGGGTCTGGAGGAGGGGGTTCCGGGGGGGGAGGTTCCACGA GCGATATAGTTATGACGCAAACGCCCTTGAGCCTCAGTGTTACA CCCGGTCAACCTGCCTCTATTAGCTGTCGCTCCTCCCAATCAAT TGTGCATAGCAATGGAAATACCTACCTTGAATGGTATCTCCAAA AGCCCGGGCAGAGTCCTCAACTTCTCATCTACAAAGTATCCAAT CGATTCAGTGGCGTTCCTGACAGGTTCAGCGGAAGTGGGTCAGG GACCGATTTTACCCTCAAAATTAGTCGCGTCGAAGCTGAGGATG

		TTGGGGTGTATTACTGCTTCCAAGGGTCACACGTACCACGCACA TTCGGGGGAGGCACGAAGGTTGAAATTAAG
H1_k3_NT	143	ATGGATTTCCAAGTCCAAATATTCAGTTTCCTTTTGATAAGTGC TTCAGTTATCATGTCCCGAGCAAGCCAGGTACAGCTTGTGCAAA GCGGGGCGGAAGTTAAGAAACCGGGAGCCTCAGTTAAAGTATCT TGCAAAGCCAGCGGTTATACATTCACTTCATACCACATACAGTG GGTGCGCCAAGCACCGGGGCAGAGACTGGAATGGATGGGATGGA TTTATCCCGGTGATGGTAGTACGCAATACAATGAAAAATTCAAA GGAAGGGTGACTATCACGCGAGACACAAGCGCGTCCACGGCCTA TATGGAACTGAGCAGTCTGAGATCCGAGGACACCGCTGTGTATT ATTGCGCCCGAGAGGGGACCTACTACGCCATGGATTATTGGGGT CAAGGGACCACTGTGACAGTCTCTTCTGTCGATTCCAGCGGCGG AGGAGGGAGTGGAGGGGGTGGGTCCGGGGGGGGAGGGTCTACGA GCGACATTGTCATGACACAGACGCCGCTTAGCTCCCCAGTTACA CTCGGACAGCCCGCAAGTATTAGTTGCAGAAGTAGCCAGTCTAT CGTACATTCAAATGGAAACACCTATTTGGAATGGTATCAACAAC GGCCTGGACAGCCGCCCAGGCTGCTCATATACAAAGTCTCCAAC CGCTTCAGCGGAGTACCCGACCGCTTTTCCGGCTCCGGAGCAGG AACTGACTTTACCTTGAAAATTAGTAGGGTCGAAGCAGAGGATG TCGGGGTATATTATTGTTTCCAAGGTAGTCATGTCCCACGGACG TTTGGTGGTGGGACGAAGGTTGAGATCAAA
H1_k4_NT	144	ATGGACTTCCAGGTGCAGATATTTTCCTTTCTTCTCATATCTGC ATCTGTAATAATGTCAAGGGCCAGCCAGGTCCAGCTCGTTCAAA GCGGAGCCGAGGTAAAAAAACCAGGCGCTTCCGTCAAGGTATCA TGCAAAGCGTCCGGCTATACCTTCACAAGTTACCATATCCAATG GGTTCGACAGGCACCCGGACAAAGACTTGAATGGATGGGTTGGA TATACCCCGGAGACGGCTCCACTCAGTATAACGAAAAGTTTAAG GGGAGAGTCACGATCACTAGGGACACATCAGCTTCTACGGCGTA TATGGAACTCAGTTCTTTGCGATCCGAGGATACTGCCGTATATT ACTGCGCCAGAGAAGGCACGTACTACGCAATGGATTACTGGGGG CAAGGGACAACTGTTACCGTCTCAAGCGTCGATTCATCAGGAGG CGGAGGGTCCGGAGGTGGGGGATCTGGCGGTGGGGGTTCTACGT CCGATGTTGTGATGACACAGTCCCCACTCTCTCTTCCAGTGACG CTGGGACAGCCCGCGAGCATCTCCTGTCGCAGCTCTCAGTCCAT AGTACACAGTAATGGTAACACCTATCTTGAGTGGTATCAGCAAC GACCCGGTCAGTCTCCCAGGTTGCTTATTTATAAGGTTAGTAAC CGCTTTTCAGGTGTTCCAGACAGATTTAGCGGGAGTGGTTCCGG TACGGATTTCACATTGAAAATCAGCCGCGTCGAAGCCGAGGACG TGGGTGTTTACTACTGCTTCCAGGGATCTCACGTACCGAGGACC TTCGGCGGAGGAACGAAAGTAGAAATTAAG
H1_k5_NT	145	ATGGATTTTCAAGTACAAATCTTTTCCTTCCTGCTTATTTCCGC AAGCGTTATTATGAGTAGAGCCAGCCAAGTACAACTGGTACAGT CCGGCGCGGAGGTGAAGAAGCCCGGAGCAAGTGTGAAGGTATCT TGCAAGGCGTCAGGCTATACCTTTACTTCATACCATATACAGTG GGTGCGACAGGCTCCCGGCCAGCGACTCGAATGGATGGGCTGGA

		TTTATCCCGGAGATGGATCTACGCAGTATAATGAGAAATTCAAG GGTCGGGTCACGATTACACGAGACACGAGTGCTTCCACAGCTTA TATGGAACTTTCTAGCCTGAGGTCTGAGGATACTGCCGTTTACT ATTGTGCACGAGAAGGGACATACTATGCGATGGATTACTGGGGA CAGGGCACCACTGTCACAGTTTCCAGCGTGGACTCAAGTGGAGG CGGTGGATCTGGTGGTGGCGGGTCCGGGGGGGGAGGCAGCACCA GTGACATTGTAATGACTCAAACACCTCTCAGTAGCCCAGTCACT CTCGGTCAGCCGGCGAGTATCTCTTTTAGGTCCTCACAATCTAT AGTGCACTCTAACGGCAATACTTATCTTGAATGGTATCAACAAA GACCGGGGCAGCCACCTCGCCTTCTCATCTACAAAGTAAGCAAT CGCTTCTCCGGTGTCCCCGATCGCTTCTCCGGTTCAGGAGCAGG AACTGACTTCACATTGAAGATTTCCAGAGTGGAGGCCGAAGACG TAGGGGTATATTATTGCTTTCAAGGGTCCCATGTGCCCAGAACC TTTGGGGGAGGAACGAAAGTTGAGATTAAA
H3_k2_NT	146	ATGGATTTCCAAGTGCAGATTTTCTCTTTTCTCCTCATAAGCGC CTCCGTAATTATGTCTAGAGCTAGTCAAGTCCAATTGGTGCAAT CCGGTGCCGAGGTTAAAAAGCCCGGCGCAAGTGTAAAAGTCTCC TGTAAGGCCAGTGGCTACACTTTCACCAGCTACCATATACAGTG GGTGCGGCAGGCGCCTGGTCAGGGTCTGGAGTGGATGGGTATTT GGATTTATCCCGGAGATGGAAGTACTCAATACAATGAGAAATTC AAGGGTGTCACTATGACAAGGGATACGAGCACTTCTACCGTATA TATGGAGTTGTCATCTTTGCGATCAGAGGATACCGCTGTATATT ATTGCGCACGGGAAGGTACATATTATGCCATGGACTACTGGGGC CAAGGAACCACCGTGACGGTAAGCTCTGTCGATTCTAGCGGTGG CGGGGGCTCTGGCGGTGGGGGTAGCGGGGGTGGCGGATCTACAT CAGATATTGTAATGACACAGACCCCTCTTTCACTTTCCGTAACG CCAGGACAGCCGGCATCAATAAGTTGCCGATCAAGCCAGTCTAT CGTACACTCCAATGGTAACACATACTTGGAATGGTATCTTCAAA AGCCCGGCCAGAGCCCGCAGCTTTTGATATATAAAGTGTCCAAC AGATTCAGTGGGGTGCCGGACCGCTTTAGTGGATCTGGTTCAGG AACGGACTTCACATTGAAAATTAGTAGAGTTGAAGCGGAAGACG TGGGAGTCTACTACTGTTTCCAGGGTTCACATGTGCCTCGGACC TTTGGGGGAGGCACCAAGGTTGAGATAAAA
H3_k3_NT	147	ATGGACTTCCAAGTCCAAATCTTTTCTTTTTTGTTGATAAGCGC ATCAGTTATTATGTCTCGCGCCAGTCAAGTACAACTGGTGCAGT CCGGAGCTGAAGTGAAAAAACCAGGAGCAAGCGTGAAAGTAAGT TGTAAGGCAAGTGGTTACACTTTCACAAGCTACCATATTCAATG GGTCCGACAGGCTCCTGGACAGGGCTTGGAGTGGATGGGCATAT GGATTTACCCTGGTGACGGGTCCACCCAGTATAATGAAAAGTTC AAGGGAGTCACGATGACCAGGGACACCTCTACATCTACCGTGTA TATGGAGCTCTCTAGTTTGCGATCCGAAGACACTGCCGTTTATT ACTGTGCAAGAGAAGGAACTTATTACGCGATGGACTACTGGGGT CAGGGGACAACAGTCACCGTTAGCTCCGTCGATTCCAGCGGGGG AGGTGGCTCAGGCGGGGGTGGTTCTGGGGGGGGCGGGAGCACTT CAGATATTGTAATGACCCAAACCCCACTGAGTAGTCCAGTCACG CTTGGTCAACCGGCAAGCATTTCTTGCAGGAGCTCTCAGAGTAT

		TGTCCACTCTAACGGGAATACATATTTGGAGTGGTATCAGCAAA GACCGGGCCAACCACCACGCCTCTTGATTTATAAGGTGAGCAAT AGGTTTTCAGGCGTGCCAGATAGGTTCTCAGGCTCCGGAGCGGG AACCGACTTCACCCTCAAGATAAGTCGGGTGGAAGCCGAAGACG TAGGAGTTTACTACTGCTTTCAAGGATCTCATGTTCCACGAACG TTTGGAGGAGGAACCAAGGTGGAAATAAAA
H3_k4_NT	148	ATGGACTTTCAGGTCCAAATTTTTTCCTTCTTGCTCATATCCGC GAGTGTCATCATGTCAAGAGCAAGTCAAGTTCAACTCGTTCAAT CAGGAGCTGAGGTGAAAAAACCAGGGGCGTCTGTCAAAGTAAGC TGCAAAGCATCAGGGTATACGTTCACGAGTTATCATATCCAGTG GGTTAGGCAGGCGCCAGGGCAGGGATTGGAATGGATGGGTATCT GGATTTACCCGGGTGACGGCAGCACTCAATACAATGAGAAATTC AAAGGCGTAACAATGACAAGGGACACGAGCACAAGCACAGTGTA CATGGAGCTTAGCTCTTTGAGGTCAGAGGATACCGCTGTTTACT ATTGTGCTCGGGAGGGTACTTACTATGCAATGGACTACTGGGGG CAAGGCACGACCGTTACAGTGAGTAGCGTAGATTCCTCCGGGGG TGGCGGTTCAGGCGGCGGAGGCTCAGGCGGAGGAGGGTCAACAT CCGATGTCGTAATGACTCAGTCCCCTCTGTCATTGCCGGTGACT TTGGGACAGCCAGCGTCTATATCTTGTAGGTCCTCTCAATCAAT AGTGCATTCCAACGGTAACACCTATCTGGAATGGTATCAGCAAA GGCCAGGACAAAGTCCACGCCTGCTTATATATAAGGTGTCTAAT CGATTCAGTGGGGTTCCCGATAGGTTTTCCGGCTCTGGTAGCGG GACTGATTTCACGTTGAAAATATCACGCGTGGAAGCGGAAGATG TTGGGGTCTATTACTGCTTTCAGGGTAGTCATGTCCCTCGAACT TTTGGCGGTGGTACAAAGGTAGAAATCAAA
H3_k5_NT	149	ATGGATTTTCAGGTACAGATATTCTCATTTCTCCTTATCTCAGC TAGTGTCATAATGTCCAGGGCGAGTCAAGTACAACTTGTCCAGT CAGGCGCAGAGGTCAAGAAGCCGGGCGCAAGCGTTAAGGTTTCC TGCAAAGCATCCGGCTATACATTCACGTCCTATCACATCCAATG GGTCAGGCAAGCACCCGGTCAAGGACTTGAGTGGATGGGCATCT GGATTTACCCTGGAGATGGCAGTACTCAGTACAACGAAAAATTC AAAGGTGTAACCATGACCCGCGACACATCTACTTCCACAGTTTA TATGGAACTCAGCAGTTTGCGGAGCGAAGATACCGCTGTTTACT ACTGTGCCCGAGAGGGAACTTACTACGCCATGGACTATTGGGGT CAAGGAACGACAGTAACAGTTAGTTCTGTAGATTCCAGTGGCGG CGGTGGGAGCGGGGGTGGGGGATCTGGCGGAGGCGGAAGTACAA GTGACATCGTTATGACTCAGACACCCCTTAGTAGTCCCGTTACG TTGGGCCAACCCGCGAGCATTTCCTTTCGATCCTCTCAGTCTAT AGTTCACTCAAATGGGAATACTTATTTGGAGTGGTATCAACAGC GCCCCGGACAACCACCAAGGCTCCTGATATACAAGGTGTCCAAT CGATTCTCTGGGGTGCCTGATAGATTTAGCGGAAGTGGAGCCGG TACAGATTTTACCCTGAAAATATCACGGGTAGAAGCCGAAGATG TCGGCGTCTACTACTGTTTCCAGGGTTCCCATGTACCGCGAACG TTCGGGGGCGGAACAAAAGTTGAGATCAAG
H4_k2_NT	150	ATGGATTTCCAGGTTCAGATATTTAGTTTCCTCTTGATTTCTGC CAGTGTCATCATGAGCAGGGCTTCCCAAGTTCAGTTGGTGCAAA

		GTGGCGCTGAAGTCAAAAAACCTGGGGCTTCCGTTAAAGTATCT TGCAAGGCGTCCGGCTACACTTTCACATCCTACCACATTCAATG GGTCCGGCAAGCGCCCGGTCAGGGGCTCGAATGGATGGGGTGGA TATACCCAGGAGATGGATCTACTCAGTACAACGAGAAATTTAAA GGACGGGTGACGATGACGCGCGACACTTCAATAAGCACTGCATA CATGGAACTGTCCCGGCTTAGGTCAGATGACACCGCGGTCTACT ATTGTGCGAGAGAGGGTACTTACTATGCTATGGACTACTGGGGG CAAGGCACGACGGTTACAGTTTCCTCAGTCGATAGTTCAGGCGG AGGCGGCTCCGGGGGCGGTGGTAGTGGAGGGGGTGGATCTACTT CCGACATTGTCATGACCCAGACCCCGTTGAGCCTTTCAGTGACG CCCGGTCAACCCGCCAGCATAAGTTGTCGATCAAGCCAGTCTAT TGTACACTCCAATGGAAACACATATTTGGAGTGGTATCTCCAAA AACCCGGCCAAAGCCCTCAACTGCTCATCTACAAGGTCTCAAAC AGGTTTAGCGGGGTCCCGGATCGCTTCTCAGGGTCAGGATCTGG TACGGACTTTACACTGAAAATTTCCCGAGTCGAAGCGGAAGACG TGGGTGTATATTACTGCTTCCAGGGGAGTCATGTTCCAAGAACC TTTGGGGGAGGTACAAAGGTCGAAATAAAA
H4_k3_NT	151	ATGGATTTTCAGGTCCAAATTTTTTCCTTCTTGCTTATCAGCGC AAGTGTAATCATGTCCCGCGCGTCCCAAGTACAACTTGTGCAAT CTGGCGCGGAGGTGAAAAAACCTGGAGCTTCCGTCAAGGTTTCT TGTAAGGCCTCTGGCTACACCTTCACGTCCTACCACATTCAGTG GGTTCGACAGGCGCCGGGCCAAGGACTGGAGTGGATGGGATGGA TATATCCAGGAGATGGTTCTACTCAGTATAATGAGAAATTCAAG GGTCGCGTAACAATGACGAGGGATACATCAATCTCCACCGCGTA CATGGAACTTTCAAGACTCCGGTCAGATGACACGGCGGTTTACT ACTGTGCTCGGGAGGGCACTTACTATGCTATGGACTACTGGGGG CAAGGGACAACGGTAACGGTATCTAGTGTGGATTCTAGTGGCGG CGGCGGTTCAGGAGGAGGTGGTTCAGGCGGGGGGGGTAGTACAA GTGATATTGTGATGACCCAAACACCCCTTTCTAGCCCTGTTACT CTGGGTCAACCCGCGTCCATAAGTTGTCGAAGTAGTCAATCCAT CGTGCATAGCAACGGCAACACTTACCTTGAATGGTATCAACAAC GACCCGGACAGCCCCCGCGACTGCTTATCTATAAAGTATCAAAC AGGTTCAGTGGCGTGCCAGATCGATTCTCCGGCTCTGGGGCAGG CACAGATTTCACGTTGAAAATTTCTCGGGTCGAGGCCGAGGACG TGGGCGTTTATTACTGTTTCCAGGGGAGTCACGTCCCCAGGACG TTCGGAGGAGGAACTAAAGTCGAAATAAAG
H4_k4_NT	152	ATGGACTTCCAGGTCCAAATATTCAGCTTCCTCCTCATTTCCGC CAGTGTAATAATGTCCAGAGCCTCACAAGTACAGTTGGTTCAGA GCGGGGCTGAGGTTAAGAAACCAGGCGCGAGCGTCAAGGTATCC TGCAAGGCGAGTGGTTATACTTTCACTAGTTATCACATTCAGTG GGTCCGACAGGCCCCCGGTCAAGGCCTGGAGTGGATGGGGTGGA TATATCCGGGAGATGGTTCTACCCAATATAATGAGAAGTTTAAG GGGAGAGTCACAATGACAAGGGACACCAGTATTAGCACCGCGTA TATGGAGCTTTCCCGCCTGCGATCAGATGACACGGCCGTGTACT ACTGTGCTAGGGAGGGAACCTATTATGCGATGGATTACTGGGGA CAGGGTACTACAGTCACGGTCTCTAGCGTGGACAGTTCCGGGGG

		CGGTGGAAGCGGTGGTGGCGGTTCAGGTGGAGGAGGCTCTACGA GTGATGTTGTGATGACTCAGTCCCCGCTTTCACTTCCCGTCACC CTTGGGCAACCCGCAAGCATCTCATGCCGATCCTCCCAGTCTAT AGTACATAGTAATGGCAACACATATCTTGAATGGTATCAGCAGA GGCCGGGTCAGTCTCCCCGACTCCTTATATATAAAGTGAGCAAC AGATTCTCCGGAGTACCGGATAGATTTTCCGGCTCTGGGAGCGG CACCGACTTTACACTGAAAATTTCACGGGTTGAAGCTGAAGATG TTGGGGTATACTATTGTTTCCAGGGTTCTCACGTCCCGAGGACA TTCGGGGGAGGAACGAAAGTCGAAATAAAG
H4_k5_NT	153	ATGGATTTTCAGGTACAAATCTTCAGCTTCCTGCTCATCTCCGC GAGCGTAATCATGTCTAGGGCGTCCCAGGTGCAGTTGGTGCAAT CAGGTGCAGAGGTGAAGAAGCCTGGTGCATCCGTTAAAGTAAGT TGTAAGGCAAGCGGATATACTTTTACATCCTATCATATTCAATG GGTCAGACAAGCACCTGGACAGGGTCTTGAGTGGATGGGCTGGA TCTATCCAGGCGATGGCTCAACTCAATATAACGAGAAGTTCAAG GGGAGGGTTACTATGACCAGGGATACGTCTATTTCCACTGCGTA CATGGAACTCTCCAGGTTGAGAAGTGATGATACCGCGGTTTACT ACTGCGCTAGAGAAGGAACGTACTACGCTATGGATTACTGGGGG CAGGGTACAACTGTCACCGTCTCAAGTGTGGATTCTTCTGGGGG TGGGGGATCAGGAGGGGGAGGCTCCGGTGGGGGCGGGTCAACCA GCGACATTGTCATGACTCAAACCCCCCTGAGCAGCCCTGTCACC CTGGGTCAGCCTGCCTCAATATCCTTTAGAAGCTCCCAAAGCAT CGTCCATTCAAATGGTAATACCTATCTGGAGTGGTATCAGCAAA GGCCTGGTCAACCCCCGCGCCTTCTCATTTACAAGGTGTCAAAC AGGTTCTCCGGCGTACCGGATAGGTTTTCCGGAAGCGGTGCTGG AACCGACTTTACTCTCAAAATCTCTAGGGTGGAAGCTGAGGACG TCGGTGTATACTATTGTTTTCAAGGCTCCCATGTTCCCAGGACA TTTGGTGGGGGAACGAAGGTAGAAATCAAG
H5_k2_NT	154	ATGGACTTTCAGGTTCAGATTTTCTCTTTCTTGTTGATCTCCGC TAGTGTCATAATGTCACGGGCAAGTCAGGTACAACTCGTTCAGA GTGGTGCCGAAGTGAAGAAACCGGGTGCCTCCGTAAAGGTGTCA TGTAAAGCTAGTGGCTATACATTCACAAGTTATCATATCCAATG GGTACGACAAGCACCGGGACAGCGACTGGAATGGATGGGATGGA TCTATCCTGGGGACGGATCTACACAGTACAATGAGAAATTTAAG GGACGGGTCACGATAACCAGGGACACATCTGCTTCCACGGCTTA CATGGAGCTTTCCTCCCTGCGGAGCGAGGACATGGCTGTTTACT ATTGCGCTCGCGAAGGGACATACTACGCAATGGATTATTGGGGC CAAGGCACTACCGTGACGGTCTCTTCTGTCGATAGTTCCGGAGG AGGTGGTTCAGGGGGAGGCGGTTCAGGTGGGGGTGGATCTACCT CAGATATTGTCATGACACAGACACCTTTGTCCTTGAGTGTGACA CCGGGTCAACCGGCGAGTATAAGCTGTCGCAGCTCACAATCTAT TGTGCATAGCAACGGGAATACATATCTCGAATGGTATCTCCAAA AGCCGGGCCAATCCCCCCAACTTCTCATTTACAAAGTTTCTAAT CGATTTTCAGGTGTACCAGATCGGTTTTCCGGGTCTGGCTCAGG TACTGACTTCACCTTGAAAATATCAAGGGTTGAAGCTGAGGATG TAGGTGTGTACTATTGCTTCCAGGGGTCTCACGTTCCTCGGACT

		TTTGGGGGGGGCACAAAAGTAGAGATTAAA
H5_k3_NT	155	ATGGATTTCCAGGTGCAAATCTTCTCATTTCTTTTGATAAGTGC GTCAGTGATAATGTCTCGGGCCAGTCAAGTACAGCTTGTCCAAA GTGGCGCTGAAGTCAAGAAGCCGGGAGCCTCAGTTAAGGTTAGC TGCAAGGCCTCAGGGTATACTTTTACCTCCTATCATATACAGTG GGTACGACAAGCACCGGGACAGCGACTGGAGTGGATGGGTTGGA TATATCCGGGAGATGGTTCAACCCAGTATAATGAGAAGTTCAAG GGGCGAGTTACGATAACCCGCGATACGAGTGCATCAACAGCGTA CATGGAGTTGAGTTCCCTCCGCAGCGAGGACATGGCGGTATACT ATTGTGCCAGGGAGGGGACTTATTATGCCATGGACTACTGGGGG CAGGGCACAACCGTAACAGTCTCTTCTGTAGACAGTTCAGGAGG GGGCGGAAGTGGAGGTGGCGGATCTGGTGGAGGTGGATCTACTT CCGACATCGTTATGACCCAAACACCACTTTCATCTCCCGTTACT CTCGGGCAACCTGCTAGTATTTCCTGTAGATCCTCACAATCTAT AGTACATAGCAATGGCAATACCTACCTGGAGTGGTATCAACAAC GCCCAGGCCAACCACCTCGCCTGCTTATCTATAAAGTAAGCAAT AGATTCAGTGGTGTACCGGATAGGTTCTCTGGTTCCGGAGCAGG AACTGACTTTACACTCAAAATCAGTAGGGTGGAGGCGGAAGACG TGGGAGTATATTATTGCTTTCAAGGTTCACATGTACCTCGAACA TTTGGCGGAGGAACTAAGGTTGAGATTAAA
H5_k4_NT	156	ATGGATTTCCAAGTCCAGATATTCAGTTTTCTTTTGATAAGCGC TTCTGTAATCATGTCTCGGGCGTCCCAAGTACAACTGGTGCAAT CAGGGGCAGAAGTGAAAAAACCAGGTGCATCCGTTAAGGTGAGT TGCAAGGCTTCCGGCTATACCTTTACATCATATCATATTCAATG GGTCAGGCAAGCACCTGGTCAGCGATTGGAATGGATGGGTTGGA TATATCCTGGTGATGGGTCTACACAATATAACGAAAAATTCAAG GGGCGAGTGACCATCACAAGAGATACATCAGCGTCAACAGCGTA TATGGAACTGTCATCCCTTAGATCAGAGGACATGGCGGTCTATT ACTGTGCCAGAGAAGGCACTTATTATGCAATGGATTATTGGGGA CAAGGAACCACTGTCACTGTTTCCAGCGTAGACTCCTCCGGTGG TGGTGGAAGTGGCGGCGGTGGGTCAGGAGGGGGTGGGTCAACTT CTGATGTAGTGATGACACAGAGCCCTCTGAGCTTGCCTGTGACC TTGGGTCAGCCGGCCTCAATAAGTTGTCGATCTAGTCAGTCAAT CGTCCATAGTAATGGGAACACATACCTTGAATGGTATCAGCAAA GACCTGGACAATCTCCACGACTCCTTATATACAAAGTTAGCAAC CGATTTAGCGGAGTGCCAGACCGCTTTTCTGGTTCCGGGTCTGG CACAGATTTTACCCTTAAGATCTCCCGCGTGGAGGCGGAAGACG TTGGTGTTTACTATTGCTTCCAGGGGTCACACGTTCCACGCACC TTTGGAGGAGGTACGAAGGTCGAGATTAAG
H5_k5_NT	157	ATGGATTTTCAAGTACAGATCTTCTCTTTCTTGCTTATTTCAGC GAGCGTAATCATGAGTAGGGCATCTCAAGTTCAACTCGTTCAGT CAGGTGCTGAGGTAAAAAAACCAGGGGCTTCCGTTAAAGTTAGC TGTAAGGCATCTGGGTACACATTTACTAGCTACCATATCCAGTG GGTGCGACAAGCCCCGGGGCAGCGCTTGGAATGGATGGGCTGGA TTTACCCAGGTGACGGCTCCACGCAATATAATGAGAAATTTAAG

		GGTAGAGTTACTATTACCAGGGACACAAGTGCTTCAACTGCCTA TATGGAACTGAGCAGCCTTCGGAGTGAAGATATGGCCGTATATT ACTGCGCAAGGGAGGGGACTTACTATGCAATGGACTACTGGGGT CAGGGAACGACTGTGACCGTGTCCTCAGTTGACTCCAGCGGTGG TGGCGGCTCTGGAGGTGGGGGTTCCGGCGGAGGCGGAAGCACAT CTGATATAGTGATGACGCAAACGCCTCTTTCTTCCCCGGTAACT CTGGGACAGCCAGCGTCAATTTCATTTAGGTCCTCCCAGTCAAT CGTACATAGTAATGGAAATACTTACCTGGAATGGTATCAACAAC GACCAGGGCAACCGCCCCGATTGTTGATCTATAAAGTGAGCAAT CGCTTTTCTGGCGTGCCCGATCGGTTCTCAGGGTCTGGAGCTGG GACTGACTTCACATTGAAAATTTCCAGGGTTGAGGCCGAGGATG TGGGGGTTTATTACTGCTTCCAAGGCTCCCACGTCCCCCGCACC TTCGGAGGGGGAACCAAAGTCGAAATAAAG
H6_k2_NT	158	ATGGATTTTCAAGTTCAGATATTCTCATTTTTGCTTATATCAGC CTCCGTAATTATGTCACGGGCAAGTCAAGTTCAGTTGGTGCAGT CCGGAGCAGAAGTTAAGAAGCCCGGTGCTTCTGTGAAAGTCTCC TGCAAAGCGTCTGGGTACACCTTCACGAGCTACCATATACAGTG GGTCCGGCAAGCGCCTGGGCAGAGGCTGGAGTGGATGGGCTGGA TTTACCCAGGAGATGGGAGTACAAAGTATAGTCAGAAGTTTCAA GGGCGAGTGACGATAACCAGAGATACGAGTGCAAGTACTGCATA CATGGAACTGAGCTCCTTGAGGTCCGAGGATACAGCGGTGTACT ATTGCGCTCGGGAAGGGACATATTATGCTATGGACTATTGGGGA CAAGGGACAACGGTAACGGTGAGTTCCGTCGATTCCTCAGGTGG CGGAGGCAGTGGGGGCGGGGGTTCCGGCGGTGGCGGGTCCACGA GTGATATAGTTATGACACAGACCCCCCTCAGCCTTTCTGTGACC CCAGGACAACCCGCTAGTATCTCTTGCCGCAGTTCTCAGTCCAT AGTACACAGTAACGGAAATACCTATCTTGAGTGGTATCTTCAAA AGCCCGGCCAGAGCCCTCAACTCTTGATATATAAAGTGTCAAAT CGATTTTCAGGTGTGCCTGATCGATTCTCAGGGTCTGGTTCAGG GACAGATTTCACGCTTAAGATAAGCAGAGTAGAGGCTGAAGACG TGGGAGTCTACTATTGTTTTCAGGGGTCACACGTTCCCCGCACT TTTGGTGGGGGAACCAAGGTGGAAATCAAA
CD8 hinge	159	TCTTCAGCGCTGAGCAACTCCATCATGTACTTCAGCCACTTCGT GCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGC GACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCC CTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCA CACGAGGGGGCTGGAC
CD28 TM	160	TTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAG CTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTG
CD28 IC	161	AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACAT GACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCAGCCCT ATGCCCCACCACGCGACTTCGCAGCCTATCGCTCC
CD3z	162	AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCA GGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAG

		AGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAG ATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTA CAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGA TTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGC CTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGC CCTTCACATGCAGGCCCTGCCCCCTCGC
CAR_H1_k1_NT	163	ATGGATTTCCAAGTTCAAATCTTCAGTTTCTTGCTTATCAGTGC TTCTGTTATTATGTCACGAGCAAGTCAAGTTCAACTCGTACAGT CTGGAGCCGAGGTGAAAAAACCGGGAGCGTCCGTGAAAGTGAGT TGCAAGGCGAGTGGATACACCTTCACTTCATACCATATACAATG GGTTCGGCAGGCGCCTGGTCAACGGCTGGAATGGATGGGCTGGA TTTATCCCGGAGATGGTTCCACGCAGTACAATGAGAAATTCAAA GGGAGAGTGACAATCACCCGAGATACCAGTGCCTCTACGGCATA TATGGAACTGAGTAGTCTGCGGTCTGAAGATACGGCGGTGTATT ATTGTGCGAGAGAAGGGACGTACTACGCCATGGATTATTGGGGA CAAGGAACAACAGTCACAGTCTCCAGCGTTGATTCCTCAGGCGG GGGCGGAAGTGGGGGCGGCGGATCTGGCGGCGGCGGGTCTACGT CTGACGTGGTCATGACCCAATCTCCATTGTCTTTGCCAGTTACT CTGGGACAGCCTGCAAGTATCAGTTGCCGATCCTCCCAATCTAT CGTCCATTCAAACGGGAACACTTATTTGGAATGGTTTCAACAGA GACCTGGGCAAAGTCCGCGCCGACTGATATATAAGGTCAGTAAC CGCTTTTCAGGCGTCCCCGATCGATTCAGTGGATCTGGGTCAGG GACTGACTTCACTCTGAAAATATCAAGAGTCGAAGCTGAAGATG TCGGAGTATATTACTGTTTCCAGGGGTCTCACGTCCCTCGGACG TTTGGAGGCGGAACTAAGGTTGAGATAAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H1_k2_NT	164	ATGGATTTTCAGGTTCAAATCTTTAGCTTTCTCTTGATTTCCGC CTCCGTAATAATGAGTCGGGCCAGTCAGGTACAGCTCGTTCAAT CTGGGGCTGAAGTAAAAAAGCCTGGAGCGTCTGTAAAGGTATCT

		TGCAAAGCGAGCGGCTACACATTCACAAGTTATCACATCCAATG GGTGAGACAGGCCCCAGGACAACGCTTGGAGTGGATGGGGTGGA TTTACCCTGGCGACGGCAGCACACAGTACAATGAGAAATTTAAA GGCCGGGTGACTATCACTCGGGACACCTCCGCCAGCACGGCTTA TATGGAGCTTAGCAGTTTGAGATCCGAAGATACAGCGGTATATT ACTGCGCGAGAGAAGGAACGTACTACGCTATGGACTATTGGGGT CAGGGCACAACCGTTACAGTCTCCTCTGTGGACAGCTCCGGAGG TGGGGGTTCAGGAGGGGGTGGAAGCGGTGGTGGTGGCAGTACAA GCGATATAGTAATGACCCAAACCCCGCTCTCTCTGAGCGTCACG CCAGGACAACCAGCATCAATCTCTTGCCGCAGTAGTCAATCCAT CGTTCACTCTAATGGAAACACATACCTTGAGTGGTATCTTCAGA AACCAGGTCAGAGCCCTCAGCTCCTCATCTATAAAGTCTCTAAC CGGTTCTCAGGTGTTCCGGACCGGTTCAGTGGTTCCGGCTCAGG AACAGACTTCACCTTGAAGATCAGTCGAGTAGAAGCCGAAGACG TGGGTGTATATTATTGCTTTCAGGGTTCCCACGTTCCGCGCACC TTCGGCGGCGGGACCAAAGTTGAGATCAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H2_k1_NT	165	ATGGACTTTCAAGTCCAAATCTTCTCATTCCTCCTGATCTCTGC GTCAGTAATCATGTCCAGAGCGTCACAAGTGCAACTCGTTCAAT CCGGAGCTGAGGTAAAGAAGCCCGGCGCCAGCGTGAAAGTCTCC TGCAAAGCGAGCGGCTACACGTTCACCTCATATCACATTCAATG GGTAAGACAAGCACCTGGGCAACGACTCGAGTGGATGGGGTGGA TCTACCCTGGGGACGGGAGCACGCAGTATAATGAGAAATTCAAA GGCAGGGTTACAATTACAGCCGATACCAGTGCATCTACGGCTTA TATGCTCCTCTCCTCACTCCGGTCTGAGGACACAGCGGTTTATT ATTGCGCACGGGAGGGAACGTACTACGCGATGGACTATTGGGGG CAAGGCACCACAGTTACAGTGAGCTCAGTTGACTCATCAGGAGG CGGAGGATCAGGGGGAGGTGGTAGTGGGGGCGGTGGGAGCACAT CAGATGTTGTCATGACTCAGAGCCCACTTTCTTTGCCGGTGACG

		CTGGGGCAGCCCGCTTCAATCTCTTGCCGCTCATCACAGTCTAT CGTTCATAGCAATGGTAACACTTACTTGGAATGGTTCCAACAAA GACCGGGTCAAAGTCCACGGCGCTTGATATATAAAGTATCAAAT AGATTTTCAGGGGTGCCTGATCGGTTCAGCGGTTCTGGATCTGG CACCGACTTCACGCTTAAAATAAGTAGGGTAGAAGCCGAAGACG TGGGAGTGTATTATTGTTTCCAGGGGTCACACGTCCCTCGCACG TTCGGCGGAGGCACTAAAGTGGAGATCAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H2_k2_NT	166	ATGGACTTTCAAGTCCAAATCTTCAGCTTTCTCCTTATATCTGC GTCTGTCATTATGAGTAGAGCAAGTCAAGTCCAGCTCGTACAAA GTGGAGCTGAGGTGAAAAAGCCGGGCGCGAGTGTGAAAGTCTCA TGCAAGGCGAGTGGATACACCTTTACCTCTTACCACATTCAATG GGTGCGGCAGGCGCCTGGGCAGCGCTTGGAATGGATGGGCTGGA TATATCCTGGCGACGGAAGTACCCAGTACAACGAAAAATTCAAA GGTAGGGTTACCATCACTGCTGATACCTCCGCGTCCACTGCTTA TATGCTTCTTAGCTCCTTGCGAAGCGAGGATACAGCCGTGTATT ATTGTGCCAGAGAGGGGACTTATTATGCCATGGACTATTGGGGT CAGGGTACAACGGTCACTGTCTCATCTGTTGACAGTAGCGGGGG AGGGGGGTCTGGAGGAGGGGGTTCCGGGGGGGGAGGTTCCACGA GCGATATAGTTATGACGCAAACGCCCTTGAGCCTCAGTGTTACA CCCGGTCAACCTGCCTCTATTAGCTGTCGCTCCTCCCAATCAAT TGTGCATAGCAATGGAAATACCTACCTTGAATGGTATCTCCAAA AGCCCGGGCAGAGTCCTCAACTTCTCATCTACAAAGTATCCAAT CGATTCAGTGGCGTTCCTGACAGGTTCAGCGGAAGTGGGTCAGG GACCGATTTTACCCTCAAAATTAGTCGCGTCGAAGCTGAGGATG TTGGGGTGTATTACTGCTTCCAAGGGTCACACGTACCACGCACA TTCGGGGGAGGCACGAAGGTTGAAATTAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG

		CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H1_k3_NT	167	ATGGATTTCCAAGTCCAAATATTCAGTTTCCTTTTGATAAGTGC TTCAGTTATCATGTCCCGAGCAAGCCAGGTACAGCTTGTGCAAA GCGGGGCGGAAGTTAAGAAACCGGGAGCCTCAGTTAAAGTATCT TGCAAAGCCAGCGGTTATACATTCACTTCATACCACATACAGTG GGTGCGCCAAGCACCGGGGCAGAGACTGGAATGGATGGGATGGA TTTATCCCGGTGATGGTAGTACGCAATACAATGAAAAATTCAAA GGAAGGGTGACTATCACGCGAGACACAAGCGCGTCCACGGCCTA TATGGAACTGAGCAGTCTGAGATCCGAGGACACCGCTGTGTATT ATTGCGCCCGAGAGGGGACCTACTACGCCATGGATTATTGGGGT CAAGGGACCACTGTGACAGTCTCTTCTGTCGATTCCAGCGGCGG AGGAGGGAGTGGAGGGGGTGGGTCCGGGGGGGGAGGGTCTACGA GCGACATTGTCATGACACAGACGCCGCTTAGCTCCCCAGTTACA CTCGGACAGCCCGCAAGTATTAGTTGCAGAAGTAGCCAGTCTAT CGTACATTCAAATGGAAACACCTATTTGGAATGGTATCAACAAC GGCCTGGACAGCCGCCCAGGCTGCTCATATACAAAGTCTCCAAC CGCTTCAGCGGAGTACCCGACCGCTTTTCCGGCTCCGGAGCAGG AACTGACTTTACCTTGAAAATTAGTAGGGTCGAAGCAGAGGATG TCGGGGTATATTATTGTTTCCAAGGTAGTCATGTCCCACGGACG TTTGGTGGTGGGACGAAGGTTGAGATCAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG

		CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H1_k4_NT	168	ATGGACTTCCAGGTGCAGATATTTTCCTTTCTTCTCATATCTGC ATCTGTAATAATGTCAAGGGCCAGCCAGGTCCAGCTCGTTCAAA GCGGAGCCGAGGTAAAAAAACCAGGCGCTTCCGTCAAGGTATCA TGCAAAGCGTCCGGCTATACCTTCACAAGTTACCATATCCAATG GGTTCGACAGGCACCCGGACAAAGACTTGAATGGATGGGTTGGA TATACCCCGGAGACGGCTCCACTCAGTATAACGAAAAGTTTAAG GGGAGAGTCACGATCACTAGGGACACATCAGCTTCTACGGCGTA TATGGAACTCAGTTCTTTGCGATCCGAGGATACTGCCGTATATT ACTGCGCCAGAGAAGGCACGTACTACGCAATGGATTACTGGGGG CAAGGGACAACTGTTACCGTCTCAAGCGTCGATTCATCAGGAGG CGGAGGGTCCGGAGGTGGGGGATCTGGCGGTGGGGGTTCTACGT CCGATGTTGTGATGACACAGTCCCCACTCTCTCTTCCAGTGACG CTGGGACAGCCCGCGAGCATCTCCTGTCGCAGCTCTCAGTCCAT AGTACACAGTAATGGTAACACCTATCTTGAGTGGTATCAGCAAC GACCCGGTCAGTCTCCCAGGTTGCTTATTTATAAGGTTAGTAAC CGCTTTTCAGGTGTTCCAGACAGATTTAGCGGGAGTGGTTCCGG TACGGATTTCACATTGAAAATCAGCCGCGTCGAAGCCGAGGACG TGGGTGTTTACTACTGCTTCCAGGGATCTCACGTACCGAGGACC TTCGGCGGAGGAACGAAAGTAGAAATTAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H1_k	169	ATGGATTTTCAAGTACAAATCTTTTCCTTCCTGCTTATTTCCGC AAGCGTTATTATGAGTAGAGCCAGCCAAGTACAACTGGTACAGT

5_NT		CCGGCGCGGAGGTGAAGAAGCCCGGAGCAAGTGTGAAGGTATCT TGCAAGGCGTCAGGCTATACCTTTACTTCATACCATATACAGTG GGTGCGACAGGCTCCCGGCCAGCGACTCGAATGGATGGGCTGGA TTTATCCCGGAGATGGATCTACGCAGTATAATGAGAAATTCAAG GGTCGGGTCACGATTACACGAGACACGAGTGCTTCCACAGCTTA TATGGAACTTTCTAGCCTGAGGTCTGAGGATACTGCCGTTTACT ATTGTGCACGAGAAGGGACATACTATGCGATGGATTACTGGGGA CAGGGCACCACTGTCACAGTTTCCAGCGTGGACTCAAGTGGAGG CGGTGGATCTGGTGGTGGCGGGTCCGGGGGGGGAGGCAGCACCA GTGACATTGTAATGACTCAAACACCTCTCAGTAGCCCAGTCACT CTCGGTCAGCCGGCGAGTATCTCTTTTAGGTCCTCACAATCTAT AGTGCACTCTAACGGCAATACTTATCTTGAATGGTATCAACAAA GACCGGGGCAGCCACCTCGCCTTCTCATCTACAAAGTAAGCAAT CGCTTCTCCGGTGTCCCCGATCGCTTCTCCGGTTCAGGAGCAGG AACTGACTTCACATTGAAGATTTCCAGAGTGGAGGCCGAAGACG TAGGGGTATATTATTGCTTTCAAGGGTCCCATGTGCCCAGAACC TTTGGGGGAGGAACGAAAGTTGAGATTAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H3_k2_NT	170	ATGGATTTCCAAGTGCAGATTTTCTCTTTTCTCCTCATAAGCGC CTCCGTAATTATGTCTAGAGCTAGTCAAGTCCAATTGGTGCAAT CCGGTGCCGAGGTTAAAAAGCCCGGCGCAAGTGTAAAAGTCTCC TGTAAGGCCAGTGGCTACACTTTCACCAGCTACCATATACAGTG GGTGCGGCAGGCGCCTGGTCAGGGTCTGGAGTGGATGGGTATTT GGATTTATCCCGGAGATGGAAGTACTCAATACAATGAGAAATTC AAGGGTGTCACTATGACAAGGGATACGAGCACTTCTACCGTATA TATGGAGTTGTCATCTTTGCGATCAGAGGATACCGCTGTATATT ATTGCGCACGGGAAGGTACATATTATGCCATGGACTACTGGGGC CAAGGAACCACCGTGACGGTAAGCTCTGTCGATTCTAGCGGTGG CGGGGGCTCTGGCGGTGGGGGTAGCGGGGGTGGCGGATCTACAT

		CAGATATTGTAATGACACAGACCCCTCTTTCACTTTCCGTAACG CCAGGACAGCCGGCATCAATAAGTTGCCGATCAAGCCAGTCTAT CGTACACTCCAATGGTAACACATACTTGGAATGGTATCTTCAAA AGCCCGGCCAGAGCCCGCAGCTTTTGATATATAAAGTGTCCAAC AGATTCAGTGGGGTGCCGGACCGCTTTAGTGGATCTGGTTCAGG AACGGACTTCACATTGAAAATTAGTAGAGTTGAAGCGGAAGACG TGGGAGTCTACTACTGTTTCCAGGGTTCACATGTGCCTCGGACC TTTGGGGGAGGCACCAAGGTTGAGATAAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H3_k3_NT	171	ATGGACTTCCAAGTCCAAATCTTTTCTTTTTTGTTGATAAGCGC ATCAGTTATTATGTCTCGCGCCAGTCAAGTACAACTGGTGCAGT CCGGAGCTGAAGTGAAAAAACCAGGAGCAAGCGTGAAAGTAAGT TGTAAGGCAAGTGGTTACACTTTCACAAGCTACCATATTCAATG GGTCCGACAGGCTCCTGGACAGGGCTTGGAGTGGATGGGCATAT GGATTTACCCTGGTGACGGGTCCACCCAGTATAATGAAAAGTTC AAGGGAGTCACGATGACCAGGGACACCTCTACATCTACCGTGTA TATGGAGCTCTCTAGTTTGCGATCCGAAGACACTGCCGTTTATT ACTGTGCAAGAGAAGGAACTTATTACGCGATGGACTACTGGGGT CAGGGGACAACAGTCACCGTTAGCTCCGTCGATTCCAGCGGGGG AGGTGGCTCAGGCGGGGGTGGTTCTGGGGGGGGCGGGAGCACTT CAGATATTGTAATGACCCAAACCCCACTGAGTAGTCCAGTCACG CTTGGTCAACCGGCAAGCATTTCTTGCAGGAGCTCTCAGAGTAT TGTCCACTCTAACGGGAATACATATTTGGAGTGGTATCAGCAAA GACCGGGCCAACCACCACGCCTCTTGATTTATAAGGTGAGCAAT AGGTTTTCAGGCGTGCCAGATAGGTTCTCAGGCTCCGGAGCGGG AACCGACTTCACCCTCAAGATAAGTCGGGTGGAAGCCGAAGACG TAGGAGTTTACTACTGCTTTCAAGGATCTCATGTTCCACGAACG TTTGGAGGAGGAACCAAGGTGGAAATAAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA

		GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H3_k4_NT	172	ATGGACTTTCAGGTCCAAATTTTTTCCTTCTTGCTCATATCCGC GAGTGTCATCATGTCAAGAGCAAGTCAAGTTCAACTCGTTCAAT CAGGAGCTGAGGTGAAAAAACCAGGGGCGTCTGTCAAAGTAAGC TGCAAAGCATCAGGGTATACGTTCACGAGTTATCATATCCAGTG GGTTAGGCAGGCGCCAGGGCAGGGATTGGAATGGATGGGTATCT GGATTTACCCGGGTGACGGCAGCACTCAATACAATGAGAAATTC AAAGGCGTAACAATGACAAGGGACACGAGCACAAGCACAGTGTA CATGGAGCTTAGCTCTTTGAGGTCAGAGGATACCGCTGTTTACT ATTGTGCTCGGGAGGGTACTTACTATGCAATGGACTACTGGGGG CAAGGCACGACCGTTACAGTGAGTAGCGTAGATTCCTCCGGGGG TGGCGGTTCAGGCGGCGGAGGCTCAGGCGGAGGAGGGTCAACAT CCGATGTCGTAATGACTCAGTCCCCTCTGTCATTGCCGGTGACT TTGGGACAGCCAGCGTCTATATCTTGTAGGTCCTCTCAATCAAT AGTGCATTCCAACGGTAACACCTATCTGGAATGGTATCAGCAAA GGCCAGGACAAAGTCCACGCCTGCTTATATATAAGGTGTCTAAT CGATTCAGTGGGGTTCCCGATAGGTTTTCCGGCTCTGGTAGCGG GACTGATTTCACGTTGAAAATATCACGCGTGGAAGCGGAAGATG TTGGGGTCTATTACTGCTTTCAGGGTAGTCATGTCCCTCGAACT TTTGGCGGTGGTACAAAGGTAGAAATCAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA

		GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H3_k5_NT	173	ATGGATTTTCAGGTACAGATATTCTCATTTCTCCTTATCTCAGC TAGTGTCATAATGTCCAGGGCGAGTCAAGTACAACTTGTCCAGT CAGGCGCAGAGGTCAAGAAGCCGGGCGCAAGCGTTAAGGTTTCC TGCAAAGCATCCGGCTATACATTCACGTCCTATCACATCCAATG GGTCAGGCAAGCACCCGGTCAAGGACTTGAGTGGATGGGCATCT GGATTTACCCTGGAGATGGCAGTACTCAGTACAACGAAAAATTC AAAGGTGTAACCATGACCCGCGACACATCTACTTCCACAGTTTA TATGGAACTCAGCAGTTTGCGGAGCGAAGATACCGCTGTTTACT ACTGTGCCCGAGAGGGAACTTACTACGCCATGGACTATTGGGGT CAAGGAACGACAGTAACAGTTAGTTCTGTAGATTCCAGTGGCGG CGGTGGGAGCGGGGGTGGGGGATCTGGCGGAGGCGGAAGTACAA GTGACATCGTTATGACTCAGACACCCCTTAGTAGTCCCGTTACG TTGGGCCAACCCGCGAGCATTTCCTTTCGATCCTCTCAGTCTAT AGTTCACTCAAATGGGAATACTTATTTGGAGTGGTATCAACAGC GCCCCGGACAACCACCAAGGCTCCTGATATACAAGGTGTCCAAT CGATTCTCTGGGGTGCCTGATAGATTTAGCGGAAGTGGAGCCGG TACAGATTTTACCCTGAAAATATCACGGGTAGAAGCCGAAGATG TCGGCGTCTACTACTGTTTCCAGGGTTCCCATGTACCGCGAACG TTCGGGGGCGGAACAAAAGTTGAGATCAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC

CAR_H4_k2_NT	174	ATGGATTTCCAGGTTCAGATATTTAGTTTCCTCTTGATTTCTGC CAGTGTCATCATGAGCAGGGCTTCCCAAGTTCAGTTGGTGCAAA GTGGCGCTGAAGTCAAAAAACCTGGGGCTTCCGTTAAAGTATCT TGCAAGGCGTCCGGCTACACTTTCACATCCTACCACATTCAATG GGTCCGGCAAGCGCCCGGTCAGGGGCTCGAATGGATGGGGTGGA TATACCCAGGAGATGGATCTACTCAGTACAACGAGAAATTTAAA GGACGGGTGACGATGACGCGCGACACTTCAATAAGCACTGCATA CATGGAACTGTCCCGGCTTAGGTCAGATGACACCGCGGTCTACT ATTGTGCGAGAGAGGGTACTTACTATGCTATGGACTACTGGGGG CAAGGCACGACGGTTACAGTTTCCTCAGTCGATAGTTCAGGCGG AGGCGGCTCCGGGGGCGGTGGTAGTGGAGGGGGTGGATCTACTT CCGACATTGTCATGACCCAGACCCCGTTGAGCCTTTCAGTGACG CCCGGTCAACCCGCCAGCATAAGTTGTCGATCAAGCCAGTCTAT TGTACACTCCAATGGAAACACATATTTGGAGTGGTATCTCCAAA AACCCGGCCAAAGCCCTCAACTGCTCATCTACAAGGTCTCAAAC AGGTTTAGCGGGGTCCCGGATCGCTTCTCAGGGTCAGGATCTGG TACGGACTTTACACTGAAAATTTCCCGAGTCGAAGCGGAAGACG TGGGTGTATATTACTGCTTCCAGGGGAGTCATGTTCCAAGAACC TTTGGGGGAGGTACAAAGGTCGAAATAAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H4_k3_NT	175	ATGGATTTTCAGGTCCAAATTTTTTCCTTCTTGCTTATCAGCGC AAGTGTAATCATGTCCCGCGCGTCCCAAGTACAACTTGTGCAAT CTGGCGCGGAGGTGAAAAAACCTGGAGCTTCCGTCAAGGTTTCT TGTAAGGCCTCTGGCTACACCTTCACGTCCTACCACATTCAGTG GGTTCGACAGGCGCCGGGCCAAGGACTGGAGTGGATGGGATGGA TATATCCAGGAGATGGTTCTACTCAGTATAATGAGAAATTCAAG GGTCGCGTAACAATGACGAGGGATACATCAATCTCCACCGCGTA CATGGAACTTTCAAGACTCCGGTCAGATGACACGGCGGTTTACT ACTGTGCTCGGGAGGGCACTTACTATGCTATGGACTACTGGGGG

		CAAGGGACAACGGTAACGGTATCTAGTGTGGATTCTAGTGGCGG CGGCGGTTCAGGAGGAGGTGGTTCAGGCGGGGGGGGTAGTACAA GTGATATTGTGATGACCCAAACACCCCTTTCTAGCCCTGTTACT CTGGGTCAACCCGCGTCCATAAGTTGTCGAAGTAGTCAATCCAT CGTGCATAGCAACGGCAACACTTACCTTGAATGGTATCAACAAC GACCCGGACAGCCCCCGCGACTGCTTATCTATAAAGTATCAAAC AGGTTCAGTGGCGTGCCAGATCGATTCTCCGGCTCTGGGGCAGG CACAGATTTCACGTTGAAAATTTCTCGGGTCGAGGCCGAGGACG TGGGCGTTTATTACTGTTTCCAGGGGAGTCACGTCCCCAGGACG TTCGGAGGAGGAACTAAAGTCGAAATAAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H4_k4_NT	176	ATGGACTTCCAGGTCCAAATATTCAGCTTCCTCCTCATTTCCGC CAGTGTAATAATGTCCAGAGCCTCACAAGTACAGTTGGTTCAGA GCGGGGCTGAGGTTAAGAAACCAGGCGCGAGCGTCAAGGTATCC TGCAAGGCGAGTGGTTATACTTTCACTAGTTATCACATTCAGTG GGTCCGACAGGCCCCCGGTCAAGGCCTGGAGTGGATGGGGTGGA TATATCCGGGAGATGGTTCTACCCAATATAATGAGAAGTTTAAG GGGAGAGTCACAATGACAAGGGACACCAGTATTAGCACCGCGTA TATGGAGCTTTCCCGCCTGCGATCAGATGACACGGCCGTGTACT ACTGTGCTAGGGAGGGAACCTATTATGCGATGGATTACTGGGGA CAGGGTACTACAGTCACGGTCTCTAGCGTGGACAGTTCCGGGGG CGGTGGAAGCGGTGGTGGCGGTTCAGGTGGAGGAGGCTCTACGA GTGATGTTGTGATGACTCAGTCCCCGCTTTCACTTCCCGTCACC CTTGGGCAACCCGCAAGCATCTCATGCCGATCCTCCCAGTCTAT AGTACATAGTAATGGCAACACATATCTTGAATGGTATCAGCAGA GGCCGGGTCAGTCTCCCCGACTCCTTATATATAAAGTGAGCAAC AGATTCTCCGGAGTACCGGATAGATTTTCCGGCTCTGGGAGCGG CACCGACTTTACACTGAAAATTTCACGGGTTGAAGCTGAAGATG TTGGGGTATACTATTGTTTCCAGGGTTCTCACGTCCCGAGGACA

		TTCGGGGGAGGAACGAAAGTCGAAATAAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H4_k5_NT	177	ATGGATTTTCAGGTACAAATCTTCAGCTTCCTGCTCATCTCCGC GAGCGTAATCATGTCTAGGGCGTCCCAGGTGCAGTTGGTGCAAT CAGGTGCAGAGGTGAAGAAGCCTGGTGCATCCGTTAAAGTAAGT TGTAAGGCAAGCGGATATACTTTTACATCCTATCATATTCAATG GGTCAGACAAGCACCTGGACAGGGTCTTGAGTGGATGGGCTGGA TCTATCCAGGCGATGGCTCAACTCAATATAACGAGAAGTTCAAG GGGAGGGTTACTATGACCAGGGATACGTCTATTTCCACTGCGTA CATGGAACTCTCCAGGTTGAGAAGTGATGATACCGCGGTTTACT ACTGCGCTAGAGAAGGAACGTACTACGCTATGGATTACTGGGGG CAGGGTACAACTGTCACCGTCTCAAGTGTGGATTCTTCTGGGGG TGGGGGATCAGGAGGGGGAGGCTCCGGTGGGGGCGGGTCAACCA GCGACATTGTCATGACTCAAACCCCCCTGAGCAGCCCTGTCACC CTGGGTCAGCCTGCCTCAATATCCTTTAGAAGCTCCCAAAGCAT CGTCCATTCAAATGGTAATACCTATCTGGAGTGGTATCAGCAAA GGCCTGGTCAACCCCCGCGCCTTCTCATTTACAAGGTGTCAAAC AGGTTCTCCGGCGTACCGGATAGGTTTTCCGGAAGCGGTGCTGG AACCGACTTTACTCTCAAAATCTCTAGGGTGGAAGCTGAGGACG TCGGTGTATACTATTGTTTTCAAGGCTCCCATGTTCCCAGGACA TTTGGTGGGGGAACGAAGGTAGAAATCAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC

		GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H5_k2_NT	178	ATGGACTTTCAGGTTCAGATTTTCTCTTTCTTGTTGATCTCCGC TAGTGTCATAATGTCACGGGCAAGTCAGGTACAACTCGTTCAGA GTGGTGCCGAAGTGAAGAAACCGGGTGCCTCCGTAAAGGTGTCA TGTAAAGCTAGTGGCTATACATTCACAAGTTATCATATCCAATG GGTACGACAAGCACCGGGACAGCGACTGGAATGGATGGGATGGA TCTATCCTGGGGACGGATCTACACAGTACAATGAGAAATTTAAG GGACGGGTCACGATAACCAGGGACACATCTGCTTCCACGGCTTA CATGGAGCTTTCCTCCCTGCGGAGCGAGGACATGGCTGTTTACT ATTGCGCTCGCGAAGGGACATACTACGCAATGGATTATTGGGGC CAAGGCACTACCGTGACGGTCTCTTCTGTCGATAGTTCCGGAGG AGGTGGTTCAGGGGGAGGCGGTTCAGGTGGGGGTGGATCTACCT CAGATATTGTCATGACACAGACACCTTTGTCCTTGAGTGTGACA CCGGGTCAACCGGCGAGTATAAGCTGTCGCAGCTCACAATCTAT TGTGCATAGCAACGGGAATACATATCTCGAATGGTATCTCCAAA AGCCGGGCCAATCCCCCCAACTTCTCATTTACAAAGTTTCTAAT CGATTTTCAGGTGTACCAGATCGGTTTTCCGGGTCTGGCTCAGG TACTGACTTCACCTTGAAAATATCAAGGGTTGAAGCTGAGGATG TAGGTGTGTACTATTGCTTCCAGGGGTCTCACGTTCCTCGGACT TTTGGGGGGGGCACAAAAGTAGAGATTAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG

		GCCCTGCCCCCTCGC
CAR_H5_k3_NT	179	ATGGATTTCCAGGTGCAAATCTTCTCATTTCTTTTGATAAGTGC GTCAGTGATAATGTCTCGGGCCAGTCAAGTACAGCTTGTCCAAA GTGGCGCTGAAGTCAAGAAGCCGGGAGCCTCAGTTAAGGTTAGC TGCAAGGCCTCAGGGTATACTTTTACCTCCTATCATATACAGTG GGTACGACAAGCACCGGGACAGCGACTGGAGTGGATGGGTTGGA TATATCCGGGAGATGGTTCAACCCAGTATAATGAGAAGTTCAAG GGGCGAGTTACGATAACCCGCGATACGAGTGCATCAACAGCGTA CATGGAGTTGAGTTCCCTCCGCAGCGAGGACATGGCGGTATACT ATTGTGCCAGGGAGGGGACTTATTATGCCATGGACTACTGGGGG CAGGGCACAACCGTAACAGTCTCTTCTGTAGACAGTTCAGGAGG GGGCGGAAGTGGAGGTGGCGGATCTGGTGGAGGTGGATCTACTT CCGACATCGTTATGACCCAAACACCACTTTCATCTCCCGTTACT CTCGGGCAACCTGCTAGTATTTCCTGTAGATCCTCACAATCTAT AGTACATAGCAATGGCAATACCTACCTGGAGTGGTATCAACAAC GCCCAGGCCAACCACCTCGCCTGCTTATCTATAAAGTAAGCAAT AGATTCAGTGGTGTACCGGATAGGTTCTCTGGTTCCGGAGCAGG AACTGACTTTACACTCAAAATCAGTAGGGTGGAGGCGGAAGACG TGGGAGTATATTATTGCTTTCAAGGTTCACATGTACCTCGAACA TTTGGCGGAGGAACTAAGGTTGAGATTAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H5_k4_NT	180	ATGGATTTCCAAGTCCAGATATTCAGTTTTCTTTTGATAAGCGC TTCTGTAATCATGTCTCGGGCGTCCCAAGTACAACTGGTGCAAT CAGGGGCAGAAGTGAAAAAACCAGGTGCATCCGTTAAGGTGAGT TGCAAGGCTTCCGGCTATACCTTTACATCATATCATATTCAATG GGTCAGGCAAGCACCTGGTCAGCGATTGGAATGGATGGGTTGGA TATATCCTGGTGATGGGTCTACACAATATAACGAAAAATTCAAG GGGCGAGTGACCATCACAAGAGATACATCAGCGTCAACAGCGTA TATGGAACTGTCATCCCTTAGATCAGAGGACATGGCGGTCTATT

		ACTGTGCCAGAGAAGGCACTTATTATGCAATGGATTATTGGGGA CAAGGAACCACTGTCACTGTTTCCAGCGTAGACTCCTCCGGTGG TGGTGGAAGTGGCGGCGGTGGGTCAGGAGGGGGTGGGTCAACTT CTGATGTAGTGATGACACAGAGCCCTCTGAGCTTGCCTGTGACC TTGGGTCAGCCGGCCTCAATAAGTTGTCGATCTAGTCAGTCAAT CGTCCATAGTAATGGGAACACATACCTTGAATGGTATCAGCAAA GACCTGGACAATCTCCACGACTCCTTATATACAAAGTTAGCAAC CGATTTAGCGGAGTGCCAGACCGCTTTTCTGGTTCCGGGTCTGG CACAGATTTTACCCTTAAGATCTCCCGCGTGGAGGCGGAAGACG TTGGTGTTTACTATTGCTTCCAGGGGTCACACGTTCCACGCACC TTTGGAGGAGGTACGAAGGTCGAGATTAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H5_k5_NT	181	ATGGATTTTCAAGTACAGATCTTCTCTTTCTTGCTTATTTCAGC GAGCGTAATCATGAGTAGGGCATCTCAAGTTCAACTCGTTCAGT CAGGTGCTGAGGTAAAAAAACCAGGGGCTTCCGTTAAAGTTAGC TGTAAGGCATCTGGGTACACATTTACTAGCTACCATATCCAGTG GGTGCGACAAGCCCCGGGGCAGCGCTTGGAATGGATGGGCTGGA TTTACCCAGGTGACGGCTCCACGCAATATAATGAGAAATTTAAG GGTAGAGTTACTATTACCAGGGACACAAGTGCTTCAACTGCCTA TATGGAACTGAGCAGCCTTCGGAGTGAAGATATGGCCGTATATT ACTGCGCAAGGGAGGGGACTTACTATGCAATGGACTACTGGGGT CAGGGAACGACTGTGACCGTGTCCTCAGTTGACTCCAGCGGTGG TGGCGGCTCTGGAGGTGGGGGTTCCGGCGGAGGCGGAAGCACAT CTGATATAGTGATGACGCAAACGCCTCTTTCTTCCCCGGTAACT CTGGGACAGCCAGCGTCAATTTCATTTAGGTCCTCCCAGTCAAT CGTACATAGTAATGGAAATACTTACCTGGAATGGTATCAACAAC GACCAGGGCAACCGCCCCGATTGTTGATCTATAAAGTGAGCAAT CGCTTTTCTGGCGTGCCCGATCGGTTCTCAGGGTCTGGAGCTGG GACTGACTTCACATTGAAAATTTCCAGGGTTGAGGCCGAGGATG

		TGGGGGTTTATTACTGCTTCCAAGGCTCCCACGTCCCCCGCACC TTCGGAGGGGGAACCAAAGTCGAAATAAAGAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H6_k2_NT	182	ATGGATTTTCAAGTTCAGATATTCTCATTTTTGCTTATATCAGC CTCCGTAATTATGTCACGGGCAAGTCAAGTTCAGTTGGTGCAGT CCGGAGCAGAAGTTAAGAAGCCCGGTGCTTCTGTGAAAGTCTCC TGCAAAGCGTCTGGGTACACCTTCACGAGCTACCATATACAGTG GGTCCGGCAAGCGCCTGGGCAGAGGCTGGAGTGGATGGGCTGGA TTTACCCAGGAGATGGGAGTACAAAGTATAGTCAGAAGTTTCAA GGGCGAGTGACGATAACCAGAGATACGAGTGCAAGTACTGCATA CATGGAACTGAGCTCCTTGAGGTCCGAGGATACAGCGGTGTACT ATTGCGCTCGGGAAGGGACATATTATGCTATGGACTATTGGGGA CAAGGGACAACGGTAACGGTGAGTTCCGTCGATTCCTCAGGTGG CGGAGGCAGTGGGGGCGGGGGTTCCGGCGGTGGCGGGTCCACGA GTGATATAGTTATGACACAGACCCCCCTCAGCCTTTCTGTGACC CCAGGACAACCCGCTAGTATCTCTTGCCGCAGTTCTCAGTCCAT AGTACACAGTAACGGAAATACCTATCTTGAGTGGTATCTTCAAA AGCCCGGCCAGAGCCCTCAACTCTTGATATATAAAGTGTCAAAT CGATTTTCAGGTGTGCCTGATCGATTCTCAGGGTCTGGTTCAGG GACAGATTTCACGCTTAAGATAAGCAGAGTAGAGGCTGAAGACG TGGGAGTCTACTATTGTTTTCAGGGGTCACACGTTCCCCGCACT TTTGGTGGGGGAACCAAGGTGGAAATCAAAAACCGGATCCGTGG GGTCACCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCA GCCACTTCGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACG CCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCA GCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACCCCTTTGGGTTTTGGGTG CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGT AACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCA

		GGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCC GGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCCTCGAGAGAGTGAGAGTGAAGTTCA GCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT TTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGC CGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAG AAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGG CGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTC TCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAG GCCCTGCCCCCTCGC
CAR_H1_K2_NT	214	ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCT TCTTCATGCCGCCAGACCATCTCAGGTCCAGCTAGTACAAAGCG GCGCCGAAGTAAAGAAACCTGGTGCCTCTGTGAAGGTGAGCTGC AAGGCCAGCGGCTACACCTTCACCAGCTACCACATCCAGTGGGT TCGACAGGCCCCTGGACAGAGACTAGAGTGGATGGGCTGGATCT ATCCTGGCGACGGCAGCACCCAGTACAACGAGAAGTTCAAGGGC AGAGTTACCATCACCCGAGACACCAGCGCCAGCACAGCCTATAT GGAGCTGAGCAGCCTGCGAAGCGAGGACACAGCTGTTTACTATT GTGCCAGAGAGGGCACCTACTACGCAATGGATTATTGGGGCCAG GGGACCACCGTGACCGTTTCTTCTGGAGGCGGAGGTTCTGGCGG CGGAGGAAGTGGTGGCGGAGGCTCAGATATTGTAATGACCCAGA CACCTCTGTCCCTGTCTGTGACACCTGGACAGCCTGCAAGCATC AGCTGTCGGAGCAGCCAGAGCATCGTTCACAGCAACGGCAACAC CTACCTGGAATGGTATCTGCAGAAGCCCGGACAGTCCCCCCAGC TGCTGATCTACAAGGTGTCCAACCGCTTCAGTGGAGTACCCGAT AGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAAGAT CTCCAGAGTAGAAGCAGAGGACGTTGGAGTGTACTACTGCTTCC AAGGCAGCCATGTGCCAAGAACCTTTGGTGGAGGCACAAAGGTG GAAATCAAGCGGACAACAACACCTGCTCCTCGGCCTCCTACACC AGCTCCTACAATTGCCAGCCAGCCACTGTCTCTGAGGCCCGAAG CTTGCAGGCCTGCTGCTGGCGGAGCCGTGCATACAAGAGGACTG GATTTCGCCTGCGACATCTACATCTGGGCACCTCTGGCTGGAAC CTGTGGCGTGCTGCTGCTGAGCCTGGTCATCACCCTGTATTGCC GGAGCAAGAGAAGCAGACTGCTGCACAGCGACTACATGAACATG ACCCCTAGACGGCCCGGACCTACCAGAAAGCACTACCAGCCTTA CGCTCCTCCTAGAGACTTCGCCGCCTACAGATCCAGAGTGAAGT TCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAAC CAGCTCTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGA CGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCA AGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTATAATGAGCTA CAGAAAGACAAGATGGCAGAGGCCTACAGCGAGATCGGAATGAA GGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGTACCAGG GCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATG CAGGCCCTGCCTCCAAGA
4-1BB NT	215	AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATT

(SEQ ID NO: 17 of US9,102,760)		TATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCT GCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG
CD8 hinge NT (SEQ ID NO: 15 of US9,102,760)	217	ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCAT CGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAG CGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGT GAT
CD8_hinge NT	220	ACAACAACACCTGCTCCTCGGCCTCCTACACCAGCTCCTACAAT TGCCAGCCAGCCACTGTCTCTGAGGCCCGAAGCTTGCAGGCCTG CTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTGC GAC
CD8 transmembrane_NT (SEQ ID NO: 16 of US9,102,760)	222	ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCT CCTGTCACTGGTTATCACCCTTTACTGC
CD8 transmembrane_NT	224	ATCTACATCTGGGCACCTCTGGCTGGAACCTGTGGCGTGCTGCT GCTGAGCCTGGTCATCACCCTGTATTGC

Table 5

	Kabat	Chothia
BB7.2 VH CDR1	SYHIQ (SEQ ID NO: 183)	GYTFTSY (SEQ ID NO: 184)
BB7.2 VH CDR2	WIYPGDGSTQYNEKFKG (SEQ ID NO: 185)	YPGDGS (SEQ ID NO: 186)
BB7.2 VH CDR3	EGTYYAMDY (SEQ ID NO: 187)	EGTYYAMDY (SEQ ID NO: 187)

Table 6

	Kabat	Chothia
BB7.2 VL CDR1	RSSQSIVHSNGNTYLE (SEQ ID NO: 188)	RSSQSIVHSNGNTYLE (SEQ ID NO: 188)
BB7.2 VL CDR2	KVSNRFS (SEQ ID NO: 189)	KVSNRFS (SEQ ID NO:189)
BB7.2 VL CDR3	FQGSHVPRT (SEQ ID NO: 190)	FQGSHVPRT (SEQ ID NO: 190)

Table 7

SEQ Name	SEQ ID	AA
BB7.2_AA VH	191	QVQLQQSGPELVKPGASVKMSCKASGYTFTSYHIQWVKQRPGQGLE WIGWIYPGDGSTQYNEKFKGKTTLTADKSSSTAYMLLSSLTSEDSA IYFCAREGTYYAMDYWGQGTSVTVSS
BB7.2_AA VL	192	DVLMTQTPLSLPVSLGDQVSISCRSSQSIVHSNGNTYLEWYLQKPG QSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYY CFQGSHVPRTFGGGTKLEIK
BB7.2_AA scFv (without leader)	193	QVQLQQSGPELVKPGASVKMSCKASGYTFTSYHIQWVKQRPGQGLE WIGWIYPGDGSTQYNEKFKGKTTLTADKSSSTAYMLLSSLTSEDSA IYFCAREGTYYAMDYWGQGTSVTVSSVDSSGGGGSGGGGSGGGGST SDVLMTQTPLSLPVSLGDQVSISCRSSQSIVHSNGNTYLEWYLQKP GQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVY YCFQGSHVPRTFGGGTKLEIK
BB7.2CAR (without leader)	194	QVQLQQSGPELVKPGASVKMSCKASGYTFTSYHIQWVKQRPGQGLE WIGWIYPGDGSTQYNEKFKGKTTLTADKSSSTAYMLLSSLTSEDSA IYFCAREGTYYAMDYWGQGTSVTVSSVDSSGGGGSGGGGSGGGGST SDVLMTQTPLSLPVSLGDQVSISCRSSQSIVHSNGNTYLEWYLQKP GQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVY YCFQGSHVPRTFGGGTKLEIKLEQKLISEEDLNRIRGVTVSSALSN SIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRP AAGGAVHTRGLDPFGFWVLVVVGGVLACYSLLVTVAFIIFWVRSKR SRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSLERVRVKFS RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
BB7.2CAR (with leader)	195	MDFQVQIFSFLLISASVIMSRASQVQLQQSGPELVKPGASVKMSCK ASGYTFTSYHIQWVKQRPGQGLEWIGWIYPGDGSTQYNEKFKGKTT LTADKSSSTAYMLLSSLTSEDSAIYFCAREGTYYAMDYWGQGTSVT VSSVDSSGGGGSGGGGSGGGGSTSDVLMTQTPLSLPVSLGDQVSIS CRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFS

		GSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPRTFGGGTKLEIKLE QKLISEEDLNRIRGVTVSSALSNSIMYFSHFVPVFLPAKPTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDPFGFWVLVVVG GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRSLERVRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
SEQ Name	SEQ ID	NT
BB7.2CAR (with leader)	196	ATGGACTTCCAGGTGCAGATCTTCAGCTTCCTGCTGATCAGCGCCA GCGTGATCATGAGCCGCGCTAGCCAGGTGCAGCTGCAGCAGAGCGG CCCCGAGCTGGTGAAGCCCGGCGCCAGCGTGAAGATGAGCTGCAAG GCCAGCGGCTACACCTTCACCAGCTACCACATCCAGTGGGTGAAGC AGCGCCCCGGCCAGGGCCTGGAGTGGATCGGCTGGATCTACCCCGG CGACGGCAGCACCCAGTACAACGAGAAGTTCAAGGGCAAGACCACC CTGACCGCCGACAAGAGCAGCAGCACCGCCTACATGCTGCTGAGCA GCCTGACCAGCGAGGACAGCGCCATCTACTTCTGCGCCCGCGAGGG CACCTACTACGCCATGGACTACTGGGGCCAGGGCACCAGCGTGACC GTGAGCAGCGTCGACAGCAGCGGCGGCGGCGGCAGCGGCGGCGGCG GCAGCGGCGGCGGCGGCAGCACTAGTGACGTGCTGATGACCCAGAC CCCCCTGAGCCTGCCCGTGAGCCTGGGCGACCAGGTGAGCATCAGC TGCCGCAGCAGCCAGAGCATCGTGCACAGCAACGGCAACACCTACC TGGAGTGGTACCTGCAGAAGCCCGGCCAGAGCCCCAAGCTGCTGAT CTACAAGGTGAGCAACCGCTTCAGCGGCGTGCCCGACCGCTTCAGC GGCAGCGGCAGCGGCACCGACTTCACCCTGAAGATCAGCCGCGTGG AGGCCGAGGACCTGGGCGTGTACTACTGCTTCCAGGGCAGCCACGT GCCCCGCACCTTCGGCGGCGGCACCAAGCTGGAGATCAAGCTCGAG CAGAAGCTGATCAGCGAGGAGGACCTGAACCGGATCCGTGGGGTCA CCGTCTCTTCAGCGCTGAGCAACTCCATCATGTACTTCAGCCACTT CGTGCCGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCG CGACCACCAACACCGGCGCCCACCATCGCGTCGCAGCCCCTGTCCC TGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACAC GAGGGGGCTGGACCCCTTTGGGTTTTGGGTGCTGGTGGTGGTTGGT GGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTA TTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTA CATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTAC CAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCCCTCG AGAGAGTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTA CCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGA AGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTG AGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTA CAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATT GGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTT ACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCA CATGCAGGCCCTGCCCCCTCGCTAA

EXAMPLES

Methods

Generation of humanized HLA-A*02-specific scFvs. The humanized genes were codon optimized using the codon optimizer from Invitrogen GeneArt Gene Synthesis service using the settings for Homo sapiens. gBlocks® Gene Fragments were ordered from Integrated DNA Technologies (Coralville, Iowa) such that the 5' region of the chimeric antigen receptor contained a Kozak sequence, and a 36 NT overlap with a pcDNA3 plasmid. The 3' end contains a BamHI site and an overlap with a CD8 hinge sequence to facilitate Gibson assembly into the plasmid in frame with the CD8 hinge and the intracellular signaling domains of the chimeric antigen receptor.

Generation of hA2-CARs. The scFv variants were fused to a stalk region from human CD8α, the transmembrane and intracellular domains of human CD28, and human CD3ζ as described (Sadelain et al., 2013). The resulting cDNAs were cloned into a lentiviral vector that encodes cell-surface expression of a truncated nerve-growth-factor (TrkA) receptor (ΔNGFR) as a marker (Figure 1). Surface expression was determined by flow cytometry with transiently transfected HEK 293T cells (jetPRIME®, Polyplus Transfection). Viral particles were produced as described (Allan et al., 2008).

Generation of HLA-expressing K562 cell lines. CD64-expressing K562 cells (K562.64) were a gift from James Riley, University of Pennsylvania, Philadelphia, USA. cDNA for HLA-A*02:01 and A*24:02 were isolated from mRNA of peripheral blood mononuclear cells from a donor homozygous for A*02:01 or A*24:02, respectively, on the HLA-A locus using the primer sequences: 5'-TTTTCTAGACGCGTGCCACCATGGCCGTCATGGCGCC-3' (forward) (SEQ ID NO: 197) and 5'-AAGTCGACGCTAGCTCACACTTTACAAGCTGTGAGAGACA-3' (reverse) (SEQ ID NO: 198). The resulting sequence was confirmed by Sanger sequencing, aligned to the expected sequences from the IPD and IMGT/HLA Database (Robinson et al., 2015) and transduced into K562 cells, respectively, using a lentiviral expression vector. To generate A25- and A68-K562 cells, HLA sequences for A*25:01, A*68:01 were scraped from the IPD and IMGT/HLA Database (Robinson et al., 2015), codon optimized using the codon optimizer tool (set to Homo sapiens) from ThermoFisher Invitrogen GeneArt Gene Synthesis service, then cloned into a

lentiviral expression vector and transduced into K562 cells. The resulting K562 cell lines were then sorted on a FACSAria II (BD Biosciences) using anti-HLA-ABC (ThermoFisher, 12-9983-41) to ensure equivalent surface expression of the transduced HLA and anti-HLA-A2 (BB7.2) (ThermoFisher, 17-9876-42) to ensure purity.

Treg sorting, transduction, and expansion. $CD4^+$ T cells were isolated from HLA-$A2^-$ donors via RosetteSep (Stemcell) and enriched for $CD25^+$ cells (Miltenyi) prior to sorting live $CD4^+CD25^{hi}CD127^{lo}$Tregs (for in vitro and luciferase experiments) or $CD4^+CD127^{lo}CD25^{hi}$ $CD45RA^+$ Tregs (for xenogeneic GvHD and skin transplant experiments) using a MoFlo® Astrios (Beckman Coulter) or FACSAria II (BD Biosciences), respectively. Sorted Tregs were stimulated with L cells and αCD3 mAb (OKT3; 100ng/mL) in immunocult-XF T cell expansion media (STEMCELL Technologies) with 1000U/ml of IL-2 (Proleukin). One day later, cells were transduced with lentivirus at a multiplicity of infection of 10 virus particles:1 cell. At day 7, $\Delta NGFR^+$ cells were purified with magnetic selection (Miltenyi) then used in assays or re-stimulated with L cells as above and expanded for 5 days for in vivo experiments. To test the effects of HLA-A2-mediated stimulation, Tregs were cultured with limiting IL-2 (100U/mL) for 24 hours, then re-counted and co-cultured with irradiated anti-CD3/anti-CD28 loaded K562.64 cells or HLA-A*02:01, A*24:02, A*25:01 or A*68:01-expressing K562.64 cells at a 1:2 (K562:T cell) ratio for 24 hours.

Flow cytometry. For phenotypic analysis, cells were stained with fixable viability dye (FVD, 65-0865-14, ThermoFisher; 423102, Biolegend) and for surface markers before fixation and permeabilization with eBioscience FOXP3/Transcription Factor Staining Buffer Set (ThermoFisher) and staining for intracellular proteins. Samples were read on a Cytoflex (Beckman-Coulter) and results analyzed using FlowJo Software version 9.9.4 and 10.3 (Tree Star).

Surface staining was performed for ΔNGFR (130-091-885, Miltenyi), CD3 (564465, BD Biosciences), CD4 (317410, Biolegend), CD25 (130-091-024, Miltenyi), LAP (25-9829-42, ThermoFisher), CD69 (310946, Biolegend), CD154 (555702, BD Biosciences), CD71 (BD Biosciences, 563768), and CD127 (48-1278-42, ThermoFisher). Tetramer staining was performed with HLA-A*02:01 monomers made into tetramers with streptavidin-

allophycocyanin (PJ27S, Prozyme). Intracellular staining was performed for CTLA-4 (369606, Biolegend).

For in vivo experiments, 50uL of blood was collected weekly and at endpoints. Ammonium chloride was used for red blood cell lysis. Cells were resuspended in PBS with anti-mouse CD16/32 (ThermoFisher, 14-0161-82) and stained for extracellular markers using fixable viability dye (FVD; ThermoFisher, 65-0865-14), anti-mouse CD45 (ThermoFisher, 25-0451-82), and anti-human CD45 (BD Biosciences, 560777), CD4 (Biolegend, 300554, 317434), CD8 (ThermoFisher, 48-0087-42), anti-human CD271 (NGFR; BD Biosciences, 557196) HLA-A2 (BD Biosciences, 551285). Intracellular staining for FOXP3 (ThermoFisher, 12-4777-42) was done with the eBioscience FOXP3/Transcription Factor Staining Buffer Set (ThermoFisher, 00-5523-00). 10,000 counting beads were added to every sample (ThermoFisher, 01-1234-42). The gating strategies for the xenogeneic GvHD, luciferase and skin transplant experiments are illustrated in Figures 8D, 10A and 12A, respectively.

HLA allele cross reactivity assay. 0.25×10^6 CAR Tregs (prepared as above, after 7 days of culture) were incubated with individual FlowPRA Single Antigen Antibody beads panels (FL1HD01, FL1HD02, FL1HD03, FL1HD04, FL1HD06 and FL1HD08, One Lambda) and fixable viability dye (FVD, 65-0865-14, ThermoFisher) for 30 minutes at room temperature, then washed, fixed with 0.5% formaldehyde and analyzed via flow cytometry. Two hundred negative control beads were acquired per sample. For analysis, dead cells were first eliminated using the fixable viability dye. Single beads were then gated after exclusion of dead cells and doublets. Then, the number of beads for each HLA antigen was determined by their respective PE intensity. Data were normalized by dividing the number of negative beads in the sample by the number of negative beads in the Treg-NGFR sample, multiplied by the number of negative beads in the Treg-NGFR specimen. Percent relative binding is the number of beads in the NGFR specimen for one specific HLA minus the normalized number of beads in the specimen for that HLA, divided by the number of beads in the NGFR specimen multiplied by 100.

Suppression of mixed lymphocyte reactions. Adherent cells from PBMCs from HLA-$A2^+$ healthy donors were differentiated into monocyte-derived dendritic cells as described (Dijke et al., 2015). For mixed lymphocyte reactions, HLA-$A2^-$ PBMC responder cells were labeled with cell proliferation dye eF450 (65-0842-85, ThermoFisher), then plated with $5x10^4$ HLA-$A2^+$

monocyte-derived dendritic cells and increasing ratios of expanded ΔNGFR- or hA2-CAR-expressing Tregs labelled with cell proliferation dye e670 (65-0840-90, ThermoFisher). After 6 days, division of HLA-A2$^-$ CD4$^+$ responder T cells was measured by flow cytometry. Percent suppression was calculated based on the proliferation index of a given cell combination and ratio versus the positive control (HLA-A2+ monocyte-derived dendritic cells with HLA-A2- CD4$^+$ responder T cells only) as described (McMurchy and Levings, 2012). Data were normalized by first calculating percent suppression as follows: $\%\ suppression = 100 - 100 \times \frac{proliferation\ index\ (sample)}{proliferation\ index\ (DC+responder\ control)}$, then normalizing the resulting values from 0-100%, according to the formula for each independent experiment: $normalized\ \%\ suppression = 100 \times \frac{\%\ suppression\ (sample)}{\%\ suppression\ (DC+responder\ control)}$.

Suppression of xenogeneic graft-versus-host disease in vivo. 8- to 12-week-old female NSG mice (The Jackson Laboratory, bred in house) received whole-body irradiation (150 cGy, RS-2000 Pro Biological System) 1 day before injection of 8 x 10^6 HLA-A2$^+$ PBMCs with or without 4 x 10^6 hA2-CAR Tregs intravenously into the tail vein. Saline-injected mice served as controls. hA2-CAR Tregs were generated from four different healthy donors. GVHD was scored based on weight, fur texture, posture, activity level, and skin integrity, with 0 to 2 points per category as described (Cooke, et al. 1996; Hill et al. 1997). GVHD scoring was performed by two blinded investigators. Peripheral blood from the saphenous vein was centrifuged; then erythrocytes were lysed and leukocytes were measured by flow cytometry.

Study Approval. For human PBMCs, healthy volunteers gave written informed consent according to protocols approved by the University of British Columbia Clinical Research Ethics Board and Canadian Blood Services. Samples of human skin discarded from plastic surgery were obtained from the Harvard Skin Resource Centre, Skin Works or the Cambie Surgery Clinic according to University of British Columbia Clinical Research Ethics Board-approved protocols. Animal protocols were approved by the UBC Animal Care Committee.

Skin transplantation. To evaluate A2-CAR Treg homing and capacity to inhibit skin rejection, 8- to 12-week-old female NSG mice (The Jackson Laboratory, bred in house) were transplanted with skin from transgenic HLA-A2$^+$ NSG mice (The Jackson Laboratory, bred in house) and NSG skin (HLA-A2 negative), For mouse skin transplants, skin was cut into circular pieces

utilizing 8mm biopsy punch and skin was placed onto fresh plates with PBS and kept at low temperature (4-8°C) until transplanted (~1–4 hr). HLA-A2 expression of human skin was assessed by flow cytometry and qPCR. Split-thickness explants were generated by trimming fat from the specimen. Explants were rinsed with sterile PBS and stored in RPMI. Quality skin explants were cut into 1 cm^2 pieces, placed onto fresh plates with PBS and kept at low temperature (4-8°C) until transplanted (~1–4 hr). For both mouse and human skin transplants, previously shaved mice were anesthetized, dorsal skin was cut near the shoulder and mouse skin of similar size was removed, then grafts were placed on the exposed area and stabilized with steri-strips (3M, Nexcare). Grafts were covered with a Vaseline gauze and wrapped with a 2 cm wide CoFlex bandage (3M, Nexcare) to secure graft for up to 14 days prior to cell injection.

Histology

Human skin graft and surrounding mouse skin was harvested 27 days post-cell injection, fixed overnight at 4°C in 10% formalin (1:10 v/v ratio of tissue to formalin), and stored in 70% ethanol before paraffin-embedding. Paraffin sections (5-µm thickness) and H&E staining were prepared by BC Children's Hospital Research Institute Histology Services (Vancouver, British Columbia, Canada). For immunostaining, sections were deparaffinized and rehydrated using a series of xylene washes (×3), graded alcohol solutions (2× 100% ethanol, 1× 95% ethanol and 1× 70% ethanol), and 1×PBS. Heat-induced epitope retrieval (HIER) was performed on slides using a microwave to reach 93-95°C (5 min, high power followed by 20 min, low power) in 10 mM sodium citrate buffer (0.5% Tween-20, pH 6.0). Following HIER, slides were washed using running tap water, deionized water and PBS. Sections were incubated with DAKO® Protein Block, Serum-Free (Dako, Burlington, Canada, X0909) to limit non-specific antibody staining. Sections were then incubated at 4°C overnight with the following primary antibodies: FOXP3 (Invitrogen, clone PCH101, 14-4776-82), CD45 (eBioscience, clone H130, 17-0459), Ki67 (eBioscience, clone 20Raji, 17-5699), Involucrin (Abcam, ab53112). The following day, sections were gently rinsed with PBS several times, then stained for 1 hour RT with the following secondary antibodies: donkey anti-rat 488 (Life Tech, A11006), goat anti-mouse APC (Invitrogen, 1834696), donkey anti-rabbit 488 (Life Tech, A21206). Finally, sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI) to identify cell nuclei and

mounted using VECTASHIELD® Mounting Medium with DAPI (Vector Laboratories, Burlingame, California, USA, H-1200). All antibodies were diluted in Antibody Diluent (Dako, Burlington, Canada, S3022). Images were captured using the Olympus BX61 Fluorescence and Bright Field Automated Upright Microscope with QImaging Retiga Exi camera and Olympus DP71 color camera. Quantitative analysis of fluorescence images performed using Fiji with Olympus viewer Plugin (Schneider et al., 2012; Eliceiri et al., 2012).

H&E-stained slides were evaluated by a blinded clinical pathologist using a scoring system defined by 8 factors each graded from 0 to 3-4; Lerner grade (0, 1 - focal or diffuse vacuolar degeneration, 2 - dyskeratosis, 3 - clefts in basal or superficial layers, 4 - frank loss of epidermis), spongiosis (0, 1 - basal layer only, 2 - up to half way, 3 - full thickness), necrotic keratinocytes (0, 1 - rare (1/hpf), 2 - occasional (2-3/hpf), 3 - many (>4/hpf)), necrotic keratinocyte location (0, 1 - basal only, 2 - up to upper half, 3 - full thickness), satellitetosis (0, 1 - 1 only, 2 - 2-3/hpf, 3 - >4/hpf), exocytosis (0, 1 - focal, 2 - <50% biopsy, 3 - >50% biopsy), adnexal involvement (0, 1 - minor involvement of any adnexa, 2 - marked involvement of <50% adnexa, 3 - marked involvement of > 50% adnexa) and lymphoid cuffs in dermis (0, 1 - slight, 2 - abundant, 3 - band like) (Massi et al., 1999; Fischer et al., 2015, Kanitakis et al., 2008).

qPCR. RNA was harvested from human skin samples according to the manufacturer's instructions (RNeasy Plus Mini Kit; Qiagen) and converted to complementary DNA (cDNA). qPCR was performed using SYBR-green (Biorad) and primers for *IL17, IL6, IL1B, DEFB4, IFNg, TNFa, 18S* ribosomal RNA (Table 8). Melt curve and SYBR-green emission data were collected. Relative concentrations were calculated using a standard curve and values were normalized to amplification products of *18S* ribosomal RNA. Log_2(RQ) values for each sample were obtained using the double delta Ct (ΔΔCt) method (Schmittgen et al., 2008). Each sample's ΔCt value was obtained by calculating averaged Ct (gene of interest) – Ct (housekeeping gene). To obtain ΔΔCt, the ΔCt of the control sample was subtracted from the ΔCt of the treated sample. Fold gene expression was then calculated by $2^{-(\Delta\Delta Ct)}$.

Table 8: Primers used in qPCR analysis

Oligo name	**Direction** Forward (FWD) or Reverse (RVS)	**Oligo sequence (5' to 3')**
human *IL17*	FWD	TCA ACC CGA TTG TCC ACC AT (SEQ ID NO: 199)
human *IL17*	RVS	GAG TTT AGT CCG AAA TGA GGC TG (SEQ ID NO: 200)
human *IL6*	FWD	TCC AAA GAT GTA GCC GCC CCA (SEQ ID NO: 201)
human *IL6*	RVS	CCA GTG CCT CTT TGC TGC TTT CA (SEQ ID NO: 202)
human *IL1B*	FWD	CTG AGC TCG CCA GTG AAA TGA TG (SEQ ID NO: 203)
human *IL1B*	RVS	TGC TGT AGT GGT GGT CGG AGA (SEQ ID NO: 204)
human *DEFB4*	FWD	ACC TGC CTT AAG AGT GGA GCC A (SEQ ID NO: 205)
human *DEFB4*	RVS	ACA TGT CGC ACG TCT CTG ATG A (SEQ ID NO: 206)
human *IFNG*	FWD	TGC CCA GAG CAT CCA AAA GA (SEQ ID NO:207)
human *IFNG*	RVS	TGT ATT GCT TTG CGT TGG AC (SEQ ID NO: 208)
human *TNFA*	FWD	AGG CGC TCC CCA AGA AGA CA (SEQ ID NO: 209)
human *TNFA*	RVS	GGG CTG ATT AGA GAG AGG TCC CT (SEQ ID NO: 210)
18S ribosomal RNA	FWD	CAA GAC GGA CCA GAG CGA AA (SEQ ID NO: 211)
18S ribosomal RNA	RVS	GGC GGG TCA TGG GAA TAA C (SEQ ID NO: 212)

Luciferase. To evaluate Treg homing towards HLA-A2-expressing mouse skin grafts in vivo, sorted Tregs ($CD4^+CD25^{hi}CD127^{lo}$) were stimulated with L-cells as described above. The next day, cells were transduced with HER2-CAR, mA2-CAR or H1k2 hA2-CAR lentivirus at an MOI of 10 and 8h later with luciferase-GFP-lentivirus at an MOI of 5. The lentiviral plasmid encoding a beetle luciferase-GFP-fusion protein (pELNS.CBG-T2A-GFP (CBR)) was used as described previously (Barrett et al., 2014). After 7 days of culture, double-transduced GFP+ΔNGFR+ Tregs expressing the CAR and luciferase were sorted before restimulation with L-cells as described above. On day 12 of the culture, 1-3 $x10^6$ luciferase-CAR Tregs and $6x10^6$ human allogeneic HLA-A2- PBMCs were injected intravenously into skin-transplanted NSG mice. For bioluminescent imaging, D-luciferin potassium salt (150mg/kg, Gold Bio) was injected i.p. immediately before anesthesia with isoflurane and images were acquired within 15-20min on Ami-X (Spectral Instruments Imaging). Data were analyzed with AmiView software (Spectral Instruments Imaging, version 1.7.06) and the luminescent signal was quantified as the ratio of photons/sec/cm^2/steradian in the HLA-A2+ over the HLA-A2- skin graft. At experimental endpoint, skin-draining axillar lymph nodes and spleen were harvested, placed on a 70μm cell strainer (BD Falcon), then fragmented and filtered through using the plunger of a 1cc syringe. Cells were then stained for flow cytometry.

Statistical analysis. All statistics were done using Prism 7.0b. IBM*SPSS Statistics Version 24.0.0.0 was used for Figure 9.

Results

Construction of humanized HLA-A2-specific CARs

The amino acid sequence of the variable regions of the heavy and light chains from the mouse BB7.2 mAbs were aligned to the human immunoglobulin sequences obtained from the international ImMunoGeneTics information system® (IMGT®) database using the IgBLAST tool available from the National Center for Biotechnology Information (NCBI). The V-gene delimitation system was set to the Kabat sequences to obtain the Kabat defined CDRs (Kabat et al., 1991). In addition, the Chothia definition (Chothia et al., 1987) was identified.

A number of different human germline genes were tested as framework sequences for the CDR grafting. In addition to identifying the human germline sequences that were most

comparable to the mouse sequence, the CDR lengths were also considered to maintain the structure as much as possible. The human CDRs (Kabat numbering) were replaced with the mouse counterpart CDRs from the BB7.2 antibody. Also, additional CDR amino acids of the heavy chain were replaced by combining the Chothia and Kabat numbering. Ultimately 6 different humanized heavy chains and 5 different humanized light chains were generated, and a total of 20 different chimeric antigen receptors were generated by combining the humanized heavy and light chains.

Expression and antigen specificity of humanized HLA-A2-specific CARs

To test for antigen specificity, the various hA2-CAR constructs were transiently transfected into 293T cells, stained with HLA-A*02:01 tetramers and analyzed by flow cytometry. Eleven hA2-CAR constructs were expressed and bound to the A*02:01 tetramer (Figure 2).

Next we compared the strength of binding of the various hA2-CAR constructs. Transiently transfected 293T cells were stained with increasing dilutions of A*02:01 tetramer, then analyzed by flow cytometry. As shown in Figure 3 (A, B and C), eleven hA2-CAR constructs bind A*02:01 tetramer to a high degree in a tetramer concentration-dependent manner.

hA2-CAR-mediated stimulation activates Tregs

To test the function of the A2-CAR in Tregs, $CD4^{+}CD25^{hi}CD127^{lo}$ cells were sorted from peripheral blood. As shown in Figure 4 (A, B and C), Tregs were stimulated, transduced and cultured for an additional 6-7 days. At the end of 7-8 days of culture, cells were purified as $NGFR^{+}$ cells and cell surface expression of hA2-CAR variant was tested with flow cytometry. As shown in Figure 4, Tregs transduced with hA2-CAR constructs showed positive staining by the HLA-A2 tetramer as compared to Tregs transduced with the ΔNGFR control construct, indicating a preservation of antigen specificity for Tregs expressing the hA2-CAR. Technical difficulties were encountered with producing high titer lentivirus for (H1k3) and this CAR was not tested in Tregs in this experiment. CAR cell surface expression and specificity was tested by staining with an A*02:01 tetramer revealing that H2k2, H4k4 and H5k4 had a bimodal

pattern of expression in Tregs, with a lower mean fluorescence intensity (MFI) of A2-tetramer$^+$ cells (Figure 4D and 4E).

We next investigated if stimulation via hA2-CAR activated Tregs (hA2-CAR Tregs) in comparison to Tregs expressing only the truncated NGFR transduction marker (NGFR Tregs). NGFR and hA2-CAR Tregs were left unstimulated, stimulated via the CAR with K562 cells expressing HLA-A*02:01 or stimulated via the TCR with K562 cells expressing CD64, a high affinity Fc receptor, that was pre-loaded with anti-CD3/anti-CD28 antibodies. Comparison between NGFR Tregs and hA2-CAR Tregs showed much higher CAR-stimulated expression of CD69 and CD71 in most hA2-CAR Tregs over multiple time points. In contrast, when stimulated via the TCR, both NGFR and hA2-CAR Tregs were able to upregulate CD69, indicating that they retain their ability to signal via the TCR after transduction of CAR DNA (as shown in Figure 13). hA2-CAR-mediated stimulation of Tregs also caused significantly greater upregulation of proteins associated with Treg function, demonstrating that stimulation through the hA2-CAR preserves the expected high expression of CTLA-4 and LAP (Figures 5A-F).

Cross reactivity of hA2-CAR constructs on other class I HLA alleles

There are many different alleles of HLA that have evolved over time from a smaller number of ancestor alleles. Consequently, there are allele families that may differ by only a few amino acids and a single anti-HLA antibody may recognize multiple alleles within an evolutionarily-related family. The mouse monoclonal antibody (BB7.2) is known to have cross reactivity to additional HLA-A alleles (Hilton & Parham, 2013.). Specifically, when tested in a solid-phase assay, the BB7.2 antibody was found to recognize five subtypes of HLA-A2 (*02:01, *02:02, *02:03, *02:05, *02:06) and to be cross reactive with HLA-A*69:01, and when tested at high concentrations, also with HLA-A*23:01, A*24:02, A*24:03, A*68:01, and A*68:02 (Hilton & Parham, 2013.). In the context of transplantation, knowledge of alloAg specificity is required to ensure specific targeting to allogenic cells, tissues and/or organs. The traditional way to measure T cell alloreactivity is imprecise and non-quantitative as it involves functional MLRs with large banks of haplotyped PBMCs. To assess the cross reactivity of humanized anti-HLA-A2 antibodies of the invention and compared to the BB7.2 antibody, we adapted the ONE Lambda solid phase assay, which is designed for measuring anti-HLA-

antibodies in serum, to measure HLA-coated bead binding to the humanized CARs of interest. The BB7.2 CAR was generated as described in MacDonald, K.G. (2016). NGFR+ Tregs expressing the indicated humanized CARs were incubated with Flow Panel Reactive Single Antigen beads, with binding to a single class of beads quantified as the loss of signal in the bead gate in the forward/side scatter plot (see methods for details). The data were normalized to the number of negative control beads in the sample, and the amount of relative binding to hCAR-expressing Tregs was calculated in relation to the amount of binding by NGFR-control Tregs by the number of negative beads in the NGFR specimen multiplied by the number of negative beads in the NGFR specimen. To validate the methodology, the relative binding of each m/hA2-CAR construct to HLA-A*02:01 as determined by the FlowPRT cell assay (Figure 6B) was compared to the MFI of tetramer binding (Figure 6C). This analysis revealed a strong, direct correlation between the two methods of detecting A*02:01 binding. We further asked if amount of A*02:01 binding, as determined by the FlowPRT cell assay, correlated with the biological effect of exposure to A*02:01. Indeed, we found there was a direct correlation between the amount of A*02:01 binding quantified by FlowPRT cell assay and stimulation of Treg activation, as judged by CD69 upregulation following exposure to A*02:01-expressing APCs (Figure 6D). These data demonstrate the utility of the FlowPRT cell method to measure the ability of alloAg-specific CARs to bind to different HLA alleles.

As shown in Figure 6 and Table 9, all hA2-CAR constructs significantly bound HLA-A*02:01, confirming the tetramer binding assays. When we tested BB7.2 antibody binding in the single antigen FlowPRA assay, we confirmed high binding to A*69:01 but could not confirm cross-reactivity to A*23:01 or A*24:02 (Figure 6). When testing the relative ability of the m/hA2-CAR Tregs to bind to various HLA-A alleles, we found the mA2-CAR-Tregs bound significantly to HLA-A*03:01, A*25:01, A*29:02, A*30:01. A*31:01, A*33:01, A*36:01, A*68:01 and A*69:01 (Figure 6, Table 9). In contrast, all variants of hA2-CAR Tregs surprisingly displayed reduced cross reactivity compared to mA2-CAR Tregs (Figure 6, Table 9). As expected, all CAR constructs bound to HLA-A*69:01, a variant of A*02:01 differing by only 6 amino acids. None of the CAR-constructs displayed any significant binding to HLA-B (Figure 6).

The relationship between the degree of CAR-Treg Ag binding and biological activity is unknown. To define the biological significance of HLA cross reactivity we generated APCs expressing HLA-A*24:02, A*25:01 or A*68:01; all cells had comparable levels of HLA-A expression (data not shown). We found that only co-culture with HLA-A*02:01-expressing cells resulted in significant activation of m/hCAR-Tregs, as judged by upregulated expression of CD69, CD71, LAP, CTLA-4 (Figure 6I) or CD40L (data not shown). These data suggest that effective CAR-mediated activation of Tregs requires high affinity and/or avidity interactions. Accordingly, while some hA2-CARs show binding A*25:01 and A*68:01 in the FlowPRT assay, the strength of binding is insufficient for cellular activation.

Table 9. Binding interactions between m/hA2-CARs and HLA-A/B protein found to be statistically significant (2-way ANOVA, Dunnett post-test).

HLA-Allele	**CAR**	**p value**
A*02:01	mA2-CAR	0.0001
	H1k2	0.0001
	H2K2	0.0001
	H4k2	0.0001
	H5k2	0.0001
	H6k2	0.0001
	H4k3	0.0001
	H5k3	0.0001
	H3k4	0.0001
	H4k4	0.0006
	H5k4	0.0001
A*25:01	mA2-CAR	0.0001
	H1K2	0.0061
A*69:01	mA2-CAR	0.0001
	H4k2	0.0001
	H5k2	0.0001
	H1k2	0.0001
	H3k4	0.0013

	H2k2	0.0084
	H5k3	0.0001
	H4k3	0.0001
	H6k2	0.0060
A*03:01	mA2-CAR	0.0315
A*29:02	mA2-CAR	0.0001
A*30:01	mA2-CAR	0.0001
A*31:01	mA2-CAR	0.0001
	H1k2	0.0077
A*33:01	mA2-CAR	0.0019
A*36:01	mA2-CAR	0.0035
	H1k2	0.0082
A*68:01	mA2-CAR	0.0001
	H1k2	0.0001
	H5k2	0.0337
	H6k2	0.0009

We developed a new way to systematically test the specificity of CARs for alloantigens, creating a new platform with which to comprehensively identify constructs with defined allele specificity. Surprisingly, we discovered that in comparison to the mA2-CAR, CAR humanization decreased cross-reactivity to several HLA-A allelic variants.

hA2-CAR Tregs mediate HLA-A2-specific suppression in vitro

To test the ability of a hA2-CAR to stimulate antigen-specific suppressive activity in Tregs, we used mixed lymphocyte reactions (MLRs) according to the experimental design shown in Figure 7A. Specifically, proliferation of HLA-A*02:01 negative T cells was stimulated by co-culture with mature HLA-A*02:01-positive dendritic cells in the absence or presence of increasing ratios of Tregs which were either untransduced, or transduced with the control NGFR lentivirus or lentivirus encoding the H1k2 hA2-CAR. As shown in Figures 7B-E, hA2-CAR-expressing Tregs were significantly better able to suppress alloantigen-stimulated

proliferation of $CD4^+$ T cells in comparison to control Tregs transduced with the NGFR control lentiviral construct.

hA2-CAR Tregs mediate HLA-A2-specific suppression of xenogeneic GVHD in vivo

To test the functional capacity of hA2-CAR Tregs *in vivo*, we used a mouse model in which human PBMCs engrafted into immunodeficient NSG mice caused xenogeneic GVHD. 8 x 10^6 PBMCs from an HLA-$A2^+$ donor were injected into irradiated NSG mice with or without Tregs expressing the H1k2 hA2-CAR. The ratio of PBMC to hA2-CAR Tregs tested was 1:2 (i.e. 8 x 10^6 PBMCs to 4 x 10^6 Tregs). Mice were monitored for 7 weeks by clinical score as described in the Methods. In alignment with the *in vitro* data in Figure 7, the mice receiving hA2-CAR-expressing Tregs had improved survival, reduced weight loss and delayed onset of xenoGVHD in comparison to mice that did not receive hA2-CAR Tregs (solid grey line) (Figures 8A, B and C). While the biological effect of the Tregs was observed, we did not detect circulating m/hA2-CAR Tregs, measured from 14 days post injection (Figure 8D and 8E).

hA2-CAR Tregs traffic to HLA-$A2^+$ skin grafts in vivo

To test how CAR-directed specificity affected Treg trafficking, we performed side-by-side skin transplants from NSG or NSG-A*02:01-transgenic mice onto NSG mice (Figure 9A). After graft recovery, PBMCs were injected in the absence or presence of m/hA2-CAR Tregs or with HER2-CAR Tregs as a non-specific polyclonal Treg control (MacDonald et al., 2016). In addition to the CAR, Tregs were co-transduced with a lentivirus encoding a luciferase-GFP fusion protein. Bioluminescent imaging was performed after D-luciferin injection up to 21 days post-Treg injection (Barrett et al., 2014). Polyclonal HER2-CAR Tregs showed a directed pattern of trafficking toward the allografts but were equally distributed between the A2-positive and -negative grafts. These polyclonal Tregs may be migrating in response to the inflammatory signals emanating from post-operative skin because in unmanipulated immunodeficient mice, human T cells typically traffic to the lung (Nervi et al. 2007). In contrast to polyclonal HER2-CAR Tregs which trafficked equally to A2-negative and -positive skin, both m/hA2-CAR Tregs rapidly trafficked to the A2-expressing skin. In addition, m/hA2-CAR Tregs persisted longer than non-specific HER2-CAR Tregs. Whereas HER2-CAR Tregs were undetectable by day 21, a strong m/hA2-CAR Tregs signal remained (Figure 9B). Quantification of the ratio of

luminescence in the A2-positive versus A2-negative graft revealed significant Ag-driven trafficking of both H1k2 and mA2-CAR Tregs to the A2-expressing graft (Figure 9C and 9D).

In addition to graft-localized m/hA2-CAR Tregs, at day 21 we noted an adjacent signal consistent with the location of a local draining lymph node. Upon sacrifice, graft draining lymph nodes were collected and flow cytometric analysis revealed a significant proportion of $hCD4^+FOXP3^+\Delta NGFR^+A2\text{-tetramer}^+$ CAR Tregs. In contrast, very few of these cells were detected in the spleen (Figure 10B and C). This two-step migratory process from graft to lymph node has previously been reported to be necessary for tolerance induction (Zhang et al. 2009). Importantly, lymph node-homing CAR Tregs maintained expression of FOXP3 and CAR, illustrating their stable phenotype.

hA2-CAR Tregs prevent human skin allograft rejection

To evaluate the immunoregulatory potential of hA2-CAR Tregs in a solid organ transplant model, we used a humanized model of skin transplantation in which NSG mice were transplanted with human $HLA\text{-}A2^{pos}$ skin grafts. After 6 weeks, mice were injected with $HLA\text{-}A2^{neg}$ PBMCs with or without autologous H1k2 hA2-CAR Tregs. Four weeks after cell injection, mice were sacrificed, and the skin graft was collected for evaluation of pathology and inflammatory cytokine expression. All mice maintained stable body weight, indicating a lack of xenogeneic GvHD (Figure 11A), with similar levels of human leukocyte engraftment in blood and spleens (Figure 11B). H&E sections were evaluated using a 25-point scale, revealing a significant decrease in the cumulative pathological rejection score in mice that received H1k2 hA2-CAR Tregs versus PBMC (Figure 11C). Immunostaining revealed that in comparison to mice receiving PBMCs alone, mice receiving PBMCs and H1k2 hA2-CAR Tregs had a significant reduction in $Ki67^+$ keratinocytes, and diminished involucrin destruction (Figure 11D). qPCR quantification also showed a general reduction in inflammatory cytokines within the graft of H1k2 hA2-CAR Treg-treated mice (Figure 11E).

As for the xenogeneic GVHD model, while PBMCs were detectable in blood, CAR Tregs were not (Figure 12). However, immunostaining revealed that in comparison to mice receiving PBMCs alone, mice receiving PBMCs and H1k2 hA2-CAR Tregs had a significantly higher proportion of $FOXP3^+$ cells in the graft (Figure 11F). The presence of $FOXP3^+$ cells was unique to the transplanted skin graft as they were undetectable in the intestine, liver or lung

(Figure 11G). These data show that, as for the model with A2-transgenic NSG skin, H1k2 hA2-CAR Tregs specifically traffic to human $A2^+$ skin allograft, where they persist indefinitely.

This application contains a sequence listing in electronic form as a text file, "CDRD-003WO_SEQ LISTING_ST25" created on September 18, 2018 and having a size of 320 KB. The contents of the text file are incorporated by reference herein in their entirety.

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While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques and apparatus described above can be used in various combinations. All publications, patents, patent applications, and/or other documents

cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually indicated to be incorporated by reference for all purposes.

CLAIMS:

1. A humanized anti-HLA-A2 antibody, wherein said antibody is capable of constituting an antigen binding domain of a chimeric antigen receptor (CAR), wherein said CAR is capable of being expressed in a human cell such that said CAR specifically binds to HLA-A2.

2. The humanized anti-HLA-A2 antibody of claim 1, wherein said antibody competes for binding to HLA-A2 with an antibody comprising:

a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183;

a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185;

a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187;

a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188;

a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and

a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190.

3. A humanized anti-HLA-A2 antibody, wherein said antibody competes for binding to HLA-A2 with an antibody comprising:

a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183;

a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185;

a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187;

a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188;

a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and

a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190.

4. The humanized anti-HLA-A2 antibody of any one of claims 1 to 3, wherein said antibody binds to the same HLA-A2 epitope as an antibody comprising:

a heavy chain complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID NO: 183;

a heavy chain complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID NO: 185;

a heavy chain complementarity determining region 3 (HCDR3) having the amino acid sequence of SEQ ID NO: 187;

a light chain complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID NO: 188;

a light chain complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID NO: 189; and

a light chain complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID NO: 190.

5. The humanized anti-HLA-A2 antibody of any one of claims 1 to 4, wherein said antibody has less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 antibody.

6. The humanized anti-HLA-A2 antibody of any one of claims 1 to 4, wherein said antibody has less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a BB7.2 scFv.

7. The humanized anti-HLA-A2 antibody of any one of claims 1 to 6, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: SYHIQ (SEQ ID NO: 1) and GYTFTSY (SEQ ID NO: 2).

8. The humanized anti-HLA-A2 antibody of any one of claims 1 to 7, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: YPGDGS (SEQ ID NO: 4) and WIYPGDGSTX^{10}YX^{12}X^{13}KFX^{16}G (SEQ ID NO: 10), wherein X^{10} is Q or K, X^{12} is N or S, X^{13} is E or Q, and X^{16} is K or Q.

9. The humanized anti-HLA-A2 antibody of claim 8, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of: WIYPGDGSTQYNEKFKG (SEQ ID NO: 3) and YPGDGS (SEQ ID NO: 4).

10. The humanized anti-HLA-A2 antibody of claim 8, wherein said antibody comprises a heavy chain variable region comprising the amino acid sequence WIYPGDGSTKYSQKFQG (SEQ ID NO: 5).

11. The humanized anti-HLA-A2 antibody of any one of claims 1 to 10, wherein said antibody comprises a heavy chain variable region comprising the amino acid sequence EGTYYAMDY (SEQ ID NO: 6).

12. The humanized anti-HLA-A2 antibody of any one of claims 1 to 11, wherein said antibody comprises a light chain variable region comprising the amino acid sequence RSSQSIVHSNGNTYLE (SEQ ID NO: 7).

13. The humanized anti-HLA-A2 antibody of any one of claims 1 to 12, wherein said antibody comprises a light chain variable region comprising the amino acid sequence KVSNRFS (SEQ ID NO: 8).

14. The humanized anti-HLA-A2 antibody of any one of claims 1 to 13, wherein said antibody comprises a light chain variable region comprising the amino acid sequence FQGSHVPRT (SEQ ID NO: 9).

15. The humanized anti-HLA-A2 antibody of any one of claims 1 to 14, wherein said antibody comprises a heavy chain variable region comprising a framework region 1 (VH FR1) comprising an amino acid sequence selected from the group consisting of:

QVQLVQSGAEVKKPGASVKVSCKAS (SEQ ID NO: 11) and

QVQLVQSGAEVKKPGASVKVSCKASGYTFT (SEQ ID NO: 12).

16. The humanized anti-HLA-A2 antibody of any one of claims 1 to 15, wherein said antibody comprises a heavy chain variable region comprising a framework region 2 (VH FR2) comprising an amino acid sequence selected from the group consisting of:

WVRQAPGQX^{9}LEWMGX^{15} (SEQ ID NO: 13),

WVRQAPGQX^{9}LEWMGX^{15}WI (SEQ ID NO: 17),

HIQWVRQAPGQX^{12}LEWMGX^{18}WI (SEQ ID NO: 21), and

HIQWVRQAPGQX^{12}LEWMGX^{18} (SEQ ID NO: 25), wherein:

X^{9} is R or G and X^{15} is I or absent in SEQ ID NO: 13;

X^{9} is R or G, and X^{15} is I or absent in SEQ ID NO: 17;

X^{12} is R or G, and X^{18} is I or absent in SEQ ID NO: 21; and

X^{12} is R or G, and X^{18} is I or absent in SEQ ID NO: 25.

17. The humanized anti-HLA-A2 antibody of any one of claims 1 to 16, wherein said antibody comprises a heavy chain variable region comprising a framework region 3 (VH FR3) comprising an amino acid sequence selected from the group consisting of:

$X^1VTX^4TX^6DTSX^{10}STAYMX^{16}LSX^{19}LRSX^{23}DX^{25}AVYYCAR$ (SEQ ID NO: 29),

$TX^2YX^4X^5KFX^8GX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}$

AVYYCAR (SEQ ID NO: 35),

$TQYNEKFKGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}$

AVYYCAR (SEQ ID NO: 36), and

$TKYSQKFQGX^{10}VTX^{13}TX^{15}DTSX^{19}STAYMX^{25}LSX^{28}LRSX^{32}DX^{34}AVYYCAR$ (SEQ ID NO: 37), wherein:

X^1 is R or absent, X^4 is I or M, X^6 is R or A, X^{10} is A, T or I, X^{16} is E or L, X^{19} is S or R, X^{23} is E or D, and X^{25} is T or M in SEQ ID NO: 29;

X^2 is Q or K, X^4 is N or S, X^5 is E or Q, X^8 is K or Q, X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 35;

X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 36; and

X^{10} is R or absent, X^{13} is I or M, X^{15} is R or A, X^{19} is A, T or I, X^{25} is E or L, X^{28} is S or R, X^{32} is E or D, and X^{34} is T or M in SEQ ID NO: 37.

18. The humanized anti-HLA-A2 antibody of any one of claims 1 to 17, wherein said antibody comprises a heavy chain variable region comprising a framework region 4 (VH FR4) comprising the amino acid sequence WGQGTTVTVSS (SEQ ID NO: 44).

19. The humanized anti-HLA-A2 antibody of any one of claims 1 to 6, wherein said antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 61-66.

20. The humanized anti-HLA-A2 antibody of any one of claims 1 to 19, wherein said antibody comprises a light chain variable region comprising a framework region 1 (VL FR1) comprising the amino acid sequence $DX^2VMTQX^7PLSX^{11}X^{12}VTX^{15}GQPASISX^{23}$ (SEQ ID NO: 46), wherein X^2 is V or I, X^7 is S or T, X^{11} is L or S, X^{12} is P or S, X^{15} is L or P, and X^{23} is C or F.

21. The humanized anti-HLA-A2 antibody of any one of claims 1 to 20, wherein said antibody comprises a light chain variable region comprising a framework region 2 (VL FR2) comprising the amino acid sequence $WX^2X^3QX^5PGQX^9PX^{11}X^{12}LIY$ (SEQ ID NO: 51), wherein X^2 is F or Y, X^3 is Q or L, X^5 is R or K, X^9 is S or P, X^{11} is R or Q, and X^{12} is R or L.

22. The humanized anti-HLA-A2 antibody of any one of claims 1 to 21, wherein said antibody comprises a light chain variable region comprising a framework region 3 (VL FR3) comprising the amino acid sequence $GVPDRFSGSGX^{11}GTDFTLKISRVEAEDVGVYYC$ (SEQ ID NO: 56), wherein X^{11} is S or A.

23. The humanized anti-HLA-A2 antibody of any one of claims1 to 22, wherein said antibody comprises a light chain variable region comprising a framework region 4 (VL FR4) comprising the amino acid sequence FGGGTKVEIK (SEQ ID NO: 59).

24. The humanized anti-HLA-A2 antibody of any one of claims 1 to 6 and 19, wherein said antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 67-71.

25. The humanized anti-HLA-A2 antibody of any one of claims 1 to 24, wherein said antibody is an scFv.

26. The humanized anti-HLA-A2 antibody of any one of claims 1 to 6, wherein said antibody is an scFv comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-91.

27. The humanized anti-HLA-A2 antibody of any one of claims 1 to 26, wherein said human cell is an immune cell.

28. The humanized anti-HLA-A2 antibody of any one of claims 1 to 27, wherein said antibody is capable of constituting an antigen binding domain of a chimeric antigen receptor (CAR), wherein said CAR is capable of being expressed in a T regulatory cell (Treg) such that said CAR specifically binds to HLA-A2.

29. The humanized anti-HLA-A2 antibody of any one of claims 1 to 27, wherein said antibody is capable of constituting an antigen binding domain of a chimeric antigen receptor (CAR), wherein said CAR is capable of being expressed in an immune cell such that said immune cell is activated by HLA-A2.

30. The humanized anti-HLA-A2 antibody of claim 29, where said immune cell is a T regulatory cell (Treg).

31. A nucleic acid encoding the humanized anti-HLA-A2 antibody of any one of claims 1 to 30.

32. An expression vector comprising the nucleic acid of claim 31.

33. A host cell comprising the expression vector of claim 32.

34. A chimeric antigen receptor (CAR) comprising:

(i) an extracellular domain comprising the humanized anti-HLA-A2 antibody according to any one of claims 1 to 30;

(ii) a transmembrane domain; and

(iii) a cytoplasmic domain comprising an intracellular signaling domain;

wherein said CAR is capable of being expressed in an immune cell such that said CAR specifically binds to HLA-A2.

35. The CAR according to claim 34, wherein said CAR has less reactivity to at least one HLA-A subtype selected from one or more of HLA-A*25, HLA-A*29, HLA-A*30, as compared to a CAR comprising a BB7.2 antibody.

36. The CAR according to claim 34 or 35, wherein said CAR is capable of being expressed in an immune cell such that said immune cell is activated by HLA-A2.

37. The CAR according to any one of claims 34 to 36, where said immune cell is a T regulatory cell (Treg).

38. The CAR of any one of claims 34 to 37, further comprising a hinge region.

39. The CAR of claim 38, wherein said hinge region comprises a stalk region of CD8α.

40. The CAR of any one of claims 34 to 39, wherein said transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of: CD3 gamma, CD3

delta, CD3 epsilon, CD3 zeta, the alpha chain of the T-cell receptor, the beta chain of the T-cell receptor, the gamma chain of the T-cell receptor, the delta chain of the T-cell receptor, CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, and any combination thereof.

41. The CAR of claim 40, wherein said transmembrane domain comprises a transmembrane domain of CD28.

42. The CAR of any one of claims 34 to 41, wherein said intracellular signaling domain comprises a functional signaling domain of a protein selected from the group consisting of: CD3 gamma, CD3 delta, CD3 epsilon, CD3 zeta, FcR gamma, FcR alpha, FcR epsilon, CD5, CD22, CD79a, CD79b, and CD66d, and any combination thereof.

43. The CAR of claim 42, wherein said intracellular signaling domain comprises a functional signaling domain of CD3 zeta.

44. The CAR of any one of claims 34 to 43, wherein said intracellular signaling domain further comprises a costimulatory domain.

45. The CAR of claim 44, wherein said costimulatory domain comprises a functional signaling domain of a protein selected from the group consisting of OX40, CD27, CD28, lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18), TNFR1 (CD120a/TNFRSF1A), TNFR2 (CD120b/TNFRSF1B), CTLA-4 (CD152), CD95, ICOS (CD278), 4-1BB (CD137), CD2, CD30, CD40, PD-1, CD7, LIGHT, NKG2C, B7-H3, ICAM-1, a ligand that specifically binds with CD83, IL2ra (CD25), IL6Ra (CD126), IL-7Ra (CD127), IL-13RA1, IL-13RA2, IL-33R(IL1RL1), IL-10RA, IL-10RB, IL-4R, IL-5R (CSF2RB), ARHR, BAFF receptor, IL-21R, TGFbR1, TGFbR2, TGFbR3, common gamma chain, and any combination thereof.

46. The CAR of claim 44 or 45, wherein said costimulatory domain comprises a functional signaling domain of a protein selected from CD28 and 4-1BB.

47. The CAR of claim 46, wherein said costimulatory domain comprises a functional signaling domain of CD28.

48. A modified immune cell, comprising the CAR according to any one of claims 34 to 47.

49. The modified immune cell of claim 48, wherein said modified immune cell is a T regulatory cell (Treg).

50. A nucleic acid encoding the CAR according to any one of claims 34 to 47.

51. An expression vector comprising the nucleic acid according to claim 50.

52. An immune cell comprising the expression vector according to claim 51.

53. The immune cell of claim 52, wherein said immune cell is a T regulatory cell (Treg).

54. A pharmaceutical composition comprising a plurality of the modified immune cell according to claim 48 or 49.

55. A pharmaceutical composition comprising a plurality of the immune cell according to claim 52 or 53.

56. A method of making the modified immune cell according to claim 48 or 49, comprising transducing an immune cell with the expression vector according to claim 51, thereby generating said modified immune cell.

57. A method of promoting immune tolerance in a subject, the method comprising administering to said subject the pharmaceutical composition according to claim 54 or 55.

58. The method according to claim 57, wherein said immune tolerance is tolerance to a transplanted organ or tissue.

59. A method of preventing or treating graft versus host disease (GVHD) in a subject, the method comprising administering to said subject the pharmaceutical composition according to claim 54 or 55.

60. The method according to claim 59, wherein said subject is undergoing or has undergone a hematopoietic stem cell transplant.

61. A method of preventing or treating organ or tissue transplant rejection in a subject, the method comprising administering to said subject the pharmaceutical composition according to claim 54 or 55.

62. The method according to any one of claims 57 to 61, wherein said subject is human.

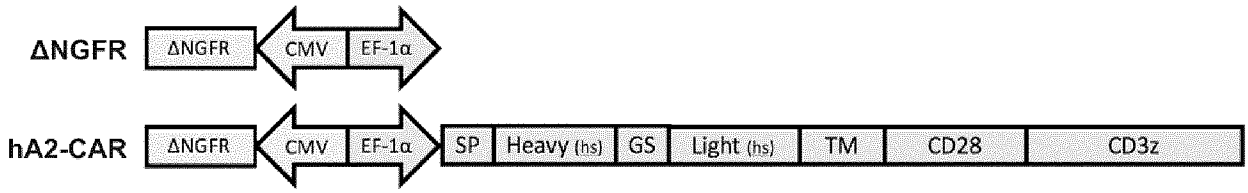

ΔNGFR
ΔNGFR
CMV
EF-1α
hA2-CAR
ΔNGFR
CMV
EF-1α
SP
Heavy (hs)
GS
Light (hs)
TM
CD28
CD3z

Fig. 1

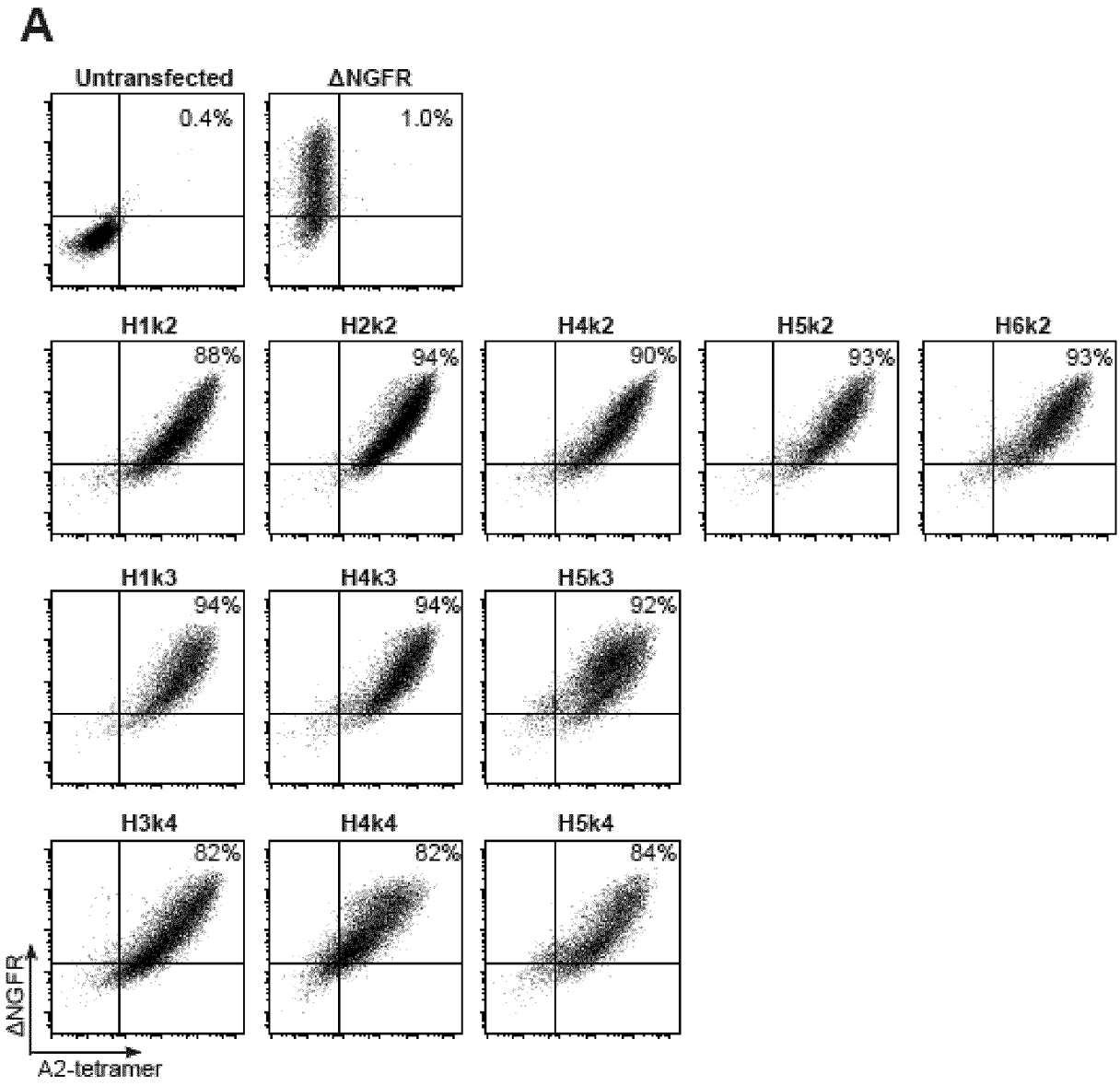

A
Untransfected
0.4%
ΔNGFR
1.0%
H1k2
88%
H2k2
94%
H4k2
90%
H5k2
93%
H6k2
93%
H1k3
94%
H4k3
94%
H5k3
92%
H3k4
82%
H4k4
82%
H5k4
84%
ΔNGFR
A2-tetramer

Fig. 2

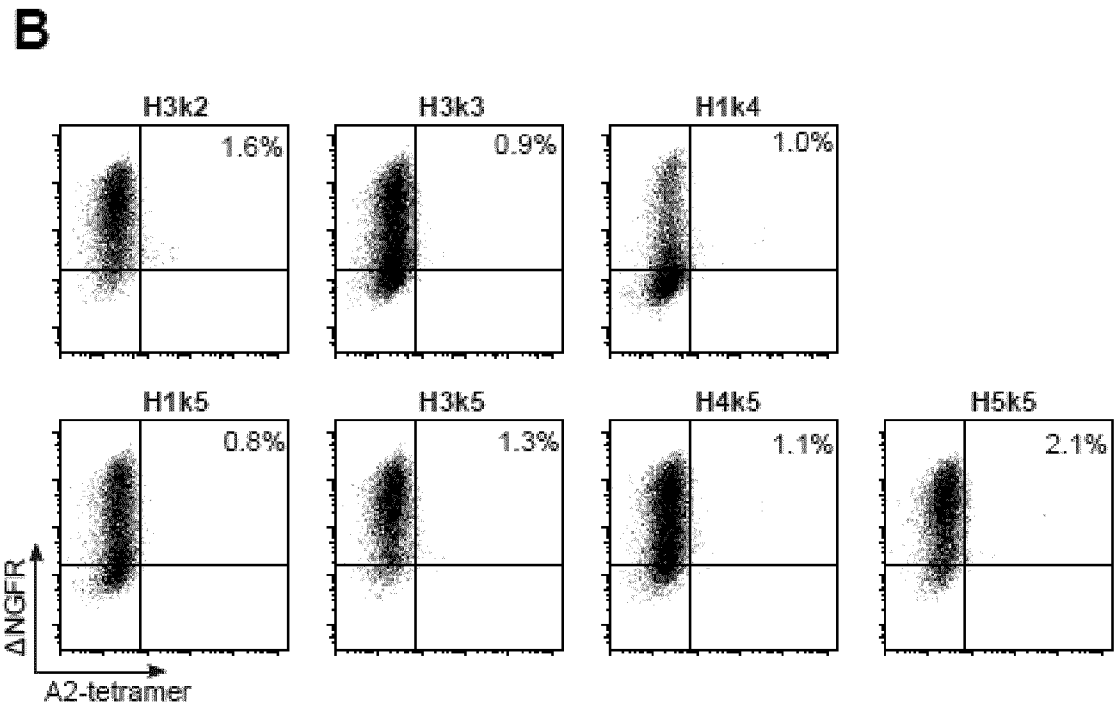
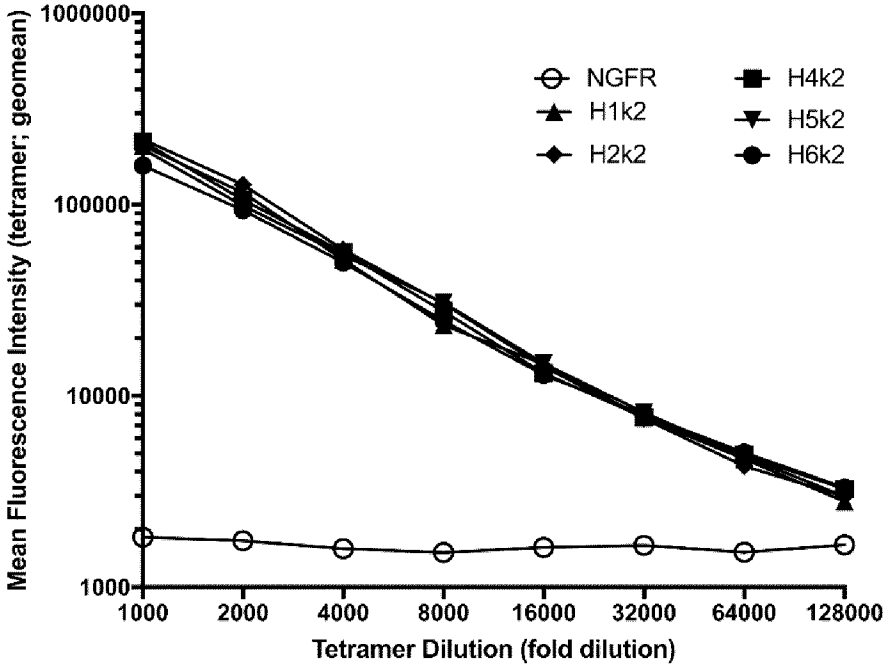

B
H3k2
1.6%
H3k3
0.9%
H1k4
1.0%
H1k5
0.8%
H3k5
1.3%
H4k5
1.1%
H5k5
2.1%
ΔNGFR
A2-tetramer

Fig. 2 cont'd

A


Mean Fluorescence Intensity (tetramer; geomean)
1000000
100000
10000
1000
NGFR
H1k2
H2k2
H4k2
H5k2
H6k2
1000
2000
4000
8000
16000
32000
64000
128000
Tetramer Dilution (fold dilution)

B

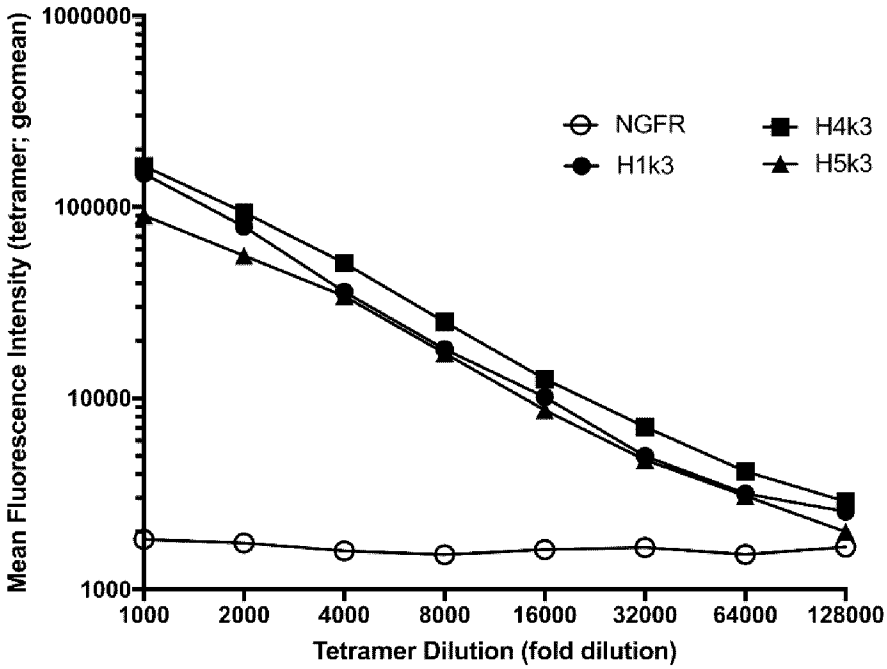

Mean Fluorescence Intensity (tetramer; geomean)
1000000
100000
10000
1000
NGFR
H1k3
H4k3
H5k3
1000
2000
4000
8000
16000
32000
64000
128000
Tetramer Dilution (fold dilution)

Fig. 3

C

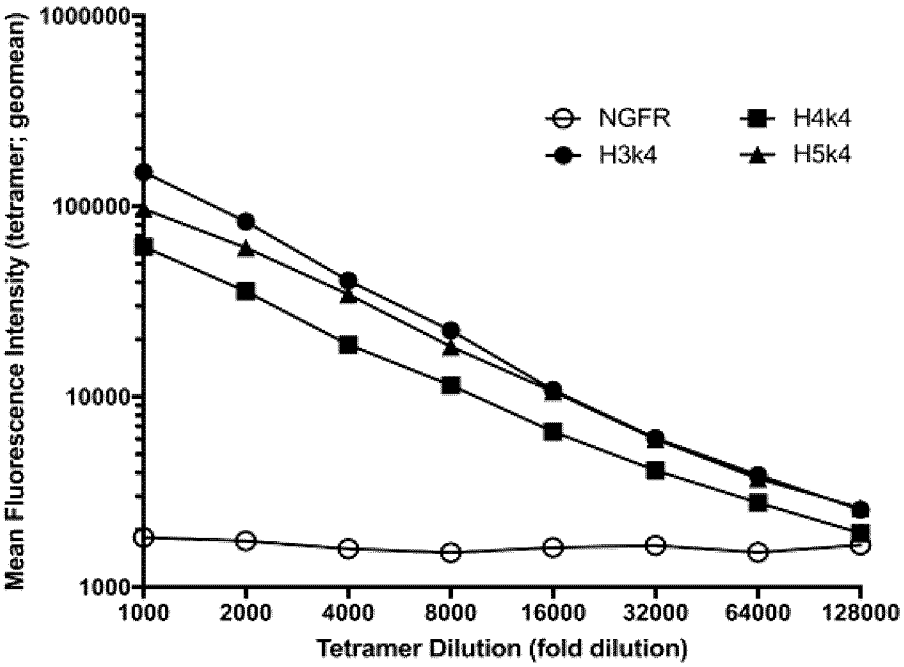

Mean Fluorescence Intensity (tetramer; geomean)
1000000
100000
10000
1000
NGFR
H3k4
H4k4
H5k4
1000
2000
4000
8000
16000
32000
64000
128000
Tetramer Dilution (fold dilution)

Fig. 3 cont'd

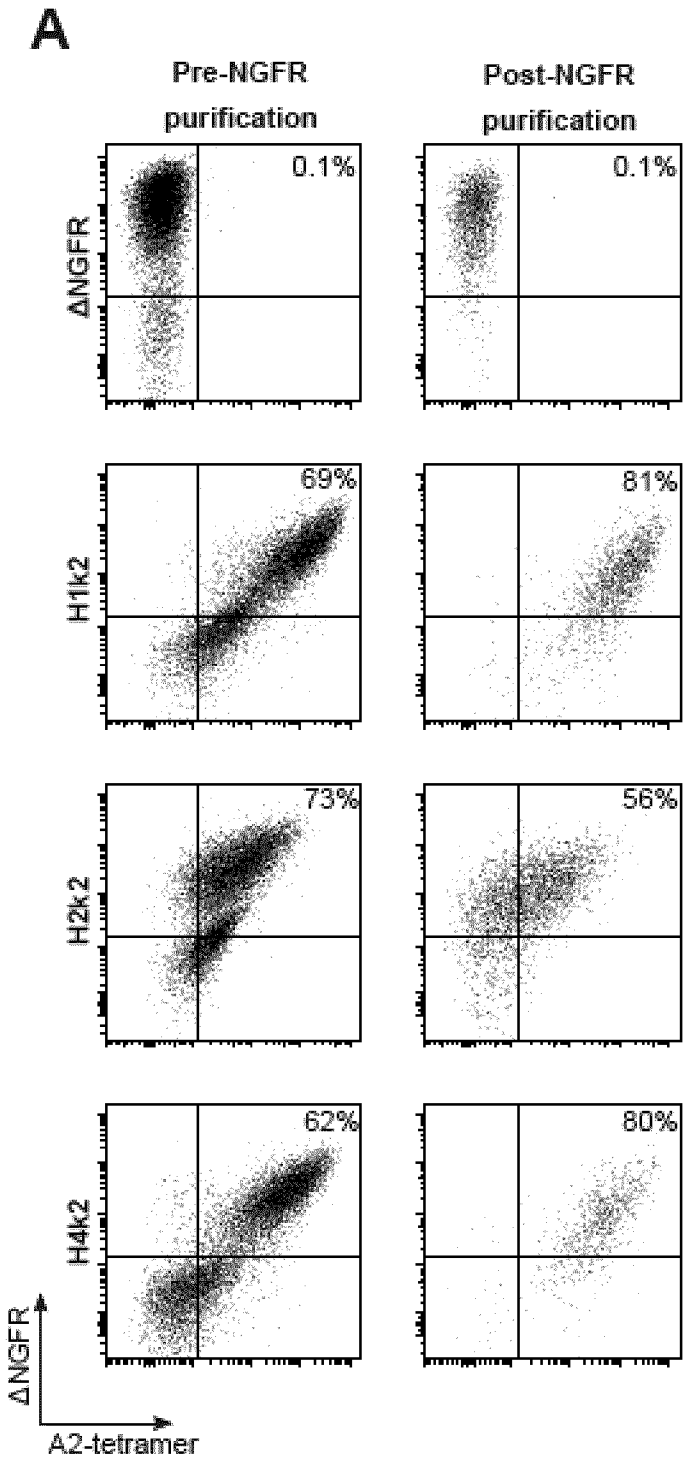

A
Pre-NGFR purification
Post-NGFR purification
0.1%
0.1%
ΔNGFR
69%
81%
H1k2
73%
56%
H2k2
62%
80%
H4k2
ΔNGFR
A2-tetramer

Fig. 4

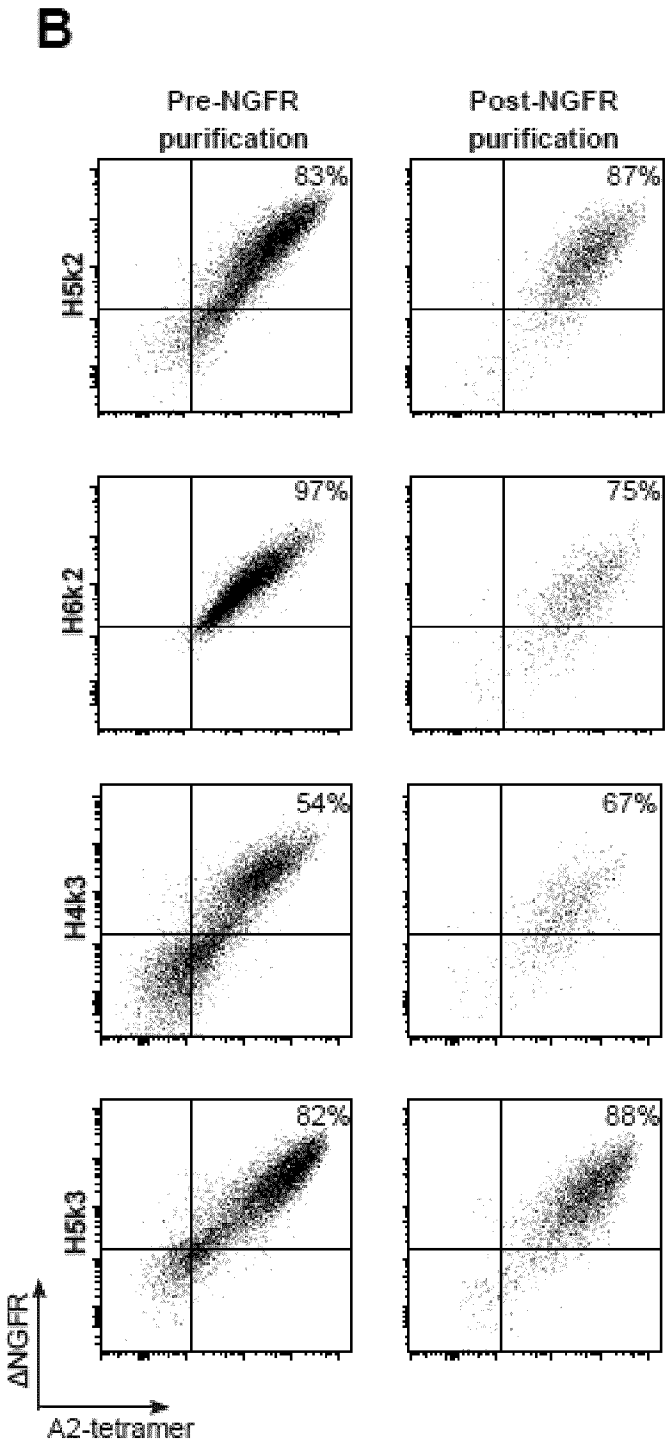

B
Pre-NGFR purification
Post-NGFR purification
H5k2
83%
87%
H6k2
97%
75%
H4k3
54%
67%
H5k3
82%
88%
ΔNGFR
A2-tetramer

Fig. 4 cont'd

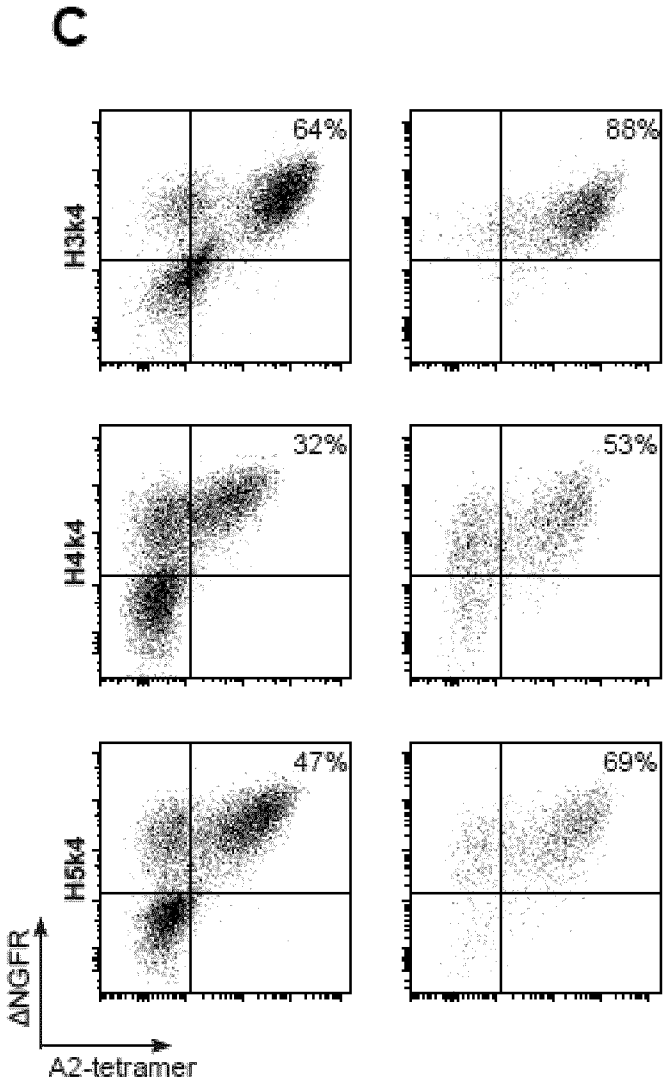


Fig. 4 cont'd

D

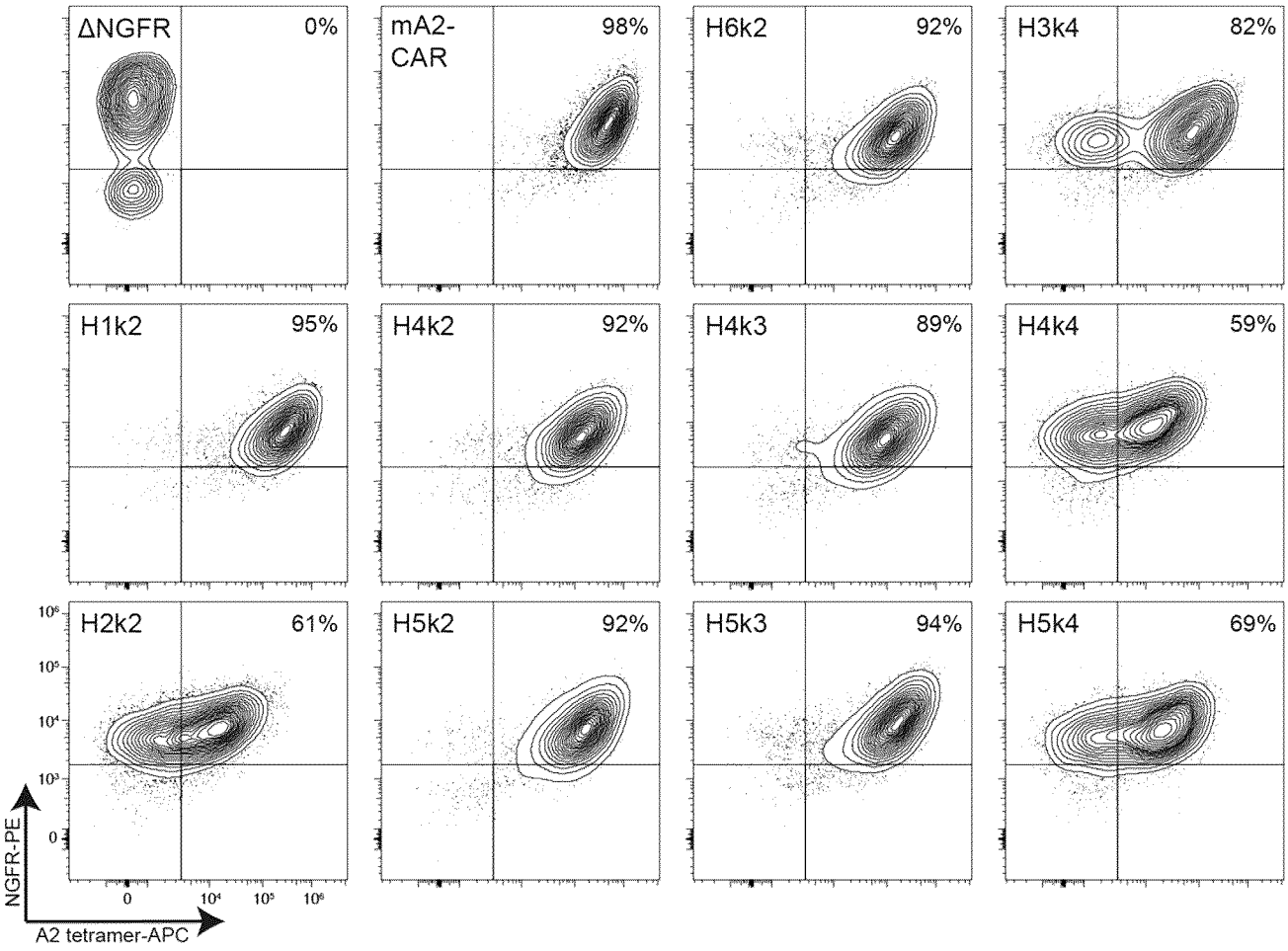

ΔNGFR
0%
mA2-CAR
98%
H6k2
92%
H3k4
82%
H1k2
95%
H4k2
92%
H4k3
89%
H4k4
59%
H2k2
61%
H5k2
92%
H5k3
94%
H5k4
69%
NGFR-PE
A2 tetramer-APC

Fig. 4 cont'd

E

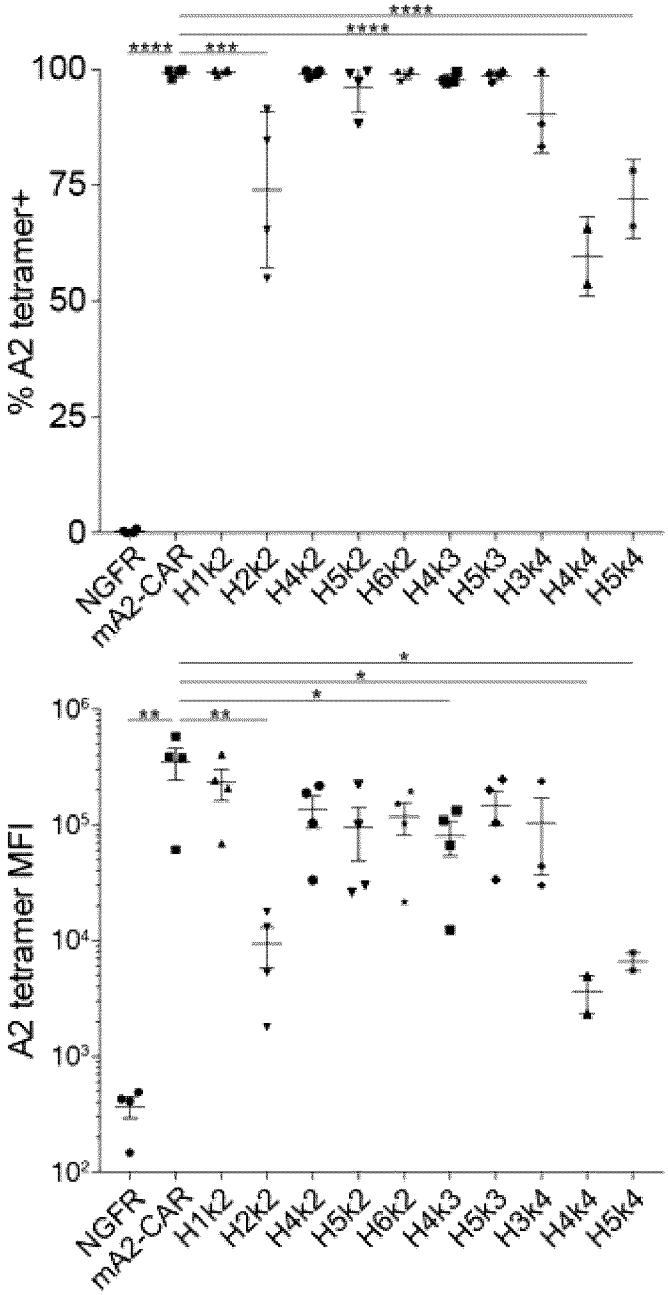

% A2 tetramer+
100
75
50
25
0
****
****
****
***
NGFR
mA2-CAR
H1K2
H2K2
H4K2
H5K2
H6K2
H4K3
H5K3
H3K4
H4K4
H5K4
A2 tetramer MFI
10^6
10^5
10^4
10^3
10^2
*
*
*
**
**

Fig. 4 cont'd

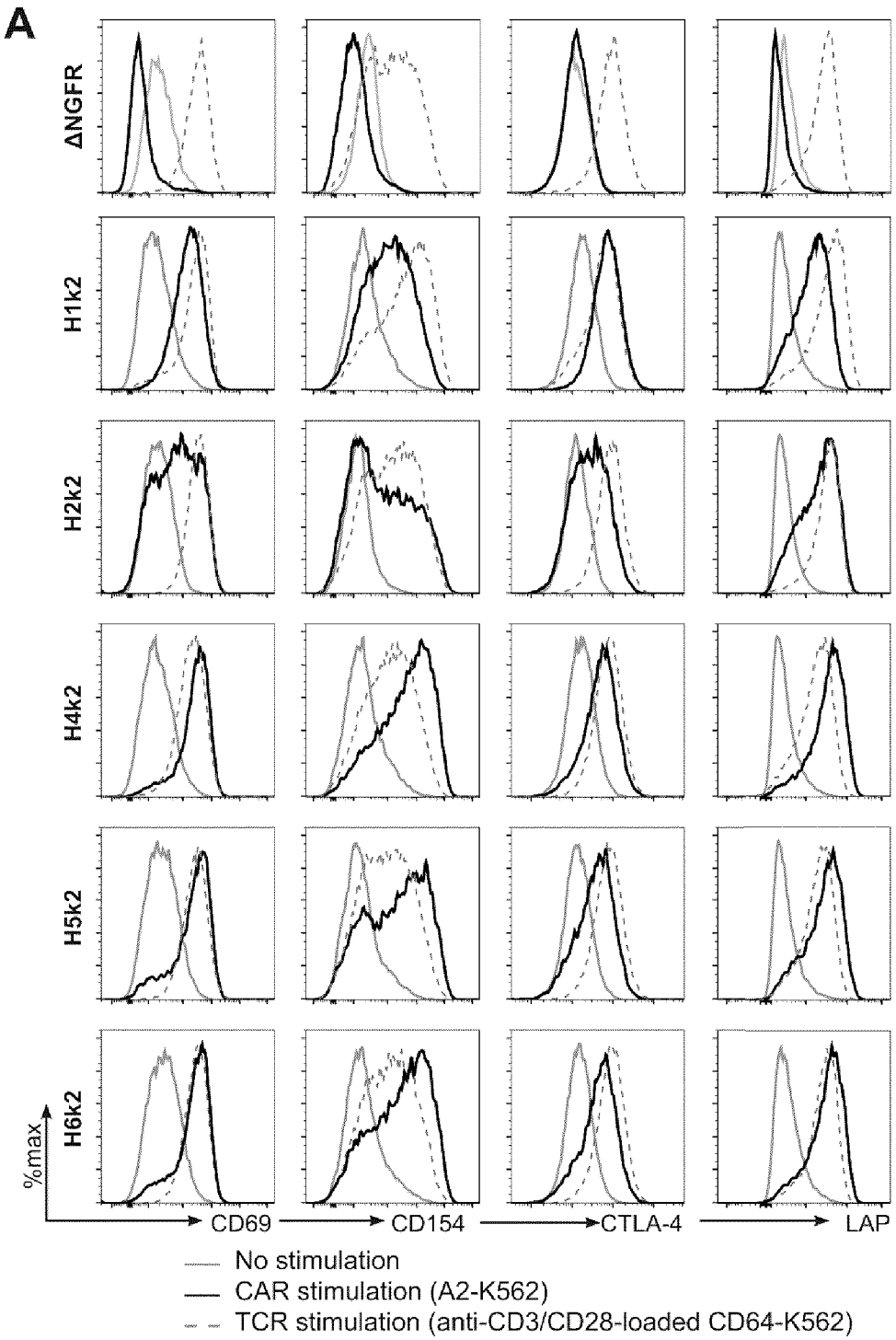

A
ΔNGFR
H1k2
H2k2
H4k2
H5k2
H6k2
%max
CD69
CD154
CTLA-4
LAP
No stimulation
CAR stimulation (A2-K562)
TCR stimulation (anti-CD3/CD28-loaded CD64-K562)

Fig. 5

B

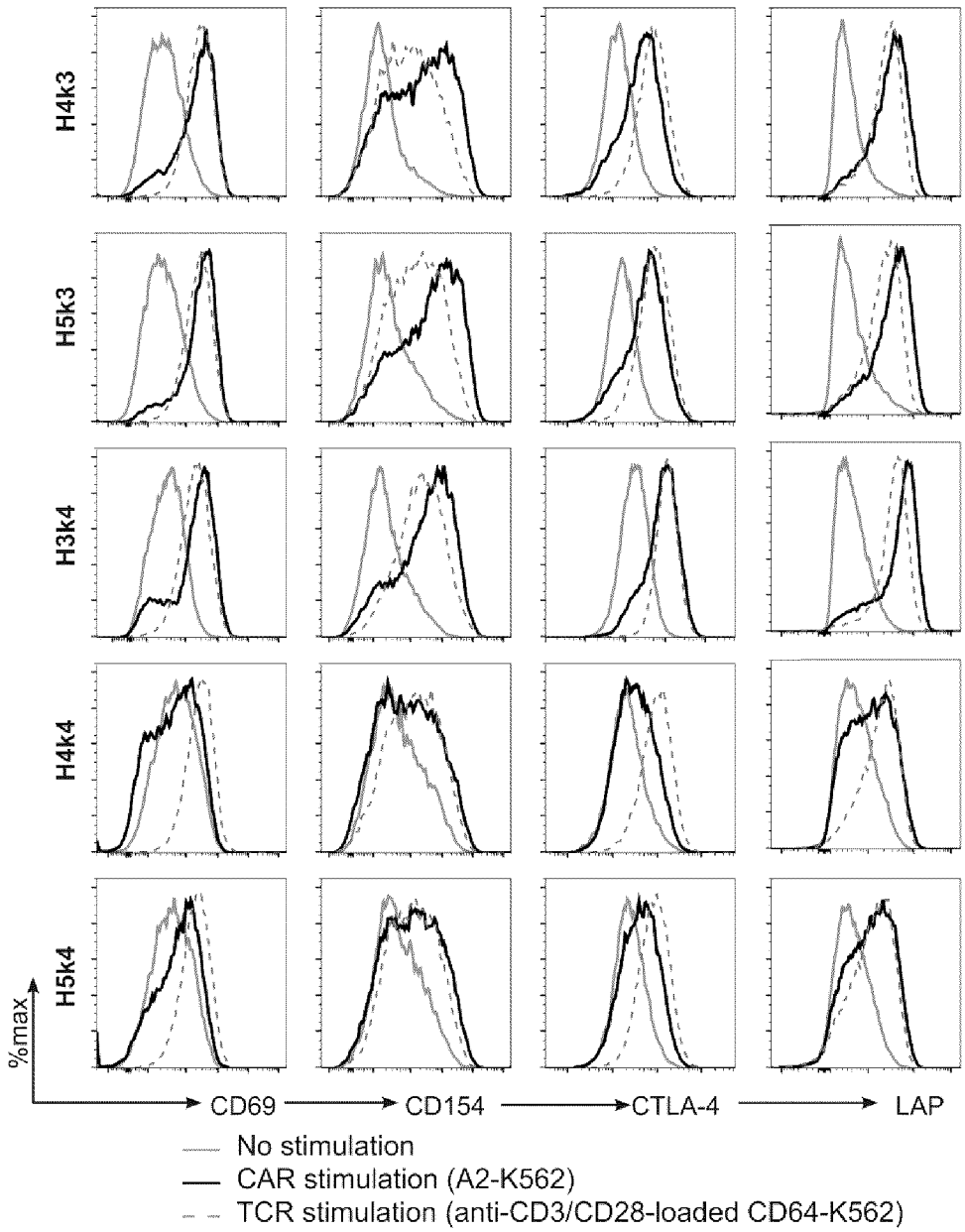

H4k3
H5k3
H3k4
H4k4
H5k4
%max
CD69
CD154
CTLA-4
LAP
No stimulation
CAR stimulation (A2-K562)
TCR stimulation (anti-CD3/CD28-loaded CD64-K562)

Fig. 5 cont'd

C

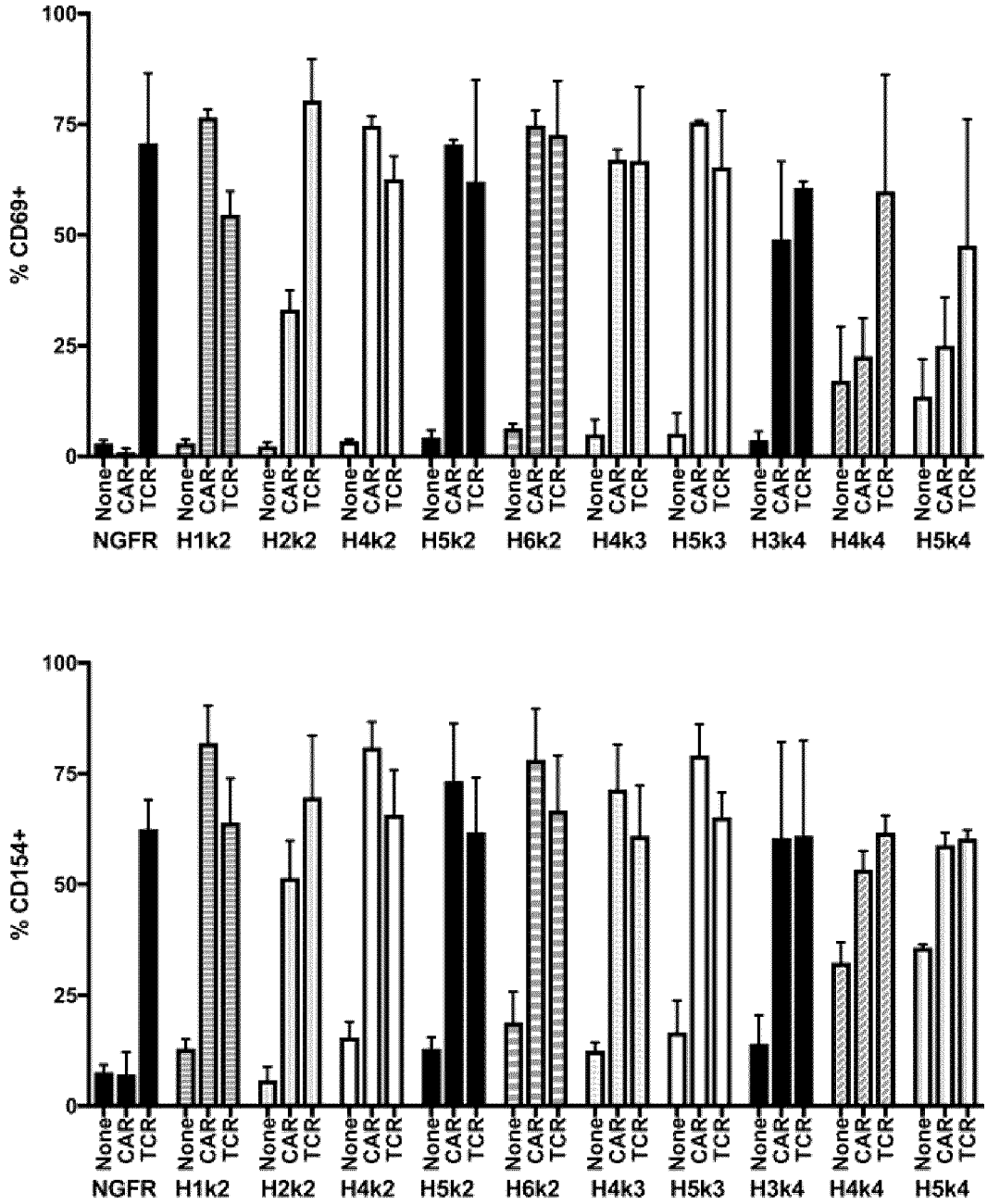

100
75
50
25
0
% CD69+
None
CAR
TCR
NGFR
H1k2
H2k2
H4k2
H5k2
H6k2
H4k3
H5k3
H3k4
H4k4
H5k4
100
75
50
25
0
% CD154+
None
CAR
TCR
NGFR
H1k2
H2k2
H4k2
H5k2
H6k2
H4k3
H5k3
H3k4
H4k4
H5k4

Fig. 5 cont'd

D

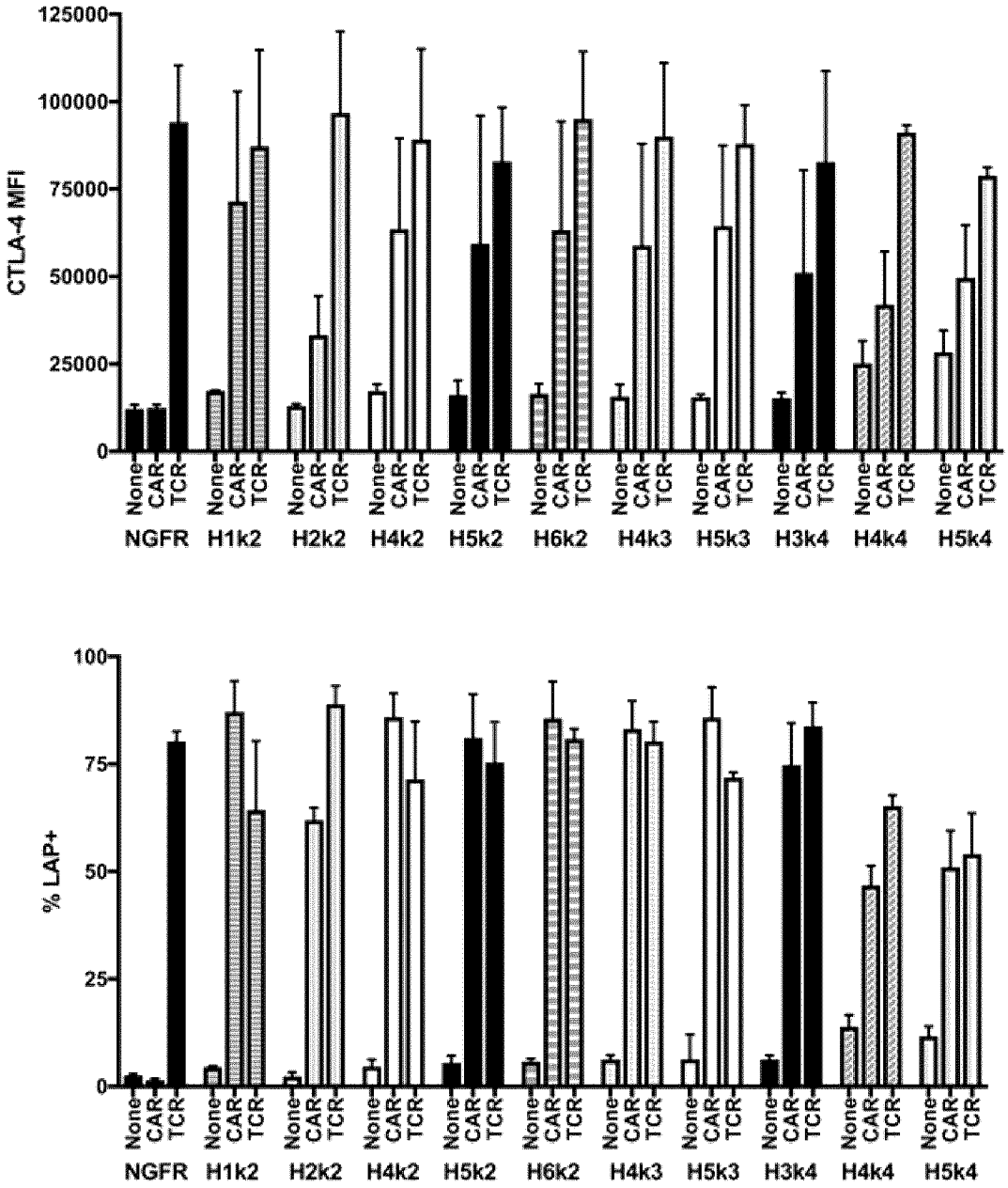

CTLA-4 MFI
125000
100000
75000
50000
25000
0
% LAP+
100
75
50
25
0
None
CAR
TCR
NGFR
H1k2
H2k2
H4k2
H5k2
H6k2
H4k3
H5k3
H3k4
H4k4
H5k4

Fig. 5 cont'd

E

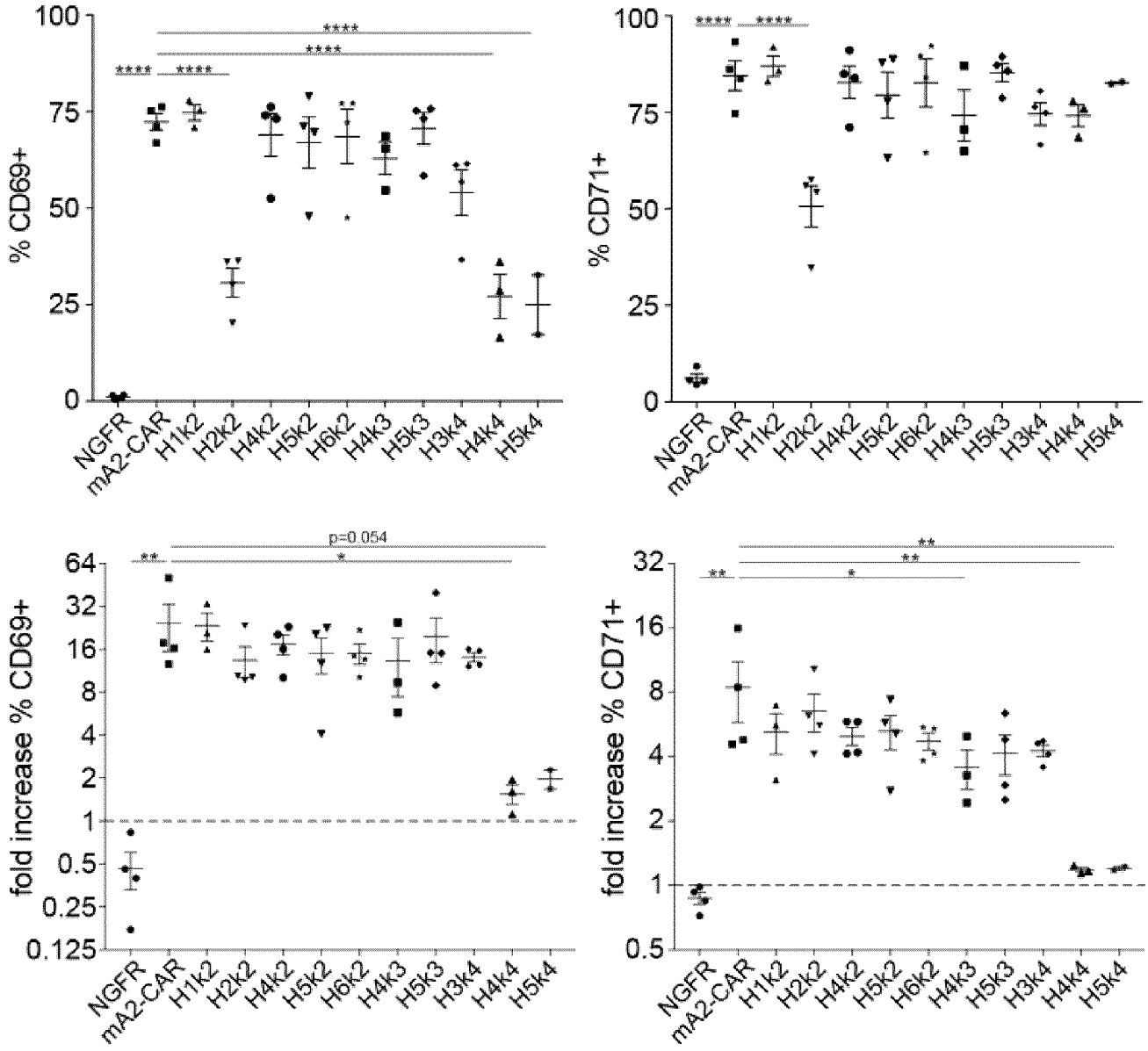

% CD69+
% CD71+
fold increase % CD69+
fold increase % CD71+
p=0.054
NGFR
mA2-CAR
H1k2
H2k2
H4k2
H5k2
H6k2
H4k3
H5k3
H3k4
H4k4
H5k4

Fig. 5 cont'd

F

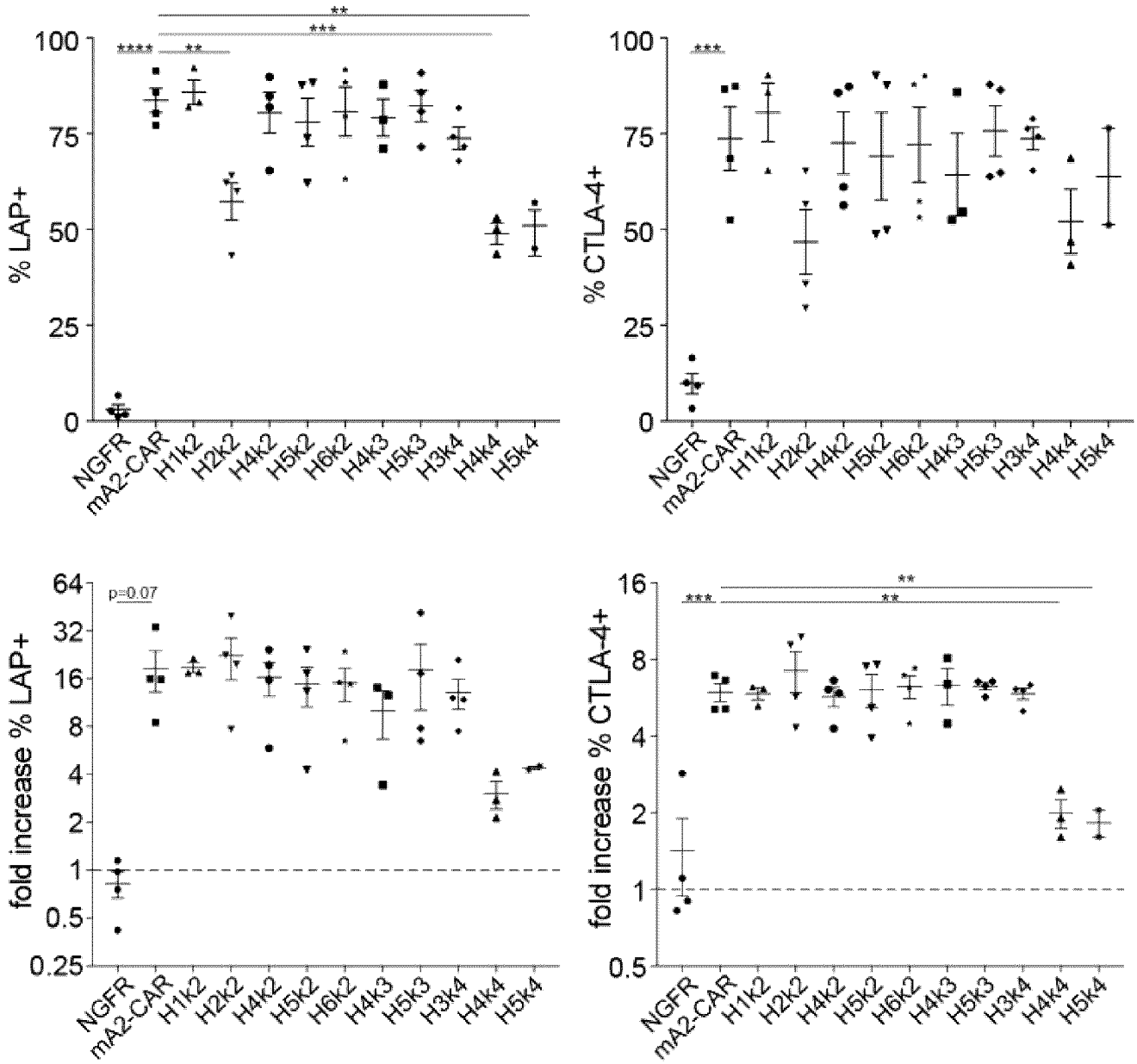
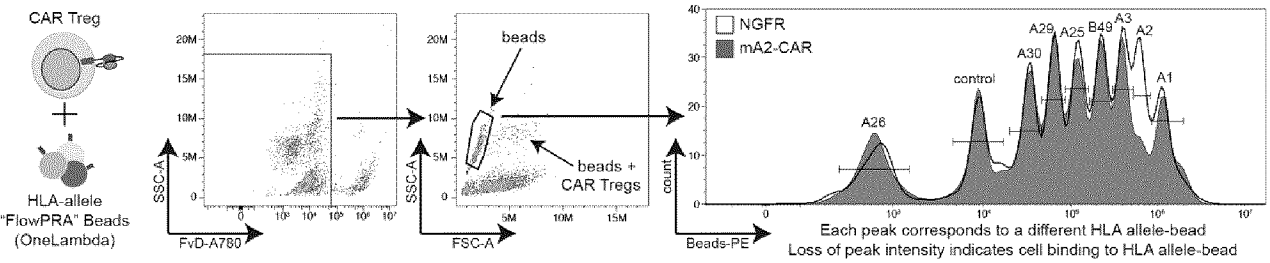

% LAP+
% CTLA-4+
fold increase % LAP+
fold increase % CTLA-4+
p=0.07
NGFR
mA2-CAR
H1K2
H2K2
H4K2
H5K2
H6K2
H4K3
H5K3
H3K4
H4K4
H5K4

Fig. 5 cont'd

A


CAR Treg
HLA-allele
"FlowPRA" Beads
(OneLambda)
SSC-A
FvD-A780
beads
beads +
CAR Tregs
FSC-A
NGFR
mA2-CAR
A26
control
A30
A29
A25
B49
A3
A2
A1
count
Beads-PE
Each peak corresponds to a different HLA allele-bead
Loss of peak intensity indicates cell binding to HLA allele-bead

B

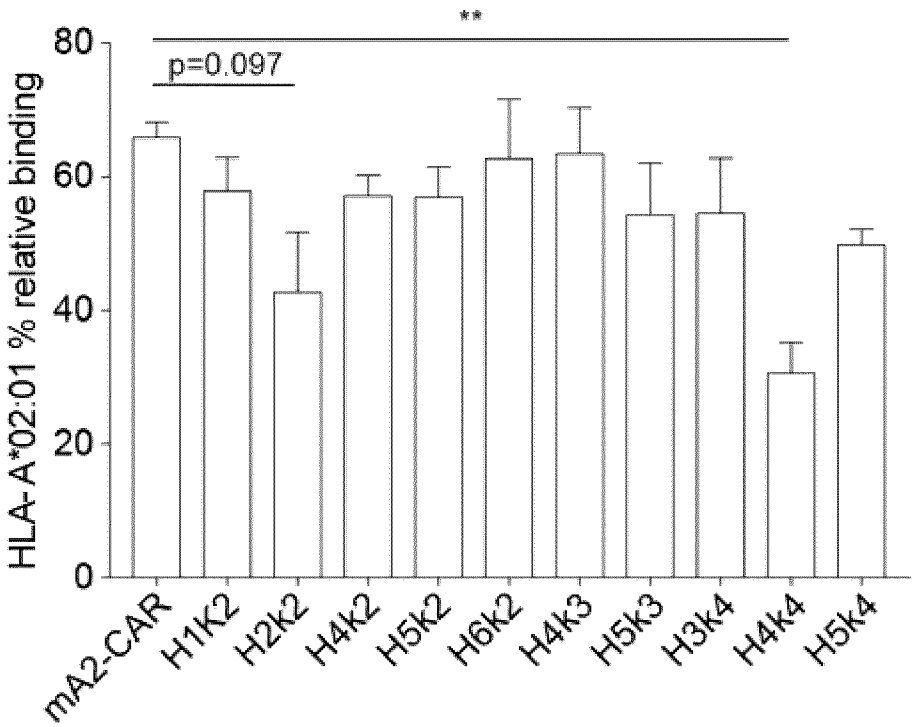
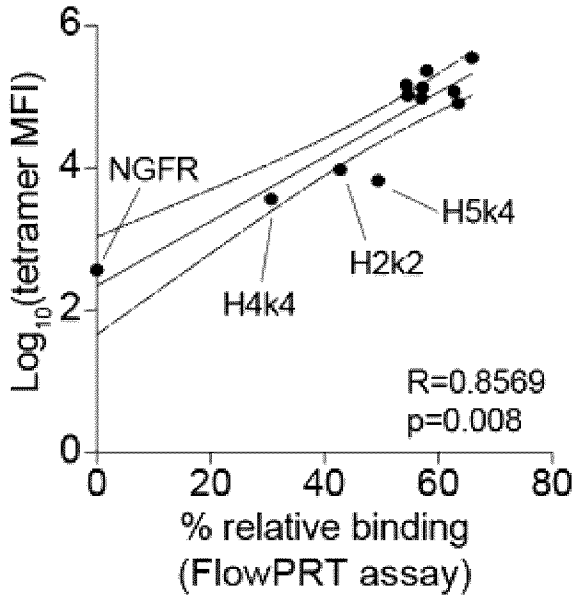

**
p=0.097
HLA-A*02:01 % relative binding
mA2-CAR
H1K2
H2K2
H4K2
H5K2
H6K2
H4K3
H5K3
H3K4
H4K4
H5K4

Fig. 6

C



D

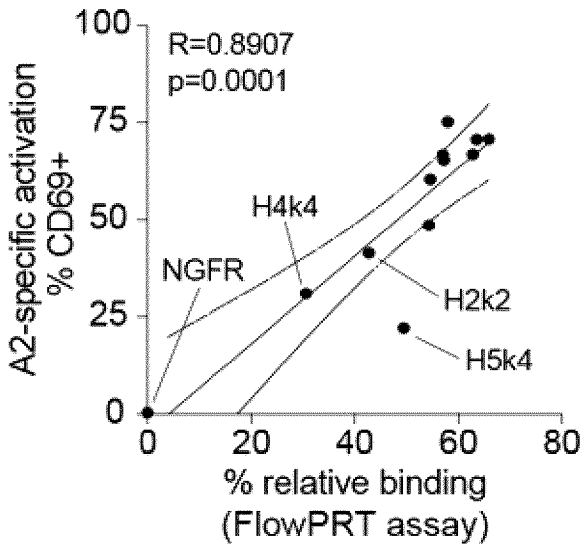


Fig. 6 cont'd

E

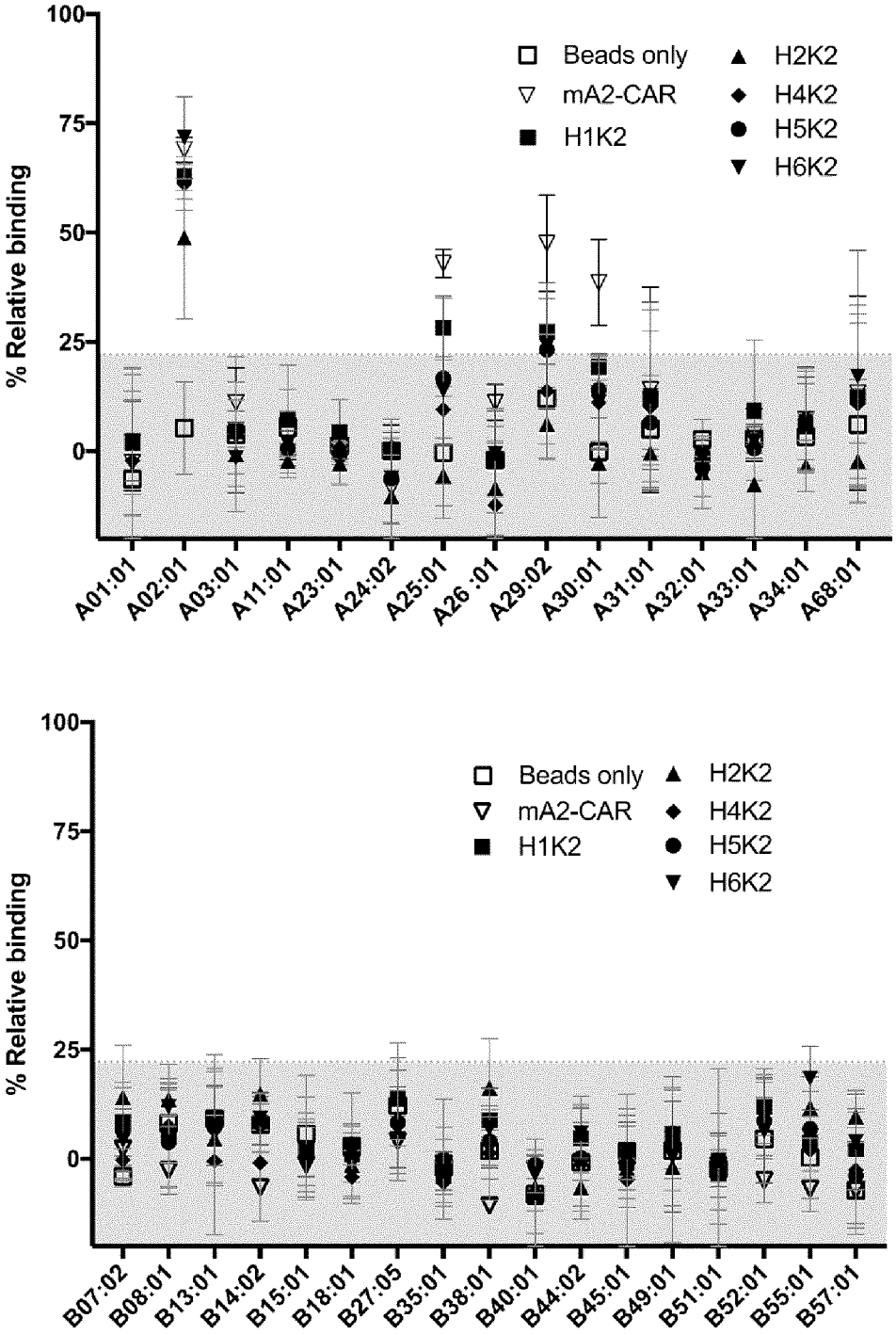

100
75
50
25
0
% Relative binding
Beads only
mA2-CAR
H1K2
H2K2
H4K2
H5K2
H6K2
A01:01
A02:01
A03:01
A11:01
A23:01
A24:02
A25:01
A26 :01
A29:02
A30:01
A31:01
A32:01
A33:01
A34:01
A68:01
100
75
50
25
0
% Relative binding
Beads only
mA2-CAR
H1K2
H2K2
H4K2
H5K2
H6K2
B07:02
B08:01
B13:01
B14:02
B15:01
B18:01
B27:05
B35:01
B38:01
B40:01
B44:02
B45:01
B49:01
B51:01
B52:01
B55:01
B57:01

Fig. 6 cont'd

F

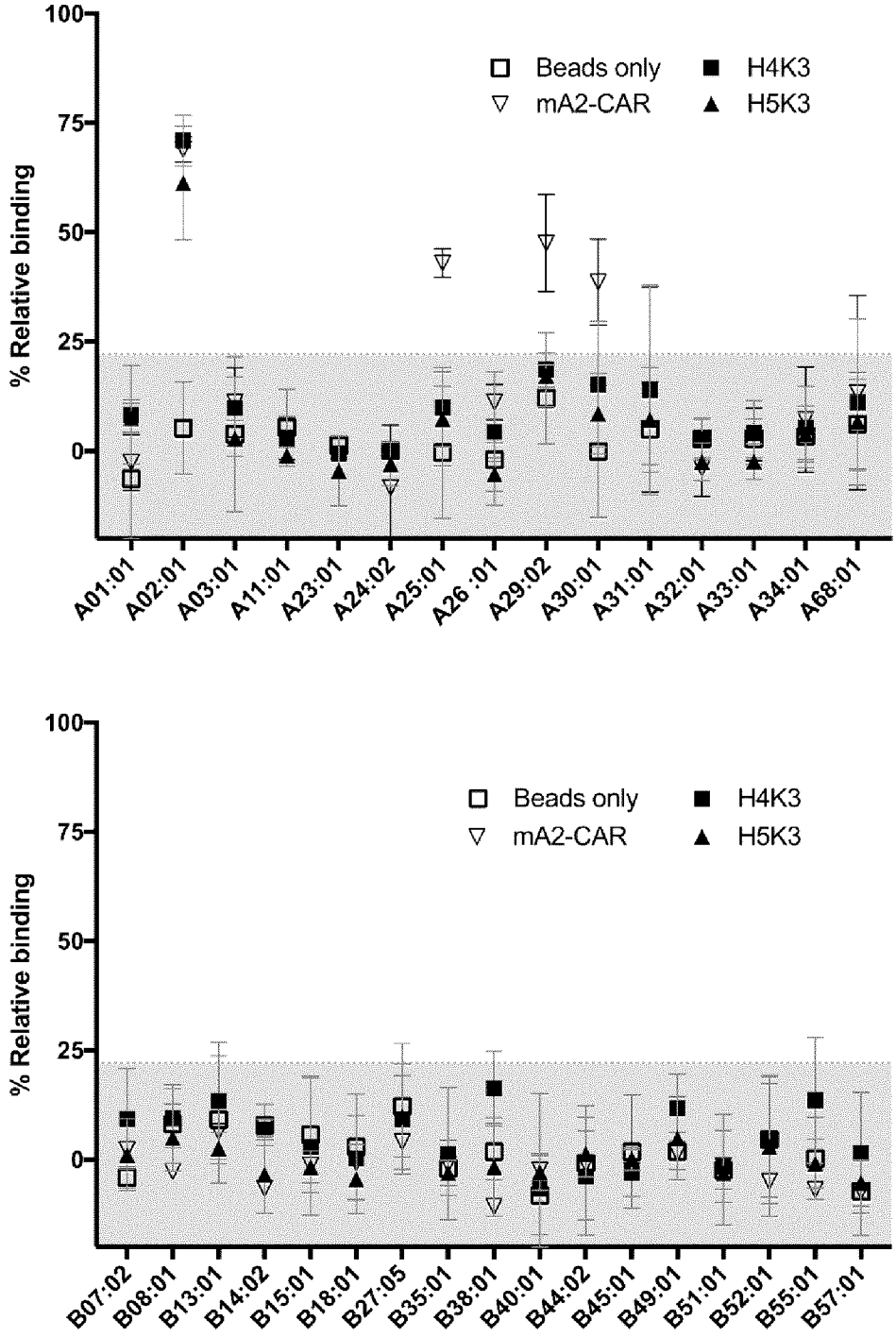

100
75
50
25
0
% Relative binding
Beads only
mA2-CAR
H4K3
H5K3
A01:01
A02:01
A03:01
A11:01
A23:01
A24:02
A25:01
A26 :01
A29:02
A30:01
A31:01
A32:01
A33:01
A34:01
A68:01
B07:02
B08:01
B13:01
B14:02
B15:01
B18:01
B27:05
B35:01
B38:01
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B57:01

Fig. 6 cont'd

G

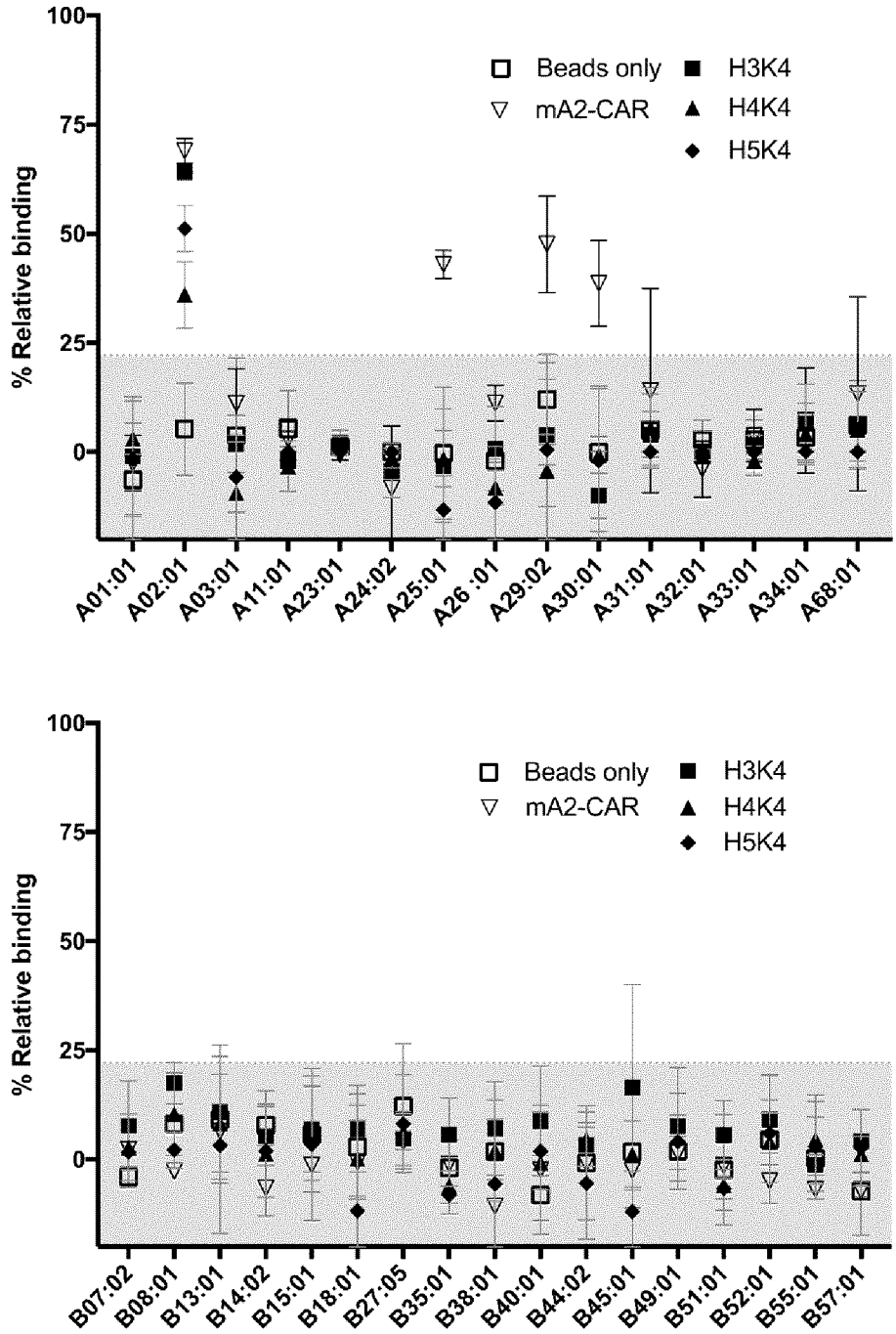
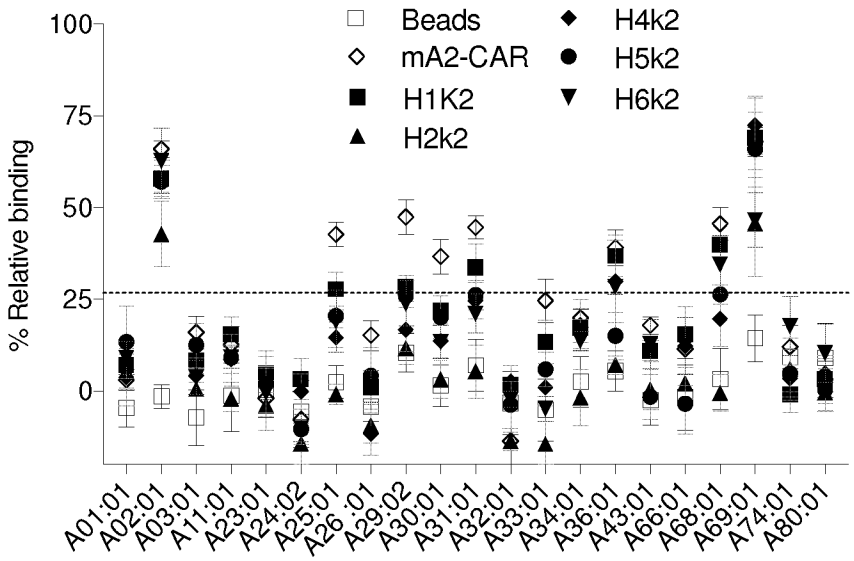

100
75
50
25
0
% Relative binding
Beads only
mA2-CAR
H3K4
H4K4
H5K4
A01:01
A02:01
A03:01
A11:01
A23:01
A24:02
A25:01
A26 :01
A29:02
A30:01
A31:01
A32:01
A33:01
A34:01
A68:01
B07:02
B08:01
B13:01
B14:02
B15:01
B18:01
B27:05
B35:01
B38:01
B40:01
B44:02
B45:01
B49:01
B51:01
B52:01
B55:01
B57:01

Fig. 6 cont'd

H


Beads
mA2-CAR
H1K2
H2k2
H4k2
H5k2
H6k2
% Relative binding
100
75
50
25
0
A01:01
A02:01
A03:01
A11:01
A23:01
A24:02
A25:01
A26 :01
A29:02
A30:01
A31:01
A32:01
A33:01
A34:01
A36:01
A43:01
A66:01
A68:01
A69:01
A74:01
A80:01

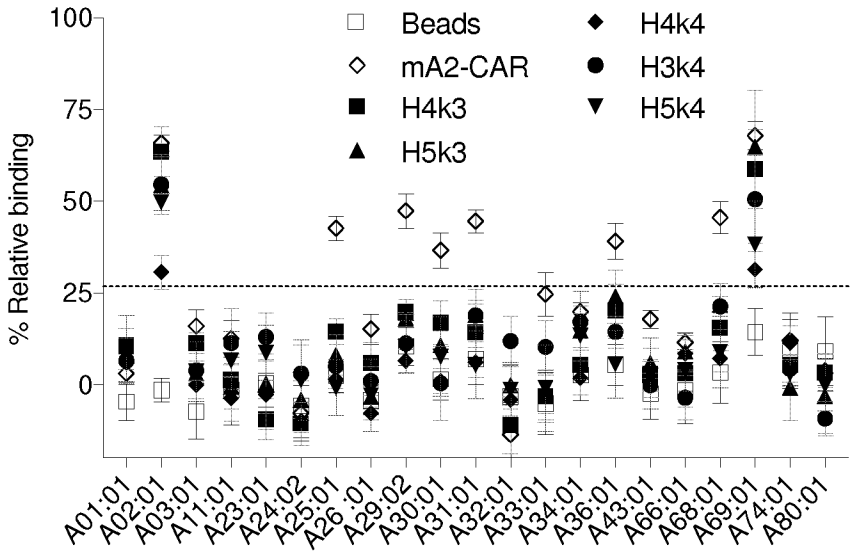

Beads
mA2-CAR
H4k3
H5k3
H4k4
H3k4
H5k4
% Relative binding
100
75
50
25
0
A01:01
A02:01
A03:01
A11:01
A23:01
A24:02
A25:01
A26 :01
A29:02
A30:01
A31:01
A32:01
A33:01
A34:01
A36:01
A43:01
A66:01
A68:01
A69:01
A74:01
A80:01

Fig. 6 cont'd

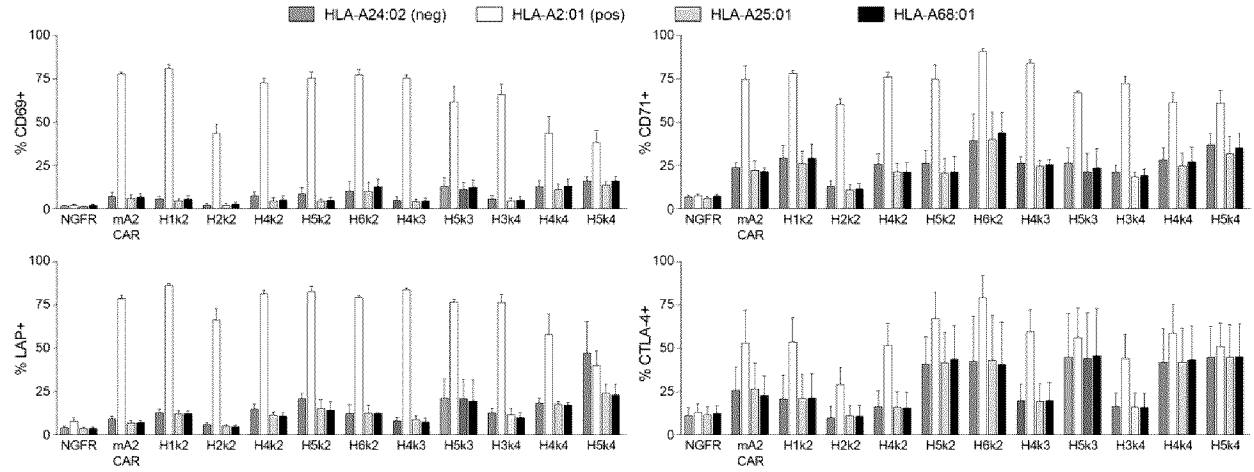

HLA-A24:02 (neg)
HLA-A2:01 (pos)
HLA-A25:01
HLA-A68:01
% CD69+
% CD71+
% LAP+
% CTLA-4+
100
75
50
25
0
NGFR
mA2 CAR
H1k2
H2k2
H4k2
H5k2
H6k2
H4k3
H5k3
H3k4
H4k4
H5k4

Fig. 6 cont'd

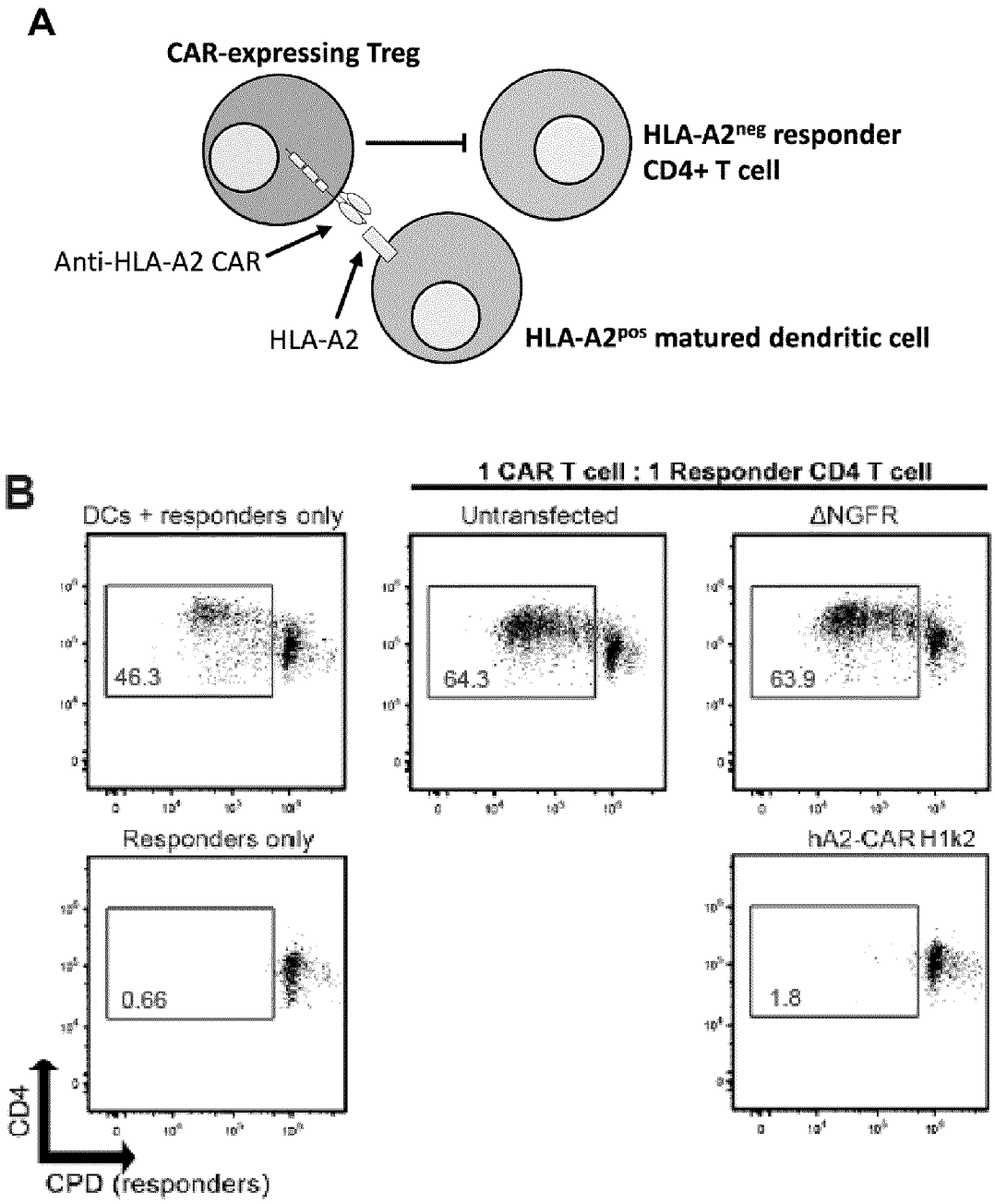
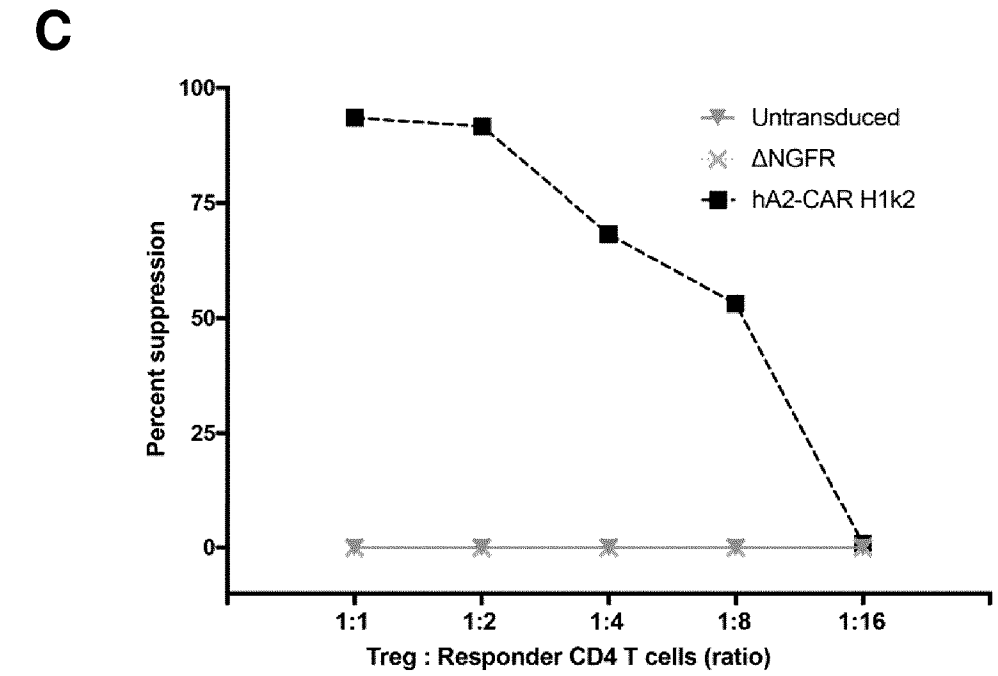

A
CAR-expressing Treg
HLA-A2neg responder
CD4+ T cell
Anti-HLA-A2 CAR
HLA-A2
HLA-A2pos matured dendritic cell
B
1 CAR T cell : 1 Responder CD4 T cell
DCs + responders only
Untransfected
ΔNGFR
46.3
64.3
63.9
Responders only
hA2-CAR H1k2
0.66
1.8
CD4
CPD (responders)

Fig. 7


C
100
75
50
25
0
Percent suppression
Untransduced
ΔNGFR
hA2-CAR H1k2
1:1
1:2
1:4
1:8
1:16
Treg : Responder CD4 T cells (ratio)

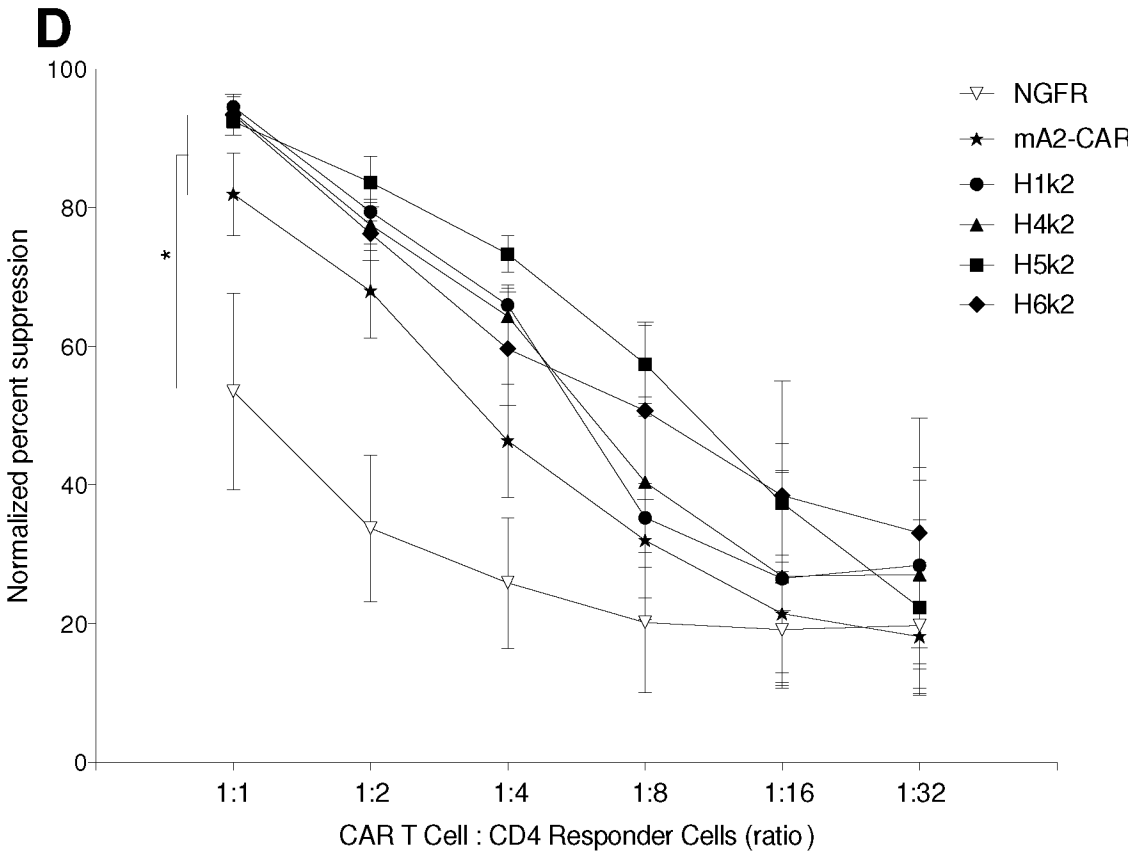

D
100
80
60
40
20
0
Normalized percent suppression
*
NGFR
mA2-CAR
H1k2
H4k2
H5k2
H6k2
1:1
1:2
1:4
1:8
1:16
1:32
CAR T Cell : CD4 Responder Cells (ratio)

Fig. 7 cont'd

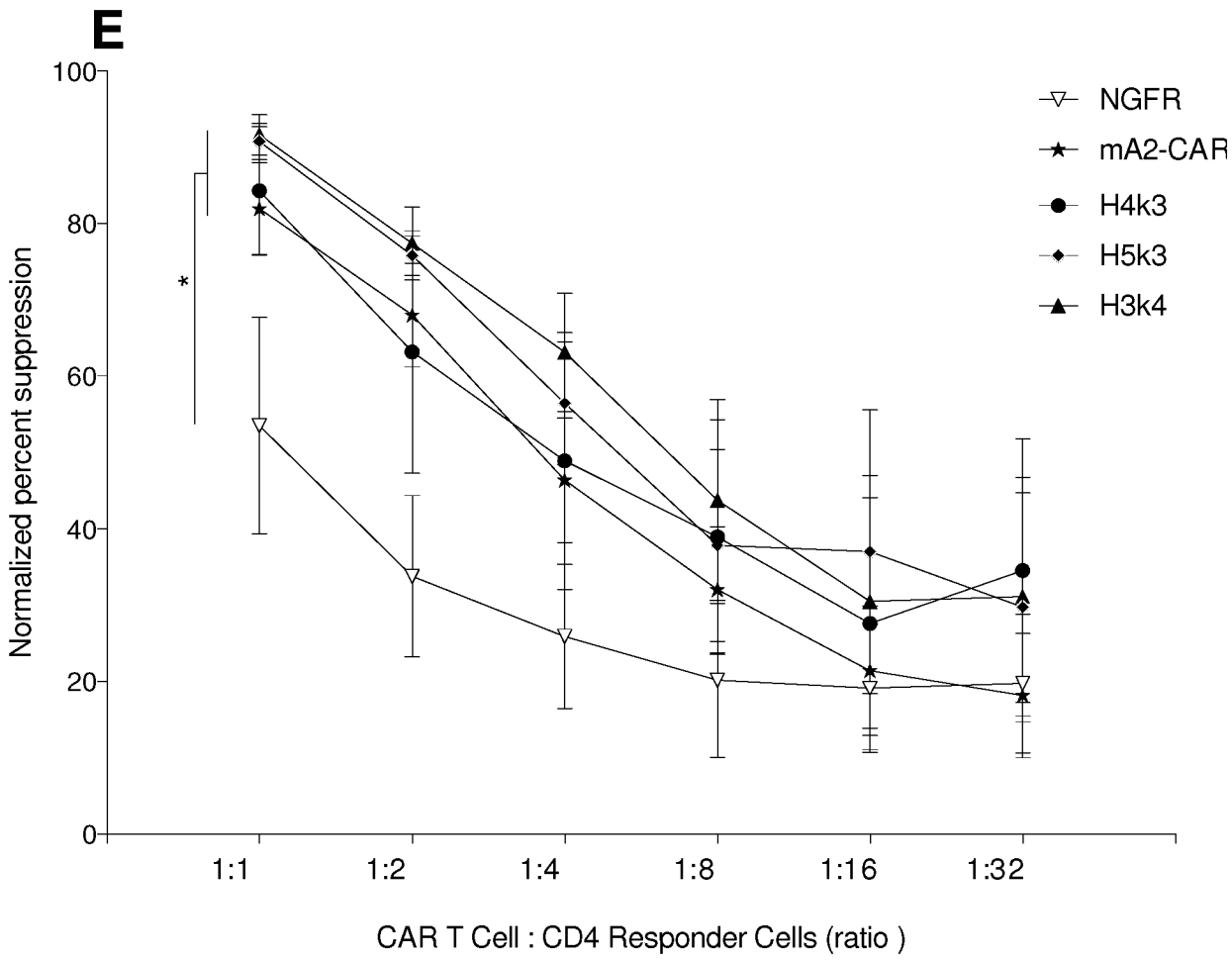

E
100
80
60
40
20
0
Normalized percent suppression
*
NGFR
mA2-CAR
H4k3
H5k3
H3k4
1:1
1:2
1:4
1:8
1:16
1:32
CAR T Cell : CD4 Responder Cells (ratio)

Fig. 7 cont'd

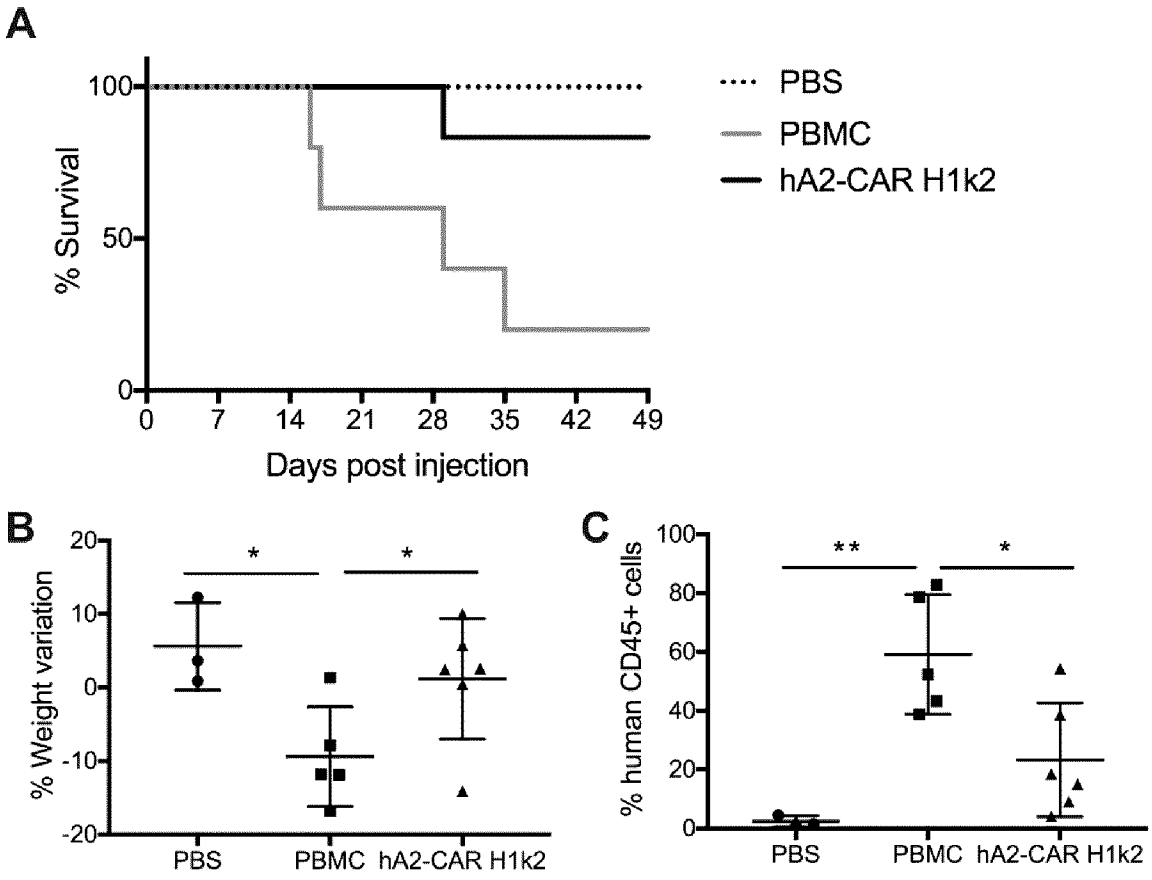
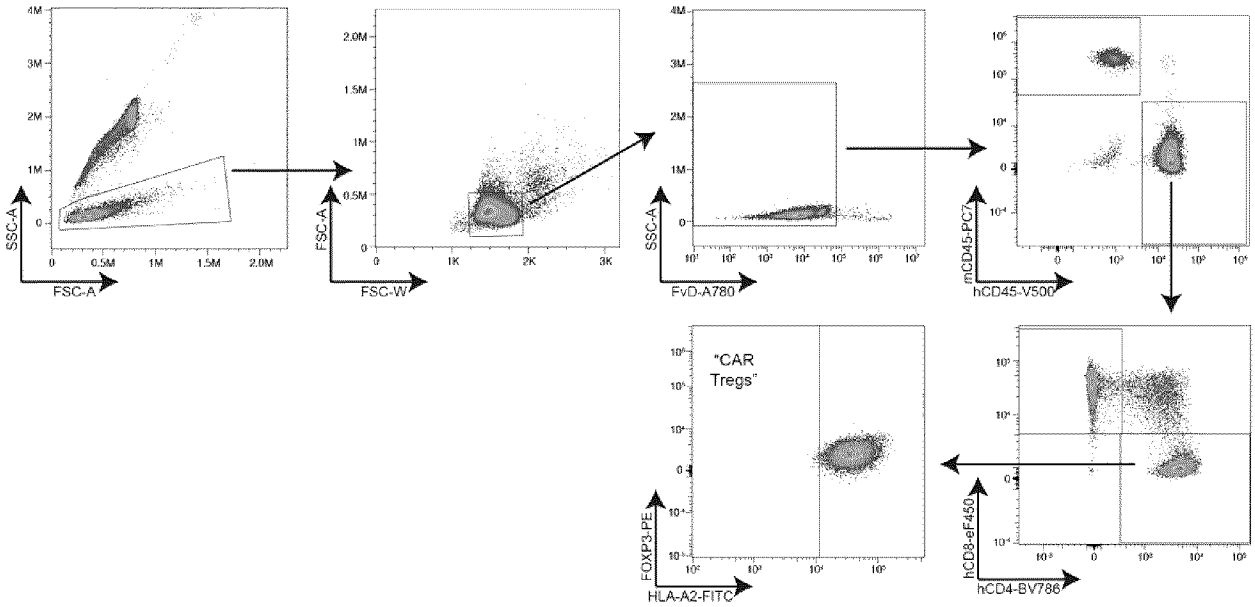

A
100
50
0
% Survival
0 7 14 21 28 35 42 49
Days post injection
PBS
PBMC
hA2-CAR H1k2
B
20
10
0
-10
-20
% Weight variation
*
*
PBS
PBMC
hA2-CAR H1k2
C
100
80
60
40
20
0
% human CD45+ cells
**
*
PBS
PBMC
hA2-CAR H1k2

Fig. 8

D



E

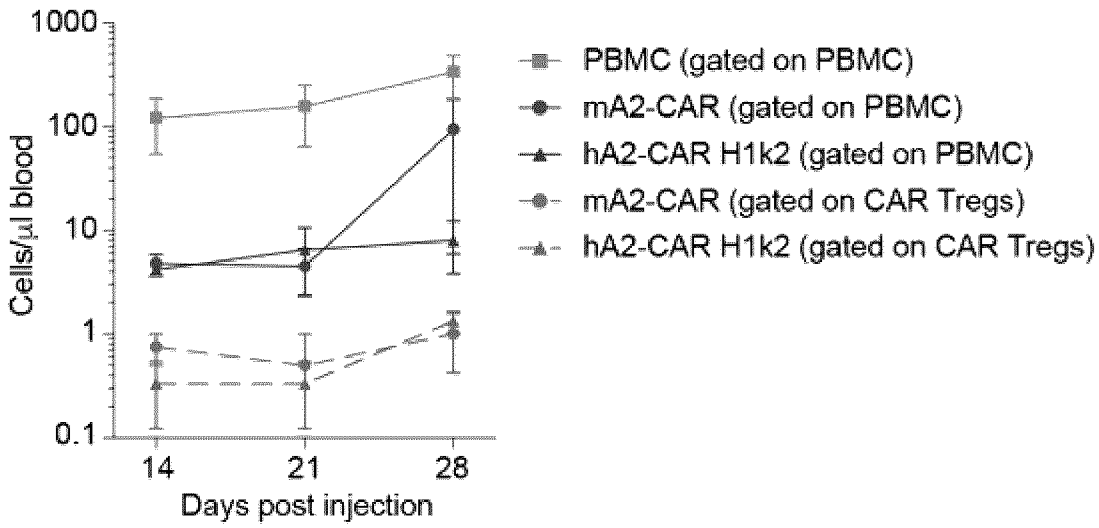


Fig. 8 cont'd

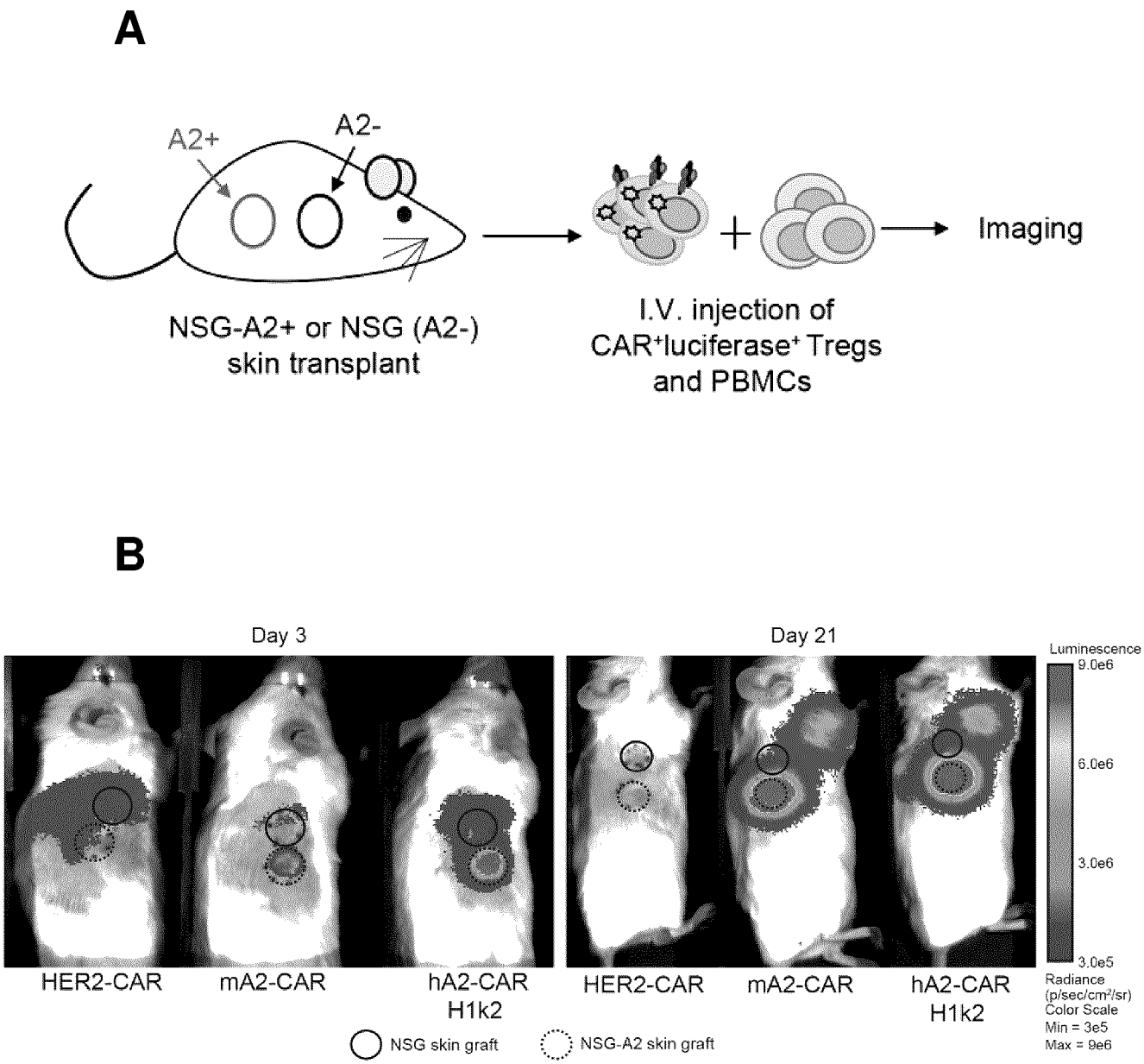
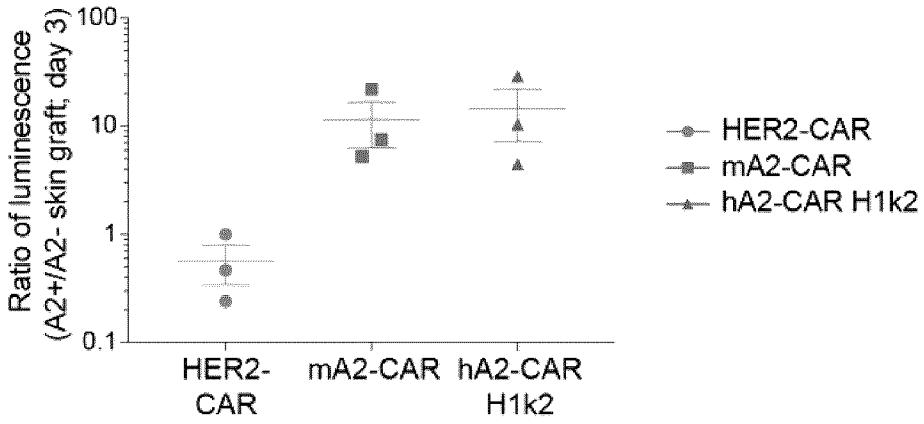

A
A2+
A2-
NSG-A2+ or NSG (A2-)
skin transplant
I.V. injection of
CAR+luciferase+ Tregs
and PBMCs
Imaging
B
Day 3
Day 21
Luminescence
9.0e6
6.0e6
3.0e6
3.0e5
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 3e5
Max = 9e6
HER2-CAR
mA2-CAR
hA2-CAR
H1k2
HER2-CAR
mA2-CAR
hA2-CAR
H1k2
NSG skin graft
NSG-A2 skin graft

Fig. 9

C



D

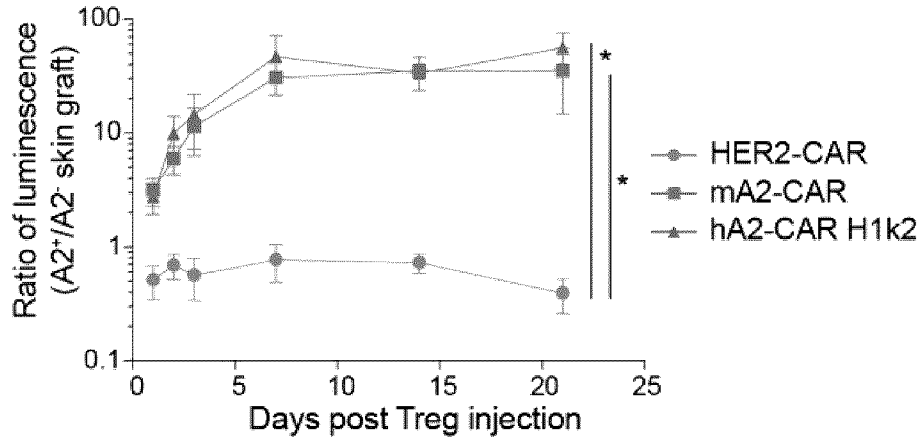
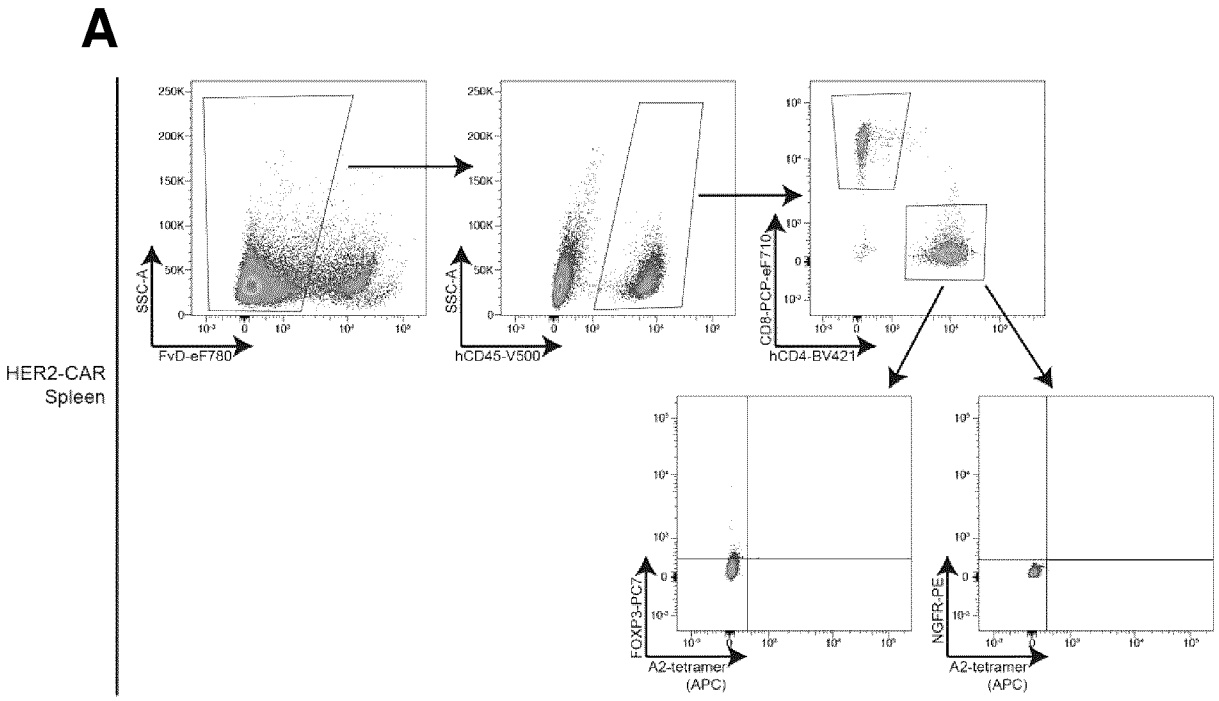


Fig. 9 cont'd


A
HER2-CAR
Spleen
SSC-A
FvD-eF780
SSC-A
hCD45-V500
CD8-PCP-eF710
hCD4-BV421
FOXP3-PC7
A2-tetramer
(APC)
NGFR-PE
A2-tetramer
(APC)

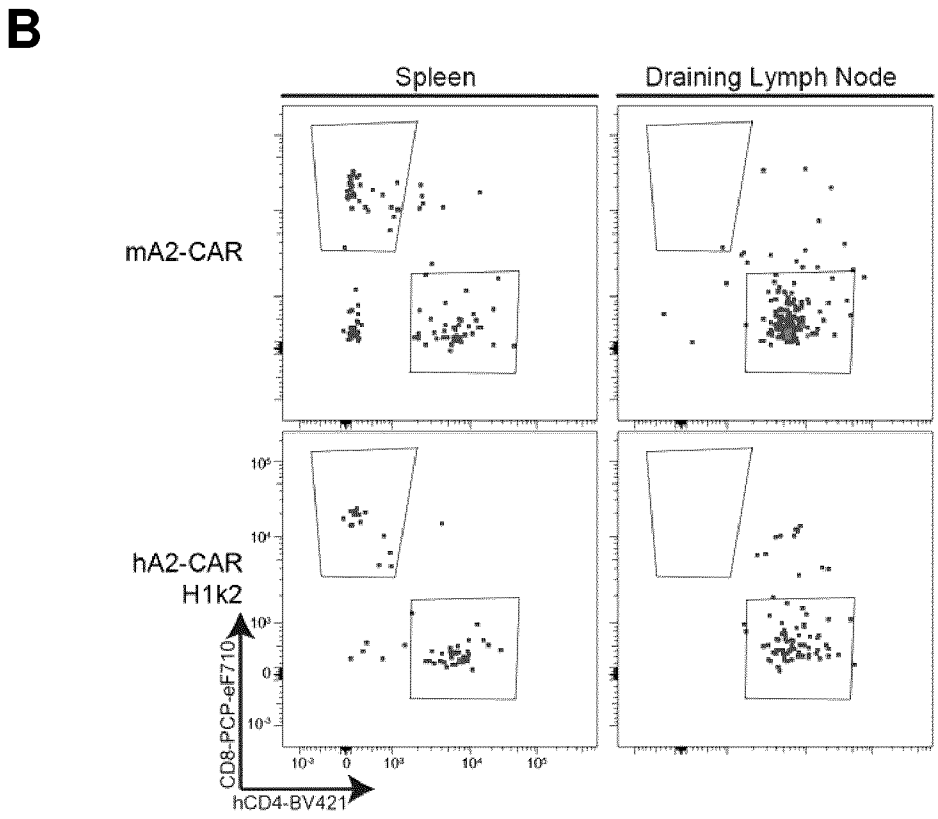

B
Spleen
Draining Lymph Node
mA2-CAR
hA2-CAR
H1k2
CD8-PCP-eF710
hCD4-BV421

Fig. 10

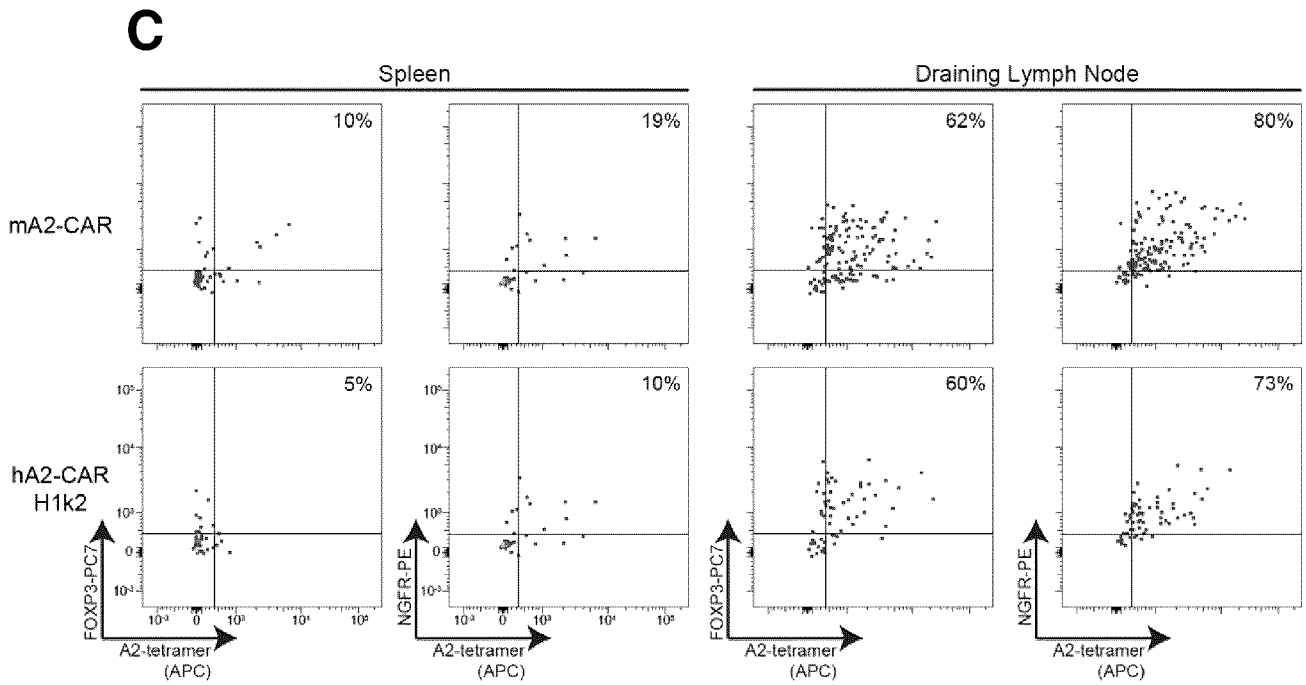

C
Spleen
Draining Lymph Node
mA2-CAR
hA2-CAR
H1k2
10%
19%
62%
80%
5%
10%
60%
73%
FOXP3-PC7
NGFR-PE
A2-tetramer
(APC)

Fig. 10 cont'd

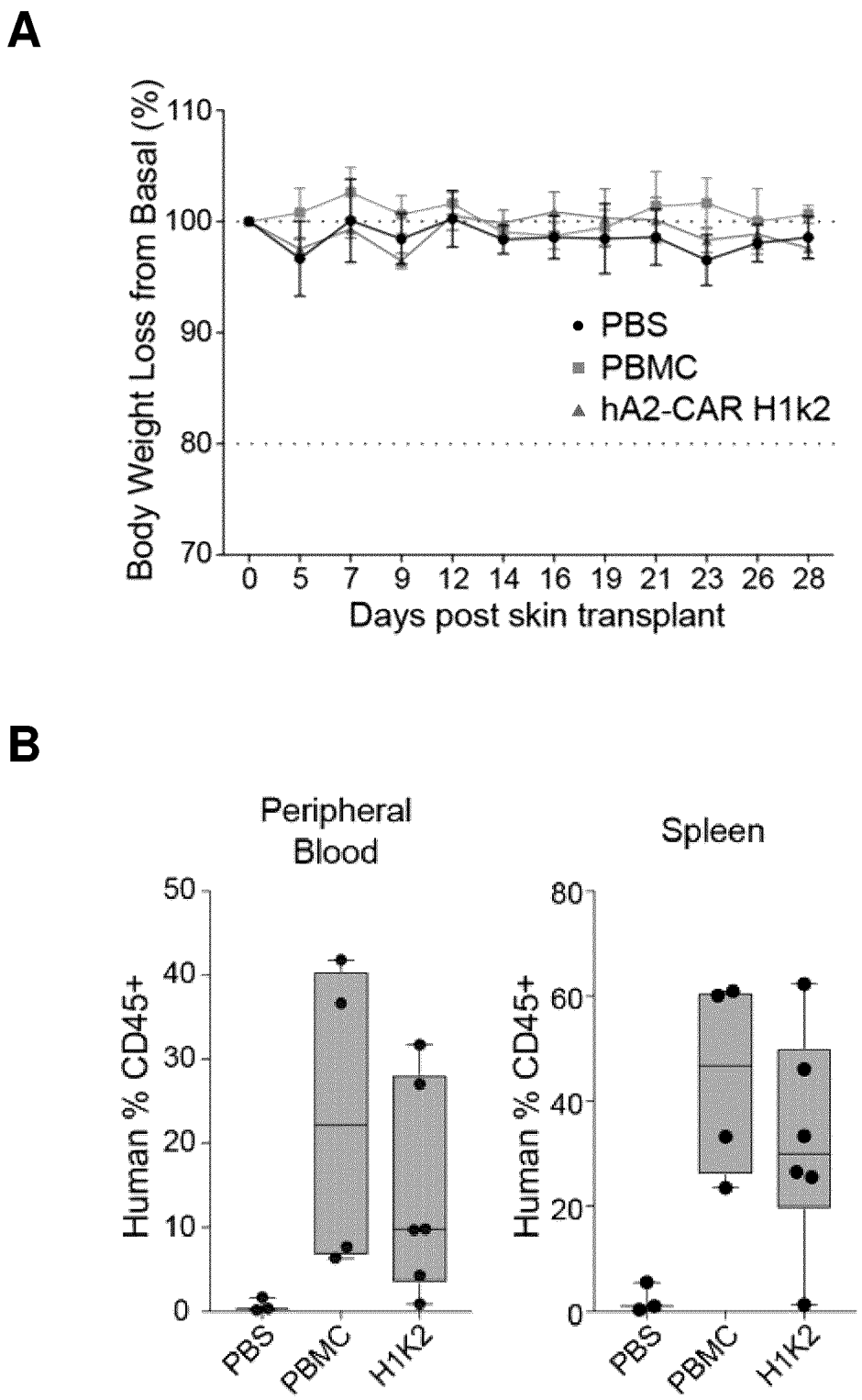
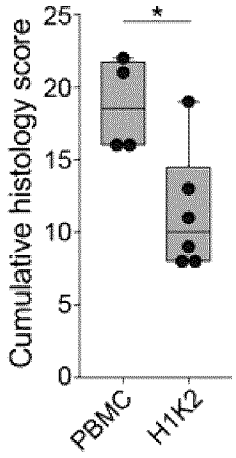

A
Body Weight Loss from Basal (%)
110
100
90
80
70
0 5 7 9 12 14 16 19 21 23 26 28
Days post skin transplant
PBS
PBMC
hA2-CAR H1k2
B
Peripheral Blood
Spleen
Human % CD45+
50
40
30
20
10
0
80
60
40
20
0
PBS
PBMC
H1K2

Fig. 11

C



D

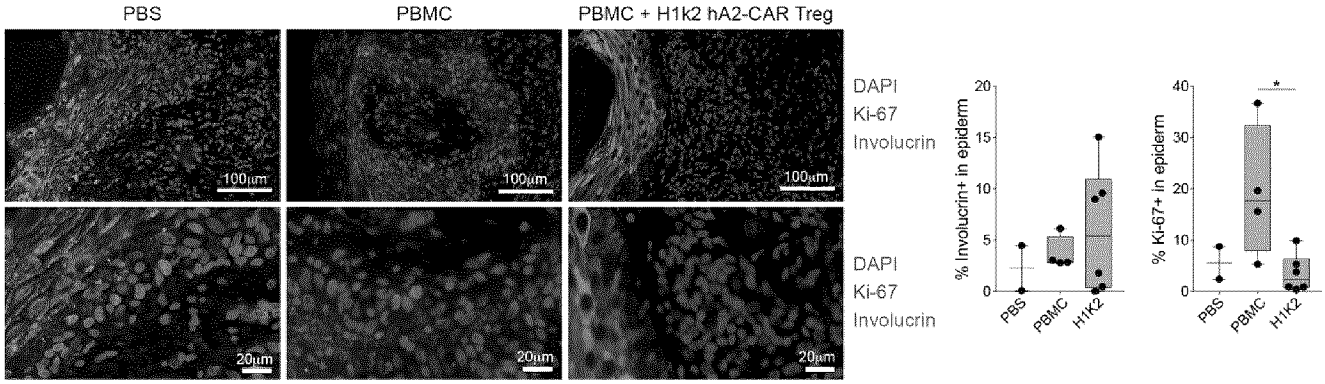


Fig. 11 cont'd

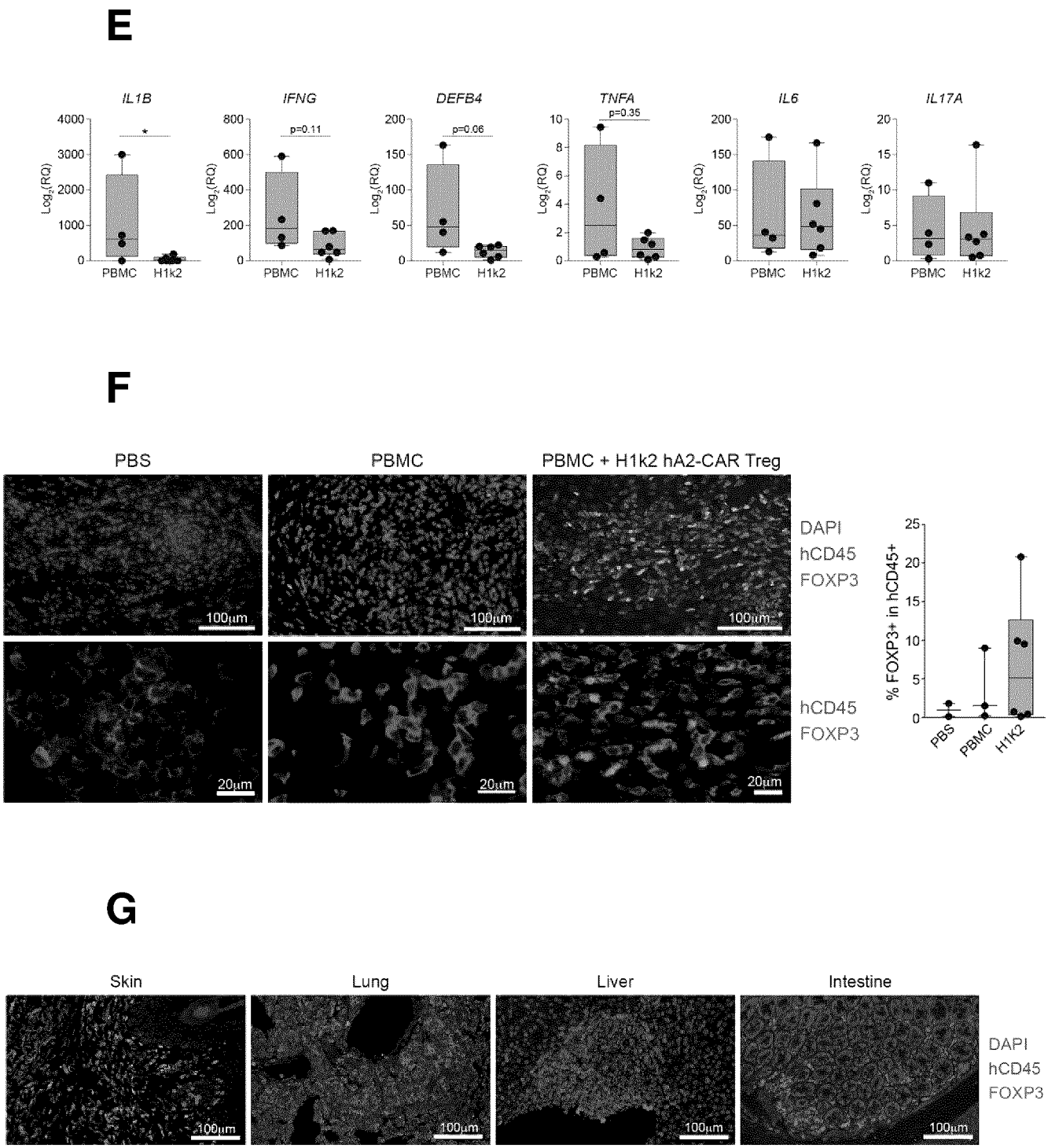
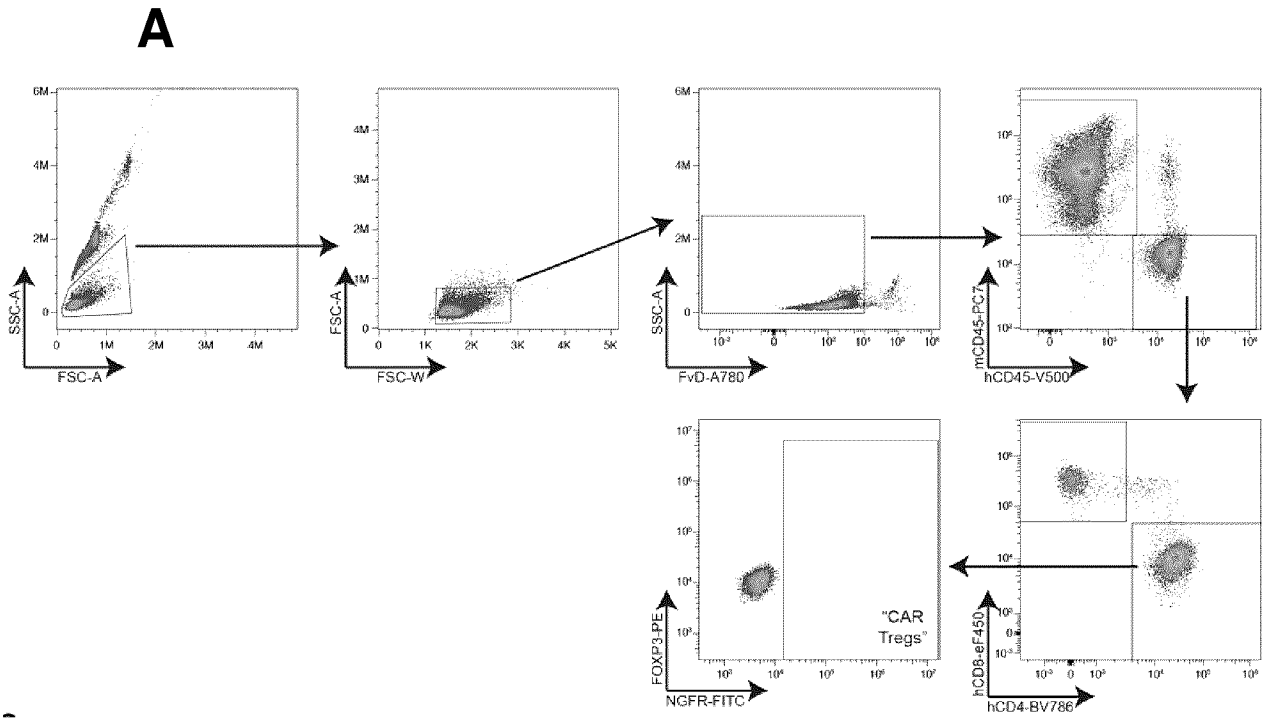

E
IL1B
IFNG
DEFB4
TNFA
IL6
IL17A
Log2(RQ)
PBMC
H1k2
p=0.11
p=0.06
p=0.35
F
PBS
PBMC
PBMC + H1k2 hA2-CAR Treg
DAPI
hCD45
FOXP3
100µm
20µm
% FOXP3+ in hCD45+
G
Skin
Lung
Liver
Intestine

Fig. 11 cont'd


A
SSC-A
FSC-A
FSC-A
FSC-W
SSC-A
FvD-A780
mCD45-PC7
hCD45-V500
FOXP3-PE
NGFR-FITC
"CAR Tregs"
hCD8-eF450
hCD4-BV786

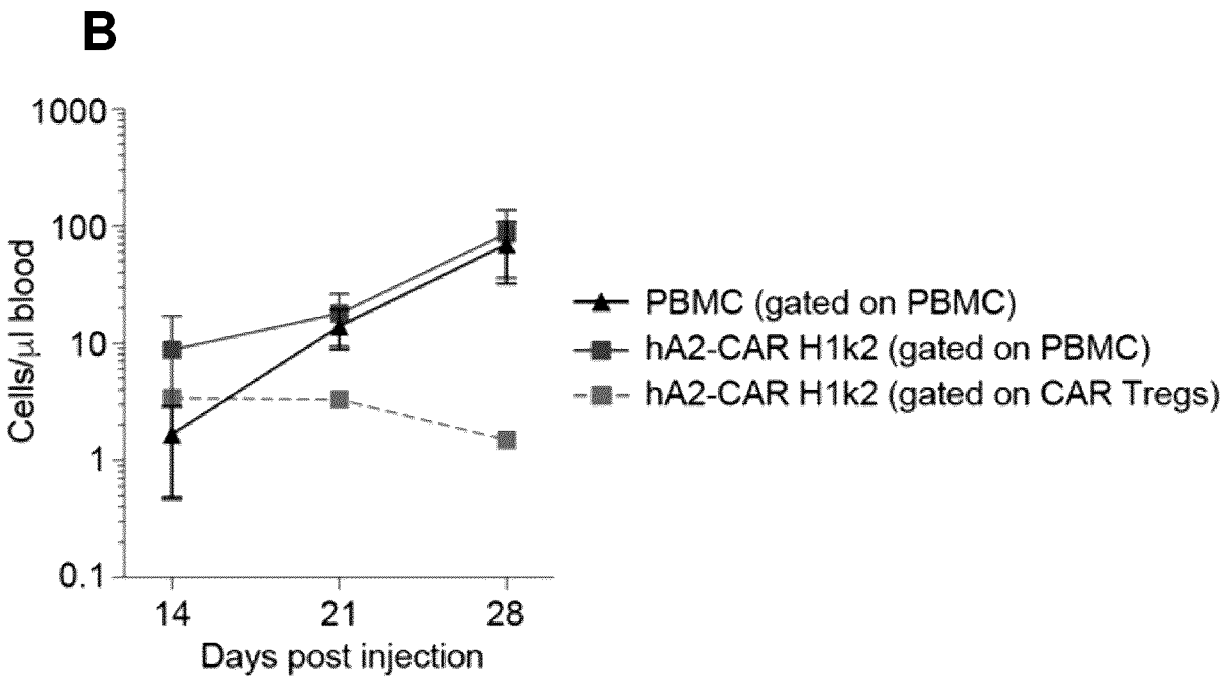

B
Cells/µl blood
1000
100
10
1
0.1
14
21
28
Days post injection
PBMC (gated on PBMC)
hA2-CAR H1k2 (gated on PBMC)
hA2-CAR H1k2 (gated on CAR Tregs)

Fig. 12

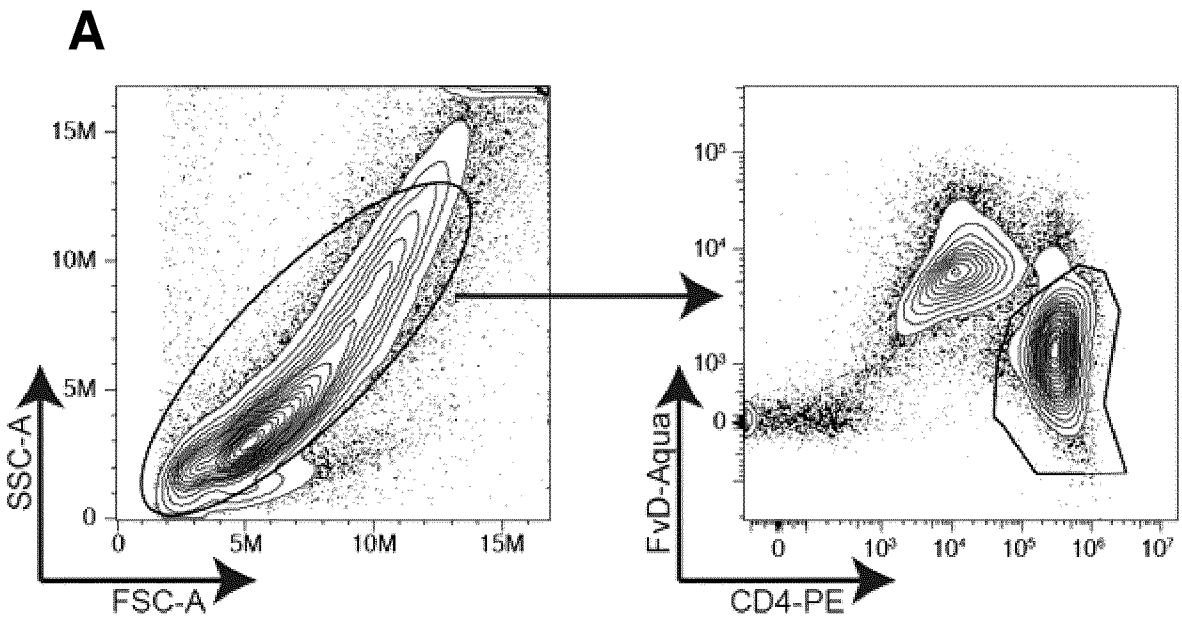

A
15M
10M
5M
0
SSC-A
0
5M
10M
15M
FSC-A
10⁵
10⁴
10³
0
FvD-Aqua
0
10³
10⁴
10⁵
10⁶
10⁷
CD4-PE

Fig. 13

B

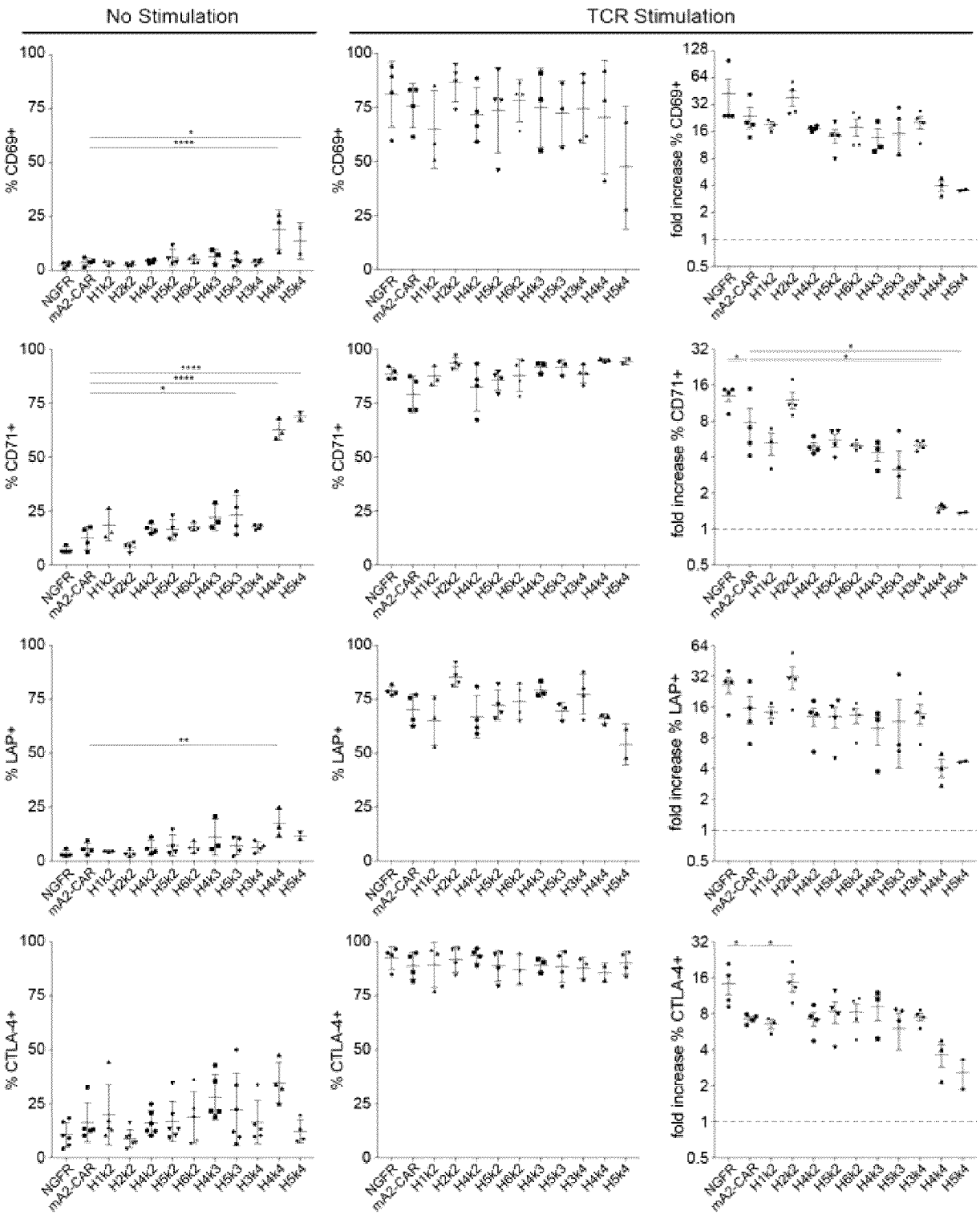


Fig. 13 cont'd

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2018/051167

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 16/46*** (2006.01), ***A61K 35/14*** (2015.01), ***A61K 35/17*** (2015.01), ***A61P 37/02*** (2006.01), ***A61P 37/06*** (2006.01), ***C07K 14/705*** (2006.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 16/46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel, CIPO Library Discovery Tool, GQ-Pat, Geneseq, Protein Data Bank, Genpept, Genbank: anti-HLA-A2 antibody, BB7.2, chimeric antigen receptor, SEQ ID NOs: 61-91, 183, 185, 187-190

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2011058321 (MOHAMMAD, I.) 19 May 2011 (19-05-2011)	1-4, 7-25 and 27-33
X	BOARDMAN, D.A. et al. Expression of a Chimeric Antigen Receptor Specific for Donor HLA Class I Enhances the Potency of Human Regulatory T Cells in Preventing Human Skin Transplant	1, 5, 6, 25, 27-38, and 40-62
Y	Rejection. Am J Transplant. 1 February 2017 (01-02-2017), Vol. 17, No. 4, pages 931-943.	39
Y	US9102760 (JUNE, C.H. et al.) 11 August 2015 (11-08-2015)	39
X	WATKINS, N.A. et al. The isolation and characterization of human monoclonal HLA-A2 antibodies from an immune V gene phage display library. Tissue Antigens. Mar 2000 (03-2000), Vol. 55(3), pages 219-228.	23

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 2018 (26-11-2018)	29 November 2018 (29-11-2018)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Rena Oulton (819) 639-6855

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☑ forming part of the international application as filed:

☑ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MACDONALD, K.G. et al. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. J Clin Invest. 21 March 2016 (21-03-2016), Vol. 126, No. 4, pages 1413-1424.	1-4, 7-25, 27-34 and 36-62

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2011058321A1	19 May 2011 (19-05-2011)	WO2011058321A1	19 May 2011 (19-05-2011)
		EP2498822A1	19 September 2012 (19-09-2012)
		GB0919751D0	30 December 2009 (30-12-2009)
		IL219735D0	31 July 2012 (31-07-2012)
		JP2013510836A	28 March 2013 (28-03-2013)
		JP5848254B2	27 January 2016 (27-01-2016)
		US2013078243A1	28 March 2013 (28-03-2013)
		US2018311373A1	01 November 2018 (01-11-2018)
US9102760B2	11 August 2015 (11-08-2015)	US2015093822A1	02 April 2015 (02-04-2015)
		US9102760B2	11 August 2015 (11-08-2015)
		AU2011338200A1	04 July 2013 (04-07-2013)
		AU2011338200B2	23 February 2017 (23-02-2017)
		AU2017203087A1	08 June 2017 (08-06-2017)
		AU2017225161A1	09 November 2017 (09-11-2017)
		BR112013014265A2	19 December 2017 (19-12-2017)
		BR112013014265A8	17 April 2018 (17-04-2018)
		CA2820681A1	14 June 2012 (14-06-2012)
		CA2820681C	23 October 2018 (23-10-2018)
		CL2013001645A1	14 February 2014 (14-02-2014)
		CN103492406A	01 January 2014 (01-01-2014)
		CN106220739A	14 December 2016 (14-12-2016)
		CN107699585A	16 February 2018 (16-02-2018)
		CN108103085A	01 June 2018 (01-06-2018)
		CO6801633A2	29 November 2013 (29-11-2013)
		CR20130269A	03 September 2013 (03-09-2013)
		CU20130079A7	27 September 2013 (27-09-2013)
		CY1119760T1	27 June 2018 (27-06-2018)
		DK2649086T3	18 September 2017 (18-09-2017)
		DOP2013000128A	16 March 2014 (16-03-2014)
		EA201390847A1	30 December 2013 (30-12-2013)
		EA027153B1	30 June 2017 (30-06-2017)
		EA201790175A1	29 September 2017 (29-09-2017)
		ECSP13012739A	31 October 2013 (31-10-2013)
		EP2649086A1	16 October 2013 (16-10-2013)
		EP2649086A4	31 December 2014 (31-12-2014)
		EP2649086B1	19 July 2017 (19-07-2017)
		EP3214091A1	06 September 2017 (06-09-2017)
		EP3214091B1	03 October 2018 (03-10-2018)
		EP3305798A1	11 April 2018 (11-04-2018)
		ES2641870T3	14 November 2017 (14-11-2017)
		GT201300150A	10 June 2014 (10-06-2014)
		HRP20171577T1	01 December 2017 (01-12-2017)
		HUE036872T2	28 August 2018 (28-08-2018)
		JP2014507118A	27 March 2014 (27-03-2014)
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