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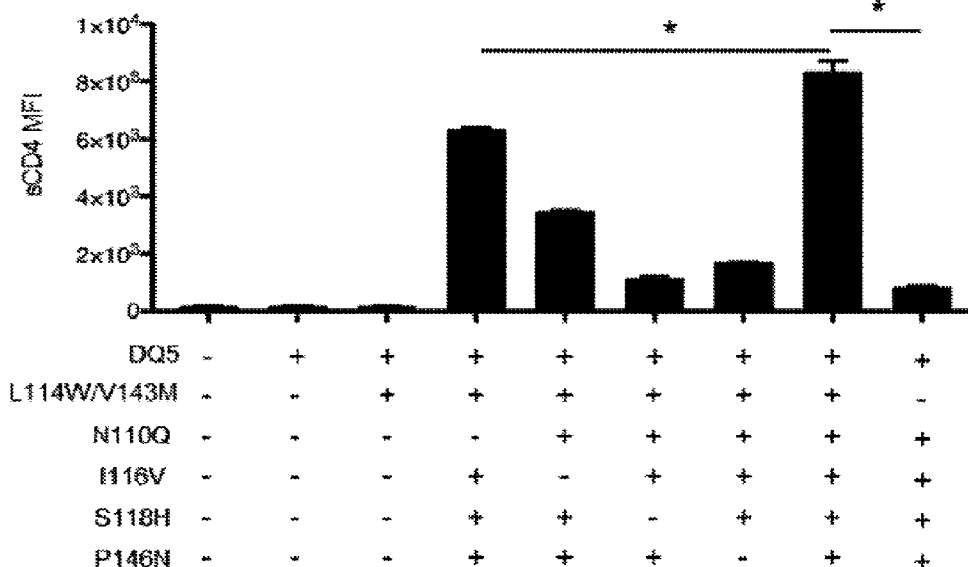
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(71) Demandeur/Applicant:
UNIVERSITY HEALTH NETWORK, CA
(72) Inventeurs/Inventors:
HIRANO, NAOTO, CA;
SUGATA, KENJI, CA
(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : MOLECULES DE CLASSE II DU CMH ET LEURS PROCEDES D'UTILISATION
(54) Title: MHC CLASS II MOLECULES AND METHODS OF USE THEREOF

FIG. 1D



(57) **Abrégé/Abstract:**

The present disclosure is directed to HLA class II molecules having a higher affinity for CD4 than naturally occurring HLA class II molecules. In certain aspects, the HLA class II molecule comprises a DQ beta chain having (i) an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, (ii) an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1, (iii) or both (i) and (ii). Certain aspects of the present disclosure are directed to nucleic acid molecules encoding the HLA class II molecules, vectors comprising the nucleic acid molecule, cells comprising the same, and methods of use thereof.

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Abstract:

The present disclosure is directed to HLA class II molecules having a higher affinity for CD4 than naturally occurring HLA class II molecules. In certain aspects, the HLA class II molecule comprises a DQ beta chain having (i) an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, (ii) an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1, (iii) or both (i) and (ii). Certain aspects of the present disclosure are directed to nucleic acid molecules encoding the HLA class II molecules, vectors comprising the nucleic acid molecule, cells comprising the same, and methods of use thereof.

MHC CLASS II MOLECULES AND METHODS OF USE THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This PCT application claims the priority benefit of U.S. Provisional Application Nos. 62/880,501, filed July 30, 2019, and 63/029,114, filed May 22, 2020, each of which is incorporated herein by reference in its entirety.

**REFERENCE TO SEQUENCE LISTING SUBMITTED
ELECTRONICALLY VIA EFS-WEB**

[0002] The content of the electronically submitted sequence listing (Name: 4285-011PC02_SL_ST25.txt, Size: 34,403 bytes; and Date of Creation: July 28, 2020) is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure provides major histocompatibility complex (MHC) class II molecules with increased affinity for CD4 and uses thereof.

BACKGROUND OF THE DISCLOSURE

[0004] Immunotherapy has emerged as a critical tool in the battle against a variety of diseases, including cancer. T cell therapies are at the forefront of immunotherapeutic development, and adoptive transfer of antitumor T cells has been shown to induce clinical responses in cancer patients.

[0005] Directed T cell therapy using T cells expression T cell receptors (TCRs) specific for a target epitope expressed by tumor cells is a promising form of T cell therapy. Antigen presenting cells display peptide fragments associated with the major histocompatibility complex (MHC) on their surface to induce an immune response. It has been demonstrated that the improved presentation of endogenous peptides via class II is correlated with improved survival of cancer patients. However, the development of novel TCRs capable of specifically targeting MHC class II presented peptides is hindered by the low affinity of MHC class II proteins for CD4 expressed by T cells.

[0006] The present disclosure provides MHC class II proteins with increased affinity for CD4 and methods of using the same for the identification and development of novel MHC class II-specific TCRs.

SUMMARY OF THE DISCLOSURE

[0007] Certain aspects of the present disclosure are directed to an HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1.

[0008] Certain aspects of the present disclosure are directed to an HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises a substitution mutation at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, wherein the substitution mutation is with an amino acid other than leucine.

[0009] In some aspects, the DQ beta chain further comprises an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.

[0010] Certain aspects of the present disclosure are directed to an HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.

[0011] Certain aspects of the present disclosure are directed to an HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises a substitution mutation at a position corresponding to amino acid residue 143 of SEQ ID NO: 1, wherein the substitution mutation is with an amino acid other than valine.

[0012] In some aspects, the DQ beta chain further comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the DQ beta chain further comprises an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1. In some aspects, the DQ beta chain further comprises an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1. In some aspects, the DQ beta chain further comprises an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1. In some aspects, the DQ beta chain further comprises an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0013] In some aspects, the DQ beta chain further comprises at least three of: (i) an amino acid other than asparagine at a position corresponding to amino acid residue 110 of

SEQ ID NO: 1, (ii) an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (iv) an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0014] In some aspects, the DQ beta chain further comprises: (i) an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1, (ii) an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (iv) an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0015] In some aspects, the amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 comprises a hydrophobic side chain. In some aspects, the amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 is selected from the group consisting of an alanine, a valine, an isoleucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In some aspects, the amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a tryptophan.

[0016] In some aspects, the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 comprises a hydrophobic side chain. In some aspects, the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is selected from an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In some aspects, the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a methionine.

[0017] In some aspects, the beta chain of the MHC class II molecule comprises an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1. In some aspects, the amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1 is selected from a serine, a threonine, and a glutamine. In some aspects, the amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1 is a glutamine.

[0018] In some aspects, the beta chain of the MHC class II molecule comprises an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1. In some aspects, the amino acid other than isoleucine at a position corresponding to

amino acid residue 116 of SEQ ID NO: 1 is selected from an alanine, a valine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In some aspects, the amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1 is valine.

[0019] In some aspects, the beta chain of the MHC class II molecule comprises an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1. In some aspects, the amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1 is selected from an arginine, a histidine, and a lysine. In some aspects, the amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1 is a histidine.

[0020] In some aspects, the beta chain of the MHC class II molecule comprises an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1. In some aspects, the amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1 is selected from a serine, a threonine, an asparagine, and a glutamine. In some aspects, the amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1 is a glutamine.

[0021] In some aspects, the DQ beta chain comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to an amino acid sequence selected from SEQ ID NOs: 1, 3, 4, and 5.

[0022] In some aspects, the DQ beta chain comprises a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 and a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.

[0023] In some aspects, the beta chain of the MHC class II molecule comprises (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1; (b) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; and (c) at least one of: (i) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1; (ii) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (iv) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0024] In some aspects, the beta chain of the MHC class II molecule comprises (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1; (b) a

methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; and (c) at least two of: (i) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1; (ii) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (iv) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0025] In some aspects, the beta chain of the MHC class II molecule comprises (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1; (b) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; and (c) at least three of: (i) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1; (ii) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (iv) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0026] In some aspects, the beta chain of the MHC class II molecule comprises (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1; (b) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; (c) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1; (d) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (e) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (f) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0027] In some aspects, the DQ beta chain comprises the amino acid sequence set forth in SEQ ID NO: 3. In some aspects, the DQ beta chain comprises the amino acid sequence set forth in SEQ ID NO: 4.

[0028] In some aspects, the beta chain of the HLA class II molecule comprises a DQ2, DQ3, DQ4, DQ5, or DQ6 allele. In some aspects, the beta chain of the MHC class II molecule comprises an HLA-DQB1*02, HLA-DQB1*03, HLA-DQB1*04, HLA-DQB1*05, or HLA-DQB1*06 allele.

[0029] In some aspects, the HLA class II molecule further comprises a DQ alpha chain. In some aspects, the alpha chain of the MHC class II molecule comprises an HLA-DQA1*01, HLA-DQA1*02, HLA-DQA1*03, HLA-DQA1*04, HLA-DQA1*05, or HLA-DQA1*06 allele.

[0030] In some aspects, the DQ alpha chain comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about

96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 6 or 8. In some aspects, the DQ alpha chain comprises the amino acid sequence set forth in SEQ ID NO: 6 or 8.

[0031] In some aspects, the DQ beta chain has an increased affinity for a CD4 protein as compared to a reference HLA class II molecule, wherein the reference HLA class II molecule comprises a DQ beta chain comprising (i) a leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 and/or (ii) a valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.

[0032] In some aspects, the increased affinity is at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, or at least about 1000.

[0033] In some aspects, the DQ beta chain is bound to a membrane of a cell. In some aspects, the DQ beta chain is not bound to a membrane of a cell. In some aspects, the DQ beta chain comprises an extracellular domain of a full length DQ alpha chain. In some aspects, the DQ beta chain does not comprise a transmembrane domain of a full length DQ beta chain.

[0034] In some aspects, the DQ alpha chain is bound to a membrane of a cell. In some aspects, the DQ alpha chain is not bound to a membrane of a cell. In some aspects, the DQ alpha chain comprises an extracellular domain of a full length DQ alpha chain. In some aspects, the DQ alpha chain does not comprise a transmembrane domain of a full length DQ alpha chain.

[0035] In some aspects, the DQ beta chain is linked to or associated with an inert particle. In some aspects, the inert particle is a bead. In some aspects, the inert particle is a nanoparticle. In some aspects, the nanoparticle is selected from a pegylated iron oxide, chitosan, dextrane, gelatin, alginate, liposome, starch, branched polymer, carbon-based carrier, polylactic acid, poly(cyano)acrylate, polyethyleinamine, block copolymer, poly caprolactone, SPIONS, USPIONS, Cd/Zn-selenide, or silica nanoparticle. In some aspects, the nanoparticle is a pegylated iron oxide nanoparticle.

- [0036]** In some aspects, the DQ beta chain comprises a signal peptide. In some aspects, the DQ alpha chain comprises a signal peptide. In some aspects, the signal peptide comprises the amino acid sequence set forth in SEQ ID NO: 9.
- [0037]** Certain aspects of the present disclosure are directed to a nucleic acid molecule encoding a DQ beta chain disclosed herein. In some aspects, the nucleic acid molecule further encodes a DQ alpha chain disclosed herein.
- [0038]** In some aspects, the nucleic acid molecule comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 2.
- [0039]** Certain aspects of the present disclosure are directed to a vector comprising a nucleic acid molecule disclosed herein.
- [0040]** Certain aspects of the present disclosure are directed to a cell comprising an HLA class II molecule disclosed herein, a nucleic acid molecule disclosed herein, or a vector disclosed herein. In some aspects, the cell is a mammalian cell or an insect cell. In some aspects, the cell is selected from a K562 cell, T2, HEK293, HEK293T, A375, SK-MEL-28, Me275, COS, a fibroblast cell, a tumor cell, or any combination thereof. In some aspects, the cell lacks endogenous MHC class II DQ beta chain expression. In some aspects, the cell lacks endogenous MHC class II DQ alpha chain expression.
- [0041]** Certain aspects of the present disclosure are directed to a method of identifying a T cell receptor capable of binding an epitope in an MHC class II complex, comprising pulsing a cell disclosed herein with one or more peptide comprising the epitope, and stimulating one or more CD4⁺ T cell with the APC.
- [0042]** Certain aspects of the present disclosure are directed to a method of treating a disease or condition in a subject in need thereof, comprising administering to the subject a MHC class II molecule disclosed herein. In some aspects, the disease or condition is cancer or an infection.
- [0043]** In some aspects, the cancer is selected from the group consisting of melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, stomach cancer, uterine cancer, lung cancer, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), cancer of the esophagus, cancer of the small intestine, cancer of the urethra, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid

leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, glioma, squamous cell cancer, and combinations of said cancers.

[0044] In some aspects, the cancer is relapsed or refractory. In some aspects, the cancer is locally advanced. In some aspects, the cancer is advanced. In some aspects, the cancer is metastatic.

[0045] In some aspects, the HLA class II molecule binds CD4 with a K_D of less than about 100 μM . In some aspects, the HLA class II molecule binds CD4 with a K_D of less than about 10 μM . In some aspects, the HLA class II molecule binds CD4 with a K_D of about 8.9 μM or less.

[0046] Certain aspects of the disclosure are directed to a complex comprising an HLA class II molecule disclosed herein and a peptide, wherein the peptide comprises DDX3Y₁₇₁₋₁₉₀, HA₂₅₅₋₂₇₀, GPC3₁₃₈₋₁₅₇, or any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] FIGs. 1A-1F provide data illustrating the enhanced CD4 binding ability of modified DQ molecules. FIG. 1A is a table comparing the amino acid sequences of DPB1*04:01, DQB1*05:01, and DQB1*05:01^{L114W/V143M+4reps}, with mutated amino acids underlined. FIGs. 1B and 1C are graphical representations of data of class II-deficient K562 cells stably expressing wild-type DQ5 (DQA1*01:01/DQB1*05:01), DQ5^{L114W/V143M}, DQ5^{L114W/V143M+4reps}, wild-type DP4, or DP4^{L112W/V141M} stained with sCD4, as shown in FIG. 1A. FIG. 1D shows the CD4 binding ability of a series of K562 derivatives individually expressing DQ5^{L114W/V143M+4reps} mutants with a single amino acid reversal at one of the four positions, similarly stained with sCD4. FIG. 1E is a table listing the amino acid sequences of DPB1*04:01, DQB1*02:01, DQB1*04:02 and DQB1*06:01 with replaced amino acids underlined. Note that unlike DQB1*05:01, DQB1*02:01, DQB1*04:02 and DQB1*06:01 encode Val at position 116, similar to DPB1*04:01, which codes for Val at position 114. FIG. 1F provides graphical representations of data showing that the L114W/V143M+3reps replacements in the β chains enhanced the binding of DQ2, DQ4, and DQ6 to CD4. At least 2 independent experiments were performed. *, $P < 0.05$ by Student's t-test. Bars and error bars represent the mean $\pm$ SD of results in triplicate experiments.

- [0048]** FIGs. 2A-2B are graphical representations illustrating that affinity-matured DQ dimers detected cognate TCRs expressed in human primary CD4⁺ T cells. DQ5 (DQA1*01:01-DQB1*05:01)-restricted DDX3Y-specific TCR (E6) (FIG. 2A) and DQ6 (DQA1*01:02-DQB1*06:02)-restricted influenza virus HA-specific TCR (DM2) (FIG. 2B) were reconstituted in human primary CD4⁺ T cells and stained by DQ5^{L114W/V143M+4reps} and DQ6^{L114W/V143M+3reps} dimers, respectively. At least 2 independent experiments were performed.
- [0049]** FIGs. 3A-Q are graphical representations of histograms illustrating the comparable expression levels of HLA class II genes. HLA-DQ and their derivatives were reconstituted in K562 cells and stained with anti-HLA class II monoclonal antibodies. The surface expression of each DQ2, DQ5, and DQ6 allele was detected using the anti-HLA class II monoclonal antibody clone 9-49(I3) (DQ5 and DQ6) or the anti-class II monoclonal antibody clone Tü39 (DQ2 and DQ4). Open histograms represent the isotype control staining.
- [0050]** FIGs. 4A-4L are graphical representations showing that DQ5^{L114W/V143M+4reps} dimers robustly stained E6-transduced CD4⁺ T cells. E6 was reconstituted in CD4⁺ T cells, which were then stained with wild-type DQ5 (FIGs. 4D and 4J), DQ5^{L114W/V143M} (FIGs. 4E and 4K), and DQ5^{L114W/V143M+4reps} (FIGs. 4F and 4L) CLIP control dimers (FIGs. 4D-4F) and dimers specific to DDX3Y₁₇₁₋₁₉₀ (FIGs. 4J-4L). Control cells not transduced with a TCR are shown in FIGs. 4A-4C and 4G-4I.
- [0051]** FIGs. 5A-5G are graphical representations showing cloning of DQ5-restricted TCR using affinity matured dimer. Primary CD4⁺ T cells were purified from a DQ5.1⁺ melanoma patient and stimulated with irradiated GPC3₁₃₈₋₁₅₇-pulsed aAPCs expressing DQ5.1. Two weeks later, stimulated CD4⁺ T cells were stained with cognate GPC3₁₃₈₋₁₅₇-DQ5^{L114W/V143M+4reps} dimers (FIGs. 5A-5B). The GPC3 specific TCR was reconstituted in TCR-defective Jurkat 76/CD4 cells, and stained by the respective DQ5^{L114W/V143M+4reps} dimers (FIG. 5C (E6/Control); FIG. 5D (E6/GPC3₁₃₈₋₁₅₇); FIG. 5E (DQ5-06-GPC3₁₃₈₋₁₅₇/Control); and FIG. 5F (DQ5-06-GPC3₁₃₈₋₁₅₇/GPC3₁₃₈₋₁₅₇)). Jurkat 76/CD4 cells expressing the GPC3 specific TCR were stimulated by DQ5-K562 cells pulsed with the respective peptides in IL-2 ELISPOT assays (FIG. 5G).

DETAILED DESCRIPTION OF THE DISCLOSURE

- [0052]** The present disclosure is directed to MHC class II molecules with increased affinity for CD4. In some aspects, the present disclosure is directed to MHC class II

molecules comprising an HLA-DQ (DQ) beta chain, wherein the DQ beta chain has increased affinity for CD4.

[0053] The present disclosure is further directed to MHC class II molecules comprising a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the DQ beta chain further comprises an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.

[0054] The present disclosure is further directed to MHC class II molecules comprising a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1. In some aspects, the DQ beta chain further comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1.

[0055] In some aspects, the DQ beta chain further comprises at least three of: (i) an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1, (ii) an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (iv) an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

I. Terms

[0056] In order that the present disclosure can be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0057] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0058] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as

"A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0059] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower).

[0060] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0061] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0062] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0063] "Administering" refers to the physical introduction of an agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Exemplary routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular,

intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some aspects, the formulation is administered via a non-parenteral route, *e.g.*, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0064] The term "HLA," as used herein, refers to the human leukocyte antigen. HLA genes encode the major histocompatibility complex (MHC) proteins in humans. MHC proteins are expressed on the surface of cells, and are involved in activation of the immune response. HLA class II genes encode MHC class II proteins which are expressed on the surface of professional antigen presenting cells (APCs). Non-limiting examples of professional APCs include monocytes, macrophages, dendritic cells (DCs), and B lymphocytes. Some endothelial and epithelial cells can also express MHC class II molecules after inflammatory signals are activated. Humans lacking functional MHC class II molecules are extremely susceptible to an array of infectious diseases and typically die at a young age.

[0065] As used herein, an "HLA class II molecule" or "MHC class II molecule" refers to a protein product of a wild-type or variant HLA class II gene encoding an MHC class II molecule. Accordingly, "HLA class II molecule" and "MHC class II molecule" are used interchangeably herein. A typical MHC Class II molecule comprises two protein chains: an alpha chain and a beta chain. In general, naturally occurring alpha chains and beta chains each comprise a transmembrane domain, which anchors the alpha/beta chain to the cell surface, and an extracellular domain, which carries the antigen and interacts with a TCR and/or CD4 expressed on a T cell.

[0066] Both the MHC Class II alpha and beta chains are encoded by the HLA gene complex. The HLA complex is located within the 6p21.3 region on the short arm of human chromosome 6 and contains more than 220 genes of diverse function. The HLA gene complex is highly variant, with over 20,000 HLA alleles and related alleles, including over 250 MHC class II alpha chain alleles and 5,000 MHC class II beta chain alleles, known in the art, encoding thousands of MHC class II proteins (*see, e.g.,* hla.alleles.org, last visited May 20, 2019, which is incorporated by reference herein in its entirety). For example one such

HLA-DP allele, DP4 is the most frequently found allele in many ethnic groups. Each alpha chain and beta chain is typically expressed as a proprotein, which further comprises a signal peptide that is cleaved off. Any number of naturally occurring signal peptides can be used to facilitate expression and localization of the alpha chains and beta chains disclosed herein. One such example is SEQ ID NO: 9.

[0067] Three loci in the HLA complex encode MHC Class II proteins: HLA-DP, HLA-DQ, and HLA-DR. HLA-DO and HLA-DM encode proteins that associate with the MHC class II molecule and support its configuration and function. Representative HLA-DQ sequences are provided in Table 1.

Table 1: DQ Beta chain and alpha chain amino acid and nucleotide sequences.

Beta Chain
<u>DQB1*05:01 Extracellular Domain (SEQ ID NO: 1)</u> RDSPEDFVYQFKGLCYFTNGTERVRGVTRHIYNREEYVRFDSVGVYRAVTPQGRPVAEYWNSQKEVLEGARAS VDRVCRHNYEVAYRGILQRRVEPTVTISPSRTEALNHHNLLICSVTDFYPSQIKVRWFRNDQEETAGVVSTPLI RNGDWTFFQILVMLEMT PQRGDVYTCHVEHPSLQSPITVEWRAQSESAQSK
<u>DQB1*05:01 Extracellular Domain (SEQ ID NO: 2)</u> AGAGACTCTCCCGAGGATTTTCGTGTACCACTTAAAGGGCCTGTGCTACTTCACCAACGGGACGGAGCGCGTGCG GGGTGTGACCAGACACATCTATAACCGAGAGGAGTACGTGCGCTTCGACAGCGACGTGGGGGTGTACCGGGCAG TGACGCCGCGAGGGGCGGCCCTGTTGCCGAGTACTGGAACAGCCAGAAGGAAGTCTGGAGGGGGCCCGGGCGTCG GTGGACAGGGTGTGCAGACACAACACTACGAGGTGGCGTACCGCGGGATCCTGCAGAGGAGAGTGGAGCCCACAGT GACCATCTCCCATCCAGGACAGAGGCCCTCAACCACCACAACCTGCTGATCTGCTCGGTGACAGATTTCTATC CAAGCCAGATCAAAGTCCGGTGGTTTCGGAATGATCAGGAGGAGACAGCCGGCGTTGTGTCCACCCCCCTCATT AGGAACGGTGACTGGACCTTCCAGATCCTGGTGATGCTGGAAATGACTCCCCAGCGTGGAGATGTCTACACCTG CCACGTGGAGCACCCAGCCTCCAGAGCCCCATCACCGTGGAGTGGCGGGCTCAGTCTGAATCTGCCAGAGCA AG
<u>DQB1*05:01 L114W/V143M Extracellular Domain (SEQ ID NO: 11)</u> RDSPEDFVYQFKGLCYFTNGTERVRGVTRHIYNREEYVRFDSVGVYRAVTPQGRPVAEYWNSQKEVLEGARAS VDRVCRHNYEVAYRGILQRRVEPTVTISPSRTEALNHHNWLICSVTDFYPSQIKVRWFRNDQEETAGVMSTPLI RNGDWTFFQILVMLEMT PQRGDVYTCHVEHPSLQSPITVEWRAQSESAQSK
<u>DQB1*05:01 L114W/V143M + 4 Reps Extracellular Domain (SEQ ID NO: 3)</u> RDSPEDFVYQFKGLCYFTNGTERVRGVTRHIYNREEYVRFDSVGVYRAVTPQGRPVAEYWNSQKEVLEGARAS VDRVCRHNYEVAYRGILQRRVEPTVTISPSRTEALQHNNWLCHVTDFYPSQIKVRWFRNDQEETAGVMSTNLI RNGDWTFFQILVMLEMT PQRGDVYTCHVEHPSLQSPITVEWRAQSESAQSK
<u>Signal Peptide; DQB1*05:01 Domain; Gly/Ser Linker; Zip Sequences and His tag sequences) (SEQ ID NO: 12)</u> MMRPIVLVLLFATSALARDS PEDFVYQFKGLCYFTNGTERVRGVTRHIYNREEYVRFDSVGVYRAVTPQGRPV AEYWNSQKEVLEGARASVDRVCRHNYEVAYRGILQRRVEPTVTISPSRTEALNHHNLLICSVTDFYPSQIKVRW FRNDQEETAGVVSTPLIRNGDWTFFQILVMLEMT PQRGDVYTCHVEHPSLQSPITVEWRAQSESAQSKGGGGSLE IEAAFLERENTALET RVAELRQRVQRLNRVSOYRTRYGPLGGGK
<u>Signal Peptide; DQB1*05:01 L114W/V143M Domain; Gly/Ser Linker; Zip Sequences and His tag sequences) (SEQ ID NO: 13)</u> MMRPIVLVLLFATSALARDS PEDFVYQFKGLCYFTNGTERVRGVTRHIYNREEYVRFDSVGVYRAVTPQGRPV AEYWNSQKEVLEGARASVDRVCRHNYEVAYRGILQRRVEPTVTISPSRTEALNHHNWLICSVTDFYPSQIKVRW FRNDQEETAGVMSTPLIRNGDWTFFQILVMLEMT PQRGDVYTCHVEHPSLQSPITVEWRAQSESAQSKGGGGSLE IEAAFLERENTALET RVAELRQRVQRLNRVSOYRTRYGPLGGGK

<u>Signal Peptide; DQB1*05:01 L114W/V143M + 4 Reps Extracellular Domain; Gly/Ser Linker; Zip Sequences and His tag sequences) (SEQ ID NO: 4)</u> <u>MMRPVLVLLFATSALARDS PEDFVYQFKGLCYFTNGTERVGRVTRHIYNREEYVRFDS DVG VYRAVTPQGRPV AEYWNSQKEVLEGARASVDRVCRHNYEVAYRGILQRRVEPTVTISPSRTEALQHHNWL VCHVTDFYPSQIKVRW FRNDQEETAGVMSTNLIRNGDWTFFQILVMLEMPQRGDVYTCHVEHPSLQSPITVEWRAQSESAQSKGGGSLE IEAAFLERENTALETRVAELRQRVQRLRNRVSQYRTRYGPLGGGK</u>
Full-length wild-type DQB1*05:01 (SEQ ID NO: 5) MSWKKSLRIPGDLRVATVTLMLAILSSSLAEGRDSPEDFVYQFKGLCYFTNGTERVGRVTRHIYNREEYVRFDS DVG VYRAVTPQGRPVAEYWNSQKEVLEGARASVDRVCRHNYEVAYRGILQRRVEPTVTISPSRTEALNHHNLLI CSVTDFYPSQIKVRWFRNDQEETAGVVSTPLIRNGDWTFFQILVMLEMPQRGDVYTCHVEHPSLQSPITVEWRA QSESAQSKMLSGVGGFVLGLIFLGLGLIIRQSRKGLLH
Alpha Chain
<u>DQA1*01:01 Extracellular Domain (SEQ ID NO: 6)</u> EDIVADHVASC GVNLYQFYGPSGQYTHEFDGDEEFYVDLERKETAWRWPEFSKFGGFDPQGALRNMAVAKHNLN IMIKRYNSTAATNEVPEVTVFSSKSPVTLGQPNTLICLVDNIFPPVVNITWLSNGQSVTEGVSETSFLSKSDHSF FKISYLTFLPSADEIYDCKVEHWGLDQPLLKHWEPEIPAPMSELTET
<u>DQA1*01:01 Extracellular Domain (SEQ ID NO: 7)</u> GAGGACATCGTGGCCGATCACGTGGCAAGCTGCGGCGTGAACCTGTACAGTTCTACGGCCCCCTCTGGCCAGTA CACCCATGAATTTGATGGAGATGAGGAGTTCTACGTGGACCTGGAGAGGAAGGAGACTGCCTGGCGGTGGCCTG AGTTCAGCAAATTTGGAGGTTTTGACCCGCAGGGTGCCTGAGAAACATGGCTGTGGCAAACACAACCTGAAC ATCATGATTAAACGCTACAACCTCTACCGCTGCTACCAATGAGGTTCTGAGGTCACAGTGTTTTCCAAGTCTCC CGTGACACTGGGTGAGCCCAACACCCTCATTTGTCTTGTGGACAACATCTTTCCTCTGTGGTCAACATCACAT GGCTGAGCAATGGGCAGTCAGTCACAGAAGGTGTTTCTGAGACCAGCTTCTCTCCAAGAGTGATCATTCTCTT TCAAGATCAGTTACCTCACCTTCTCCCTTCTGCTGATGAGATTTATGACTGCAAGGTGGAGCACTGGGGCCT GGACCAGCCTCTTCTGAAACACTGGGAGCTGAGATTCAGCCCTATGTGTCAGAGCTCACAGAGACT
<u>Signal Peptide; DQA1*01:01 Extracellular Domain; Gly/Ser Linker, Zip Sequences and His tag sequences) (SEQ ID NO: 8)</u> <u>MMRPVLVLLFATSALAE DIVADHVASC GVNLYQFYGPSGQYTHEFDGDEEFYVDLERKETAWRWPEFSKFGGF DPQGALRNMAVAKHNLNIMIKRYNSTAATNEVPEVTVFSSKSPVTLGQPNTLICLVDNIFPPVVNITWLSNGQSV TEGVSETSFLSKSDHSFFKISYLTFLPSADEIYDCKVEHWGLDQPLLKHWEPEIPAPMSELTETGGGGSLEIRA AFLRQNTALRTEVAELEQEVQRLNEVVSQYETRYGPLGGGKGSHHHHH</u>
Signal Peptide (Fibroin light chain-derived)
MMRPVLVLLFATSALA (SEQ ID NO: 9)

[0068] When the MHC class II molecule is complexed with an antigen peptide, the 10-30 amino acid long antigen peptide binds the peptide-binding groove and is presented extracellularly to CD4⁺ cells. Both the alpha- and beta-chains fold into two separate domains; alpha-1 and alpha-2 for the alpha polypeptide, and beta-1 and beta-2 for the beta polypeptide. The invariant residues at L114, V116, V143, L158, and M160 that are recognized and bound by CD4 are located in the beta-2 domain of the beta polypeptide. The open-ended peptide-binding groove which holds the presented antigen is found between the alpha-1 and beta-1 domains. Upon interaction with a CD4⁺ T cell, the MHC class II complex interacts with a T cell receptor (TCR) expressed on the surface of the T cell. In addition, the beta chain of the MHC class II molecule weakly interacts ($K_D > 2$ mM) with CD4 expressed on the surface of

the T cell. The canonical CD4 amino acid sequence (UniProt - P01730) is provided in Table 2 (SEQ ID NO: 10).

Table 2: Human CD4 Amino Acid Sequence

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MNRGVPFRHLLLVQLALLPAATQGKKVVLGKKGDTVELTCTASQKKSIOFHWKNSNQIKILGNQGSFLTSGPSK
LNDRADSRRLWDQGNFPLIIKNLKIEDSDTYICEVEDQKEEVQLLVFGLTANS DTHLLQGQSLTLTLESPPGSS
PSVQCRSPRGKNIQGGKTLVSQLELQDSGTWTCTVLQNKQKVEFKIDIVVLAFOKASSIVYKKEGEQVEFSFPL
AFTVEKLTGSGELWWQAERASSSKSWITFDLKNKEVSVKRVTDPKLQMGKKLPLHLTLPQALPQYAGSGNLTLA
LEAKTGKLHQEVNLVVMRATQLQKNLTCEVWGPTSPKLMLSLKLENKEAKVSKREKAVVVLNPEAGMWQCLLSDS
GOVLLESNIKVLPTWSTFPVQPMALIVLGGVAGLLFLIGLGIFFCVRCRHRRRQAERMSQIKRLLSEKKTCQCPHR
FQKTCSPI (SEQ ID NO: 10)

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[0069] The term "T cell receptor" (TCR), as used herein, refers to a heteromeric cell-surface receptor capable of specifically interacting with a target antigen. As used herein, "TCR" includes but is not limited to naturally occurring and non-naturally occurring TCRs, full-length TCRs and antigen binding portions thereof, chimeric TCRs, TCR fusion constructs, and synthetic TCRs. In human, TCRs are expressed on the surface of T cells, and they are responsible for T cell recognition and targeting of antigen presenting cells. Antigen presenting cells (APCs) display fragments of foreign proteins (antigens) complexed with the major histocompatibility complex (MHC class I or MHC class II; also referred to herein as complexed with an HLA molecule, *e.g.*, an HLA class II molecule). A TCR recognizes and binds to the peptide:HLA complex and recruits CD8 (for MHC Class I molecules) or CD4 (for MHC class II molecules) expressed by T cells, activating the TCR. The activated TCR initiates downstream signaling and an immune response, including the destruction of the APC.

[0070] In general, a TCR can comprise two chains, an alpha chain and a beta chain (or less commonly a gamma chain and a delta chain), interconnected by disulfide bonds. Each chain comprises a variable domain (alpha chain variable domain and beta chain variable domain) and a constant region (alpha chain constant region and beta chain constant region). The variable domain is located distal to the cell membrane, and the variable domain interacts with an antigen. The constant region is located proximal to the cell membrane. A TCR can further comprises a transmembrane region and a short cytoplasmic tail. As used herein, the term "constant region" encompasses the transmembrane region and the cytoplasmic tail, when present, as well as the traditional "constant region."

[0071] The variable domains can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each alpha chain variable domain and beta chain

variable domain comprises three CDRs and four FRs: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Each variable domain contains a binding domain that interacts with an antigen. Though all three CDRs on each chain are involved in antigen binding, CDR3 is believed to be the primary antigen binding region, while CDR1 and CDR2 are believed to primarily recognize the HLA molecule.

[0072] Where not expressly stated, and unless the context indicates otherwise, the term "TCR" also includes an antigen-binding fragment or an antigen-binding portion of any TCR disclosed herein, and includes a monovalent and a divalent fragment or portion, and a single chain TCR. The term "TCR" is not limited to naturally occurring TCRs bound to the surface of a T cell. As used herein, the term "TCR" further refers to a TCR described herein that is expressed on the surface of a cell other than a T cell (*e.g.*, a cell that naturally expresses or that is modified to express CD4, as described herein), or a TCR described herein that is free from a cell membrane (*e.g.*, an isolated TCR or a soluble TCR).

[0073] An "antigen binding molecule," "portion of a TCR," or "TCR fragment" refers to any portion of an TCR less than the whole. An antigen binding molecule can include the antigenic CDRs.

[0074] An "antigen" refers to any molecule, *e.g.*, a peptide, that provokes an immune response or is capable of being bound by a TCR. An "epitope," as used herein, refers to a portion of a polypeptide that provokes an immune response or is capable of being bound by a TCR. The immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. A person of skill in the art would readily understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. An antigen and/or an epitope can be endogenously expressed, *i.e.* expressed by genomic DNA, or can be recombinantly expressed. An antigen and/or an epitope can be specific to a certain tissue, such as a diseased cell, *e.g.*, a cancer cell, or it can be broadly expressed. In addition, fragments of larger molecules can act as antigens. In one aspect, antigens are tumor antigens. An epitope can be present in a longer polypeptide (*e.g.*, in a protein), or an epitope can be present as a fragment of a longer polypeptide. In some aspects, an epitope is complexed with a major histocompatibility complex (MHC; also referred to herein as complexed with an HLA molecule, *e.g.*, an HLA class 1 molecule).

[0075] The term "autologous" refers to any material derived from the same individual to which it is later to be re-introduced. For example, an autologous T cell therapy comprises

administering to a subject a T cell that was isolated from the same subject. The term "allogeneic" refers to any material derived from one individual which is then introduced to another individual of the same species. For example, an allogeneic T cell transplantation comprises administering to a subject a T cell that was obtained from a donor other than the subject.

[0076] A "cancer" refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor. Examples of cancers that can be treated by the methods of the present invention include, but are not limited to, cancers of the immune system including lymphoma, leukemia, and other leukocyte malignancies. In some aspects, the methods of the present invention can be used to reduce the tumor size of a tumor derived from, for example, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, stomach cancer, uterine cancer, lung cancer, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), cancer of the esophagus, cancer of the small intestine, cancer of the urethra, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, glioma, squamous cell cancer, and combinations of said cancers. The particular cancer can be responsive to chemo- or radiation therapy or the cancer can be refractory. A refractory cancer refers to a cancer that is not amendable to surgical intervention, and the cancer is either initially unresponsive to chemo- or radiation therapy or the cancer becomes unresponsive over time.

[0077] The term "progression-free survival," which can be abbreviated as PFS, as used herein refers to the time from the treatment date to the date of disease progression per the revised IWG Response Criteria for Malignant Lymphoma or death from any cause.

[0078] The term "overall survival," which can be abbreviated as OS, is defined as the time from the date of treatment to the date of death.

[0079] The term "infection," as used herein refers to any type of invasion of one or more tissue of the body by a foreign agent. The term "infection" includes without limitation

infection by a virus (including viroids and prions), a bacterium, a fungus, a parasite, and any combination thereof.

[0080] The term "lymphocyte" as used herein includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represents a major component of the inherent immune system. NK cells reject tumors and cells infected by viruses. It works through the process of apoptosis or programmed cell death. They were termed "natural killers" because they do not require activation in order to kill cells. T-cells play a major role in cell-mediated-immunity (no antibody involvement). T-cell receptors (TCR) differentiate T cells from other lymphocyte types. The thymus, a specialized organ of the immune system, is primarily responsible for the T cell's maturation. There are six types of T-cells, namely: Helper T-cells (*e.g.*, CD4⁺ cells), Cytotoxic T-cells (also known as TC, cytotoxic T lymphocyte, CTL, T-killer cell, cytolytic T cell, CD8⁺ T-cells or killer T cell), Memory T-cells ((i) stem memory T_{SCM} cells, like naive cells, are CD45RO⁻, CCR7⁺, CD45RA⁺, CD62L⁺ (L-selectin), CD27⁺, CD28⁺ and IL-7Rα⁺, but they also express large amounts of CD95, IL-2Rβ, CXCR3, and LFA-1, and show numerous functional attributes distinctive of memory cells); (ii) central memory T_{CM} cells express L-selectin and the CCR7, they secrete IL-2, but not IFNγ or IL-4, and (iii) effector memory T_{EM} cells, however, do not express L-selectin or CCR7 but produce effector cytokines like IFNγ and IL-4), Regulatory T-cells (Tregs, suppressor T cells, or CD4⁺CD25⁺ regulatory T cells), Natural Killer T-cells (NKT) and Gamma Delta T-cells. B-cells, on the other hand, play a principal role in humoral immunity (with antibody involvement). A B cell makes antibodies and antigens and performs the role of antigen-presenting cells (APCs) and turns into memory B-cells after activation by antigen interaction. In mammals, immature B-cells are formed in the bone marrow, where its name is derived from.

[0081] The terms "modified" and "mutated," when used herein to refer to a nucleotide or amino acid sequence, refers to a change in the sequence relative to a wild-type sequence or a specified reference sequence. The terms "modified" and "mutated" do not require a step in a process for making the modified or mutated sequence (*e.g.*, the modified beta chain sequence), unless otherwise specified. Rather, these terms indicate that there is a variation in the modified or mutated sequence relative to a reference sequence, *e.g.*, a wild-type sequence. For example, a DQ beta chain comprising a substitution mutation at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 does not require that a wild-type DQ beta chain

has been physically altered to arrive at the recited DQ beta chain; but rather that, when properly aligned, the recited DQ beta chain comprises an amino acid residue at the recited position (residue 114) that is different from the amino acid residue at the corresponding position in a wild-type or reference DQ beta chain.

[0082] The term "any amino acid," as used herein, means any known amino acid. Amino acids are organic compounds comprising (i) an amine (-NH₂) functional group, (ii) a carboxyl (-COOH) functional group, and (iii) a side chain (R group), wherein the side chain is specific to each amino acid. This includes but is not limited to any naturally occurring amino acid, as well as any modifications and variants thereof. There are about 500 naturally occurring amino acids, 20 of which are encoded by the genetic code. Amino acids with positively charged side chains include arginine (Arg; R), histidine (His; H), and lysine (Lys; K). Amino acids with a negatively charged side chain include aspartic acid (Asp; D) and glutamic acid (Glu; E). Amino acids with a polar uncharged side chain include serine (Ser; S), threonine (Thr; T), glutamine (Gln; Q), and asparagine (Asn; N). Amino acids with a hydrophobic side chain include alanine (Ala; A), isoleucine (Ile; I), leucine (Leu; L), methionine (Met; M), phenylalanine (Phe; F), valine (Val; V), Tryptophan (Trp; W), Tyrosine (Tyr; Y). Tryptophan (Trp; W), tyrosine (Tyr; Y), and methionine (Met; M) can also be classified as polar and/or amphipathic, in that these amino acids can often be found at the surface of proteins or lipid membranes. Additional amino acids include cysteine (Cys; C), selenocysteine (Sec; U), glycine (Gly; G) and proline (Pro; P).

[0083] As used herein "at a position corresponding to" is used as a means to identify a particular amino acid residue, *e.g.*, a specific amino acid position, in a polynucleotide or a particular nucleic acid, *e.g.*, a specific nucleic acid position, in a polypeptide. The position can be determined by properly aligning the sequence in question with the referenced sequence. A person of skill in the art would readily understand how to align to sequences to determine the relative position. For example, various alignment tools are available online, including, without limitation, "Clustal Omega Multiple Sequence Alignment," available at www.ebi.ac.uk (last visited May 25, 2019).

[0084] The term "genetically engineered" or "engineered" refers to a method of modifying the genome of a cell, including, but not limited to, deleting a coding or non-coding region or a portion thereof or inserting a coding region or a portion thereof. In some aspects, the cell that is modified is a lymphocyte, *e.g.*, a T cell or a modified cell that expresses CD4,

which can either be obtained from a patient or a donor. The cell can be modified to express an exogenous construct, such as, *e.g.*, a T cell receptor (TCR) disclosed herein, which is incorporated into the cell's genome. In some aspects, the cell is modified to express CD4.

[0085] An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0086] The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. Examples of immunotherapy include, but are not limited to, T cell therapies. T cell therapy can include adoptive T cell therapy, tumor-infiltrating lymphocyte (TIL) immunotherapy, autologous cell therapy, engineered autologous cell therapy (eACT), and allogeneic T cell transplantation.

[0087] Cells used in an immunotherapy described herein can come from any source known in the art. For example, T cells can be differentiated *in vitro* from a hematopoietic stem cell population, or T cells can be obtained from a subject. T cells can be obtained from, *e.g.*, 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 addition, the T cells can be derived from one or more T cell lines available in the art. T cells can also be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation and/or apheresis. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Publication No. 2013/0287748, which is herein incorporated by references in its entirety. An immunotherapy can also comprise administering a modified cell to a subject, wherein the modified cell expresses CD4 and a TCR disclosed herein. In some aspects, the modified cell is not a T cell.

- [0088]** A "patient" as used herein includes any human who is afflicted with a cancer (*e.g.*, a lymphoma or a leukemia). The terms "subject" and "patient" are used interchangeably herein.
- [0089]** 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. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.
- [0090]** "Stimulation," as used herein, refers to a primary response induced by binding of a stimulatory molecule with its cognate ligand, wherein the binding mediates a signal transduction event. A "stimulatory molecule" is a molecule on a T cell, *e.g.*, the T cell receptor (TCR)/ CD4 complex, that specifically binds with a cognate stimulatory ligand present on an antigen present cell. A "stimulatory ligand" is a ligand that when present on an antigen presenting cell (*e.g.*, an aAPC, a dendritic cell, a B-cell, and the like) can specifically bind with a stimulatory molecule on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands include, but are not limited to, an MHC Class II molecule loaded with a peptide, an anti-CD4 antibody, an anti-CD28 antibody, an anti-CD2 antibody, and an anti-CD3 antibody.
- [0091]** "Treatment" or "treating" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease. In one aspect, "treatment" or "treating"

includes a partial remission. In another aspect, "treatment" or "treating" includes a complete remission.

[0092] The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

[0093] The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% (*i.e.*, $\pm 10\%$). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

[0094] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[0095] Various aspects of the invention are described in further detail in the following subsections.

II. Compositions of the Disclosure

[0096] The present disclosure is directed to HLA class II molecules with enhanced CD4 binding. Certain aspects of the present disclosure are directed to HLA class II molecules comprising a beta chain, wherein the beta chain comprises one or more mutations. In certain aspects, the one or more mutations in the beta chain increase the affinity of the beta chain for CD4. In certain aspects, the beta chain is an HLA-DQ ("DQ") beta chain.

II.A. MHC Class II Molecules

[0097] The human leukocyte antigen (HLA) system (the major histocompatibility complex [MHC] in humans) is an important part of the immune system and is controlled by genes located on chromosome 6. It encodes cell surface molecules specialized to present antigenic peptides to the T-cell receptor (TCR) on T cells. (See also Overview of the Immune System.) MHC molecules that present antigen (Ag) are divided into 2 main classes: Class I MHC molecules and Class II MHC molecules.

[0098] Class II MHC molecules are present as transmembrane glycoproteins on the surface of professional antigen presenting cells (APCs). Intact class II molecules consist of an alpha chain and a beta chain. Three loci in the HLA complex encode MHC class II proteins: HLA-DP, HLA-DQ, and HLA-DR. T cells that express CD4 molecules react with class II MHC molecules. These lymphocytes often have effector and helper functions and activate a response to eliminate self-cells infected with intracellular pathogens or to destroy extracellular parasites and help other T cells such as CD8 T cells. Because only professional APCs express class II MHC molecules, only these cells present antigen for CD4 T cells (CD4 binds to the nonpolymorphic part of the alpha-2 and beta-2 domains of the alpha and beta chains of an MHC class II molecule respectively).

[0099] In some aspects, the HLA class II alpha and beta chains are selected from an HLA-DP, HLA-DQ, and HLA-DR allele. In certain aspects, the HLA class II beta chain is an HLA-DQ allele. In certain aspects, the HLA class II alpha chain is an HLA-DQ allele.

[0100] Many HLA-DQ alleles are known in the art, and any of the known alleles can be used in the present disclosure. Examples of HLA-DQ alpha chain and beta chain alleles are shown in Table 1. An updated list of HLA alleles is available at hla.alleles.org/ (last visited on July 10, 2019).

II.A.1. MHC Class II Beta Chain

[0101] In certain aspects, the HLA class II molecule comprises a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. Any amino acid other than leucine can be present at the position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the amino acid other than leucine is an amino acid comprising a hydrophobic side chain. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is an amino acid selected from an alanine, a valine, an

isoleucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is an alanine. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a valine. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is an isoleucine. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a methionine. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a phenylalanine. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a tyrosine. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a tryptophan.

[0102] In some embodiments, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 consists of more than one amino acid, *e.g.*, two amino acids, three amino acids, four amino acids, five amino acids, or more. In some aspects at least one of the more than one amino acids comprises a hydrophobic side chain. In certain aspects, the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 consists of a series, *e.g.*, at least 2, at least 3, at least 4, or at least 5, amino acids, wherein each of the series of amino acids comprises a hydrophobic side chain.

[0103] In certain aspects, the HLA class II molecule comprises a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1. Any amino acid other than valine can be present at the position corresponding to amino acid residue 143 of SEQ ID NO: 1. In some aspects, the amino acid other than valine is an amino acid comprising a hydrophobic side chain. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 is an amino acid selected from an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 is an alanine. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 is an isoleucine. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO:

1 is a leucine. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a methionine. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a phenylalanine. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a tyrosine. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a tryptophan.

[0104] In some aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 consists of more than one amino acid, *e.g.*, two amino acids, three amino acids, four amino acids, five amino acids, or more. In some aspects at least one of the more than one amino acids comprises a hydrophobic side chain. In certain aspects, the amino acid other than valine at the position corresponding to amino acid residue 143 of SEQ ID NO: 1 consists of a series, *e.g.*, at least 2, at least 3, at least 4, or at least 5, amino acids, wherein each of the series of amino acids comprises a hydrophobic side chain.

[0105] In certain aspects, the HLA class II molecule comprises a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1. Any amino acid other than asparagine can be present at the position corresponding to amino acid residue 110 of SEQ ID NO: 1. In some aspects, the amino acid other than asparagine is an amino acid comprising a polar uncharged side chain. In certain aspects, the amino acid other than asparagine at the position corresponding to amino acid residue 110 of SEQ ID NO: 1 is an amino acid selected from a serine, a threonine, and a glutamine. In certain aspects, the amino acid other than asparagine at the position corresponding to amino acid residue 110 of SEQ ID NO: 1 is a serine. In certain aspects, the amino acid other than asparagine at the position corresponding to amino acid residue 110 of SEQ ID NO: 1 is a threonine. In certain aspects, the amino acid other than asparagine at the position corresponding to amino acid residue 110 of SEQ ID NO: 1 is a glutamine.

[0106] In some aspects, the amino acid other than asparagine at the position corresponding to amino acid residue 110 of SEQ ID NO: 1 consists of more than one amino acid, *e.g.*, two amino acids, three amino acids, four amino acids, five amino acids, or more. In some aspects at least one of the more than one amino acids comprises a polar uncharged side chain. In certain aspects, the amino acid other than asparagine at the position corresponding

to amino acid residue 110 of SEQ ID NO: 1 consists of a series, *e.g.*, at least 2, at least 3, at least 4, or at least 5, amino acids, wherein each of the series of amino acids comprises a polar uncharged side chain.

[0107] In certain aspects, the HLA class II molecule comprises a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1. Any amino acid other than isoleucine can be present at the position corresponding to amino acid residue 116 of SEQ ID NO: 1. In some aspects, the amino acid other than isoleucine is an amino acid comprising a hydrophobic side chain. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is an amino acid selected from an alanine, a valine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is an alanine. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is a valine. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is a leucine. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is a methionine. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is a phenylalanine. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is a tyrosine. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 is a tryptophan.

[0108] In some aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 consists of more than one amino acid, *e.g.*, two amino acids, three amino acids, four amino acids, five amino acids, or more. In some aspects at least one of the more than one amino acids comprises a hydrophobic side chain. In certain aspects, the amino acid other than isoleucine at the position corresponding to amino acid residue 116 of SEQ ID NO: 1 consists of a series, *e.g.*, at least 2, at least 3, at least 4, or at least 5, amino acids, wherein each of the series of amino acids comprises a hydrophobic side chain.

- [0109]** In certain aspects, the HLA class II molecule comprises a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1. Any amino acid other than serine can be present at the position corresponding to amino acid residue 118 of SEQ ID NO: 1. In some aspects, the amino acid other than serine is an amino acid comprising an electrically charged side chain. In certain aspects, the amino acid other than serine at the position corresponding to amino acid residue 118 of SEQ ID NO: 1 is an amino acid selected from an arginine, a histidine, and a lysine. In certain aspects, the amino acid other than serine at the position corresponding to amino acid residue 118 of SEQ ID NO: 1 is an arginine. In certain aspects, the amino acid other than serine at the position corresponding to amino acid residue 118 of SEQ ID NO: 1 is a histidine. In certain aspects, the amino acid other than serine at the position corresponding to amino acid residue 118 of SEQ ID NO: 1 is a lysine.
- [0110]** In some aspects, the amino acid other than serine at the position corresponding to amino acid residue 118 of SEQ ID NO: 1 consists of more than one amino acid, *e.g.*, two amino acids, three amino acids, four amino acids, five amino acids, or more. In some aspects at least one of the more than one amino acids comprises an electrically charged side chain. In certain aspects, the amino acid other than serine at the position corresponding to amino acid residue 118 of SEQ ID NO: 1 consists of a series, *e.g.*, at least 2, at least 3, at least 4, or at least 5, amino acids, wherein each of the series of amino acids comprises an electrically charged side chain.
- [0111]** In certain aspects, the HLA class II molecule comprises a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1. Any amino acid other than proline can be present at the position corresponding to amino acid residue 146 of SEQ ID NO: 1. In some aspects, the amino acid other than proline is an amino acid comprising a polar uncharged side chain. In certain aspects, the amino acid other than proline at the position corresponding to amino acid residue 146 of SEQ ID NO: 1 is an amino acid selected from a serine, a threonine, an asparagine, and a glutamine. In certain aspects, the amino acid other than proline at the position corresponding to amino acid residue 146 of SEQ ID NO: 1 is a serine. In certain aspects, the amino acid other than proline at the position corresponding to amino acid residue 146 of SEQ ID NO: 1 is a threonine. In certain aspects, the amino acid other than proline at the position corresponding to amino acid residue 146 of SEQ ID NO: 1 is an asparagine. In

certain aspects, the amino acid other than proline at the position corresponding to amino acid residue 146 of SEQ ID NO: 1 is a glutamine.

[0112] In some aspects, the amino acid other than proline at the position corresponding to amino acid residue 146 of SEQ ID NO: 1 consists of more than one amino acid, *e.g.*, two amino acids, three amino acids, four amino acids, five amino acids, or more. In some aspects at least one of the more than one amino acids comprises a polar uncharged side chain. In certain aspects, the amino acid other than proline at the position corresponding to amino acid residue 146 of SEQ ID NO: 1 consists of a series, *e.g.*, at least 2, at least 3, at least 4, or at least 5, amino acids, wherein each of the series of amino acids comprises a polar uncharged side chain.

[0113] In certain aspects of the present disclosure, the MHC class II molecule comprises a DQ beta chain comprising more than one substitution mutation relative to the wild-type DQ beta chain. In certain aspects, the DQ beta chain comprises at least two mutations, at least three mutations, at least four mutations, at least five mutations, at least six mutations, at least seven mutations, at least eight mutations, at least nine mutations, or at least ten mutations relative to the wild-type DQ beta chain.

[0114] In certain aspects, the DQ beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 and an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1. In certain aspects, the DQ beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1; an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; and at least three of: (i) an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1, (ii) an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and (iv) an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0115] In some aspects, the DQ beta chain comprises (i) an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1; (ii) an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; (iii) an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1; (iv) an amino acid other than isoleucine at a position corresponding to

amino acid residue 116 of SEQ ID NO: 1; (v) an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1; and (vi) an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0116] In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1, (ii) the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1, or each of the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 and the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is an amino acid comprising a hydrophobic side chain.

[0117] In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is selected from an alanine, a valine, an isoleucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan; (ii) the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is selected from an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan; (iii) the amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1 is selected from a serine, a threonine, and a glutamine; (iv) the amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1 is selected from an alanine, a valine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan; (v) the amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1 is selected from an arginine, a histidine, and a lysine; and (vi) the amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1 is selected from a serine, a threonine, an asparagine, and a glutamine.

[0118] In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a tryptophan; (ii) the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is selected from an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is selected from an alanine, a valine, an isoleucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan; and (ii) the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a methionine. In some aspects, (i) the amino acid other than leucine

at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a tryptophan; and (ii) the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a methionine. In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a tryptophan; (ii) the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a methionine; (iii) the amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1 is a glutamine; (iv) the amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1 is a valine; (v) the amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1 is a histidine; and (vi) the amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1 is a glutamine.

[0119] In certain aspects, a DQ beta chain described herein has an increased affinity for a CD4 protein as compared to a reference HLA class II molecule. In some aspects, the reference HLA class II molecule is an HLA class II molecule having a wild-type DQ beta chain. In some aspects, the reference HLA class II molecule is an HLA class II molecule having a DQ beta chain comprising (i) a leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 and/or (ii) a valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1. In some aspects, the reference HLA class II molecule is an HLA class II molecule having a DQ beta chain comprising (i) a leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, (ii) a valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1, (iii) an asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1, (iv) an isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1, (iii) a serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and/or (iv) a proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

[0120] In some aspects, the increased affinity for CD4 is at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, at least about 1000-fold, at least about 1500-

fold, at least about 2000-fold, at least about 2500-fold, at least about 3000-fold, at least about 3500-fold, at least about 4000-fold, at least about 4500-fold, or at least about 4000-fold greater than the affinity of the reference HLA class II molecule for CD4.

[0121] In some aspects, the increased affinity for CD4 is at least about 1.5-fold to at least about 5000-fold, 1.5-fold to at least about 4000-fold, 1.5-fold to at least about 3000-fold, 1.5-fold to at least about 2000-fold, 1.5-fold to at least about 1000-fold, 10-fold to at least about 5000-fold, 10-fold to at least about 4000-fold, 10-fold to at least about 3000-fold, 10-fold to at least about 2000-fold, 10-fold to at least about 1000-fold, 10-fold to at least about 900-fold, 10-fold to at least about 800-fold, 10-fold to at least about 700-fold, 10-fold to at least about 600-fold, 10-fold to at least about 500-fold, 10-fold to at least about 400-fold, 10-fold to at least about 300-fold, 10-fold to at least about 200-fold, 10-fold to at least about 100-fold, 100-fold to at least about 5000-fold, 100-fold to at least about 4000-fold, 100-fold to at least about 3000-fold, 100-fold to at least about 2000-fold, 100-fold to at least about 1000-fold, 100-fold to at least about 900-fold, 100-fold to at least about 800-fold, 100-fold to at least about 700-fold, 100-fold to at least about 600-fold, 100-fold to at least about 500-fold, 100-fold to at least about 400-fold, 100-fold to at least about 300-fold, or 100-fold to at least about 200-fold greater than the affinity of the reference HLA class II molecule for CD4.

[0122] In certain aspects, the DQ beta chain comprises an allele selected from an HLA-DQB1*02, an HLA-DQB1*03, an HLA-DQB1*04, an HLA-DQB1*05, and an HLA-DQB1*06 allele. In certain aspects, the DQ beta chain comprises an HLA-DQB1*05 allele. In particular aspects, the DQ beta chain comprises an HLA-DQB1*05:01 allele.

[0123] In certain aspects, the DQ beta chain comprises an allele selected from DQB1*02:01:01, DQB1*02:01:02, DQB1*02:01:03, DQB1*02:01:04, DQB1*02:01:05, DQB1*02:01:06, DQB1*02:01:07, DQB1*02:01:08, DQB1*02:01:09, DQB1*02:01:10, DQB1*02:01:11, DQB1*02:01:12, DQB1*02:01:13, DQB1*02:01:14, DQB1*02:01:15, DQB1*02:01:16, DQB1*02:01:17, DQB1*02:01:18, DQB1*02:01:19, DQB1*02:01:20, DQB1*02:01:21, DQB1*02:01:22, DQB1*02:01:23, DQB1*02:01:24, DQB1*02:01:25, DQB1*02:01:26, DQB1*02:01:27, DQB1*02:01:28, DQB1*02:01:29, DQB1*02:01:30, DQB1*02:01:31, DQB1*02:02:01:01, DQB1*02:02:01:02, DQB1*02:02:01:03, DQB1*02:02:01:04, DQB1*02:02:02, DQB1*02:02:03, DQB1*02:02:04, DQB1*02:02:05, DQB1*02:02:06, DQB1*02:02:07, DQB1*02:02:08, DQB1*02:02:09, DQB1*02:03:01, DQB1*02:03:02, DQB1*02:04, DQB1*02:05, DQB1*02:06, DQB1*02:07:01,

DQB1*02:07:02, DQB1*02:08, DQB1*02:09, DQB1*02:10, DQB1*02:100, DQB1*02:101, DQB1*02:102, DQB1*02:103, DQB1*02:104, DQB1*02:105, DQB1*02:106, DQB1*02:107, DQB1*02:108, DQB1*02:109, DQB1*02:11, DQB1*02:110, DQB1*02:111, DQB1*02:112, DQB1*02:113, DQB1*02:114, DQB1*02:115, DQB1*02:116, DQB1*02:117, DQB1*02:118, DQB1*02:119, DQB1*02:12, DQB1*02:120, DQB1*02:121, DQB1*02:122, DQB1*02:123, DQB1*02:124, DQB1*02:125, DQB1*02:126, DQB1*02:127, DQB1*02:128, DQB1*02:129N, DQB1*02:13, DQB1*02:130, DQB1*02:131, DQB1*02:132N, DQB1*02:133, DQB1*02:134N, DQB1*02:135, DQB1*02:136, DQB1*02:137, DQB1*02:138, DQB1*02:139, DQB1*02:140, DQB1*02:141, DQB1*02:142, DQB1*02:14:01, DQB1*02:14:02, DQB1*02:15, DQB1*02:16, DQB1*02:17, DQB1*02:18N, DQB1*02:19, DQB1*02:20N, DQB1*02:21, DQB1*02:22, DQB1*02:23, DQB1*02:24, DQB1*02:25, DQB1*02:26, DQB1*02:27, DQB1*02:28, DQB1*02:29, DQB1*02:30, DQB1*02:31, DQB1*02:32, DQB1*02:33, DQB1*02:34, DQB1*02:35, DQB1*02:36, DQB1*02:37, DQB1*02:38, DQB1*02:39, DQB1*02:40, DQB1*02:41, DQB1*02:42, DQB1*02:43, DQB1*02:44, DQB1*02:45, DQB1*02:46, DQB1*02:47, DQB1*02:48, DQB1*02:49, DQB1*02:50, DQB1*02:51, DQB1*02:52, DQB1*02:53Q, DQB1*02:54, DQB1*02:55, DQB1*02:56, DQB1*02:57, DQB1*02:58N, DQB1*02:59, DQB1*02:60, DQB1*02:61, DQB1*02:62, DQB1*02:63, DQB1*02:64, DQB1*02:65, DQB1*02:66, DQB1*02:67NX, DQB1*02:68, DQB1*02:69, DQB1*02:70, DQB1*02:71, DQB1*02:72, DQB1*02:73, DQB1*02:74, DQB1*02:75, DQB1*02:76, DQB1*02:77, DQB1*02:78, DQB1*02:79, DQB1*02:80, DQB1*02:81, DQB1*02:82, DQB1*02:83, DQB1*02:84, DQB1*02:85, DQB1*02:86, DQB1*02:87, DQB1*02:88, DQB1*02:89:01, DQB1*02:89:02, DQB1*02:90, DQB1*02:91, DQB1*02:92, DQB1*02:93, DQB1*02:94, DQB1*02:95, DQB1*02:96N, DQB1*02:97, DQB1*02:98, DQB1*02:99, DQB1*03:01:01:01, DQB1*03:01:01:02, DQB1*03:01:01:03, DQB1*03:01:01:04, DQB1*03:01:01:05, DQB1*03:01:01:06, DQB1*03:01:01:07, DQB1*03:01:01:08, DQB1*03:01:01:09, DQB1*03:01:01:10, DQB1*03:01:01:11, DQB1*03:01:01:12, DQB1*03:01:01:14, DQB1*03:01:01:15, DQB1*03:01:01:16, DQB1*03:01:01:17, DQB1*03:01:01:18, DQB1*03:01:01:19, DQB1*03:01:01:20, DQB1*03:01:02, DQB1*03:01:03, DQB1*03:01:04, DQB1*03:01:05, DQB1*03:01:06, DQB1*03:01:07, DQB1*03:01:08, DQB1*03:01:09, DQB1*03:01:10, DQB1*03:01:11, DQB1*03:01:12, DQB1*03:01:13, DQB1*03:01:14, DQB1*03:01:15, DQB1*03:01:16,

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DQB1*06:53:02, DQB1*06:54N, DQB1*06:55, DQB1*06:56, DQB1*06:57, DQB1*06:58,
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DQB1*06:65, DQB1*06:66, DQB1*06:67, DQB1*06:68, DQB1*06:69:01,
DQB1*06:69:02, DQB1*06:70, DQB1*06:71, DQB1*06:72, DQB1*06:73, DQB1*06:74,
DQB1*06:75NX, DQB1*06:76, DQB1*06:77N, DQB1*06:78, DQB1*06:79:01,
DQB1*06:79:02, DQB1*06:80, DQB1*06:81, DQB1*06:82, DQB1*06:83, DQB1*06:84,
DQB1*06:85, DQB1*06:86, DQB1*06:87, DQB1*06:88, DQB1*06:89, DQB1*06:90,
DQB1*06:91, DQB1*06:92:01, DQB1*06:92:02, DQB1*06:93, DQB1*06:94,

DQB1*06:95, DQB1*06:96:01, DQB1*06:96:02, DQB1*06:97, DQB1*06:98, DQB1*06:99:01, DQB1*06:99:02, and any combination thereof.

[0124] In certain aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 3, wherein the DQ beta chain comprises a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, and wherein the DQ beta chain comprises a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1. In certain aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 3, wherein the DQ beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, (ii) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1, (iii) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1; (iv) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1; (v) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1; and (vi) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1. In certain aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence set forth in SEQ ID NO: 3.

II.A.2. MHC Class II Alpha Chain

[0125] In some aspects of the present disclosure, the MHC class II molecule further comprises an alpha chain. In some aspects, the alpha chain is a wild-type alpha chain. In some aspects, the alpha chain is a DQ alpha chain. Any DQ alpha chain can be used in the compositions and methods of the present disclosure. In some aspects, the DQ alpha chain comprises an HLA-DQA1*01, HLA-DQA1*02, HLA-DQA1*03, HLA-DQA1*04, HLA-DQA1*05, or HLA-DQA1*06 allele. In certain aspects, the DQ alpha chain comprises an HLA-DQA1*01 allele. In certain aspects, the DQ alpha chain comprises an HLA-DQA1*02 allele. In certain aspects, the DQ alpha chain comprises an HLA-DQA1*03 allele. In certain aspects, the DQ alpha chain comprises an HLA-DQA1*04 allele. In certain aspects, the DQ

alpha chain comprises an HLA-DQA1*05 allele. In certain aspects, the DQ alpha chain comprises an HLA-DQA1*06 allele.

[0126] In certain aspects, the DQ alpha chain is selected from DQA1*01:01:01:01, DQA1*01:01:01:02, DQA1*01:01:01:03, DQA1*01:01:01:05, DQA1*01:01:01:06, DQA1*01:01:02, DQA1*01:01:03, DQA1*01:01:04, DQA1*01:01:05, DQA1*01:02:01:01, DQA1*01:02:01:02, DQA1*01:02:01:03, DQA1*01:02:01:04, DQA1*01:02:01:05, DQA1*01:02:01:06, DQA1*01:02:01:07, DQA1*01:02:01:08, DQA1*01:02:01:09, DQA1*01:02:01:10, DQA1*01:02:01:11, DQA1*01:02:01:12, DQA1*01:02:02:01, DQA1*01:02:02:02, DQA1*01:02:02:03, DQA1*01:02:02:04, DQA1*01:02:03, DQA1*01:02:04, DQA1*01:03:01:01, DQA1*01:03:01:02, DQA1*01:03:01:03, DQA1*01:03:01:04, DQA1*01:03:01:05, DQA1*01:03:01:06, DQA1*01:03:01:07, DQA1*01:03:01:08, DQA1*01:03:01:09, DQA1*01:04:01:01, DQA1*01:04:01:02, DQA1*01:04:01:03, DQA1*01:04:01:04, DQA1*01:04:02, DQA1*01:05:01, DQA1*01:05:02, DQA1*01:06, DQA1*01:07Q, DQA1*01:08, DQA1*01:09, DQA1*01:10, DQA1*01:11, DQA1*01:12, DQA1*01:13, DQA1*01:14, DQA1*01:15N, DQA1*01:16N, DQA1*01:17, DQA1*01:18, DQA1*01:19, DQA1*01:20, DQA1*01:21, DQA1*01:22, DQA1*01:23, DQA1*01:24, DQA1*01:25, DQA1*01:26, DQA1*02:01:01:01, DQA1*02:01:01:02, DQA1*02:01:02, DQA1*02:02N, DQA1*02:03, DQA1*03:01:01, DQA1*03:01:03, DQA1*03:02:01:01, DQA1*03:02:01:02, DQA1*03:03:01:01, DQA1*03:03:01:02, DQA1*03:03:01:03, DQA1*03:03:01:04, DQA1*03:03:01:05, DQA1*03:03:01:06, DQA1*03:03:01:07, DQA1*03:03:02, DQA1*03:04, DQA1*03:05, DQA1*03:06, DQA1*03:07, DQA1*04:01:01:01, DQA1*04:01:01:02, DQA1*04:01:01:03, DQA1*04:01:01:04, DQA1*04:01:01:05, DQA1*04:01:01:06, DQA1*04:01:01:07, DQA1*04:01:01:08, DQA1*04:01:02:01, DQA1*04:01:02:02, DQA1*04:01:03, DQA1*04:02, DQA1*04:03N, DQA1*04:04, DQA1*04:05, DQA1*05:01:01:01, DQA1*05:01:01:02, DQA1*05:01:01:03, DQA1*05:01:01:04, DQA1*05:01:02, DQA1*05:01:04, DQA1*05:01:05, DQA1*05:01:06, DQA1*05:02, DQA1*05:03:01:01, DQA1*05:03:01:02, DQA1*05:04, DQA1*05:05:01:01, DQA1*05:05:01:02, DQA1*05:05:01:03, DQA1*05:05:01:04, DQA1*05:05:01:05, DQA1*05:05:01:06, DQA1*05:05:01:07, DQA1*05:05:01:08, DQA1*05:05:01:09, DQA1*05:05:01:10, DQA1*05:05:01:11, DQA1*05:05:01:12, DQA1*05:05:01:13, DQA1*05:05:01:14, DQA1*05:05:01:15, DQA1*05:05:01:16, DQA1*05:05:01:17, DQA1*05:05:01:18,

DQA1*05:05:01:19, DQA1*05:05:01:20, DQA1*05:06:01:01, DQA1*05:06:01:02, DQA1*05:07, DQA1*05:08, DQA1*05:09, DQA1*05:10, DQA1*05:11, DQA1*05:12, DQA1*05:13, DQA1*05:14, DQA1*05:15N, DQA1*06:01:01:01, DQA1*06:01:01:02, DQA1*06:01:01:03, DQA1*06:01:01:04, DQA1*06:01:02, DQA1*06:02, and any combination thereof.

II.A.3. Signal Peptide

[0127] In some aspects, the DQ beta chain and/or the DQ alpha chain further comprises a signal peptide. Any signal peptide known in the art can be used in the compositions and methods disclosed herein. In some aspects the DQ beta chain signal peptide is the same as the DQ alpha signal peptide. In some aspects the DQ beta chain signal peptide is different from the DQ alpha signal peptide.

[0128] In some aspects, the signal peptide is derived from a native signal peptide. In some aspects, the signal peptide is derived from a naturally occurring DQ beta chain signal peptide. In some aspects, the signal peptide comprises a naturally occurring DQ beta chain signal peptide. In some aspects, the signal peptide is derived from a naturally occurring DQ alpha chain signal peptide. In some aspects, the signal peptide comprises a naturally occurring DQ alpha chain signal peptide. In some aspects, the signal peptide is derived from a fibroin light chain (FibL) signal peptide. In some aspects, the signal peptide comprises SEQ ID NO: 9. In some aspects, the signal peptide is synthetic.

II.A.4. Transmembrane Domain

[0129] In some aspects, the DQ beta chain and/or the DQ alpha chain further comprises a transmembrane domain. The transmembrane domain can be any length and of any origin. In some aspects, the transmembrane domain is at least about 1 to at least about 50 amino acid in length. In some aspects, the transmembrane domain is derived from a naturally occurring transmembrane domain. In some aspects, the transmembrane domain comprises a naturally occurring transmembrane domain. In some aspects, the transmembrane domain is derived from a naturally occurring HLA transmembrane domain. In some aspects, the transmembrane domain comprises a naturally occurring HLA transmembrane domain. In some aspects, the transmembrane domain is derived from a naturally occurring DQ beta chain transmembrane domain. In some aspects, the transmembrane domain comprises a naturally occurring DQ beta chain transmembrane domain. In some aspects, the transmembrane domain is derived from a naturally occurring DQ alpha chain transmembrane domain. In some aspects, the

transmembrane domain comprises a naturally occurring DQ alpha chain transmembrane domain.

II.A.5. Leucine Zipper

[0130] In some aspects, the DQ beta chain and/or the DQ alpha chain further comprises one or more leucine zipper (LZip) sequences. Any LZip sequence known in the art can be used in the compositions and methods disclosed herein. In some aspects, the DQ beta chain and/or the DQ alpha chain comprises an acidic LZip (α LZip), a basic LZip (β LZip), or both. In some aspects, the one or more LZip sequences are derived from a naturally occurring LZip sequence. In some aspects, the one or more LZip sequences comprise a naturally occurring LZip sequence. In some aspects, the one or more LZip sequences are synthetic. In certain aspects, the one or more LZip sequences comprise the LZip sequences set forth in SEQ ID NO: 4 (Table 1).

II.A.6. Linker

[0131] In some aspects, the DQ beta chain and/or the DQ alpha chain useful for the disclosure further comprises a linker. Any linker known in the art can be used in the compositions and methods disclosed herein. In certain aspects, the linker comprises a Gly/Ser linker. In some aspects, the linker comprises an amino acid sequence selected from GlySer, Gly₂Ser, Gly₃Ser, and Gly₄Ser. In some aspects, the linker is positioned at the N-terminus of the extracellular domain of the DQ alpha chain or the DQ beta chain. In some aspects, the linker is positioned at the C-terminus of the extracellular domain of the DQ alpha chain or the DQ beta chain. In some aspects, the linker is positioned between the extracellular domain of the DQ alpha chain or the DQ beta chain and the transmembrane domain. In some aspects, the linker is positioned between the extracellular domain of the DQ alpha chain or the DQ beta chain and the one or more LZip sequences. In some aspects, the linker is positioned between the extracellular domain of the DQ alpha chain or the DQ beta chain and the signal peptide.

[0132] A linker of any length can be used in the compositions and methods disclosed herein. In some aspects, the linker is at least one amino acid in length. In some aspects, the linker is at least about 1 to at least about 100, at least about 1 to at least about 90, at least about 1 to at least about 80, at least about 1 to at least about 70, at least about 1 to at least about 60, at least about 1 to at least about 50, at least about 1 to at least about 40, at least about 1 to at least about 30, at least about 1 to at least about 20, at least about 1 to at least

about 15, at least about 1 to at least about 14, at least about 1 to at least about 13, at least about 1 to at least about 12, at least about 1 to at least about 11, at least about 1 to at least about 10, at least about 1 to at least about 9, at least about 1 to at least about 8, at least about 1 to at least about 7, at least about 1 to at least about 6, at least about 1 to at least about 5, at least about 1 to at least about 4, at least about 1 to at least about 3 amino acids in length.

[0133] In some aspects, the linker is at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100 amino acids in length. In certain aspects, the linker is about 3 amino acids in length. In certain aspects, the linker is about 4 amino acids in length. In certain aspects, the linker is about 5 amino acids in length.

II.B. Cells

[0134] In certain aspects of the present disclosure, the MHC class II molecule of the present disclosure is linked to or associated with a membrane of a cell. In certain aspects, the beta chain of the MHC class II molecule is linked or associated with a membrane of a cell. In certain aspects, the alpha chain of the MHC class II molecule is linked or associated with a membrane of a cell. In certain aspects, the alpha chain and the beta chain of the MHC class II molecule are linked or associated with a membrane of a cell.

[0135] Certain aspects of the present disclosure are directed to cells comprising an MHC class II molecule disclosed herein. Any cell can be used in the compositions described herein. In certain aspects the cell is a mammalian cell. In some aspects, the cell is an insect cell. In some aspects, the cell is derived from a healthy cell, *e.g.*, a health fibroblast cell. In some aspects the cell is derived from a tumor cell. Non-limiting examples of cells that are useful in the present disclosure include K562 cells, T2 cells, HEK293 cells, HEK293T cells, A375 cells, SK-MEL-28 cells, Me275 cells, COS cells, fibroblast cells, tumor cells, or any combination thereof. In certain aspects, the cell is any cell disclosed in Hasan et al., *Adv. Genet. Eng.* 4(3):130 (2015), which is incorporated by reference herein in its entirety.

[0136] In certain aspects, the cell is a professional APC. In certain aspects, the cell is a macrophage, a B cell, a dendritic cell, or any combination thereof.

[0137] In certain aspects, the cell lacks endogenous expression of one or more MHC class II allele. In some aspects the cell lacks endogenous expression of an HLA-DQ allele. In some aspects the cell lacks endogenous expression of an HLA-DQ alpha chain allele. In some aspects the cell lacks endogenous expression of an HLA-DQ beta chain allele.

II.C. Soluble MHC Class II Molecules

[0138] In certain aspects, the MHC class II molecule is not associated with a membrane of a cell, *e.g.*, the MHC class II molecule is in a soluble form. As used herein, a soluble MHC class II molecule includes any MHC class II molecule or a portion thereof, described herein, that is not associated with a cell membrane. In certain aspects, the MHC class II molecule or portion thereof is unbound to any membrane. In some aspects, the MHC class II molecule or portion thereof is bound to an inert particle. In some aspects, the MHC class II molecule or portion thereof is bound to the membrane of an extracellular vesicle. In some aspects, the MHC class II molecule is bound to an artificial membrane or an artificial surface, *e.g.*, the surface of an array plate.

[0139] Any inert particle known in the art can be used in the compositions and methods of the present disclosure. In some aspects, the inert particle is a bead. In some aspects, the bead is a glass bead, a latex bead, a metal bead, or any combination thereof. In some aspects, the inert particle is a nanoparticle (NP). Any NP known in the art can be used in the compositions and methods of the present disclosure. In certain aspects, the nanoparticle is selected from a pegylated iron oxide, chitosan, dextrane, gelatin, alginate, liposome, starch, branched polymer, carbon-based carrier, polylactic acid, poly(cyano)acrylate, polyethylenimine, block copolymer, polycaprolactone, SPIONS, USPIONS, Cd/Zn-selenide, or silica nanoparticle. In particular aspects, the nanoparticle is a pegylated iron oxide nanoparticle. Nonlimiting examples of nanoparticles useful in the compositions and methods disclosed herein include those set forth in De Jong and Borm, *Int. J. Nanomedicine* 3(2):133-49 (2008) and Umeshappa et al., *Nat. Commun.* 10(1):2150 (May 14, 2019), each of which is incorporated by reference herein in its entirety.

[0140] In some aspects, the MHC class II molecule comprises a fragment of a full length MHC class II molecule, wherein one or more amino acids of the transmembrane domain of the alpha chain and/or the transmembrane domain of the beta chain are deleted. In some aspects, the MHC class II molecule comprises the extracellular domain of the alpha chain

(*e.g.*, as set forth in SEQ ID NO: 6) and/or the extracellular domain of the beta chain (*e.g.*, as set forth in SEQ ID NO: 1 or 3). In certain aspects, the MHC class II molecule comprises a DQ alpha chain comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 6. In some aspects, the MHC class II molecule comprises a DQ alpha chain comprising an amino acid sequence set forth in SEQ ID NO: 6.

[0141] In certain aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 1. In some aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence set forth in SEQ ID NO: 1. In certain aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 3. In some aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence set forth in SEQ ID NO: 3. In certain aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 4. In some aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence set forth in SEQ ID NO: 4. In certain aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 5. In some aspects, the MHC class II molecule comprises a DQ beta chain comprising an amino acid sequence set forth in SEQ ID NO: 5.

II.D. Nucleic Acid Molecules and Vectors

[0142] Certain aspects of the present disclosure are directed to a nucleic acid molecule encoding an MHC class II molecule disclosed herein. In some aspects the nucleic acid molecule encodes an MHC class II beta chain disclosed herein. In certain aspects, the nucleic

acid molecule encoding the MHC class II beta chain comprises a nucleotide sequence having at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the sequence set forth in SEQ ID NO: 2.

[0143] In some aspects the nucleic acid molecule encodes an MHC class II alpha chain disclosed herein. In certain aspects, the nucleic acid molecule encoding the MHC class II alpha chain comprises a nucleotide sequence having at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with the sequence set forth in SEQ ID NO: 7.

[0144] In some aspects, the nucleic acid molecule encodes both an MHC class II alpha chain disclosed herein and an MHC class II beta chain disclosed herein. In some aspects, the sequence encoding the MHC class II alpha chain is under the control of the same promoter as the sequence encoding the MHC class II beta chain. In some aspects, the sequence encoding the MHC class II alpha chain is under the control of a first promoter, and the sequence encoding the MHC class II beta chain is under the control of a second promoter.

[0145] In some aspects, the present disclosure is directed to a first nucleic acid molecule encoding an MHC class II beta chain disclosed herein and a second nucleic acid molecule encoding an MHC class II alpha chain disclosed herein.

[0146] Certain aspects of the present disclosure are directed to a vector or a set of vectors comprising a nucleic acid molecule disclosed herein. In some aspects, the vector is a viral vector. In some aspects, the vector is a viral particle or a virus. In some aspects, the vector is a mammalian vector. In some aspects, the vector is a bacterial vector.

[0147] In certain aspects, the vector is a retroviral vector. In some aspects, the vector is an adenoviral vector, a lentivirus, a Sendai virus, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, or an adeno associated virus (AAV) vector. In particular aspects, the vector is an AAV vector. In some aspects, the vector is a lentivirus. In particular aspects, the vector is an adenoviral vector. In some aspects, the vector is a Sendai virus. In some aspects, the vector is a hybrid vector. Examples of hybrid vectors that can be used in the present disclosure can be found in

Huang and Kamihira, *Biotechnol. Adv.* 31(2):208-23 (2103), which is incorporated by reference herein in its entirety.

III. Methods of the Disclosure

[0148] Certain aspects of the present disclosure are directed to methods of treating a disease or condition in a subject. In some aspects, the disclosure is directed to methods of enhancing an immune response in a subject in need thereof.

III.A. Methods of Treating a Tumor

[0149] Certain aspects of the present disclosure are directed to methods of treating a cancer in a subject in need thereof, comprising administering to the subject an HLA class II molecule disclosed herein, a nucleic acid molecule disclosed herein, a vector disclosed herein, or a cell disclosed herein.

[0150] In some aspects, the cancer is selected from melanoma, bone cancer, renal cancer, prostate cancer, breast cancer, colon cancer, lung cancer, cutaneous or intraocular malignant melanoma, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of the cancers. In some aspects, the cancer is melanoma.

- [0151] In some aspects, the cancer is relapsed. In some aspects, the cancer is refractory. In some aspects, the cancer is advanced. In some aspects, the cancer is metastatic.
- [0152] In some aspects, the methods disclosed herein treat a cancer in a subject. In some aspects, the methods disclosed herein reduce the severity of one or more symptom of the cancer. In some aspects, the methods disclosed herein reduce the size or number of a tumor derived from the cancer. In some aspects, the methods disclosed herein increase the overall survival of the subject, relative to a subject not provided the methods disclosed herein. In some aspects, the methods disclosed herein increase the progressive-free survival of the subject, relative to a subject not provided the methods disclosed herein. In some aspects, the methods disclosed herein lead to a partial response in the subject. In some aspects, the methods disclosed herein lead to a complete response in the subject.
- [0153] Certain aspects of the present disclosure are directed to methods of treating an infection in a subject in need thereof, comprising administering to the subject an HLA class II molecule disclosed herein, a nucleic acid molecule disclosed herein, a vector disclosed herein, or a cell disclosed herein. Non-limiting examples of infections that can be treated using the compositions and methods disclosed herein include infection by a virus (including viroids and prions), a bacterium, a fungus, a parasite, or any combination thereof. In some aspects, the virus is herpesvirus, HIV, papovavirus, measles virus, rubella virus, human papillomavirus (HPV), human T-lymphotropic virus 1, Epstein-Barr virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, influenza virus, norovirus, and any combination thereof. In some aspects, the bacterium is selected from Streptococcus, Staphylococcus, and E. coli. In some aspects, the bacterial infection is selected from Brucellosis, Campylobacter infections, Cat-scratch disease, Cholera, Escherichia coli, Gonorrhea, Klebsiella, Enterobacter, Serratia, Legionella infections, Meningococcal infection, Pertussis, Plague, Pseudomonas infection, Salmonella infection, Shigellosis, Typhoid fever, Tularemia, Anthrax, Diphtheria, Enterococcal infection, Erysipelothricosis, Listeriosis, Nocardiosis, Pneumococcal infection, Staphylococcal infection, Streptococcal infection, and any combination thereof. In some embodiments, the parasite infection is selected from pinworm, trichomononiasis, toxoplasmosis, giardiasis, cryptosporidiosis, malaria, hookworm, ringworm, tapeworm, fluke, and any combination thereof. In some aspects, the fungal infection is selected from Candida, Malassezia furfur, dermatophytes (e.g., Epidermophyton, Microsporum, and Trichophyton), or any combination thereof.

[0154] In some aspects, the methods disclosed herein comprise treating a cancer or an infection in a subject in need thereof, comprising administering to the subject a cell described herein, wherein the cell comprises an MHC class II molecule disclosed herein, a nucleic acid molecule disclosed herein, a vector disclosed herein, or any combination thereof.

[0155] In some aspects, the cell is obtained from the subject. In some aspects, the cell is obtained from a donor other than the subject.

III.B. Methods of Enriching a Target Population of T Cells

[0156] Certain aspects of the present disclosure are directed to methods of enriching a target population of T cells obtained from a human subject. In some aspects, the method comprises contacting the T cells with an HLA class II molecule disclosed herein. In some aspects, the method comprises contacting the T cells with a cell, *e.g.*, an APC, disclosed herein. In some aspects, following the contacting, the enriched population of T cells comprises a higher number of T cells capable of binding the HLA class II molecule relative to the number of T cells capable of binding the HLA class II molecule prior to the contacting.

[0157] Some aspects of the present disclosure are directed to a method of selecting a T cell capable of targeting a diseased cell, *e.g.*, a tumor cell. In some aspects, the method comprises contacting a population of isolated T cells *in vitro* with a complex comprising an MHC class II molecule disclosed herein and a fragment of a polypeptide, *e.g.* an antigen expressed by a diseased cell, *e.g.*, a tumor-expressed polypeptide, *e.g.*, an epitope. In some aspects, the T cells are obtained from a human subject.

[0158] The T cells obtained from the human subject can be any T cells disclosed herein. In some aspects, the T cells obtained from the human subject are tumor infiltrating lymphocytes (TIL).

[0159] In some aspects, the method further comprises administering to the human subject the enriched T cells. In some aspects, the subject is preconditioned prior to receiving the T cells, as described herein.

[0160] All of the various aspects, aspects, and options described herein can be combined in any and all variations.

[0161] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication,

patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0162] Having generally described this disclosure, a further understanding can be obtained by reference to the examples provided herein. These examples are for purposes of illustration only and are not intended to be limiting.

EXAMPLES

Example 1 – Methods

[0163] *Cells*

[0164] Peripheral mononuclear cells were obtained via density gradient centrifugation (Ficoll-Paque PLUS, GE Healthcare Life Sciences, Marlborough, MA). The K562 cell line is an erythroleukemic cell line with defective HLA class I/II expression. A375 is melanoma cell lines. HEK293T cells and A375 cells were grown in DMEM supplemented with 10% FBS and 50 µg/ml gentamicin (Thermo Fisher Scientific, Waltham, MA). The K562 and Jurkat 76 cell lines were cultured in RPMI 1640 supplemented with 10% FBS and 50 µg/ml gentamicin.

[0165] *Peptides*

[0166] Synthetic peptides were purchased from Genscript (Piscataway, NJ) and dissolved at 50 µg/ml in DMSO.

[0167] *Antibodies*

[0168] The following antibodies were used for flow cytometry analysis: PE-conjugated anti-class II (9-49 (I3), Beckman Coulter, Brea, CA; Tü39), APC-Cy7-conjugated anti-CD4 (RPA-T4, Biolegend, San Diego, CA) and PE-conjugated anti-His tag (AD1.1.10, Abcam, Cambridge, MA). Dead cells were distinguished with the LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit 465 (Thermo Fisher Scientific, Waltham, MA). Stained cells were analyzed with Canto II or LSRFortessa X-20 (BD Biosciences, Franklin Lakes, NJ). Cell sorting was conducted using a FACS Aria II (BD Biosciences, Franklin Lakes, NJ). Data analysis was performed using FlowJo software (Tree Star, Ashland, OR).

[0169] *TCR transduction into primary T cells*

[0170] CD3⁺ and CD4⁺ T cells were purified using the Pan T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and CD4⁺ T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany), respectively. Purified T cells were stimulated with

aAPC/mOKT3 irradiated with 200 Gy at an E:T ratio of 20:1. Starting the following day, activated T cells were retrovirally transduced with the cloned TCR genes via centrifugation for 1 hour at $1,000 \times g$ at 32°C for 3 consecutive days or using a Retronectin-coated plate (Takara Bio, Shiga, Japan). On the following day, 100 IU/ml IL-2 and 10 ng/ml IL-15 were added to the TCR-transduced T cells. The culture medium was replenished every 2-3 days.

[0171] *Staining with soluble CD4*

[0172] The soluble CD4 (sCD4) gene was generated by fusing the human CD4 extracellular domain with a 6xHis tag via a GS linker. HEK293T cells were retrovirally transduced with the sCD4 gene, and the culture supernatant containing the sCD4 monomer was harvested. sCD4 was dimerized with a PE-labeled anti-6xHis tag mAb (AD1.1.10, Abcam, Cambridge, MA) and used. HLA class II-expressing K562 cells were stained with dimerized sCD4 in the presence of goat serum for 30 min at room temperature. The surface HLA class II expression in K562-derived cells individually expressing various class II genes was as demonstrated in Figs. 3A-3Q.

[0173] *Generation of the HLA class II monomer and dimer*

[0174] The extracellular domain of the wild-type class II α gene was fused with an acidic leucine zipper via a GGGS linker followed by a 6xHis tag via a GS linker (*see* SEQ ID NO: 8). The ectodomain of the class II β gene carrying mutations (*see* SEQ ID NO: 3) was similarly linked with a basic leucine zipper via a GGGS linker (*see* SEQ ID NO: 4). HEK293T cells and A375 cells were transfected with the α and β genes using the 293GPG cell-based retrovirus system and cultured in DMEM supplemented with 10% FBS and 50 μ g/ml gentamicin. For DQ5 dimer staining, HEK293T cells and A375 cells stably secreting soluble DQ5^{L114W/V143M+4reps} (which possesses the N110Q/I116V/S118H/P146N replacements (4reps) in addition to L114W/V143M) protein were grown until confluent, and after forty-eight hours, the medium was harvested. The soluble HLA class II-containing supernatant was then mixed with 100 μ g/ml peptide of interest for 20-24 hours at 37°C for *in vitro* peptide exchange. Monomer that was not subjected to peptide exchange was used as a control. The concentration of the monomer was measured by specific ELISA using a nickel-coated plate (XPressBio, Frederick, MD) and an anti-His tag biotinylated mAb (AD1.1.10, R&D Systems, Minneapolis, MN). Soluble HLA class II monomer was dimerized using PE-conjugated anti-His mAb (AD1.1.10, Abcam, Cambridge, MA) at a 2:1 molar ratio for 1.5 hours at 4°C for staining.

[0175] *HLA class II dimer staining*

[0176] Primary T cells transduced with exogenous TCR gene were pretreated with 50 nM dasatinib (LC Laboratories, Woburn, MA) for 30 min at 37°C⁴⁶ and stained with 5-15 µg/ml class II dimer for 4-5 hours at room temperature. After washing, cell surface molecules were counterstained with an APC-Cy7-conjugated anti-CD4 mAb.

[0177] *Statistical analysis*

[0178] Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA). Unpaired two-tailed Student's t-tests were used for two-sample comparisons. No statistical method was used to predetermine sample size. The investigators were not blinded to allocation during the experiments or outcome assessment. The experiments were not randomized.

Example 2 – DQ molecules with enhanced CD4 binding capacities

[0179] Affinity enhanced DQ molecules were generated by introducing L114W/V143M mutations, to determine if these substitutions could improve the binding of HLA-DQ molecules such as DQ5 (DQA1*01:01-DQB1*05:01) to CD4. DQB1*05:01 encodes four different amino acids at positions 110, 116, 118, and 146 in addition to 114 and 143. We therefore generated K562 cells expressing DQ5^{L114W/V143M+4reps}, which possesses the N110Q/I116V/S118H/P146N replacements (4reps) in addition to L114W/V143M (FIG. 1A), and stained the cells with sCD4. K562 cells expressing DQ5^{L114W/V143M+4reps} but not DQ5^{L114W/V143M}, DQ5^{4reps}, or wild-type DQ5 demonstrated enhanced CD4 binding (FIGs. 1B-1C). Importantly, a series of K562 cells individually expressing various DQ5^{L114W/V143M+4reps} mutants with a single amino acid reversal at one of the four positions lacked the enhanced CD4 binding capability (FIG. 1D). These results suggest that the four additional replacements at N110Q, I116V, S118H, and P146N are critical for the effectiveness of the L114W/V143M mutations in the observed enhanced DQ5:CD4 binding.

[0180] DQβ chains such as DQB1*02:01, 04:02, and 06:01 encode distinct amino acids at positions 110, 118, and 146 but not at 116 (FIG. 1E). Unlike DQB1*05:01, DQB1*02:01, 04:02, and 06:01 encode Val at position 116, similar to DPB1*04:01, which codes for Val at position 114. All the DQ2^{L114W/V143M+3reps}, DQ4^{L114W/V143M+3reps}, and DQ6^{L114W/V143M+3reps} mutants, the β chains of which carry the N110Q, S118H, and P146N replacements (3reps) along with L114W/V143M, showed enhanced CD4 binding activity (Fig. 1F).

Example 3 – Affinity-matured DQ dimers specifically and robustly stained cognate TCRs

[0181] The ability of the affinity-matured DQ dimers carrying the mutations described in example 2 were evaluated for the ability to identify antigen-specific CD4⁺ T cells. The DQ5^{L114W/V143M+4reps} and DQ6^{L114W/V143M+3reps} dimers successfully stained the DQ5-restricted DDX3Y-specific TCR (E6) and DQ6-restricted influenza virus-specific TCR (DM2), respectively (FIGs. 2A-2B).

[0182] To identify affinity-matured class II molecules, the present examples detail multiple mutations in the β -chain but not the α -chain because the β -chain has a more direct interaction with CD4 than the α chain. It is possible that additional mutations of the α - and/or β -chains can further enhance the binding between class II and CD4. However, the use of such soluble class II molecules with excessive CD4 binding capabilities may cause nonspecific staining of CD4⁺ T cells, thereby having a detrimental effect.

[0183] In conclusion, CD4⁺ T cells play a critical role in the development of autoimmune diseases and protection against pathogenic infections and cancers. The novel HLA class II multimer technology described herein may better facilitate the study of HLA class II-restricted CD4⁺ T cell responses across HLA-DQ alleles.

Example 4

[0184] Wild-type DQ5 and DQ5^{L114W/V143M} dimers (Table 3) and DQ5^{L114W/V143M+4reps} dimers were produced and their staining of TCR-transduced CD4⁺ T cells was compared. The wild-type DQ5 dimers could not detect E6-transduced CD4⁺ T cells. The DQ5^{L114W/V143M} dimers showed only weak staining of the E6-transduced CD4⁺ T cells compared to the DQ5^{L114W/V143M+4 reps} dimers, which instead showed robust staining (FIGs. 4A-4L). To validate DQ5^{L114W/V143M+4 reps} dimer staining, we cloned a DQ5-restricted TCR gene specific to GPC3₁₃₈₋₁₅₇ from dimer⁺ CD4⁺ T cells *in vitro* expanded in a peptide-specific manner. When clonotypically reconstituted in human CD4⁺ TCR-deficient T cells, the TCR was successfully stained by the cognate DQ5^{L114W/V143M+4reps} dimer and were functional in a DQ5-restricted and antigen-specific manner (FIGs. 5A-5G).

Table 3: TCR Sequences

No.	Peptide	TRAV	TRAJ	TCR α CDR3	TRBV	TRBJ	TCR β CDR3
06	GPC3 ₁₃₈₋₁₅₇	9-2*02	27*01	CALYTNAGKSTF (SEQ ID NO: 14)	15*02	2-3*01	CATSRDVSSTDTQYF (SEQ ID NO: 15)

[0185] Methods

[0186] Cells

[0187] Peripheral mononuclear cells were obtained via density gradient centrifugation.

K562-based artificial antigen presenting cells (aAPCs) individually expressing various HLA class II genes as a single HLA allele in conjunction with CD80 and CD83 have been reported previously (Butler, M.O. et al., *PLoS One* 7, e30229 (2012)). The Jurkat 76 cell line is a T cell leukemic cell line lacking endogenous TCR, CD4, and CD8 expression (*see*, Heemskerk, M.H. et al., *Blood* 102, 3530-3540 (2003)). Jurkat 76/CD4 cells were generated by retrovirally transducing the human CD4 gene. A375 cells are a melanoma cell line. HEK293T cells and A375 cells were grown in DMEM supplemented with 10% FBS and 50 μ g/ml gentamicin. The Jurkat 76 cell line was cultured in RPMI 1640 supplemented with 10% FBS and 50 μ g/ml gentamicin.

[0188] Peptides

[0189] Synthetic peptides were dissolved at 50 mg/ml in DMSO.

[0190] Genes

[0191] Novel TCR genes were cloned via 5'-rapid amplification of cDNA ends (RACE) PCR and sequenced as previously described (*see, e.g.*, Nakatsugawa, M. et al., *Sci Rep* 6, 23821 (2016); Nakatsugawa, M. et al., *J Immunol* 194, 3487-3500 (2015); and Ochi, T. et al., *Cancer Immunol Res* 3, 1070-1081 (2015); each of which is incorporated by reference herein in its entirety). All genes were cloned into the pMX retroviral vector and transduced into cell lines using the 293GPG and PG13 cell-based retrovirus system (*see, e.g.*, Hirano, N. et al., *Blood* 107, 1528-1536 (2006); Butler, M.O. et al., *Clin Cancer Res* 13, 1857-1867 (2007); Hirano, N. et al., *Clin Cancer Res* 12, 2967-2975 (2006); each of which is incorporated by reference herein in its entirety).

[0192] Antibodies

[0193] The following antibodies were used for flow cytometry analysis: APC-Cy7-conjugated anti-CD4 (RPA-T4, BIOLEGEND, San Diego, CA; *see* Wooldridge, L. et al., *Eur J Immunol* 36, 1847-1855 (2006)) and PE-conjugated anti-His tag (AD1.1.10, ABCAM, Cambridge, MA). Dead cells were distinguished with the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit. Stained cells were analyzed with FACSCanto II or LSRFortessa X-20. Cell

sorting was conducted using a FACS Aria II. Data analysis was performed using FlowJo software (version 9.9.6).

[0194] Generation of the HLA class II monomer and dimer

[0195] A375 cells were transfected with the α and β genes using the 293GPG cell-based retrovirus system (*see, e.g.,* Hirano, N. et al., *Blood* 107, 1528-1536 (2006); Butler, M.O. et al., *Clin Cancer Res* 13, 1857-1867 (2007); and Hirano, N. et al., *Blood* 108, 2662-2668 (2006); each of which is incorporated by reference herein in its entirety) and cultured in DMEM supplemented with 10% FBS and 50 μ g/ml gentamicin. After forty-eight hours, the conditioned medium was harvested and concentrated by filtration (molecular weight cut-off (MWCO) 10 kDa). The soluble HLA class II-containing supernatant was then mixed with 100 μ g/ml peptide of interest for 20-24 hours at 37°C for *in vitro* peptide exchange. The concentration of the monomer was measured by specific ELISA using a nickel-coated plate and an anti-His tag biotinylated mAb. Soluble HLA class II monomer was dimerized using PE-conjugated anti-His mAb at a 2:1 molar ratio for 1.5 hours at 4°C for staining.

[0196] Stimulation of DQ5-restricted antigen-specific CD4⁺ T cells

[0197] CD4⁺ T cells were purified and then stimulated with DQ5-expressing aAPCs pulsed with GPC3₁₃₈₋₁₅₇ at 10 μ g/ml and irradiated at 200 Gy at an E:T ratio of 20:1. After forty-eight hours, 10 IU/ml IL-2 and 10 ng/ml IL-15 were added to the CD4⁺ T cells. The culture medium supplemented with IL-2 (10 IU/ml) and IL-15 (10 ng/ml) was replenished every 2-3 days. Two weeks later, the T cells were subjected to DQ5^{L114W/V143M+4reps} dimer staining.

[0198] HLA class II dimer staining

[0199] Primary CD4⁺ T cells and Jurkat 76/CD4 T cells transduced with antigen-specific TCR genes were pretreated with 50 nM dasatinib for 30 min at 37°C and stained with 5-15 μ g/ml class II dimers for 4-5 hours at room temperature. After washing, cell surface molecules were counterstained with an APC-Cy7-conjugated anti-CD4 mAb.

[0200] ELISPOT assay

[0201] Cytokine ELISPOT assays were performed as previously reported (*see, e.g.,* Yamashita, Y. et al., *Nat Commun* 8, 15244 (2017); and Anczurowski, M. et al., *Sci Rep* 8, 4804 (2018); each of which is incorporated by reference herein in its entirety).

Claims:

1. An HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1.
2. An HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises a substitution mutation at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, wherein the substitution mutation is with an amino acid other than leucine.
3. The HLA class II molecule of claim 1 or 2, wherein the DQ beta chain further comprises an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.
4. An HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.
5. An HLA class II molecule comprising a DQ beta chain, wherein the DQ beta chain comprises a substitution mutation at a position corresponding to amino acid residue 143 of SEQ ID NO: 1, wherein the substitution mutation is with an amino acid other than valine.
6. The HLA class II molecule of claim 4 or 5, wherein the DQ beta chain further comprises an amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1.
7. The HLA class II molecule of any one of claims 1 to 6, wherein the DQ beta chain further comprises an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1.
8. The HLA class II molecule of any one of claims 1 to 7, wherein the DQ beta chain further comprises an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1.
9. The HLA class II molecule of any one of claims 1 to 8, wherein the DQ beta chain further comprises an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1.

10. The HLA class II molecule of any one of claims 1 to 9, wherein the DQ beta chain further comprises an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.
11. The HLA class II molecule of any one of claims 1 to 10, wherein the DQ beta chain further comprises at least three of:
- (i) an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1,
 - (ii) an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1,
 - (iii) an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and
 - (iv) an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.
12. The HLA class II molecule of any one of claims 1 to 11, wherein the DQ beta chain further comprises:
- (i) an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1,
 - (ii) an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1,
 - (iii) an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and
 - (iv) an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.
13. The HLA class II molecule of any one of claims 1 to 3 and 6 to 12, wherein the amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 comprises a hydrophobic side chain.

14. The HLA class II molecule of any one of claims 1 to 3 and 6 to 13, wherein the amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 is selected from the group consisting of an alanine, a valine, an isoleucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan.
15. The HLA class II molecule of any one of claims 1 to 3 and 6 to 14, wherein the amino acid other than leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a tryptophan.
16. The HLA class II molecule of any one of claims 4 to 15, wherein the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 comprises a hydrophobic side chain.
17. The HLA class II molecule of any one of claims 4 to 16, wherein the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is selected from an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan.
18. The HLA class II molecule of any one of claims 4 to 17, wherein the amino acid other than valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1 is a methionine.
19. The HLA class II molecule of any one of claims 7 to 18, wherein the beta chain of the MHC class II molecule comprises an amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1.
20. The HLA class II molecule of any one of claims 7 to 19, wherein the amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1 is selected from a serine, a threonine, and a glutamine.
21. The HLA class II molecule of any one of claims 7 to 20, wherein the amino acid other than asparagine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1 is a glutamine.

22. The HLA class II molecule of any one of claims 8 to 21, wherein the beta chain of the MHC class II molecule comprises an amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1.
23. The HLA class II molecule of any one of claims 8 to 22, wherein the amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1 is selected from an alanine, a valine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan.
24. The HLA class II molecule of any one of claims 8 to 23, wherein the amino acid other than isoleucine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1 is valine.
25. The HLA class II molecule of any one of claims 9 to 24, wherein the beta chain of the MHC class II molecule comprises an amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1.
26. The HLA class II molecule of any one of claims 9 to 25, wherein the amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1 is selected from an arginine, a histidine, and a lysine.
27. The HLA class II molecule of any one of claims 9 to 26, wherein the amino acid other than serine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1 is a histidine.
28. The HLA class II molecule of any one of claims 10 to 27, wherein the beta chain of the MHC class II molecule comprises an amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.
29. The HLA class II molecule of any one of claims 10 to 28, wherein the amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1 is selected from a serine, a threonine, an asparagine, and a glutamine.
30. The HLA class II molecule of any one of claims 10 to 29, wherein the amino acid other than proline at a position corresponding to amino acid residue 146 of SEQ ID NO: 1 is a glutamine.

31. The HLA class II molecule of any one of claims 1 to 30, wherein the DQ beta chain comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to an amino acid sequence selected from SEQ ID NOs: 1, 3, 4, and 5.
32. The HLA of any one of claims 1 to 13, wherein the DQ beta chain comprises a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 and a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.
33. The HLA class II molecule of any one of claims 1 to 32, wherein the beta chain of the MHC class II molecule comprises
- (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1;
 - (b) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; and
 - (c) at least one of:
 - (i) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1;
 - (ii) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1,
 - (iii) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and
 - (iv) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.
34. The HLA class II molecule of any one of claims 1 to 33, wherein the beta chain of the MHC class II molecule comprises
- (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1;
 - (b) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; and
 - (c) at least two of:
 - (i) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1;

- (ii) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1,
- (iii) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and
- (iv) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

35. The HLA class II molecule of any one of claims 1 to 34, wherein the beta chain of the MHC class II molecule comprises

- (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1;
- (b) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1; and
- (c) at least three of:
 - (i) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1;
 - (ii) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1,
 - (iii) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and
 - (iv) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.

36. The HLA class II molecule of any one of claims 1 to 35, wherein the beta chain of the MHC class II molecule comprises

- (a) a tryptophan at a position corresponding to amino acid residue 114 of SEQ ID NO: 1;
- (b) a methionine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1;
- (c) a glutamine at a position corresponding to amino acid residue 110 of SEQ ID NO: 1;
- (d) a valine at a position corresponding to amino acid residue 116 of SEQ ID NO: 1,
- (e) a histidine at a position corresponding to amino acid residue 118 of SEQ ID NO: 1, and

- (f) a glutamine at a position corresponding to amino acid residue 146 of SEQ ID NO: 1.
37. The HLA class II molecule of any one of claims 1 to 36, wherein the DQ beta chain comprises the amino acid sequence set forth in SEQ ID NO: 3.
38. The HLA class II molecule of any one of claims 1 to 37, wherein the DQ beta chain comprises the amino acid sequence set forth in SEQ ID NO: 4.
39. The HLA class II molecule of any one of claims 1 to 38, wherein the beta chain of the HLA class II molecule comprises a DQ2, DQ3, DQ4, DQ5, or DQ6 allele.
40. The HLA class II molecule of any one of claims 1 to 38, wherein the beta chain of the MHC class II molecule comprises an HLA-DQB1*02, HLA-DQB1*03, HLA-DQB1*04, HLA-DQB1*05, or HLA-DQB1*06 allele.
41. The HLA class II molecule of any one of claims 1 to 40, further comprising a DQ alpha chain.
42. The HLA class II molecule of any one of claim 41, wherein the alpha chain of the MHC class II molecule comprises an HLA-DQA1*01, HLA-DQA1*02, HLA-DQA1*03, HLA-DQA1*04, HLA-DQA1*05, or HLA-DQA1*06 allele.
43. The HLA class II molecule of claim 41 or 42, wherein the DQ alpha chain comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 6 or 8.
44. The HLA class II molecule of claim 43, wherein the DQ alpha chain comprises the amino acid sequence set forth in SEQ ID NO: 6 or 8.
45. The HLA class II molecule of any one of claims 1 to 44, wherein the DQ beta chain has an increased affinity for a CD4 protein as compared to a reference HLA class II molecule, wherein the reference HLA class II molecule comprises a DQ beta chain comprising (i) a leucine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1 and/or (ii) a valine at a position corresponding to amino acid residue 143 of SEQ ID NO: 1.
46. The HLA class II molecule of claim 45, wherein the increased affinity is at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least

about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 200-fold, at least about 300-fold, at least about 400-fold, at least about 500-fold, or at least about 1000.

47. The HLA class II molecule of any one of claims 1 to 46, wherein the DQ beta chain is bound to a membrane of a cell.
48. The HLA class II molecule of any one of claims 1 to 46, wherein the DQ beta chain is not bound to a membrane of a cell.
49. The HLA class II molecule of any one of claims 1 to 46, wherein the DQ beta chain comprises an extracellular domain of a full length DQ alpha chain.
50. The HLA class II molecule of claim 49, wherein the DQ beta chain does not comprise a transmembrane domain of a full length DQ beta chain.
51. The HLA class II molecule of any one of claims 41 to 50, wherein the DQ alpha chain is bound to a membrane of a cell.
52. The HLA class II molecule of any one of claims 41 to 50, wherein the DQ alpha chain is not bound to a membrane of a cell.
53. The HLA class II molecule of any one of claims 41 to 50, wherein the DQ alpha chain comprises an extracellular domain of a full length DQ alpha chain.
54. The HLA class II molecule of claim 53, wherein the DQ alpha chain does not comprise a transmembrane domain of a full length DQ alpha chain.
55. The HLA class II molecule of any one of claims 48 to 54, wherein the DQ beta chain is linked to or associated with an inert particle.
56. The HLA class II molecule of claim 55, wherein the inert particle is a bead.
57. The HLA class II molecule of claim 55, the inert particle is a nanoparticle.
58. The HLA class II molecule of claim 57, wherein the nanoparticle is selected from a pegylated iron oxide, chitosan, dextrane, gelatin, alginate, liposome, starch, branched polymer, carbon-

based carrier, polylactic acid, poly(cyano)acrylate, polyethyleinimine, block copolymer, poly caprolactone, SPIONS, USPIONS, Cd/Zn-selenide, or silica nanoparticle.

59. The HLA class II molecule of claim 57 or 58, wherein the nanoparticle is a pegylated iron oxide nanoparticle.
60. The HLA class II molecule of any one of claims 1 to 59, wherein the DQ beta chain comprises a signal peptide.
61. The HLA class II molecule of any one of claims 41 to 60, wherein the DQ alpha chain comprises a signal peptide.
62. The HLA class II molecule of claim 60 or 61, wherein the signal peptide comprises the amino acid sequence set forth in SEQ ID NO: 9.
63. A nucleic acid molecule encoding the DQ beta chain of any one of claims 1 to 62.
64. The nucleic acid molecule of claim 63, further encoding the DQ alpha chain of any of claims 41 to 63.
65. The nucleic acid molecule of claim 63 or 64, comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO: 2.
66. A vector comprising the nucleic acid molecule of any one of claims 63 to 65.
67. A cell comprising the HLA class II molecule of any one of claims 1 to 62, the nucleic acid molecule of any one of claims 63 to 65, or the vector of claim 66.
68. The cell of claim 67, which is a mammalian cell or an insect cell.
69. The cell of claim 67 or 68, which is selected from a K562 cell, T2, HEK293, HEK293T, A375, SK-MEL-28, Me275, COS, a fibroblast cell, a tumor cell, or any combination thereof.
70. The cell of any one of claims 67 to 69, which lacks endogenous MHC class II DQ beta chain expression.

71. The cell of any one of claims 67 to 70, which lacks endogenous MHC class II DQ alpha chain expression.
72. A method of identifying a T cell receptor capable of binding an epitope in an MHC class II complex, comprising pulsing the cell of any one of claims 67 to 71 with one or more peptide comprising the epitope, and stimulating one or more CD4⁺ T cell with the APC.
73. A method of treating a disease or condition in a subject in need thereof, comprising administering to the subject the MHC class II molecule of any one of claims 1 to 62.
74. The method of claim 73, wherein the disease or condition is cancer or an infection.
75. The method of claim 74, wherein the cancer is selected from the group consisting of melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, stomach cancer, uterine cancer, lung cancer, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), cancer of the esophagus, cancer of the small intestine, cancer of the urethra, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, glioma, squamous cell cancer, and combinations of said cancers.
76. The method of claim 74 or 75, wherein the cancer is relapsed or refractory.
77. The method of any one of claims 74 to 76, wherein the cancer is locally advanced.
78. The method of any one of claims 74 to 77, wherein the cancer is advanced.
79. The method of any one of claims 74 to 78, wherein the cancer is metastatic.
80. The HLA class II molecule of any one of claims 1 to 62, which binds CD4 with a K_D of less than about 100 μM.
81. The HLA class II molecule of any one of claims 1 to 62, which binds CD4 with a K_D of less than about 10 μM.
82. The HLA class II molecule of any one of claims 1 to 62, which binds CD4 with a K_D of about 8.9 μM or less.

83. A complex comprising the HLA class II molecule of any one of claims 1 to 62 and 80 to 82 and a peptide, wherein the peptide comprises DDX3Y₁₇₁₋₁₉₀, HA₂₅₅₋₂₇₀, GPC3₁₃₈₋₁₅₇, or any combination thereof.

FIG. 1A

	108	112	114
DPB1*04:01	Q	L	V
	110	114	116
DQB1*05:01	N	L	I
DQB1*05:01 ^{L114W/V143M+4reps}	Q	W	V
	116	141	144
DPB1*04:01	H	V	N
	118	143	146
DQB1*05:01	S	V	P
DQB1*05:01 ^{L114W/V143M+4reps}	H	M	N

FIG. 1B

DQ5

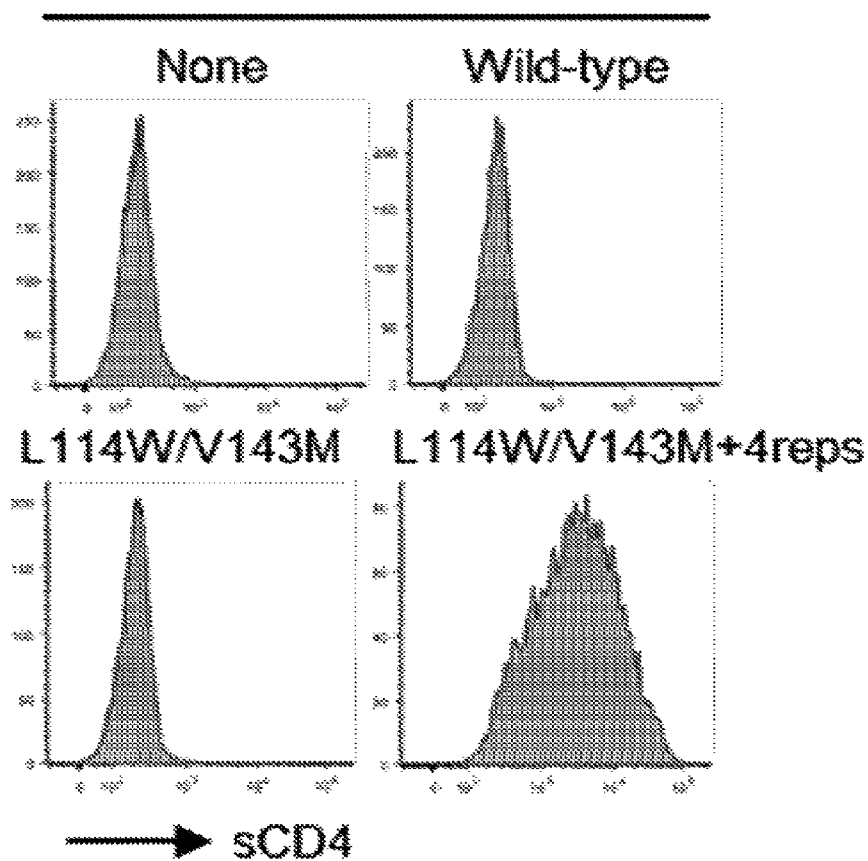


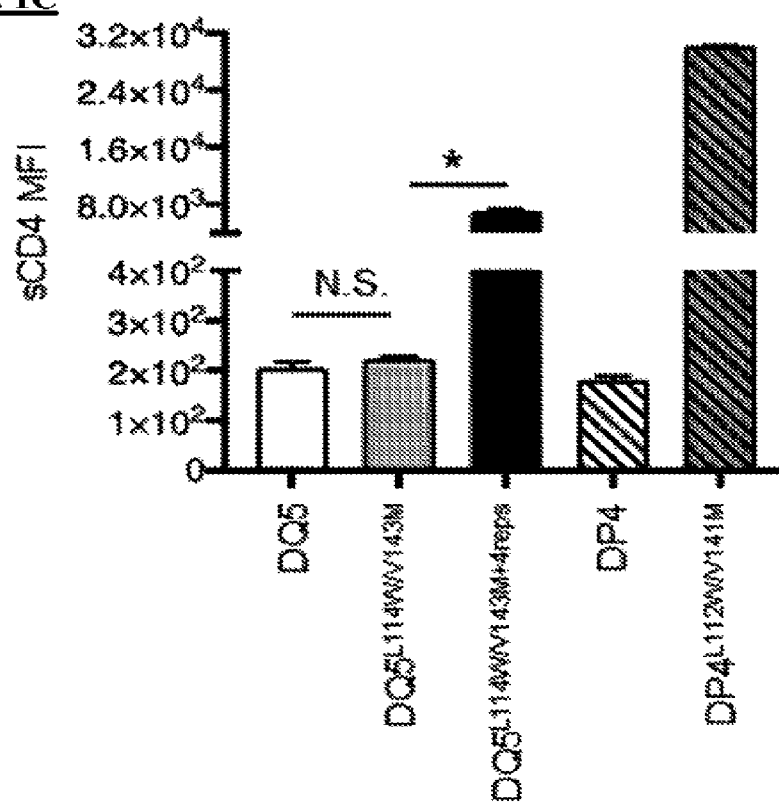
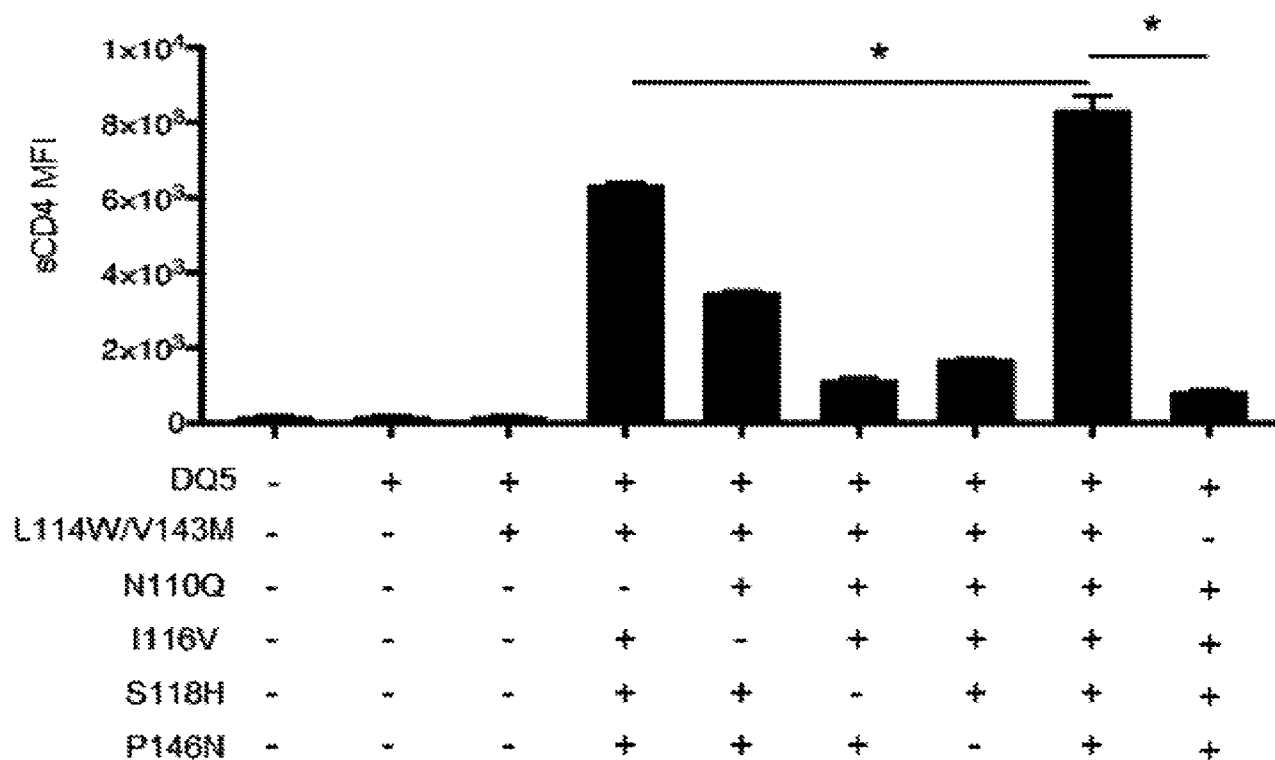
FIG. 1C**FIG. 1D**

FIG. 1E

	108	112	114
DPB1*04:01	Q	L	V
	110	114	116
DQB1*05:01	N	L	I
DQB1*02:01	N	L	V
DQB1*04:02	N	L	V
DQB1*06:01	N	L	V
DQB1 ^{L114W/V143M+3reps}	Q	W	V
	116	141	144
DPB1*04:01	H	V	N
	118	143	146
DQB1*05:01	S	V	P
DQB1*02:01	S	V	P
DQB1*04:02	S	V	P
DQB1*06:01	S	V	P
DQB1 ^{L114W/V143M+3reps}	H	M	N

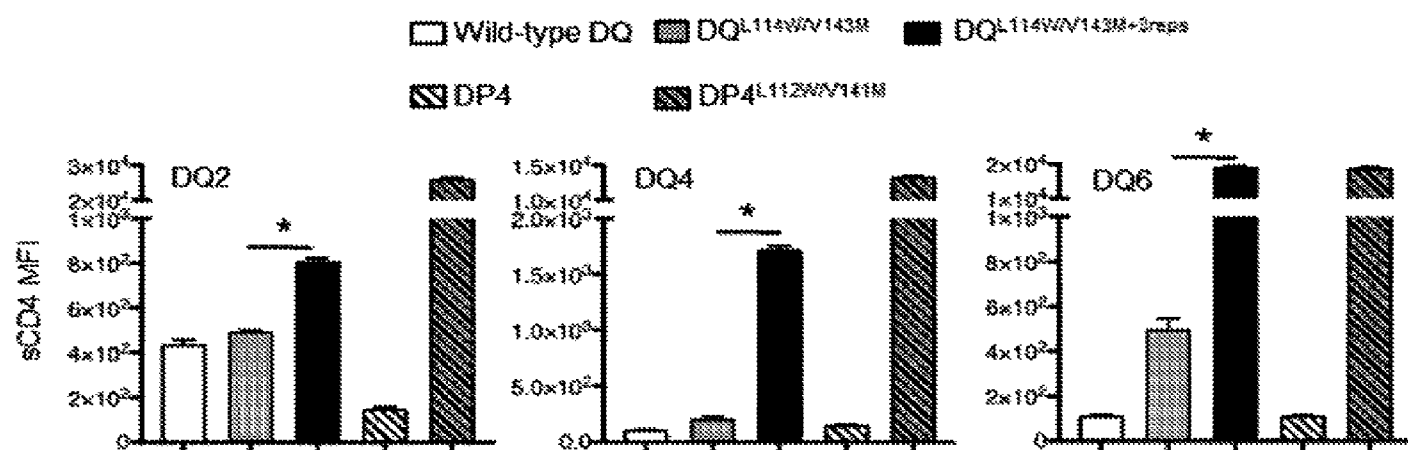
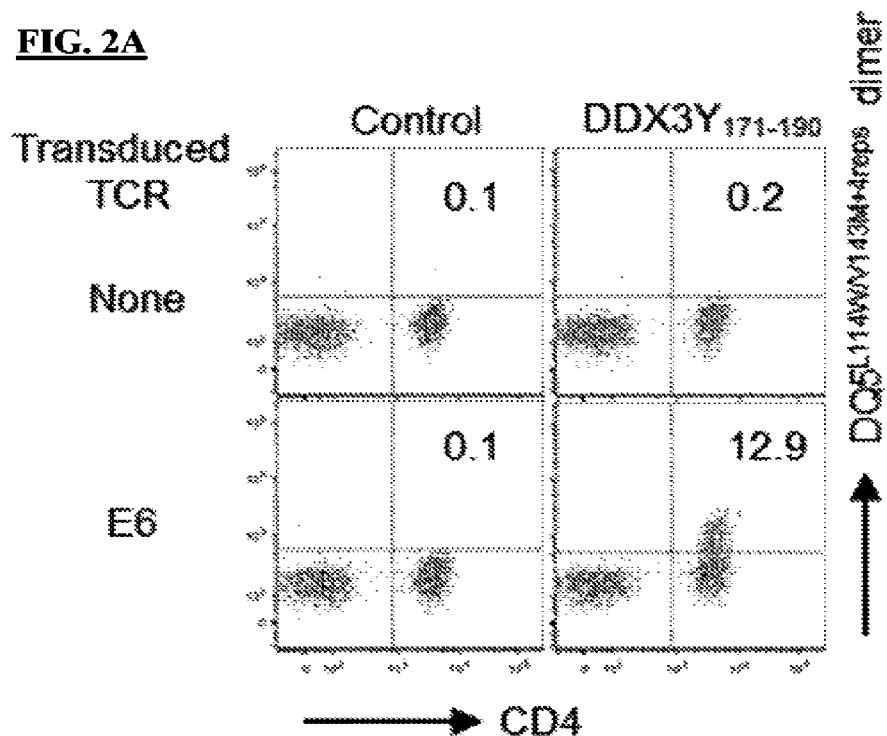
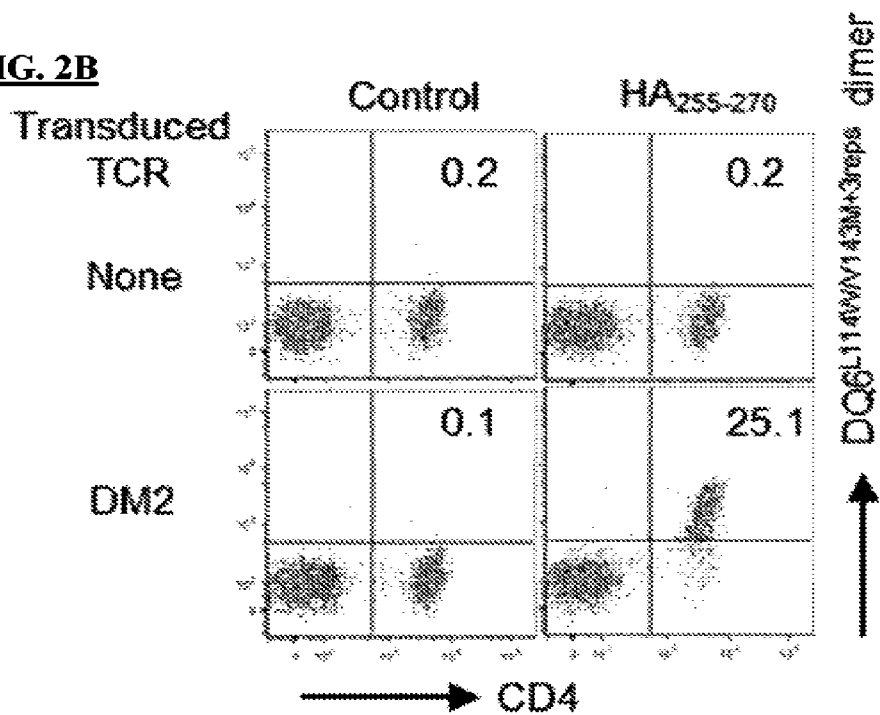
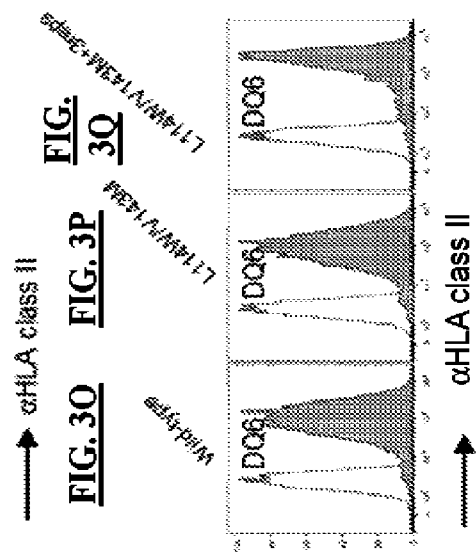
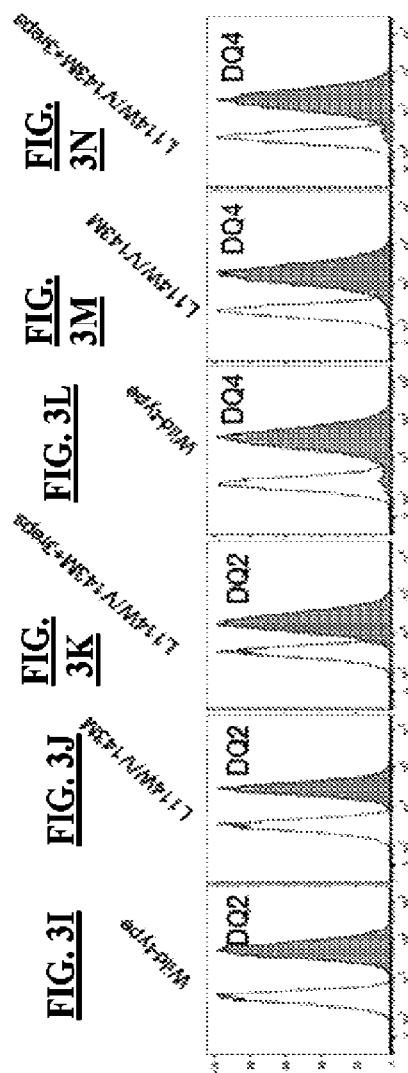
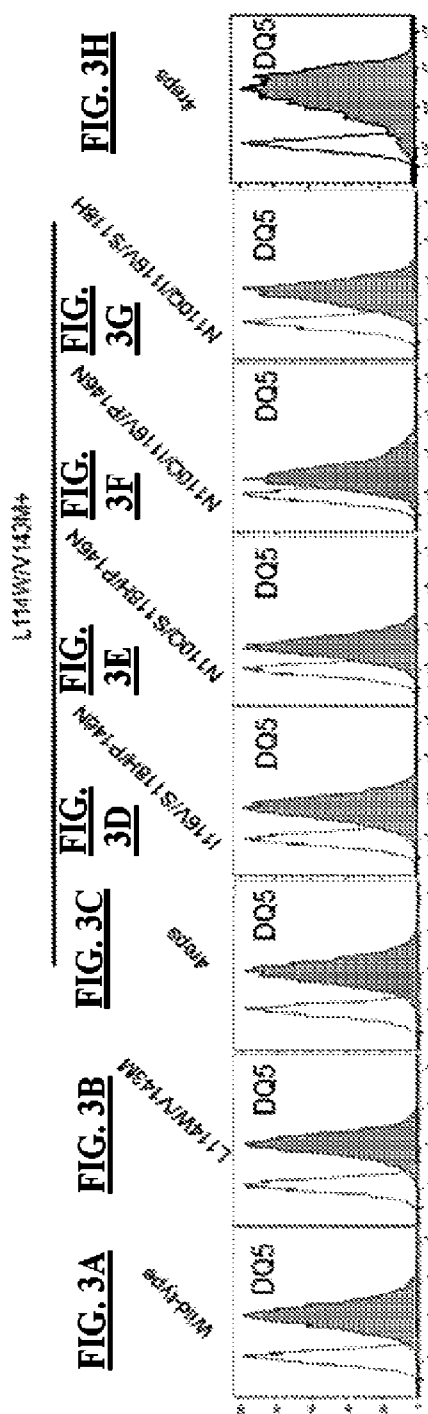
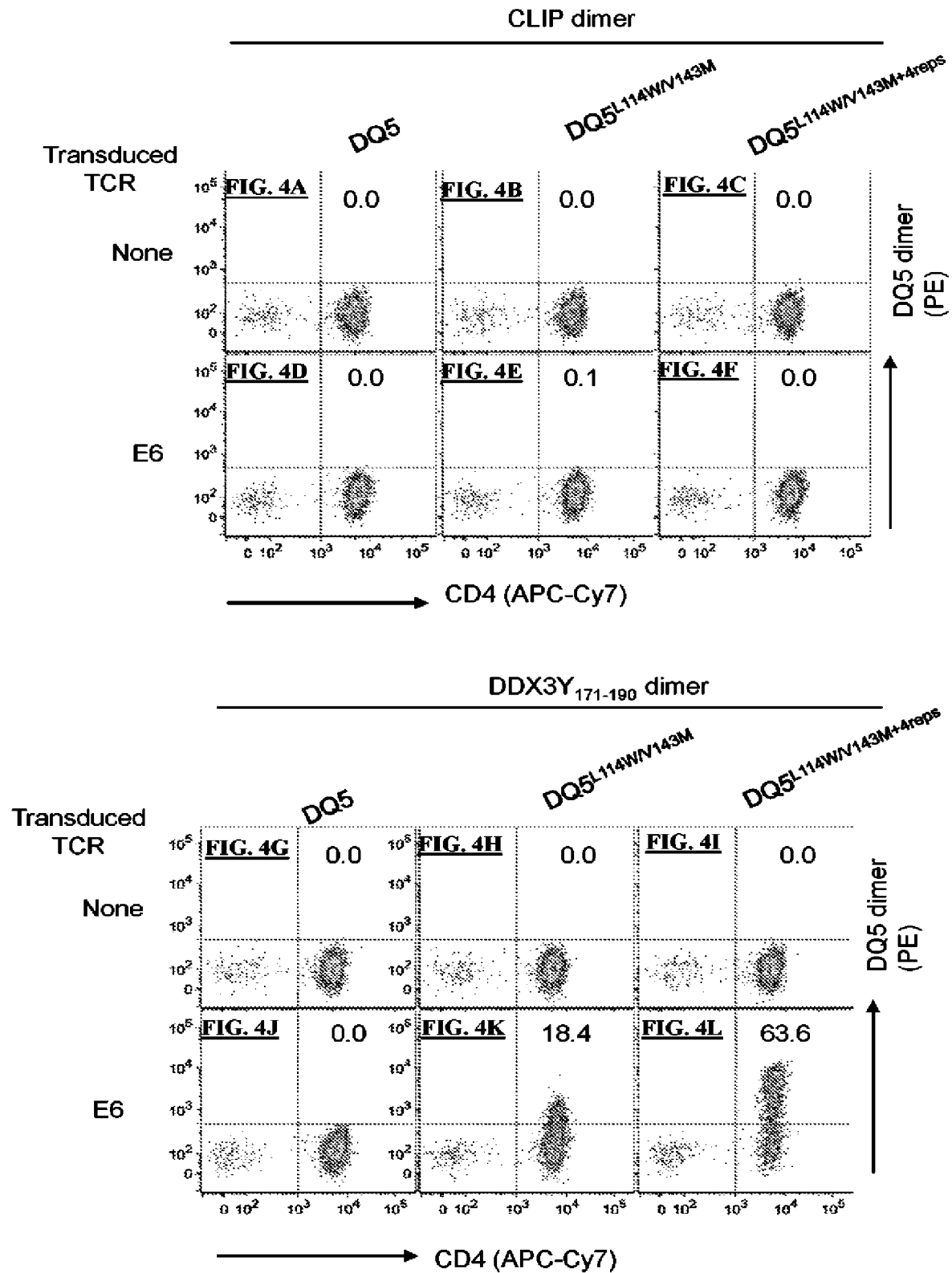
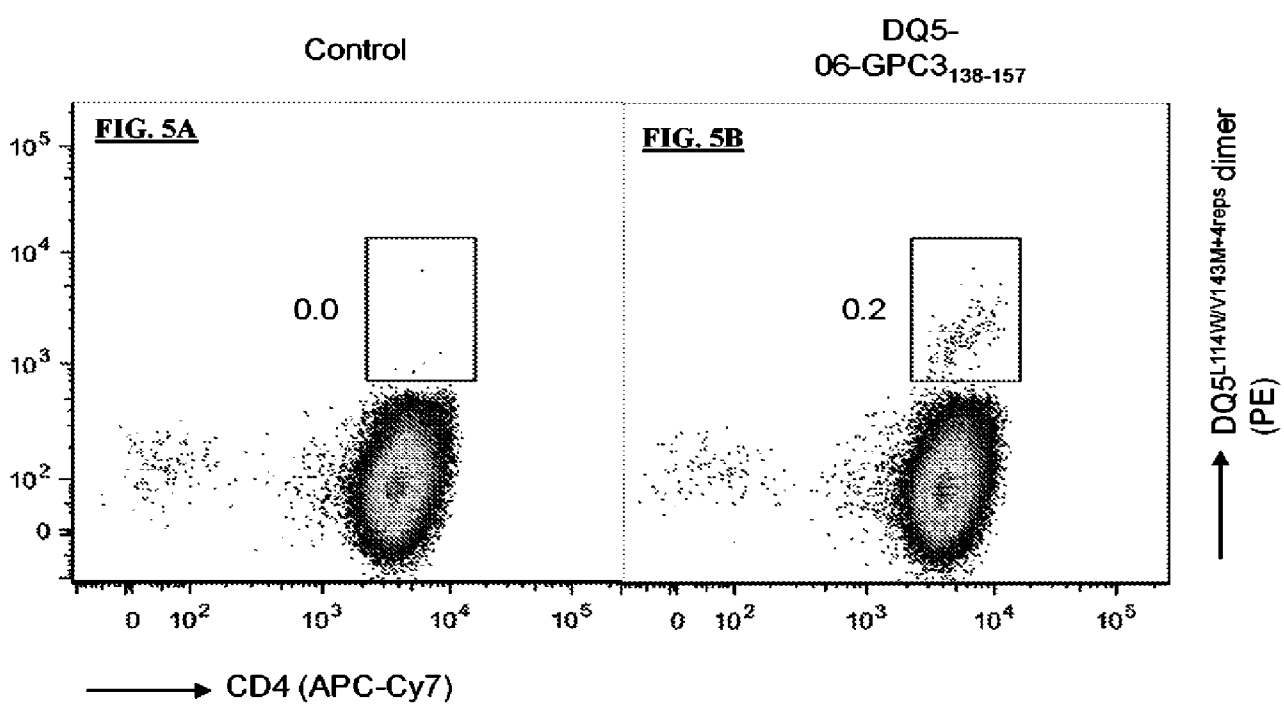
FIG. 1F

FIG. 2A**FIG. 2B**







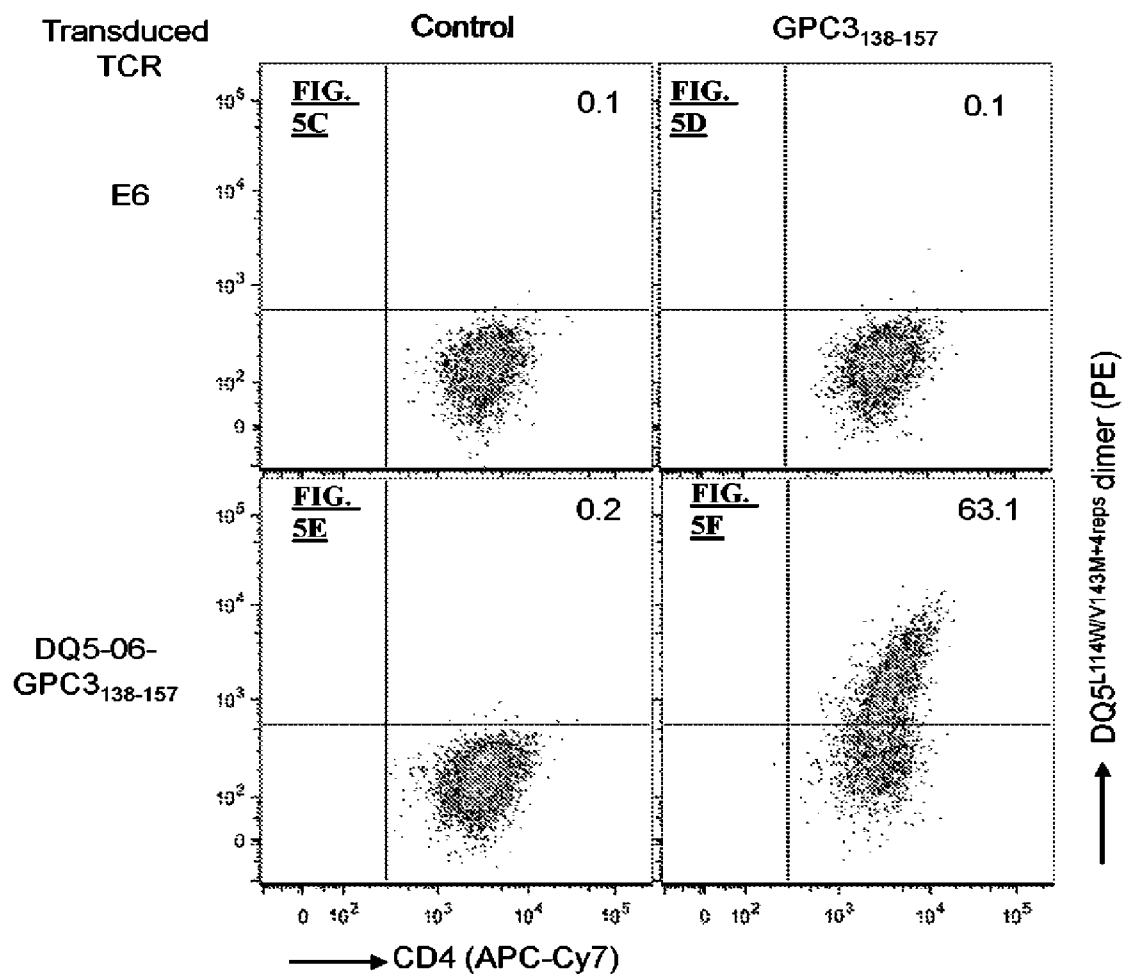


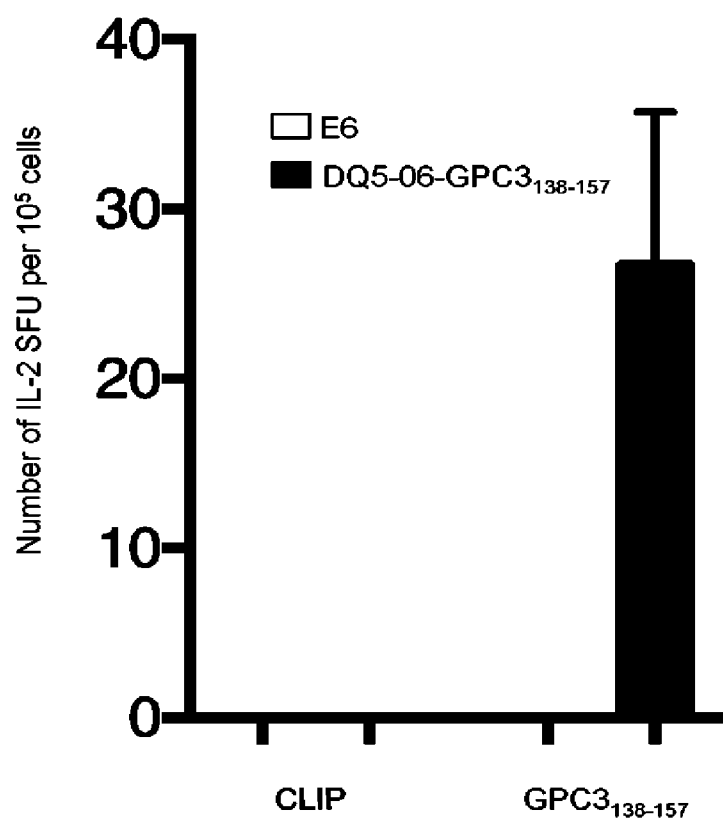
FIG. 5G

FIG. 1D

