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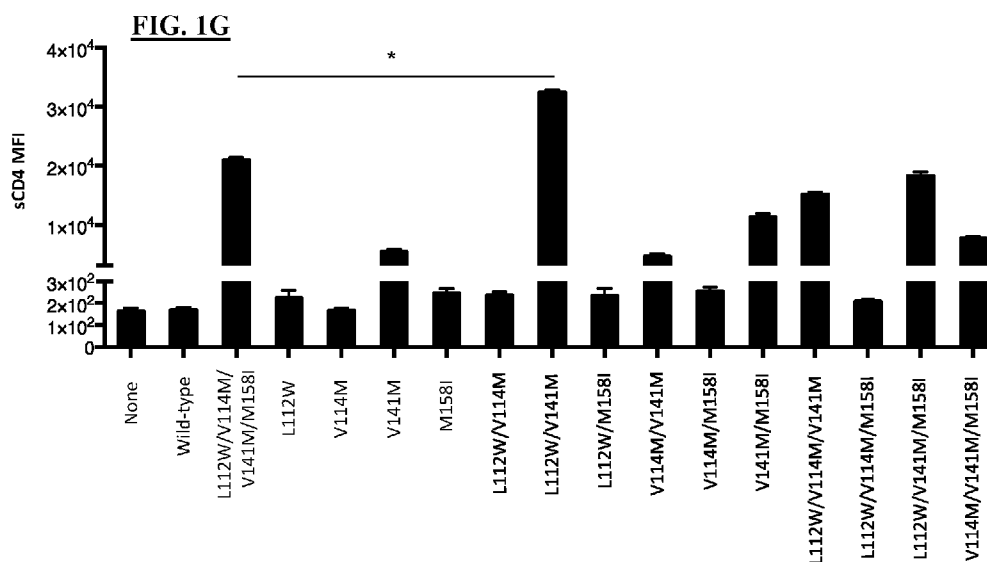
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(54) Title: MHC CLASS II MOLECULES AND METHODS OF USE THEREOF



(57) 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 DP beta chain having (i) an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) an amino acid other than valine at a position corresponding to amino acid residue 141 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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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. 62/880,496, filed July 30, 2019, and 63/029,111, 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: 4285.010PC02_SL_ST25.txt, Size: 144,830 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 DP beta chain, wherein the DP beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1.

[0008] Certain aspects of the present disclosure are directed to an HLA class II molecule comprising a DP beta chain, wherein the DP beta chain comprises a substitution mutation at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, wherein the substitution mutation is with an amino acid other than leucine.

[0009] In some aspects, the DP beta chain further comprises an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.

[0010] Certain aspects of the present disclosure are directed to an HLA class II molecule comprising a DP beta chain, wherein the DP beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.

[0011] Certain aspects of the present disclosure are directed to an HLA class II molecule comprising a DP beta chain, wherein the DP beta chain comprises a substitution mutation at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, wherein the substitution mutation is with an amino acid other than valine.

[0012] In some aspects, the DP beta chain further comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1.

[0013] In some aspects, the DP 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.

[0014] In some aspects, the amino acid other than leucine comprises a hydrophobic side chain.

[0015] In some aspects, the amino acid other than leucine is selected from the group consisting of an alanine, a valine, an isoleucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan.

- [0016] In some aspects, the amino acid other than leucine is a tryptophan.
- [0017] In some aspects, the amino acid other than valine comprises a hydrophobic side chain. In some aspects, the amino acid other than valine is selected from the group consisting of an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan. In some aspects, the amino acid other than valine is a methionine.
- [0018] In some aspects, the DP beta chain comprises a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1 and a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.
- [0019] In some aspects, the DP beta chain comprises the amino acid sequence set forth in SEQ ID NO: 3. In some aspects, the DP beta chain comprises the amino acid sequence set forth in SEQ ID NO: 4.
- [0020] In some aspects, the DP beta chain is selected from DPB1*01, DPB1*02, DPB1*03, DPB1*04, DPB1*05, DPB1*06, DPB1*08, DPB1*09, DPB1*10, DPB1*100, DPB1*101, DPB1*102, DPB1*103, DPB1*104, DPB1*105, DPB1*106, DPB1*107, DPB1*108, DPB1*109, DPB1*11, DPB1*110, DPB1*111, DPB1*112, DPB1*113, DPB1*114, DPB1*115, DPB1*116, DPB1*117, DPB1*118, DPB1*119, DPB1*120, DPB1*121, DPB1*122, DPB1*123, DPB1*124, DPB1*125, DPB1*126, DPB1*127, DPB1*128, DPB1*129, DPB1*13, DPB1*130, DPB1*131, DPB1*132, DPB1*133, DPB1*134, DPB1*135, DPB1*136, DPB1*137, DPB1*138, DPB1*139, DPB1*14, DPB1*140, DPB1*141, DPB1*142, DPB1*143, DPB1*144, DPB1*145, DPB1*146, DPB1*147, DPB1*148, DPB1*149, DPB1*15, DPB1*150, DPB1*151, DPB1*152, DPB1*153, DPB1*154, DPB1*155, DPB1*156, DPB1*157, DPB1*158, DPB1*159, DPB1*16, DPB1*160, DPB1*161, DPB1*162, DPB1*163, DPB1*164, DPB1*165, DPB1*166, DPB1*167, DPB1*168, DPB1*169, DPB1*17, DPB1*170, DPB1*171, DPB1*172, DPB1*173, DPB1*174, DPB1*175, DPB1*176, DPB1*177, DPB1*178, DPB1*179, DPB1*18, DPB1*180, DPB1*181, DPB1*182, DPB1*183, DPB1*184, DPB1*185, DPB1*186, DPB1*187, DPB1*188, DPB1*189, DPB1*19, DPB1*190, DPB1*191, DPB1*192, DPB1*193, DPB1*194, DPB1*195, DPB1*196, DPB1*197, DPB1*198, DPB1*199, DPB1*20, DPB1*200, DPB1*201, DPB1*202, DPB1*203, DPB1*204, DPB1*205, DPB1*206, DPB1*207, DPB1*208, DPB1*209, DPB1*21, DPB1*210, DPB1*211, DPB1*212, DPB1*213, DPB1*214, DPB1*215, DPB1*216, DPB1*217, DPB1*218, DPB1*219, DPB1*22, DPB1*220, DPB1*221, DPB1*222,

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DPB1*824, DPB1*825, DPB1*826, DPB1*827, DPB1*828, DPB1*829, DPB1*83,
DPB1*830, DPB1*831, DPB1*832, DPB1*833, DPB1*834, DPB1*835, DPB1*836,

DPB1*837, DPB1*838, DPB1*839, DPB1*84, DPB1*840, DPB1*841, DPB1*842, DPB1*843, DPB1*844, DPB1*845, DPB1*846, DPB1*847, DPB1*848, DPB1*849, DPB1*85, DPB1*850, DPB1*851, DPB1*852, DPB1*853, DPB1*854, DPB1*855, DPB1*856, DPB1*857, DPB1*858, DPB1*859, DPB1*86, DPB1*860, DPB1*861, DPB1*862, DPB1*863, DPB1*864, DPB1*865, DPB1*866, DPB1*867, DPB1*868, DPB1*869, DPB1*87, DPB1*870, DPB1*871, DPB1*872, DPB1*873, DPB1*874, DPB1*875, DPB1*876, DPB1*877, DPB1*878, DPB1*879, DPB1*88, DPB1*880, DPB1*881, DPB1*882, DPB1*883, DPB1*884, DPB1*885, DPB1*886, DPB1*887, DPB1*888, DPB1*889, DPB1*89, DPB1*890, DPB1*891, DPB1*892, DPB1*893, DPB1*894, DPB1*895, DPB1*896, DPB1*897, DPB1*898, DPB1*899, DPB1*90, DPB1*900, DPB1*901, DPB1*902, DPB1*903, DPB1*904, DPB1*905, DPB1*906, DPB1*907, DPB1*908, DPB1*909, DPB1*91, DPB1*910, DPB1*911, DPB1*912, DPB1*913, DPB1*914, DPB1*915, DPB1*916, DPB1*917, DPB1*918, DPB1*919, DPB1*92, DPB1*920, DPB1*921, DPB1*922, DPB1*923, DPB1*924, DPB1*925, DPB1*926, DPB1*927, DPB1*928, DPB1*929, DPB1*93, DPB1*930, DPB1*931, DPB1*932, DPB1*933, DPB1*934, DPB1*935, DPB1*936, DPB1*937, DPB1*938, DPB1*939, DPB1*94, DPB1*940, DPB1*941, DPB1*942, DPB1*943, DPB1*944, DPB1*945, DPB1*946, DPB1*947, DPB1*948, DPB1*949, DPB1*95, DPB1*950, DPB1*951, DPB1*952, DPB1*953, DPB1*954, DPB1*955, DPB1*956, DPB1*957, DPB1*958, DPB1*959, DPB1*96, DPB1*960, DPB1*961, DPB1*962, DPB1*963, DPB1*964, DPB1*965, DPB1*97, DPB1*98, and DPB1*99.

[0021] In some aspects, the DP beta chain comprises the amino acid sequence set forth in SEQ ID NO: 1.

[0022] In some aspects, the HLA class II molecule further comprises a DP alpha chain. In some aspects, the DP alpha chain is selected from DPA1*01:03:01:01, DPA1*01:03:01:02, DPA1*01:03:01:03, DPA1*01:03:01:04, DPA1*01:03:01:05, DPA1*01:03:01:06, DPA1*01:03:01:07, DPA1*01:03:01:08, DPA1*01:03:01:09, DPA1*01:03:01:10, DPA1*01:03:01:11, DPA1*01:03:01:12, DPA1*01:03:01:13, DPA1*01:03:01:14, DPA1*01:03:01:15, DPA1*01:03:01:16, DPA1*01:03:01:17, DPA1*01:03:01:18Q, DPA1*01:03:01:19, DPA1*01:03:01:20, DPA1*01:03:01:21, DPA1*01:03:01:22, DPA1*01:03:01:23, DPA1*01:03:02, DPA1*01:03:03, DPA1*01:03:04, DPA1*01:03:05, DPA1*01:03:06, DPA1*01:03:07, DPA1*01:03:08, DPA1*01:03:09, DPA1*01:04,

DPA1*01:05, DPA1*01:06:01, DPA1*01:06:02, DPA1*01:07, DPA1*01:08, DPA1*01:09, DPA1*01:10, DPA1*01:11, DPA1*01:12, DPA1*01:13, DPA1*01:14, DPA1*01:15, DPA1*01:16, DPA1*01:17, DPA1*01:18, DPA1*01:19, DPA1*02:01:01:01, DPA1*02:01:01:02, DPA1*02:01:01:03, DPA1*02:01:01:04, DPA1*02:01:01:05, DPA1*02:01:01:06, DPA1*02:01:01:07, DPA1*02:01:01:08, DPA1*02:01:01:09, DPA1*02:01:01:10, DPA1*02:01:01:11, DPA1*02:01:02:01, DPA1*02:01:02:02, DPA1*02:01:03, DPA1*02:01:04, DPA1*02:01:05, DPA1*02:01:06, DPA1*02:01:07, DPA1*02:01:08:01, DPA1*02:01:08:02, DPA1*02:02:02:01, DPA1*02:02:02:02, DPA1*02:02:02:03, DPA1*02:02:02:04, DPA1*02:02:02:05, DPA1*02:02:03, DPA1*02:02:04, DPA1*02:02:05, DPA1*02:02:06, DPA1*02:03, DPA1*02:04, DPA1*02:05, DPA1*02:06, DPA1*02:07:01:01, DPA1*02:07:01:02, DPA1*02:07:01:03, DPA1*02:08, DPA1*02:09, DPA1*02:10, DPA1*02:11, DPA1*02:12, DPA1*02:13N, DPA1*02:14, DPA1*02:15, DPA1*02:16, DPA1*03:01:01:01, DPA1*03:01:01:02, DPA1*03:01:01:03, DPA1*03:01:01:04, DPA1*03:01:01:05, DPA1*03:01:02, DPA1*03:02, DPA1*03:03, DPA1*03:04, DPA1*04:01:01:01, DPA1*04:01:01:02, and DPA1*04:01:01:03, DPA1*04:02.

[0023] In some aspects, the DP 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 DP alpha chain comprises the amino acid sequence set forth in SEQ ID NO: 6 or 8.

[0024] In some aspects, the HLA class II molecule is a DP1, DP2, DP3, DP4, DP5, DP6, DP8, or DP9 allele.

[0025] In some aspects, the DP 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 DP beta chain comprising (i) a leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1 and/or (ii) a valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.

[0026] 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.

[0027] In some aspects, the DP beta chain is bound to a membrane of a cell. In some aspects, the DP beta chain is not bound to a membrane of a cell. In some aspects, the DP beta chain comprises an extracellular domain of a full length DP alpha chain. In some aspects, the DP beta chain does not comprise a transmembrane domain of a full length DP beta chain.

[0028] In some aspects, the DP alpha chain is bound to a membrane of a cell. In some aspects, the DP alpha chain is not bound to a membrane of a cell. In some aspects, the DP alpha chain comprises an extracellular domain of a full length DP alpha chain. In some aspects, the DP alpha chain does not comprise a transmembrane domain of a full length DP alpha chain.

[0029] In some aspects, the DP 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, polyethyleinimine, block copolymer, poly caprolactone, SPIONS, USPIONS, Cd/Zn-selenide, or silica nanoparticle. In some aspects, the nanoparticle is a pegylated iron oxide nanoparticle.

[0030] In some aspects, the DP beta chain comprises a signal peptide. In some aspects, the DP alpha chain comprises a signal peptide. In some aspects, the signal peptide comprises the amino acid sequence set forth in SEQ ID NO: 9.

[0031] Certain aspects of the present disclosure are directed to a nucleic acid molecule encoding a DP beta chain disclosed herein. In some aspects, the nucleic acid molecule further encodes a DP alpha chain disclosed herein.

[0032] 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.

[0033] Certain aspects of the present disclosure are directed to a vector comprising a nucleic acid molecule disclosed herein.

- [0034] 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.
- [0035] In some aspects, the cell lacks endogenous MHC class II DP beta chain expression. In some aspects, the cell lacks endogenous MHC class II DP alpha chain expression.
- [0036] 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.
- [0037] 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 an MHC class II molecule disclosed herein. In some aspects, the disease or condition is cancer or an infection.
- [0038] 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.
- [0039] 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.
- [0040] In some aspects, the HLA class II molecule binds CD4 with a K_D of less than about 100 μ M. In some aspects, the HLA class II molecule binds CD4 with a K_D of less than about 10 μ M. In some aspects, the HLA class II molecule binds CD4 with a K_D of about 8.9 μ M or less.

[0041] 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 an amino acid sequence selected from the group consisting of SEQ ID NOs: 32-237.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] FIGs. 1A-1V are graphical representations of data illustrating that affinity-matured DP4^{L112W/V141M} molecules exhibit an enhanced CD4 binding ability. FIGs. 1A-1F are histograms showing the results of HLA class II-null K562 cells stably expressing the wild-type DP α chain (DPA1*01:03) transduced with blank, wild-type, or mutant DP β chain (DPB1*04:01) harboring L112W, V114M, V141M, and M158I substitutions (DP4^{L112W/V114M/V141M/M158I}) and stained with an anti-class II mAb and soluble CD4 (sCD4). FIG. 1G is a bar graph summarizing the binding affinity for sCD4 (MFI; y-axis) of all possible DP4 reversion mutants, which were similarly expressed and stained with sCD4 as FIGs. 1A-1F. FIG. 1H shows the affinity between DP4^{L112W/V141M} and CD4 as quantified by steady state analysis. FIG. 1I shows the results of an IL-2 EPISPOT assay of DP4/WT1 TCR, clone 9-transduced Jurkat 76 and Jurkat 76/CD4 cells stimulated by wild-type DP4 or DP4^{L112W/V141M}-expressing aAPCs pulsed with graded concentrations of the DP4/WT1 peptide. FIGs. 1J-1W are histograms representing staining of K562 cells expressing DP^{L112W/V141M} alleles (as indicated) with an anti-class II mAb and sCD4. Open histograms represent the isotype control staining. *P<0.05 by Student's t-test. Bars and error bars represent the mean $\pm$ SD of results in triplicate experiments. At least 2 independent experiments were performed. FIGs. 1X-1AA are histograms showing wild-type DP4 and DP4^{L112W/V141M} molecules on the surface of K562 cells that were detected with the indicated anti-HLA class II antibodies. Staining of control cells devoid of Class II expression is shown in solid gray. FIGs. 1AB-1BH are histograms showing aAPCs expressing the indicated DP4 or class II parental cells that were stained with sCD4 at the indicated concentrations. FIG. 1BI shows the quantification of aAPCs expressing wild-type DP4 or DP4^{L112W/V141M} at the indicated concentrations. Error bars represent the mean $\pm$ standard deviation of experiments performed in triplicate. FIG. 1BJ is a biolayer interferometry sensogram showing the interaction of biotinylated wild-type DP4 (ligand) with sCD4 (analyte) over a range of concentrations. FIG. 1BK is a biolayer interferometry sensogram showing the interaction of biotinylated DP4^{L112W/V141M} (ligand) with sCD4 (analyte) over a

range of concentrations. Experiments in FIGs. 1BJ and 1BI were performed in parallel. All data are representative of two independent experiments.

[0043] FIGs. 2A-2D are ribbon diagrams of a model structure of DP4^{L112W/V141M} and the human CD4 complex. FIGs. 2A-2B are two orientations of the ternary complex model structure of DPA1*01:03, DPB1*04:01, and CD4, as indicated. The DPB1*04:01-CD4 binding interface is enclosed in a dashed square (FIG. 2B). FIGs. 2C-2D provide close-up views of the CD4 binding interface of wild-type DP4 (FIG. 2C) and DP4^{L112W/V141M} (FIG. 2D). The side chains of interacting residues are shown as ball-and-stick representations (FIGs. 2C-2D).

[0044] FIGs. 3A-3P are graphical representations of data illustrating that DP4^{L112W/V141M} dimers stain cognate TCRs expressed in human primary CD4⁺ T cells. Primary T cells were transduced with either DP4/ MAGE-A3₂₄₃₋₂₅₈ (R12C9; FIGs. 3E-3H), DP4/ WT1₃₂₈₋₃₄₈ (clone 9; FIGs. 3I-3L), or DP4/ NY-ESO-1₁₅₇₋₁₇₀ (5B8; FIGs. 3M-3P) TCR and stained with the indicated DP4^{L112W/V141M} dimers (FIGs. 3B-3D, 3F-3H, 3J-3L, and 3N-3P).

[0045] FIGs. 4A-4D are scatter plots illustrating costaining of R12C9-transduced CD4⁺ T cells stained with DP4^{L112W/V141M} dimer and an anti-Vβ22 mAb. Note that R12C9 expresses Vβ22. FIGs. 4E-4H are scatter plots illustrating costaining of Clone 9-transduced CD4⁺ T cells double-stained with DP4^{L112W/V141M} dimer and an anti-NGFR mAb. Note that clone 9 and ΔNGFR genes are fused with P2A.

[0046] FIGs. 5A-5P are scatter plots illustrating costaining of Clone 9- (FIGs. 5A-5H) and 5B8- (FIGs. 5I-5P) transduced primary T cells stained with 5 μg/ml conventional wild-type DP4 tetramers and DP4^{L112W/V141M} dimers. At least 2 independent experiments were performed.

[0047] FIGs. 6A-6F are bar graphs illustrating the results of comprehensive screening with DP4^{L112W/V141M} dimers, which identified an array of novel DP4-restricted tumor-associated antigens. Peripheral CD4⁺ T cells were purified from six DP4⁺ melanoma patients and stimulated with DP4-expressing aAPCs individually pulsed with 196 distinct peptides derived from tumor-associated antigens and stained with cognate DP4^{L112W/V141M} dimers. The results using the 30 peptides with the highest positivity values are shown in FIGs. 6A-6B. The results for the remaining 166 peptides are shown in FIGs. 6C-6F. Each gating was set so that control dimer staining showed <0.2% positivity. Positive dimer staining was defined as

staining exceeding the control dimer staining by 3 standard deviations, as shown by the dashed line ($>0.6\%$).

[0048] FIGs. 7A-7L are graphical representations of DP4^{L112W/V141M} dimer staining of peptide-specific CD4⁺ T cells from melanoma patients. Primary CD4⁺ T cells were purified from six DP4⁺ melanoma patients and stimulated with DP4-expressing aAPCs individually pulsed with 196 distinct peptides derived from tumor-associated antigens and stained with cognate DP4^{L112W/V141M} dimers as shown in Figs. 6A-6F. Examples of DP4^{L112W/V141M} dimer staining are shown. * $P<0.05$ by Student's t-test. n.s., not significant. At least 2 independent experiments were performed.

[0049] FIGs. 8A-8X are graphical representations of data illustrating that DP4-restricted TCRs isolated from DP4^{L112W/V141M} dimer-positive cells and reconstituted in human TCR-defective CD4⁺ T cells were functional in a DP4-restricted and antigen-specific manner. 03-CCND1₂₁₉₋₂₃₈ (FIGs. 8A-8D), 05-HSD17B12₂₂₅₋₂₄₄ and 09-HSD17B12₂₂₅₋₂₄₄ (FIGs. 8E-8J), 05-LGSN₂₉₆₋₃₁₅ (FIGs. 8K-8N), 03-MAGE-A2₁₀₈₋₁₂₇ and 06-MAGE-A2₁₀₈₋₁₂₇ (FIGs. 8O-8T), and 05-MUC5AC₄₉₂₂₋₄₉₄₁ (FIGs. 8U-8X) were cloned from DP4^{L112W/V141M} dimer-positive cells, reconstituted in TCR-defective Jurkat 76/CD4 cells, and stained by the respective DP4^{L112W/V141M} dimers.

[0050] FIGs. 9A-9G are bar graphs illustrating the results of IL-2 EPISPOT assays of 03-CCND1₂₁₉₋₂₃₈ (FIG. 9A), 05-HSD17B12₂₂₅₋₂₄₄ (FIG. 9B), 09-HSD17B12₂₂₅₋₂₄₄ (FIG. 9C), 05-LGSN₂₉₆₋₃₁₅ (FIG. 9D), 03-MAGE-A2₁₀₈₋₁₂₇ (FIG. 9E), 06-MAGE-A2₁₀₈₋₁₂₇ (FIG. 9F), and 05-MUC5AC₄₉₂₂₋₄₉₄₁ (FIG. 9G) were stimulated by aAPCs pulsed with the respective peptides in IL-2 ELISPOT assays. DP4/WT1 (clone 9) TCR was used as a negative control. 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.

[0051] FIGs. 10A-10Q are graphical representations of data showing that DP4-restricted TCRs isolated from DP4^{L112W/V141M} dimer-positive cells and reconstituted in human primary CD4⁺ T cells were functional in a DP4-restricted and antigen-specific manner. 03-CCND1₂₁₉₋₂₃₈ (FIGs. 10A-10D and 10O), 03-MAGE-A2₁₀₈₋₁₂₇ and 06-MAGE-A2₁₀₈₋₁₂₇ (FIGs. 10E-10J and 10P) and 05-MUC5AC₄₉₂₂₋₄₉₄₁ (FIGs. 10K-10N and 10Q) were retrovirally transduced into human primary CD4⁺ T cells and stained with the respective DP4^{L112W/V141M} dimers (FIGs. 10A-10N). * $P<0.05$ by Student's t-test. n.s., not significant. 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.

[0052] FIGs. 11A-11E present data showing that DP4-restricted TCRs cloned from melanoma patients recognized peptides endogenously processed and presented by K562-based aAPCs. FIGs. 11A-11B are images of gel chromatography showing CCDN1 (FIG. 11A) and MAGE-A2 (FIG. 11B) endogenously expressed in K562-derived aAPC cells. FIGs. 11C-11D are bar graphs showing the results of IFN- γ ELISPOT assays of human primary T cells retrovirally transduced with 03-CCND1₂₁₉₋₂₃₈ (FIG. 11C) or 06-MAGE-A2₁₀₈₋₁₂₇ (FIG. 11D) and stimulated with peptide-unpulsed HLA-null or DP4-aAPCs (FIGs. 11C-11D). FIG. 11E is a bar graph showing the results of an IFN- γ ELISPOT assay of human primary T cells retrovirally transduced with 05-MUC5AC₄₉₂₂₋₄₉₄₁ TCR and stimulated with MUC5AC₄₉₁₄₋₄₉₄₉ minigene-transduced and peptide-unpulsed HLA-null or DP4-aAPCs. 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.

[0053] FIGs. 12A-12E present data showing that 06-MAGE-A2₁₀₈₋₁₂₇ TCR recognizes melanoma cell lines in a DP4- and MAGE-A2-dependent manner. FIG. 12A is an image of western blot showing endogenous MAGE-A2 expression in K562 cells and the indicated melanoma cell lines. FIGs. 12B-12E are bar graphs showing data from IFN- γ ELISPOT assays of primary human T cells transduced with 06-MAGE-A2₁₀₈₋₁₂₇ TCR stimulated with SK-MEL-21 (DP4⁺ MAGE-A2⁻; FIG 12B) or SK-MEL-37 (DP4⁺ MAGE-A2⁺; FIG 12C) and SK-MEL-28 (DP4⁻ MAGE-A2⁺; FIG 12D) and Me275 (DP4⁻ MAGE-A2⁺; FIG 12E) transduced with DP4. *, $P < 0.05$ by Student's t-test. Bars and error bars represent the mean $\pm$ SD of results in triplicate experiments. At least 2 independent experiments were performed.

[0054] FIGs. 13A-13Q are histograms comparing expression levels of wild-type HLADP*04:01 and derivatives thereof in K562 cells stained with the anti-HLA class II mAb clone 9-49. Open histograms represent the isotype control staining. FIGs. 14A-14D are graphical representations of data showing comparison of DP4^{L112W/V141M} dimers and dextramers for the staining of endogenous TRPC1₅₇₈₋₅₉₇-specific CD4⁺ T cells. Endogenous (non-transduced) TRPC1₅₇₈₋₅₉₇-specific CD4⁺ T cells were expanded from a melanoma patient by stimulation with peptide-pulsed and irradiated DP4⁺ artificial APCs and stained with DP4^{L112W/V141M} TRPC1₅₇₈₋₅₉₇ dimers (FIG. 14B) or a TRPC1₅₇₈₋₅₉₇ dextramer (FIG. 14D). The corresponding CLIP multimers were used as controls (FIGs. 14A and 14C).

[0055] FIGs. 15A-15F are graphical representations of data showing comparison of DP4^{L112W/V141M} dimers and conventional DP4 tetramers and dextramers for the staining of endogenous NY-ESO-1₁₅₇₋₁₇₀-specific T cells. CD4⁺ T cells were purified from DP4⁺ healthy donor No. 4 and stimulated once with NY-ESO-1₁₅₇₋₁₇₀-pulsed and irradiated DP4⁺ artificial APCs. Expanded CD4⁺ T cells were individually stained as indicated by three different DP4 multimers (DP4^{L112W/V141M} dimers (FIG. 15B), DP4 tetramers (FIG. 15D), or DP4 dextramers (FIG. 15F)).

[0056] FIGs. 16A-16Y are graphical representations of data showing pathogen-specific CD4⁺ T cells subjected to *ex vivo* staining with DP4^{L112W/V141M} dimers. Memory CD4⁺ T cells were purified from five DP4⁺ donors and subjected to *ex vivo* staining with the DP4^{L112W/V141M} dimers for the following pathogen-associated peptides without *in vitro* stimulation: TT₉₄₈₋₉₆₈ (FIGs. 16F-16J), HSV-2-UL21₂₈₃₋₃₀₂ (FIGs. 16K-16O), Flu-HA₅₂₇₋₅₄₆ (FIGs. 16P-16T), and RSV-GP₁₆₂₋₁₇₅ (FIGs. 16U-16Y). The CLIP peptide was used as a negative control (FIGs. 16A-16E).

[0057] FIGs. 17A-17W are graphical representations of data showing endogenous RSV-GP₁₆₂₋₁₇₅-specific CD4⁺ T cell clones successfully established from DP4^{L112W/V141M} dimer⁺ cells. Memory CD4⁺ T cells were purified from DP4⁺ Donor No. 06 and subjected to *ex vivo* staining with DP4^{L112W/V141M} RSV-GP₁₆₂₋₁₇₅ dimers without *in vitro* stimulation. Dimer⁺ CD4⁺ T cells were then cloned by limiting dilution. FIGs. 17A-17V are graphical representations of representative dimer staining data of 10 dimer-positive and 1 dimer-negative single-cell clones. Seventy-seven out of 84 clones (91.7%) were successfully stained with DP4^{L112W/V141M} RSV-GP₁₆₂₋₁₇₅ dimers. FIG. 17W is a bar graph showing antigen-specific IL-2 production in RSV-GP₁₆₂₋₁₇₅ dimer⁺ single-cell clones.

[0058] FIGs. 18A-18S are graphical representations of data showing endogenous DP4 TT₉₄₈₋₉₆₈-specific CD4⁺ T cell clones successfully established from DP4^{L112W/V141M} dimer⁺ cells. Memory CD4⁺ T cells were purified from DP4⁺ Donor No. 04 and subjected to *ex vivo* staining with DP4^{L112W/V141M} TT₉₄₈₋₉₆₈ dimers without *in vitro* stimulation. Dimer⁺ CD4⁺ T cells were then cloned by limiting dilution. FIGs. 18A-18R are graphical representations of representative dimer staining data of 8 dimer-positive and 1 dimer-negative single-cell clones. Twenty-six out of 29 clones (89.7%) were successfully stained with DP4^{L112W/V141M} TT₉₄₈₋₉₆₈ dimers. FIG. 18S is a bar graph showing antigen-specific IL-2 production in TT₉₄₈₋₉₆₈ dimer⁺ single-cell clones.

[0059] FIGs. 19A-19NN are graphical representations of DP4 multimer staining of RSV-GP (FIGs. 19A-19P) and TT (FIGs. 19Q-19NN) dimer⁺ single-cell clones. RSV-GP dimer⁺ single-cell clones (c6, c12, c26, and c39) were stained with either DP4^{L112W/V141M} RSV-GP₁₆₂₋₁₇₅ dimers (FIGs. 19B, 19D, 19F, and 19H) or wild-type DP4 dextramers (FIGs. 19J, 19L, 19N, and 19P). TT dimer⁺ single-cell clones (c2, c4, c6, and c9) were individually stained with three different DP4 TT₉₄₈₋₉₆₈ multimers (DP4^{L112W/V141M} dimers (FIGs. 19R, 19T, 19V, and 19X), wild-type DP4 tetramers (FIGs. 19Z, 19BB, 19DD, and 19FF), and wild-type DP4 dextramers (FIGs. 19HH, 19JJ, 19LL, and 19NN).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0060] 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-DP (DP) beta chain, wherein the DP beta chain has increased affinity for CD4.

[0061] The present disclosure is further directed to MHC class II molecules comprising a DP beta chain, wherein the DP beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1. In some aspects, the DP beta chain further comprises an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.

[0062] The present disclosure is further directed to MHC class II molecules comprising a DP beta chain, wherein the DP beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1. In some aspects, the DP beta chain further comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1.

I. Terms

[0063] 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.

[0064] 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.

[0065] 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).

[0066] 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).

[0067] 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.

[0068] 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.

[0069] 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.

[0070] "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.

[0071] 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.

[0072] 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.

[0073] 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.

[0074] 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-DP sequences are provided in Table 1.

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

Beta Chain
DPB1*04:01 Extracellular Domain (SEQ ID NO: 1) RATPENYLFQGRQECYAFNGTQRFLERYIYNREEFARFDSVGEFRAVTELGRPAAE YWNSQKDILEEKRAVPDRMCRHNYELGGPMTLQRRVQPRVNVSPSKKGPLQHHNL LVCHVTDFYPGSIQVRWFLNGQEETAGVVSTNLIRNGDWTFQILVMLEMTTPQQGDV YTCQVEHTSLDSPVTVEWKAQSDSARSK
DPB1*04:01 Extracellular Domain (SEQ ID NO: 2) AGGGCCACTCCAGAGAATTACCTTTTCCAGGGACGGCAGGAATGCTACGCGTTT AATGGGACACAGCGCTTCCTGGAGAGATACATCTACAACCGGGAGGAGTTCGCG CGCTTCGACAGCGACGTGGGGGAGTTCCGGGCGGTGACGGAGCTGGGGCGGCCT GCTGCGGAGTACTGGAACAGCCAGAAGGACATCCTGGAGGAGAAGCGGGCAGT GCCGGACAGGATGTGCAGACACAACACTACGAGCTGGGCGGGCCCATGACCCTGCA GCGCCGAGTCCAGCCTAGGGTGAATGTTTCCCCCTCCAAGAAGGGGCCCTTGCA GCACCACAACCTGCTTGTCTGCCACGTGACGGATTTCTACCCAGGCAGCATTCAA GTCCGATGGTTCCTGAATGGACAGGAGGAAACAGCTGGGGTCGTGTCCACCAAC

CTGATCCGTAATGGAGACTGGACCTTCCAGATCCTGGTGATGCTGGAAATGACCC
 CCCAGCAGGGAGATGTCTACACCTGCCAAGTGGAGCACACCAGCCTGGATAGTC
 CTGTCACCGTGGAGTGGAAAGGCACAGTCTGATTCTGCCCCGGAGTAAG

DPB1*04:01 L112W/V141M Extracellular Domain (SEQ ID NO: 3)

RATPENYLFQGRQECYAFNGTQRFLERYIYNREEFARFDSDVGEFRAVTELGRPAAE
 YWNSQKDILEEKRAVPDRMCRHNYELGGPMTLQRRVQPRVNVSPSKKGPLQHHN
 WLCHVTDFYPGSIQVRWFLNGQEETAGVMSTNLIRNGDWTFQILVMLEMTPQQG
 DVYTCQVEHTSLDSPVTVEWKAQSDSARSK

Signal Peptide; *DPB1*04:01 L112W/V141M Extracellular Domain*; Gly/Ser Linker; Zip Sequences and His tag sequences) (SEQ ID NO: 4)

MMRPIVLVLLFATSALARATPENYLFQGRQECYAFNGTQRFLERYIYNREEFARFDS
DVGEFRAVTELGRPAAEYWNSQKDILEEKRAVPDRMCRHNYELGGPMTLQRRVQPR
RVNVSPSKKGPLQHHNWLCHVTDFYPGSIQVRWFLNGQEETAGVMSTNLIRNGD
WTFQILVMLEMTPQQGDVYTCQVEHTSLDSPVTVEWKAQSDSARSKGGGGSLEIEA
AFLERENTALETRVAELRQVRQRLNRVSQYRTRYGPLGGGK

Full-length wild-type DPB1*04:01 (SEQ ID NO: 5)

MMVLQVSAAPRTVALTALLMVLLTSVVQGRATPENYLFQGRQECYAFNGTQRFLER
 RYIYNREEFARFDSDVGEFRAVTELGRPAAEYWNSQKDILEEKRAVPDRMCRHNYE
 LGGPMTLQRRVQPRVNVSPSKKGPLQHHNLLVCHVTDFYPGSIQVRWFLNGQEETA
 GVVSTNLIRNGDWTFQILVMLEMTPQQGDVYTCQVEHTSLDSPVTVEWKAQSDSAR
 SKTLTGAGGFVLGLICGVGIFMHRRSKKVQRGSA

Signal Peptide; *DPB1*04:01 Extracellular Domain*; and Gly/Ser Linker, Zip Sequences, GS linker, and biotinylation sequences) (SEQ ID NO: 238)

MMRPIVLVLLFATSALARATPENYLFQGRQECYAFNGTQRFLERYIYNREEFARFDSDVGEFRAVTELGRPAAE
YWNSQKDILEEKRAVPDRMCRHNYELGGPMTLQRRVQPRVNVSPSKKGPLQHHNLLVCHVTDFYPGSIQVRWFL
NGQEETAGVVSTNLIRNGDWTFQILVMLEMTPQQGDVYTCQVEHTSLDSPVTVEWKAQSDSARSKGGGGSLEIEA
AAFLERENTALETRVAELRQVRQRLNRVSQYRTRYGPLGGGKSGSLNDIFEAQKIEWHE

Signal Peptide; *DPB1*04:01 Extracellular Domain*; Gly/Ser Linker; Zip Sequences; GS linker and biotinylation sequences) (SEQ ID NO: 239)

ATGATGAGGCCCATCGTGCTGGTGCTGTTCCGCCACATCTGCCCTGGCCAGAGCCACCCCCGAGAAGTACCT
GTTTCAGGGCCGGCAGGAATGCTACGCCTTCAACGGCACCCAGCGGTTTCTGGAACGGTACATCTACAACCGGG
AAGAGTTCGCCAGATTGCACAGCGACGTGGGCGAGTTCAGAGCCGTGACAGAGCTGGGCAGACCTGCCGCCGAG
TACTGGAACAGCCAGAAGGACATCCTGGAAGAGAAGCGGGCCGTGCCCGACCGGATGTGCAGACACAATTACGA
GCTGGGAGGCCCATGACCTGCAGAGAAGAGTGCAGCCAGAGTGAACGTGTCCCCCAGCAAGAAGGGCCCCC
TGCAGCACCACAACCTTGCTTGTCTGCCACGTGACCGACTTCTACCCCGGCTCTATCCAAGTGCGGTGGTTCTCTG

AACGGCCAGGAAGAGACAGCCGGCGTGGTGTCCACCAACCTGATCAGAAACGGCGACTGGACCTTCCAGATCCT
CGTGATGCTGGAATGACCCCCAGCAGGGCGACGTGTACACCTGTCAGGTGGAACACACCAGCCTGGACAGCC
CCGTGACCGTGAATGGAAGGCCAGAGCGATAGCGCCAGAAGCAAAGGCGGCGGAGGCAGCCTGGAATCGAG
GCCGCCTTCTGGAAGAGAGAACACCGCCCTGGAACCCGGGTGGCCGAGCTGAGACAGAGAGTGCAGAGACT
GCGGAACCGGGTGTCCAGTACCGGACCAGATATGGCCCTCTGGGAGGCGGCAAAGGTCCGGCTTGAACGACA
TTTTTGAGGCCCAGAAGATAGAGTGGCACGAGTGA

**Signal Peptide; DPB1*04:01 L112W/V141M Extracellular Domain; Gly/Ser Linker; Zip
Sequences; GS linker and biotinylation sequences) (SEQ ID NO: 240)**

MMRPVIVLVLLFATSALARATPENYLFQGRQECYAFNGTQRFLERYIYNREEFARFDSVDVGEFRAVTELGPRPAE
YWNSQKDI LEEKRAVPDRMCRHNYELGGPMTLQRRVQPRVNVSPSKKGPLQHNNWLVCHVTDFYPGSIQVRWFL
NGQEETAGV~~MT~~SNLIRNGDWT FQILVMLEMTPQQGDVYTCQVEHTSLDSPVTVEWKAQSDSARSKGGGGSLEIE
AAFLERENTALETRVAELRQVRQRLNRVSOYRTRYGPLGGGKSGSLNDIFEAQKIEWHE

**Signal Peptide; DPB1*04:01 L112W/V141M Extracellular Domain; Gly/Ser Linker; Zip
Sequences; GS linker and biotinylation sequences) (SEQ ID NO: 241)**

ATGATGAGGCCCATCGTGCTGGTGTCTGTTCCGCACATCTGCCCTGGCCAGAGCCACCCCCGAGAACTACCT
GTTTCAGGGCCGGCAGGAATGCTACGCCTTCAACGGCACCCAGCGGTTTCTGGAACGGTACATCTACAACCGGG
AAGAGTTCGCCAGATTGCACAGCGACGTGGGCGAGTTCAGAGCCGTGACAGAGCTGGGCAGACCTGCCGCCGAG
TACTGGAACAGCCAGAAGGACATCTGGAAGAGAAGCGGGCCGTGCCCGACCGGATGTGCAGACACAATTACGA
GCTGGGAGGCCCCATGACCTGCAGAGAAGAGTGCAGCCAGAGTGAACGTGTCCCCAGCAAGAAGGGCCCCC
TGCAGCACCAACAAC~~TGG~~CCTTGCTGTGCCACGTGACCGACTTCTACCCCGGCTCTATCCAAGTGCGGTGGTTCTCTG
AACGGCCAGGAAGAGACAGCCGGCGT~~ATGT~~TCCACCAACCTGATCAGAAACGGCGACTGGACCTTCCAGATCCT
CGTGATGCTGGAATGACCCCCAGCAGGGCGACGTGTACACCTGTCAGGTGGAACACACCAGCCTGGACAGCC
CCGTGACCGTGAATGGAAGGCCAGAGCGATAGCGCCAGAAGCAAAGGCGGCGGAGGCAGCCTGGAATCGAG
GCCGCCTTCTGGAAGAGAGAACACCGCCCTGGAACCCGGGTGGCCGAGCTGAGACAGAGAGTGCAGAGACT
GCGGAACCGGGTGTCCAGTACCGGACCAGATATGGCCCTCTGGGAGGCGGCAAAGGTCCGGCTTGAACGACA
TTTTTGAGGCCCAGAAGATAGAGTGGCACGAGTGA

Alpha Chain

DPA1*01:03 Extracellular Domain (SEQ ID NO: 6)

IKADHVSTYAAFVQTHRPTGEFMFEFDEDEMFIYVDLDKKETVWHLEEFQAFSFEA
QGGLANIAILNNLNNTLIQRSNHTQATNDPPEVTVPKPEVELGQPNTLICHIDKFFPP
VLNVTWLCNGELVTEGVAESLFLPRTDYSFHKFHLYLTFVPSAEDFYDCRVEHWGLD
QPLLKHWEAQEPIQMPETTET

DPA1*01:03 Extracellular Domain (SEQ ID NO: 7)

ATCAAGGCCGACCACGTGTCCACATACGCCGCTTTCGTGCAGACCCACAGACCC
ACCGGCGAGTTCATGTTCGAGTTCGACGAGGACGAGATGTTCTACGTGGACCTG
GACAAGAAAGAAACCGTGTGGCACCTGGAAGAGTTCGGCCAGGCCTTCAGCTTT
GAGGCCCAGGGCGGACTGGCCAATATCGCCATCCTGAACAACAACCTGAACACC
CTGATCCAGCGGAGCAACCACACCCAGGCCACCAACGATCCCCCGAAGTGACC
GTGTTCCCCAAAGAACCCGTGGAACCTGGGCCAGCCCAATACCCTGATCTGCCAC
ATCGACAAGTTCTTCCCCCGGTGCTGAACGTGACCTGGCTGTGCAATGGCGAGC
TCGTGACAGAGGGCGTGGCCGAGTCTCTGTTCCTGCCCAGAACCGACTACAGCTT
CCACAAGTTCCACTACCTGACCTTCGTGCCAGCGCCGAGGACTTCTACGACTGC

AGAGTGGAACACTGGGGCCTGGACCAGCCCCTGCTGAAACATTGGGAAGCCCAG GAACCCATCCAGATGCCCCGAGACAACCGAGACA
<u>Signal Peptide; DPA1*01:03 Extracellular Domain; Gly/Ser Linker, Zip Sequences and His tag sequences</u>)(SEQ ID NO: 8) <u>MMRPIVLVLLFATSALAIKADHVSTYAAFVQTHRPTGEFMFEFDEDEMFYVDLDDKK ETVWHLEEFQAFSFEAQGGLANIAILNNNLNTLIQRSNHTQATNDPPEVTVPKEPV ELGQPNTLICHIDKFFPPVLNVTWLCNGELVTEGVAESLFLPRTDYSFHKFHLYTFVP SAEDFYDCRVEHWGLDQPLLKHWEAQEPIQMPETTETGGGGSLEIRAAFLRORNTA LRTEVAELEQEVORLENEVSQYETRYGPLGGGKGSHHHHHHH</u>
<u>Signal Peptide; DPA1*01:03 Extracellular Domain; Gly/Ser Linker, Zip Sequences, and His tag sequences (10X) (SEQ ID NO: 242)</u> <u>MMRPIVLVLLFATSALAIKADHVSTYAAFVQTHRPTGEFMFEFDEDEMFYVDLDDKKETVWHLEEFQAFSFEAQ GGLANIAILNNNLNTLIQRSNHTQATNDPPEVTVPKEPVVELGQPNTLICHIDKFFPPVLNVTWLCNGELVTEG VAESLFLPRTDYSFHKFHLYTFVPSAEDFYDCRVEHWGLDQPLLKHWEAQEPIQMPETTETGGGGSLEIRAAFL RORNTALRTEVAELEQEVORLENEVSQYETRYGPLGGGKGSHHHHHHHHHHH</u>
<u>Signal Peptide; DPA1*01:03 Extracellular Domain; Gly/Ser Linker, Zip Sequences, and His tag sequences (10X) (SEQ ID NO: 243)</u> <u>ATGATGAGGCCCATCGTGCTGGTGCTGCTGTTCCGCACATCTGCCCTGGCCATCAAGGCCGACCACGTGTCCAC ATACGCCGCCTTCGTGCAGACCCACAGACCCACCGCGAGTTCATGTTTCGAGTTCGACGAGGACGAGATGTTCT ACGTGGACCTGGACAAGAAAGAAACCGTGTGGCACCTGGAAGAGTTCGGCCAGGCCTTCAGCTTTGAGGCCAG GGCGGACTGGCCAATATCGCCATCCTGAACAACAACCTGAACACCCCTGATCCAGCGGAGCAACCACACCCAGGC CACCAACGATCCCCCGAAGTGACCGTGTTCCCCAAAGAACCCGTGGAACTGGGCCAGCCCAATACCCTGATCT GCCACATCGACAAGTTCTTCCCCCGTGCTGAACGTGACCTGGCTGTGCAATGGCGAGCTCGTGACAGAGGGC GTGGCCGAGTCTCTGTTCTGCCCAGAACCGACTACAGCTTCCACAAGTTCCACTACCTGACCTTCGTGCCAG CGCCGAGGACTTCTACGACTGCAGAGTGGAACACTGGGGCTGGACCAGCCCCTGCTGAAACATTGGGAAGCCC AGGAACCCATCCAGATGCCGAGACAACCGAGACAGGCGGCGGAGGCAGCCTGGAAATCAGAGCCGCCTTCCTG CGGCAGAGAAACACCGCCCTGAGAACCAGAGTGCCGAGCTGGAACAGGAAGTGCAGCGGCTGGAAAACGAGGT GTCCAGTACGAGACAAGATACGGCCCTCTGGGAGGCGGCAAGGGCTCTCACCACCACCATCACCATCATCATC ACCATTGA</u>
Signal Peptide (Fibroin light chain-derived)
MMRPIVLVLLFATSALA (SEQ ID NO: 9)

[0075] 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 L112, V114, V141, L156, and M158 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

MNRGVPFRHLLLVLQLALLPAATQGKKVVLGKKGDTVELTCTASQKKSIQFHWKNS NQIKILGNQGSFLT KGPSKLNDRADSRRLWDQGNFPLIKNLKI ESDTYICEVEDQK EEVQLLVFGLTANS DTHLLQGQSLTLTLESPPGSSPSVQCRSPRGKNIQGGKTL SVSQ LELQDSGTWTCTVLQNQKKVEFKIDIVVLA FQKASSIVYKKEGEQVEFSFPLAFTVEK LTGSGELWWQAERASSSKSWITFDLKNKEVSVKRVTQDPKLQMGKKLPLHLTLPQA LPQYAGSGNLT LALEAKTGK LHQEVNLVVMRATQLQKNLTCEVWGPTSPKLMLSL KLENKEAKVSKREKAVWVLNPEAGMWQCLLSDSGQVLLESNIKVLPTWSTPVQPM ALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQAERMSQIKRLLSEKKTCQC PHRFQKTC SPI (SEQ ID NO: 10)

[0076] 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.

[0077] 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."

[0078] 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.

[0079] 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).

[0080] 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.

[0081] 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 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 I molecule).

[0082] 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.

[0083] 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, 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.

- [0084] 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.
- [0085] 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.
- [0086] 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.
- [0087] 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.

[0088] 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 DP beta chain comprising a substitution mutation at a position corresponding to amino acid residue 112 of SEQ ID NO: 1 does not require that a wild-type DP beta chain has been physically altered to arrive at the recited DP beta chain; but rather that, when properly aligned, the recited DP beta chain comprises an amino acid residue at the recited position (residue 112) that is different from the amino acid residue at the corresponding position in a wild-type or reference DP beta chain.

[0089] The term "any amino acid," as used herein, means any known amino acid. Amino acids are organic compounds comprising (i) an amine ($-NH_2$) 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).

[0090] 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).

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] "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.

[0098] "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.

[0099] 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.

[0100] 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.

[0101] 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.

[0102] Various aspects of the invention are described in further detail in the following subsections.

II. Compositions of the Disclosure

[0103] 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-DP ("DP") beta chain.

II.A. MHC Class II Molecules

[0104] 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.

[0105] 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).

[0106] 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-DP allele. In certain aspects, the HLA class II alpha chain is an HLA-DP allele.

[0107] Many HLA-DP alleles are known in the art, and any of the known alleles can be used in the present disclosure. Examples of HLA-DP 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 February 27, 2019).

II.A.1. MHC Class II Beta Chain

[0108] In certain aspects, the HLA class II molecule comprises a DP beta chain, wherein the DP beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1. Any amino acid other than leucine can be present at the position corresponding to amino acid residue 112 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 112 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 112 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 112 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 112 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 112 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 112 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 112 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 112 of SEQ ID NO: 1 is a tryptophan.

[0109] In some embodiments, the amino acid other than leucine at the position corresponding to amino acid residue 112 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 112 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.

[0110] In certain aspects, the HLA class II molecule comprises a DP beta chain, wherein the DP beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1. Any amino acid other than valine can be present at the position corresponding to amino acid residue 141 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 141 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 141 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 141 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 141 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 141 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 141 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 141 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 141 of SEQ ID NO: 1 is a tryptophan.

[0111] In some aspects, the amino acid other than valine at the position corresponding to amino acid residue 141 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 141 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.

[0112] In certain aspects of the present disclosure, the MHC class II molecule comprises a DP beta chain comprising more than one substitution mutation relative to the wild-type DP beta chain. In certain aspects, the DP 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 DP beta chain.

[0113] In certain aspects, the DP beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1 and an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1. In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) the amino acid other than valine at a position

corresponding to amino acid residue 141 of SEQ ID NO: 1, or each of the amino acid other than leucine at the position corresponding to amino acid residue 112 of SEQ ID NO: 1 and the amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1 is an amino acid comprising a hydrophobic side chain. In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 112 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 141 of SEQ ID NO: 1 is selected from an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan.

[0114] In some aspects, (i) the amino acid other than leucine at the position corresponding to amino acid residue 112 of SEQ ID NO: 1 is a tryptophan; and (ii) the amino acid other than valine at a position corresponding to amino acid residue 141 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 112 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 141 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 112 of SEQ ID NO: 1 is a tryptophan; and (ii) the amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1 is a methionine.

[0115] In certain aspects, the DP beta chain further comprises an amino acid other than valine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the amino acid other than valine at the position corresponding to amino acid residue 114 of SEQ ID NO: 1 is 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 a position corresponding to amino acid residue 114 of SEQ ID NO: 1 is a methionine.

[0116] In certain aspects, the DP beta chain further comprises an amino acid other than methionine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1. In some aspects, the amino acid other than methionine at the position corresponding to amino acid residue 158 of SEQ ID NO: 1 is 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 methionine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1 is an isoleucine.

[0117] In some aspects, the DP beta chain comprises (i) an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, and (ii) an amino acid other than valine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, and (ii) an amino acid other than methionine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1.

[0118] In some aspects, the DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, and (ii) a methionine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, and (ii) a isoleucine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1.

[0119] In some aspects, the DP beta chain comprises (i) an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (ii) an amino acid other than valine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (ii) an amino acid other than methionine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1.

[0120] In some aspects, the DP beta chain comprises (i) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (ii) an amino acid other than valine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (ii) a isoleucine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1.

[0121] In some aspects, the DP beta chain comprises (i) an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1,

(iii) an amino acid other than valine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, and (iv) an amino acid other than methionine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1.

[0122] In some aspects, the DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, (iii) a methionine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, and (iv) a isoleucine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1.

[0123] In some aspects, the DP beta chain comprises a valine at a position corresponding to amino acid residue 114 SEQ ID NO: 1. In some aspects, the DP beta chain comprises a methionine at a position corresponding to amino acid residue 158 corresponding to SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (iii) a valine at a position corresponding to amino acid residue 114 SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (iii) a methionine at a position corresponding to amino acid residue 158 corresponding to SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, (iii) a valine at a position corresponding to amino acid residue 114 SEQ ID NO: 1, and (iv) a methionine at a position corresponding to amino acid residue 158 corresponding to SEQ ID NO: 1.

[0124] In some aspects, the DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (iii) a valine at a position corresponding to amino acid residue 114 SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, and (iii) a methionine at a position corresponding to amino acid residue 158

corresponding to SEQ ID NO: 1. In some aspects, the DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, (iii) a valine at a position corresponding to amino acid residue 114 SEQ ID NO: 1, and (iv) a methionine at a position corresponding to amino acid residue 158 corresponding to SEQ ID NO: 1.

[0125] In some aspects, the DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, (iii) a methionine at a position corresponding to amino acid residue 114 of SEQ ID NO: 1, and (iv) a isoleucine at a position corresponding to amino acid residue 158 of SEQ ID NO: 1.

[0126] In certain aspects, a DP 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 DP beta chain. In some aspects, the reference HLA class II molecule is an HLA class II molecule having a DP beta chain comprising (i) a leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1 and/or (ii) a valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.

[0127] 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.

[0128] 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.

[0129] In certain aspects, the DP beta chain comprises an allele selected from DPB1*01, DPB1*02, DPB1*03, DPB1*04, DPB1*05, DPB1*06, DPB1*08, DPB1*09, DPB1*10, DPB1*100, DPB1*101, DPB1*102, DPB1*103, DPB1*104, DPB1*105, DPB1*106, DPB1*107, DPB1*108, DPB1*109, DPB1*11, DPB1*110, DPB1*111, DPB1*112, DPB1*113, DPB1*114, DPB1*115, DPB1*116, DPB1*117, DPB1*118, DPB1*119, DPB1*120, DPB1*121, DPB1*122, DPB1*123, DPB1*124, DPB1*125, DPB1*126, DPB1*127, DPB1*128, DPB1*129, DPB1*13, DPB1*130, DPB1*131, DPB1*132, DPB1*133, DPB1*134, DPB1*135, DPB1*136, DPB1*137, DPB1*138, DPB1*139, DPB1*14, DPB1*140, DPB1*141, DPB1*142, DPB1*143, DPB1*144, DPB1*145, DPB1*146, DPB1*147, DPB1*148, DPB1*149, DPB1*15, DPB1*150, DPB1*151, DPB1*152, DPB1*153, DPB1*154, DPB1*155, DPB1*156, DPB1*157, DPB1*158, DPB1*159, DPB1*16, DPB1*160, DPB1*161, DPB1*162, DPB1*163, DPB1*164, DPB1*165, DPB1*166, DPB1*167, DPB1*168, DPB1*169, DPB1*17, DPB1*170, DPB1*171, DPB1*172, DPB1*173, DPB1*174, DPB1*175, DPB1*176, DPB1*177, DPB1*178, DPB1*179, DPB1*18, DPB1*180, DPB1*181, DPB1*182, DPB1*183, DPB1*184, DPB1*185, DPB1*186, DPB1*187, DPB1*188, DPB1*189, DPB1*19, DPB1*190, DPB1*191, DPB1*192, DPB1*193, DPB1*194, DPB1*195, DPB1*196, DPB1*197, DPB1*198, DPB1*199, DPB1*20, DPB1*200, DPB1*201, DPB1*202, DPB1*203, DPB1*204, DPB1*205, DPB1*206, DPB1*207, DPB1*208, DPB1*209, DPB1*21, DPB1*210, DPB1*211, DPB1*212, DPB1*213, DPB1*214, DPB1*215, DPB1*216, DPB1*217, DPB1*218, DPB1*219, DPB1*22, DPB1*220, DPB1*221, DPB1*222, DPB1*223, DPB1*224, DPB1*225, DPB1*226, DPB1*227, DPB1*228,

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[0130] In certain aspects, the MHC class II molecule comprises a DP 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 DP beta chain comprises a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, and wherein the DP beta chain comprises a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1. In certain aspects, the MHC class II molecule comprises a DP 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 DP beta chain comprises (i) a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1, (ii) a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1, (iii) a valine at a position corresponding to amino acid residue 114 SEQ ID NO: 1, and (iv) a methionine at a position corresponding to amino acid residue 158 corresponding to SEQ ID NO: 1. In certain aspects, the MHC class II molecule comprises a DP beta chain comprising an amino acid sequence set forth in SEQ ID NO: 3.

II.A.2. MHC Class II Alpha Chain

[0131] 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 DP alpha chain. Any DP alpha chain can be used in the compositions and methods of the present disclosure. In some aspects, the DP alpha chain comprises an HLA-DPA1*01, HLA-DPA1*02, HLA-DPA1*03, or HLA-DPA1*04 allele. In certain aspects, the DP alpha chain comprises an HLA-DPA1*01 allele. In certain aspects, the DP alpha chain comprises an HLA-DPA1*02 allele. In certain aspects, the DP alpha chain comprises an HLA-DPA1*03 allele. In certain aspects, the DP alpha chain comprises an HLA-DPA1*04 allele.

[0132] In certain aspects, the DP alpha chain is selected from DPA1*01:03:01:01, DPA1*01:03:01:02, DPA1*01:03:01:03, DPA1*01:03:01:04, DPA1*01:03:01:05, DPA1*01:03:01:06, DPA1*01:03:01:07, DPA1*01:03:01:08, DPA1*01:03:01:09, DPA1*01:03:01:10, DPA1*01:03:01:11, DPA1*01:03:01:12, DPA1*01:03:01:13, DPA1*01:03:01:14, DPA1*01:03:01:15, DPA1*01:03:01:16, DPA1*01:03:01:17, DPA1*01:03:01:18Q, DPA1*01:03:01:19, DPA1*01:03:01:20, DPA1*01:03:01:21, DPA1*01:03:01:22, DPA1*01:03:01:23, DPA1*01:03:02, DPA1*01:03:03, DPA1*01:03:04, DPA1*01:03:05, DPA1*01:03:06, DPA1*01:03:07, DPA1*01:03:08, DPA1*01:03:09, DPA1*01:04, DPA1*01:05, DPA1*01:06:01, DPA1*01:06:02, DPA1*01:07, DPA1*01:08, DPA1*01:09, DPA1*01:10, DPA1*01:11, DPA1*01:12, DPA1*01:13, DPA1*01:14, DPA1*01:15, DPA1*01:16, DPA1*01:17, DPA1*01:18, DPA1*01:19, DPA1*02:01:01:01, DPA1*02:01:01:02, DPA1*02:01:01:03, DPA1*02:01:01:04, DPA1*02:01:01:05, DPA1*02:01:01:06, DPA1*02:01:01:07, DPA1*02:01:01:08, DPA1*02:01:01:09,

DPA1*02:01:01:10, DPA1*02:01:01:11, DPA1*02:01:02:01, DPA1*02:01:02:02, DPA1*02:01:03, DPA1*02:01:04, DPA1*02:01:05, DPA1*02:01:06, DPA1*02:01:07, DPA1*02:01:08:01, DPA1*02:01:08:02, DPA1*02:02:02:01, DPA1*02:02:02:02, DPA1*02:02:02:03, DPA1*02:02:02:04, DPA1*02:02:02:05, DPA1*02:02:03, DPA1*02:02:04, DPA1*02:02:05, DPA1*02:02:06, DPA1*02:03, DPA1*02:04, DPA1*02:05, DPA1*02:06, DPA1*02:07:01:01, DPA1*02:07:01:02, DPA1*02:07:01:03, DPA1*02:08, DPA1*02:09, DPA1*02:10, DPA1*02:11, DPA1*02:12, DPA1*02:13N, DPA1*02:14, DPA1*02:15, DPA1*02:16, DPA1*03:01:01:01, DPA1*03:01:01:02, DPA1*03:01:01:03, DPA1*03:01:01:04, DPA1*03:01:01:05, DPA1*03:01:02, DPA1*03:02, DPA1*03:03, DPA1*03:04, DPA1*04:01:01:01, DPA1*04:01:01:02, DPA1*04:01:01:03, DPA1*04:02, or any combination thereof.

II.A.3. Signal Peptide

[0133] In some aspects, the DP beta chain and/or the DP 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 DP beta chain signal peptide is the same as the DP alpha signal peptide. In some aspects the DP beta chain signal peptide is different from the DP alpha signal peptide.

[0134] 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 DP beta chain signal peptide. In some aspects, the signal peptide comprises a naturally occurring DP beta chain signal peptide. In some aspects, the signal peptide is derived from a naturally occurring DP alpha chain signal peptide. In some aspects, the signal peptide comprises a naturally occurring DP 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

[0135] In some aspects, the DP beta chain and/or the DP 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 DP beta chain transmembrane domain. In some aspects, the transmembrane domain comprises a naturally occurring DP beta chain transmembrane domain. In some aspects, the transmembrane domain is derived from a naturally occurring DP alpha chain transmembrane domain. In some aspects, the transmembrane domain comprises a naturally occurring DP alpha chain transmembrane domain.

II.A.5. Leucine Zipper

[0136] In some aspects, the DP beta chain and/or the DP 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 DP beta chain and/or the DP 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

[0137] In some aspects, the DP beta chain and/or the DP 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 DP alpha chain or the DP beta chain. In some aspects, the linker is positioned at the C-terminus of the extracellular domain of the DP alpha chain or the DP beta chain. In some aspects, the linker is positioned between the extracellular domain of the DP alpha chain or the DP beta chain and the transmembrane domain. In some aspects, the linker is positioned between the extracellular domain of the DP alpha chain or the DP beta chain and the one or more LZip sequences. In some aspects, the linker is positioned between the extracellular domain of the DP alpha chain or the DP beta chain and the signal peptide.

[0138] 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.

[0139] 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

[0140] 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.

[0141] 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.

[0142] 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.

[0143] 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-DP allele. In some aspects the cell lacks endogenous expression of an HLA-DP alpha chain allele. In some aspects the cell lacks endogenous expression of an HLA-DP beta chain allele.

II.C. Soluble MHC Class II Molecules

[0144] 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.

[0145] 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, polyethyleinimine, 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.

[0146] 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 DP 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 DP alpha chain comprising an amino acid sequence set forth in SEQ ID NO: 6.

[0147] In certain aspects, the MHC class II molecule comprises a DP 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 DP beta chain comprising an amino acid sequence set forth in SEQ ID NO: 1. In certain aspects, the MHC class II molecule comprises a DP 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 DP beta chain comprising an amino acid sequence set forth in SEQ ID NO: 3. In certain aspects, the MHC class II molecule comprises a DP 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 DP beta chain comprising an amino acid sequence set forth in SEQ ID NO: 4. In certain aspects, the MHC class II molecule comprises a DP 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 DP beta chain comprising an amino acid sequence set forth in SEQ ID NO: 5.

II.D. Nucleic Acid Molecules and Vectors

[0148] 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.

[0149] 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.

[0150] 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.

[0151] 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.

[0152] 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.

[0153] 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

[0154] 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

[0155] 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.

[0156] 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.

[0157] 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.

[0158] 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.

[0159] 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.

[0160] 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.

[0161] 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

[0162] 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.

[0163] 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.

[0164] 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).

- [0165] 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.
- [0166] All of the various aspects, aspects, and options described herein can be combined in any and all variations.
- [0167] 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.
- [0168] 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

[0169] *Cells*

- [0170] 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. K562-based artificial APCs (aAPCs) individually expressing various HLA class II genes as a single HLA allele in conjunction with CD80 and CD83 have been reported previously (Butler 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. Jurkat 76/CD4 cells were generated by retrovirally transducing the human CD4 gene. A375, SK-MEL-21, SK-MEL-28, SK-MEL-37 and Me275 are melanoma cell lines. HEK293T cells and melanoma cell lines 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.

[0171] *Peptides*

- [0172] Synthetic peptides were purchased from Genscript (Piscataway, NJ) and dissolved at 50 µg/ml in DMSO. The peptide sequences are shown in Table 3.

Table 3: Synthetic Peptide Sequences

Name	Sequence	SEQ ID NO:	Name	Sequence	SEQ ID NO:	Name	Sequence	SEQ ID NO:
ABCC6 ₁₀ 81-1100	EVLAPVILML LNSFFNAIST	32	GPR143 120-139	AMWIQLLYSA CFWWLFCYAV	101	PAX3 ₂₁ 7-236	RKQRRSRTTFTA EQLEELER	170
ABCC6 ₁₆ 2-181	EGEISDPFRF TTFYIHFALV	33	HLA- DOB ₁₇₁ - 190	TGPIRNGDWT FQTVVMLEMT	102	PAX5 ₃₃ 2-351	LTGMVPGSEFSG SPYSHPOY	171
ABCC6 ₃₀ 1-320	KALLATFGSS FLISACFKLI	34	HPN ₃₈₇ - 406	PGVYTKVSDF REWIFQAIKT	103	PGK1 ₃₃ 5-354	VWNGPVGVFWE AFARGTKA	172
ABCC6 ₃₁ 7-336	FKLIQDLLSF INPQLLSILI	35	HSD17B 12 ₁₇ -36	GTVAYLALRI SYSFLTALRV	104	PLAC1 180-199	QAGAQEAQPLQP SHFLDISE	173
ACRBP ₄₅ 0-469	GCEDVRVSGW LQTEFLSFQD	36	HSD17B 12 ₂₂₅ - 244	VFVQSVLPYF VATKLAKIRK	105	PLIN2 18-37	NLPLVSSTYDLM SSAYLSTK	174
AFP ₂₃₉ - 258	TVTKLSQKFT KVNFTETQKL	37	Hsp70 ₂₈ 9-308	EGIDFYTSIT RARFEELCSD	106	POTEE 952-971	NERFRCPREALFQ PCFLGMES	175
AIM2 ₁₈ - 37	TDEELDRFKF FLSDEFNIAT	38	IDO1 ₄₀₃ -422	SFRDGDCKSG FFLVSLLEVI	107	PPIB ₇₉ -98	LATGEKGFYKYN SKFHRVIK	176
AIM2 ₂₀₅ -224	RIIIARYYR HSGFLEVNSA	39	IL13RA 2 ₂₁₇ -236	SSENKPIRSS YFTFQLQNI	108	PRAME 294-313	SLQCLQALYVDS LFFLRGRL	177
ALDH1A 1 ₁₄₃ -162	RTIPIDGNFF TYTRHEPIGV	40	KAAG1 ₃ - 22	DDAAPRVEGV PVAVHKHALH	109	PRDM1 659-678	YQCKVCPAKFTQ FVHLKLHK	178
ALK ₁₁₆₀ - 1179	DELDFLMEAL IISKFNHQNI	41	KDM5B ₃₈ 1-400	TFGEMADAFK SDYFNMPVHM	110	PSA ₂₈₃ -302	EVAAKTLFPYKD YFNVPYPL	179
ANKRD3 0A ₁₃₇ - 156	VYGNTALHYA VYSEILSVVA	42	KDR ₇₆₅ - 784	IIILVGTAVI AMFFWLLLEVI	111	PSA ₅₈₀ -599	KLNLGTVGFYRT QYSSAMLE	180
ANXA2 ₂₄ 1-260	LESIRKEVKG DLENAFNLV	43	KIF20A 298-317	RFSIWISFFE IYNELLYDLL	112	PSA ₆₆₇ -686	SHTDFYEEIQEF VKDVFSP	181
ARF4 ₇₃ - 92	RIRPLWKHYF QNTQGLIFVV	44	KIF2C ₃₈ 6-405	NQPCYRKLGL EVYVTFEYIY	113	PSA ₈₃₇ -856	ELYNRYQGGFLI SRLIKLSV	182
BAGE1 ₅ - 24	AVFLALSAQL LQARLMKEES	45	KLK4 ₁₀₂ -121	SVRHPEYNRP LLANDMLIK	114	PTTG1 IP ₈₁ - 100	CKLSSARWVCW VNFEALII	183
BAX ₁₃₄ - 143	RTIMGWTLDF LRERLLGWIQ	46	LGALS3 BP ₃₇₄ - 393	QKKTLLQALEF HTVPFQLLAR	115	PXDN ₁₂ 26-1245	SRLGPTLMCLLS TQFKRLRD	184

BCL2L1 13-32	LSYKLSQKGY SWSQFSDVEE	47	LGALS9 112-131	VMVNGILFVQ YFHRVPFHRV	116	RAB38 131-150	QGKDVLMNNGLK MDQFCKEH	185
BIRC5 ₈₇ -106	LSVKKQFEEL TLGEFLKLDLDR	48	LGSN ₁₈₇ -206	IAKRQLSHLQ ASGFSLLSAF	117	RCVRN 20-39	NTKFSEEEELCSW YQSFLKDC	186
BIRC7 ₁₃ 9-158	DPWTEHAKWF PSCQFLLRSK	49	LGSN ₂₈₇ -308	TGVKEVARKY NYIASFFIET	118	RGS5 ₁₆ 2-181	MEKDSLPRFVRS EFYQELIK	187
BST2 ₁₅₄ -173	YPSSQDSSSA AAPQLLIVLL	50	LGSN ₂₉₆ -315	YNYIASFFIE TGFCDSGILS	119	RGS5 ₇₃ -92	YGLASFKSFLKS EFSEENLE	188
CA9 ₃₃₁₋₃₅₀	LTPPCAQGV IWTVFNQTVM	51	LGSN ₇₈₋₉₇	QAMAKNRLQF VRFEATDLHG	120	RhoC ₁₆ -35	CGKTCLLIVFSK DQFPEVYV	189
CALCA ₁₋₂₀	MGFQKFSPFL ALSILVLLQA	52	LIMS ₁₃₄ -53	QCFVCAQCFQ QFPEGLFYEF	121	RNF43 195-214	PDYDVWILMTVV GTIFVIIL	190
CCDC11 0 ₅₈₋₇₇	VLQQQLESFQ ALRMQTLQNV	53	LY6K ₉₉₋₁₁₈	EKRFLLEPM PFFYLKCKKI	122	RPS2 ₂₅ 0-269	YLTPDLWKETVF TKSPYQEF	191
CCNA1 ₃₆ 6-385	KYVAELSLE ADPFLKYLPS	54	MAGE- A10 ₁₃₅₋₁₅₄	DEKVTDLVQF LLFKYQMKEP	123	SAGE1 857-876	KVKRQFVEFTIK EAARFKKV	192
CCNA1 ₄₃ 8-457	QQAIREKYKA SKYLCVSLME	55	MAGE- A12 ₁₀₈₋₁₂₇	ALSRKMAELV HFLLLKYRAR	124	SART1 227-246	MDQFEGVSTLVE EEFGQRRQ	193
CCND1 ₂₁ 9-238	SPNNFLSYR LTRFLSRVIK	56	MAGE- A1 ₂₃₅₋₂₅₄	PRKLLTQDLV QEKYLEYRQV	125	SART3 135-154	RQKMSEIFPLTE ELWLEWLH	194
CD274 ₁₋₂₀	MRIFAVFIFM TYWHLLNAFT	57	MAGE- A2 ₁₀₈₋₁₂₇	AISRKMVELV HFLLLKYRAR	126	SCGB2 A2 ₇₄₋₉₃	LSNVEVFMVISF SSYKLFKS	195
CD45 ₁₀₁ 2-1031	PSKYINASFI MSYWKPEVMI	58	MAGE- A2 ₁₁₁₋₁₃₀	RKMVELVHFL LLKYRAREPV	127	SCRN1 257-256	TLRDKASGVCID SEFFLTTA	196
CD45 ₁₀₃ 6-1055	PLKETIGDFW QMIFQRKVKV	59	MAGE- A4 ₂₇₀₋₂₈₉	YEFLWGPRAL AETSYVKVLE	128	SDC1 ₂₆ 2-281	VGLIFAVCLVGF MLYRMKKK	197
CD45 ₁₂₀ 4-1223	KARPGMVSTF EQYQFLYDVI	60	MAGE- A6 ₁₃₆₋₁₅₅	LGSVGNWQY FFPVIFSKAS	129	SIM2 ₁₄ 7-166	HHHLLQEYEIER SFFLRMKC	198
CDH3 ₂₁₀ -229	DHKPKFTQDT FRGSVLEGVL	61	MAGE- A6 ₂₄₂₋₂₆₁	PKLLTQYFV QENYLEYRQV	130	SLAMF 7 ₂₃₂₋₂₅₁	LLVPLLLSLFVL GLFLWFLK	199

CDKN1A 52-71	DFVTETPLEG DFAWERVRL	62	MAGE- A9 ¹⁴⁵⁻¹⁶⁴	YFPVIFGKAS EFMQVIFGTD	131	SLC45 A3 ²⁻²¹	VQRLWVSRLLRH RKAQLLLV	200
CEA ²⁶⁶⁻²⁸⁵	PAQYSWFVNG TFQOSTQELF	63	MAGE- B1 ²⁴¹⁻²⁶⁰	PRKFITQDLV QEKYLKYEQV	132	SOX10 376-395	SQIAYTSLSLPH YGSAFFSI	201
CEL ⁵³²⁻⁵⁵¹	RSLRTNFLRY WTLTYLALPT	64	MAGE- B2 ²⁴⁴⁻²⁶³	PWKLITKDLV QEKYLEYKQV	133	SOX4 ⁴¹³⁻⁴³²	NFESMSLGSFSS SSALDRDL	202
CLCA2 ¹³²⁻¹⁴¹	CGKEGKYIHF TPNFLNDNL	65	MAGE- C1 ¹²⁵⁻¹⁴⁴	QSPLQNPASS FFSSALLSIF	134	SPA17 29-48	REQPDNIPAFAA AYFESLLE	203
CNTN2 ⁴⁷⁸⁻⁴⁹⁷	ISRSDEGKYT CFAENFMGKA	66	MAGE- C1 ¹⁹⁵⁻²¹⁴	QSPLQIPVSR SFSSTLLSIF	135	SSX2 ²³⁻⁴²	KAFDDIAKYFSK EEWEKMKA	204
COTL1 ⁵⁰⁻⁶⁹	QQCTDDVRLF AFVRFTTGDA	67	MAGE- C1 ³⁷¹⁻³⁹⁰	SPLQIPGSPS FSSTLLSLFQ	136	SSX4 ²³⁻⁴²	KAFDDIAKYFSK KEWEKMKS	205
CPSF1 ²⁰²⁻²²¹	NIIDLQFLHG YYEPTLLILF	68	MAGE- C1 ⁴⁰⁶⁻⁴²⁵	SPLQIPMTSS FSSTLLSILQ	137	STEAP 1 ²⁶³⁻²⁸²	LLGTIHALIFAW NKWIDIQ	206
CPSF1 ⁴⁷⁶⁻⁴⁹⁵	ANAAVGEPAF LSEEFQNSPE	69	MAGEC2 373-392	PLSSCCSSFS WSSFSEESSS	138	STEAP 1 ²⁹⁶⁻³¹⁵	FLPIVVLIKSI LFLPCLRK	207
CSAG2 ¹¹⁻³⁰	GVKRKDQGF EKEFYHKTNI	70	MART1 ³²⁻⁵¹	ILTVILGVLL LIGCWYCRRR	139	STEAP 1 ⁷⁴⁻⁹³	PIKIAAIIASLT FLYTLLRE	208
CSF1 ¹³⁰¹⁴⁹	HDKACVRTFY ETPLQLLEKV	71	MC1R ¹³⁹⁻¹⁵⁸	AVDRYISIFY ALRYHSIVTL	140	STEAP 1 ⁷⁶⁻⁹⁵	KIAAIIASLTFL YTLLREVI	209
CSPG4 ¹⁷⁴¹⁻¹⁷⁶⁰	QRSEHDVLFQ VTQFSPRGQL	72	MC1R ²⁴⁵⁻²⁶⁴	ILLGIFFLCW GPFFLHLTLI	141	STEAP 3 ²¹⁸⁻²³⁷	LALGLFVCFYAY NFVRDVLQ	210
CSPG4 ²⁰⁰³⁻²⁰²²	FQIDQGEVVF AFTNFSSSHD	73	MDK1 ¹⁻²⁰	MQHRGFLLLT LLALLAL TSA	142	TCL1 ¹⁰⁻²⁹	AVTDHPDRLWAW EKFVYLDE	211
CSPG4 ²⁰⁰⁵⁻²⁰²⁴	IDQGEVVFAF TNFSSSHDHF	74	MDM2 ⁴⁷⁻⁶⁶	TYTMKEVLFY LGQYIMTKRL	143	TERT ⁵⁵⁷⁻⁵⁷⁶	LRSFFYVTETTF QKNRLFFY	212
CT83 ⁹⁻²⁸	SSILCALIVF WKYRRFQRNT	75	MET ¹³³⁴⁻¹³⁵³	VSRISAI FST FIGEHYVHV N	144	TERT ⁵⁵⁸⁻⁵⁷⁷	RSFFYVTETTFQ KNRLFFYR	213
CTSG ⁴⁴⁻⁶³	AGQSRCGGFL VREDFVL TAA	76	MET ³⁵⁹⁻³⁷⁸	RSAMCAFP I K YVNDFFNKIV	145	TM4SF 1 ¹²²⁻¹⁴¹	LDSLQWNYTFA STEGQYLL	214
CYP1B1 262-281	EQLNRNFSNF ILDKFLRHCE	77	MGAT5 ¹³⁻³²	KLGFFLVTFG FIWGMLLHF	146	TPBG ²⁴¹⁻²⁶⁰	LSNNSLVSLTYV SFRNLTHL	215

CYP1B1 336-355	STALQWLLLL FTRYPDVQTR	78	MMP2 ₄₇₉₋₄₉₈	AQIRGEIFFF KDRFIWRTVT	147	TRPC1 371-390	APKSQFGRIHT PFMKFIH	216
CYP1B1 9-28	DPWPLNPLSI QQTLLLLLLS	79	MMP2 ₅₂₆₋₅₄₅	APQEEKAVFF AGNEYWIYSA	148	TRPC1 388-407	IIHGASYFTFLL LLNLYSLV	217
DCT ₁₇₄₋₁₉₃	PQFANCSVYD FFVWLHYYSV	80	MMP2 ₅₇₅₋₅₉₄	SKNKKTYIFA GDKFWRYNEV	149	TRPC1 456-475	NQLSFVMNSLYL ATFALKVV	218
DCT ₁₇₇₋₁₉₆	ANCSVYDFFV WLHYYSVRDT	81	MMP2 ₆₂₃₋₆₄₂	LQGGGHSYFF KGAYYKLEN	150	TRPC1 578-597	QQSNDTFHSFIG TCFALFWY	219
DCT ₃₃₂₋₃₅₁	QKFDNPPFFQ NSTFSFRNAL	82	MMP7 ₂₉₋₄₈	ELQWEQAQDY LKRFLYLDSE	151	TYMS ₁₂₋₁₄₁	PLLTTRKRVFWKG VLEELLWF	220
DDX43 ₃₇₀₋₃₈₉	IATPGRLNDL QMSNFVNLKN	83	MOK ₁₅₆₋₁₇₅	QPYTEYISTR WYRAPECLLT	152	TYR ₃₈₃₋₄₀₂	DPIFLLHHAFVD SIFEQWLR	221
DKK1 ₁₉₅₋₂₁₄	CASGLCCARH FWSKICKPVL	84	MPO ₆₅₄₋₆₇₃	KGRVGPLLAC IIGTQFRKLR	153	UBXN1 1258-277	QRCLRDILDGFF PSELQRLY	222
EGLN3 ₈₅₋₁₀₄	EEGCEAISFL LSLIDRLVLY	85	MSH3 ₁₀₄₋₂₋₁₀₆₁	DPGAAEQVPD FVTFLYQITR	154	VENTX P1 ₁₄₋₃₃	LAAASGQNRMTQ GQHFLQKV	223
ENAH ₉₂₋₁₁₁	GSKEDANVFA SAMMHALEVL	86	MSLN ₃₃₅₋₃₅₄	QMDRVNAIPF TYEQLDVLKH	155	WDR46 273-292	RRCDRVTRLEFL PFHFLLAT	224
EPCAM ₁₇₋₂₋₁₉₁	TRYQLDPKFI TSILYENNV	87	MUC1 ₁₀₃₋₅₋₁₀₅₄	QLSTGVSFFF LSFHISNLQF	156	XAGE1 A ₃₃₋₅₂	CATWKVICKSCI SQTPGINL	225
EPHA2 ₁₂₋₅₋₁₄₄	ESDLDYGTNF QKRLFTKIDT	88	MUC16 ₁₀₋₁₄₇₋₁₀₁₆₆	SMPANFETTG FEAEFPFSLT	157	XPB1 ₁₉₋₆₋₂₁₅	LQIQSLISCWAF WTTWTQSC	226
EPHA3 ₁₂₋₆₋₁₄₅	ESDDDHGVKF REHQFTKIDT	89	MUC16 ₁₀₋₃₂₃₋₁₀₃₄₂	SLPSSTPVPF SSSTFTTDS	158	ZBTB7 A ₉₉₋₁₁₈	VSTANVGDI LSA ARLLEIPA	227
EPOR ₄₁₆₋₄₃₅	PEGASAASFE YTILDPSSQL	90	MUC16 ₁₁₋₉₈₈₋₁₂₀₀₇	AKTTTTFNTL AGSLFTPLTT	159	CLIP	LPKPPKPVSKMR MATPLLMQALPM	228
ERBB2 ₉₉₋₂₋₁₀₁₁	EDLGASPLD STFYRSLLD	91	MUC16 ₂₉₋₄₄₋₂₉₆₃	STKAISASSF QSTGFTETPE	160	MAGE- A ₃₂₄₃₋₂₅₈	KKLLTQHVFQEN YLEY	229
ERBB2 ₉₉₋₃₋₁₀₁₂	DLGPASPLDS TFYRSLLD	92	MUC2 ₉₄₋₁₁₃	ILLTIKDDTI YLTRHLAVLN	161	WT1 ₃₂₈₋₃₄₈	PGCNKRYFKLSH LQMHSRKHT	230

EXOSC5 215-234	AASQHVFRFY RESLQRRYSK	93	MUC5AC 4922-4941	SGWGDPHYIT FDGTYTFLD	162	NY- ESO- 1157-17	SLLMWITQCFLP VF	231
EZH2 ₂₈₂₋₃₀₁	HTLFCRRCFK YDCFLHPFHA	94	Nuf2 ₅₀₋₆₉	MRALQIVYGI RLEHFYMPV	163	NY- ESO- 191-110	YLAMPFATPMEA ELARRSLA	232
FGF5 ₁₋₁₉	MSLSFLLLLF FSHLILSAWA	95	OR51E2 204-223	LVMGVDVMFI SLSYFLIIRT	164	Influ enza HA ₃₀₆₋₃₁₈	PKYVKQNTLKL A	233
FOLH1 ₅₅₋₅₇₄	VYETYELVEK FYDPMFKYHL	96	p53 ₇₋₂₆	DPSVEPPLSQ ETFSDLWKLL	165	HIV Gag ₂₉₃₋₃₁₂	FRDYVDRFYKTL RAEQASQE	234
GAGE1 117-136	VAQTGILWLL MNNCFNLNLS	97	PAK2 ₃₄₄₋₃₆₃	QIAAVCRECL QALEFLHANQ	166	DDX3Y 171-190	TGSNCPPHIENF SDIDMGEI	235
GAGE3 ₁₋₂₀	MNLSRGKSTY YWPRPRRYVQ	98	PAK2 ₄₈₅₋₅₀₄	VEKRGSAKEL LQHPFLKLAK	167	Bet V 1142-153	ETLLRAVESYLL	236
gp100 ₆₂₁₋₆₄₀	MKQDFSVPQL PHSSSHWLRL	99	PAPOLA 121-140	PRHVDRSDFF TSFYDKLKLQ	168	Influ enza HA ₂₅₅₋₂₇₀	RGYFKMRTGKSS IMRS	237
GPC3 ₁₃₅₋₁₅₄	PSLTPQAFEF VGEFFTDVSL	100	PASD1 ₂₅₈₋₂₇₇	MFVDSDESTYC SSTVFLDTMP	169			

[0173] *Genes*

[0174] Novel TCR genes were cloned via 5'-rapid amplification of cDNA ends (RACE) PCR using SMARTer RACE 5'/3' Kit (Takara Bio, Shiga, Japan) and sequenced as previously described. All genes were cloned into the pMX retroviral vector and transduced into cell lines using the 293GPG and PG13 cell-based retrovirus system.

[0175] *Antibodies*

[0176] The following antibodies were used for flow cytometry analysis: PE-conjugated anti-class II (9-49 (I3)), APC-Cy7-conjugated anti-CD4 (RPA-T4, Biolegend, San Diego, CA)⁴⁴, FITC-conjugated anti-NGFR (ME20.4, Biolegend, San Diego, CA), PE-conjugated anti-His tag (AD1.1.10, Abcam, Cambridge, MA), and FITC-conjugated anti-Vβ22 (IMMU 546, Beckman Coulter, Brea, CA). Biotinylated DP4/NY-ESO1₁₅₇₋₁₇₀ and DP4/WT1₃₂₉₋₃₄₈ monomers were multimerized using PE-conjugated streptavidin (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. 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).

[0177] The following antibodies were used for immunoblot analysis: anti- β -actin (C4, Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal anti-MAGE-A2 (Abcam, Cambridge, MA), anti-CCND1 (EPR2241, Abcam, Cambridge, MA), HRP-conjugated goat anti-mouse IgG (H+L) secondary antibody (Promega, Fitchburg, WI), and HRP-conjugated anti-rabbit IgG (H+L) secondary antibody (Promega, Fitchburg, WI).

[0178] *TCR transduction into primary T cells*

[0179] 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.

[0180] *Staining with soluble CD4*

[0181] 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. 13A-13Q.

[0182] *Construction and screening of a multisite-directed DPB1*04:01 mutant cDNA library*

[0183] Multisite-directed random mutations were inserted into the DPB1*04:01 cDNA by using PCR and the following primer sets: forward: 5'-CACCACAACNNNCTTNNNTGCCACGTG-3' (SEQ ID NO: 12) and reverse: 5'-CACGTGGCANNNAAGNNNGTTGTGGTG-3' (SEQ ID NO: 13) for L112 and V114;

forward: 5'- ACAGCTGGGGTCNNNTCCACCAACCTG-3' (SEQ ID NO: 14) and reverse: 5'- CAGGTTGGTGGANNNGACCCCAGCTGT-3' (SEQ ID NO: 15) for V141; forward: 5'- CAGATCNNNGTGNNNCTGGAAATGACC-3' (SEQ ID NO: 16) and reverse: 5'- GGTCATTTCAGNNNCACNNNGATCTG-3' (SEQ ID NO: 17) for L156 and M158. N stands for any nucleotide. The resultant PCR fragments were fused to each other to construct a mutant full-length DPB1*04:01 cDNA expression library carrying random mutations at the positions L112, V114, V141, L156, and M158. K562 cells stably expressing the DPA1*01:03 gene were infected with recombinant retroviruses produced using the packaging cell line 293GPG at a transduction efficiency of less than 30%. The infected K562 cells were stained with soluble CD4 dimer, and the dimer-positive cells were collected using a flow cytometry cell sorter. The mutant DPB1*04:01 gene was cloned from the collected cells and retrovirally transduced into K562 cells along with the wild-type DPA1*01:03 gene as described above.

[0184] *Generation of the HLA class II monomer and dimer*

[0185] 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 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 DP4 dimer staining, HEK293T cells stably secreting soluble DP4^{L112W/V141M} protein were grown until confluent, and the medium was changed to serum-free 293 SFM II medium (Thermo Fisher Scientific, Waltham, MA). After forty-eight hours, the conditioned medium was harvested and concentrated using Amicon Ultra filters (molecular weight cut-off (MWCO) 10 kDa) (MilliporeSigma, Burlington, MA). 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.

[0186] *Stimulation of DP4-restricted antigen-specific CD4⁺ T cells*

- [0187] CD4⁺ T cells were purified using a CD4⁺ T Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Purified T cells were stimulated with DP4-expressing aAPCs pulsed with DP4-restricted peptides at 10 µg/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. After 2 weeks of stimulation, the T cells were subjected to DP4_{L112W/V141M} dimer staining.
- [0188] ***HLA class II dimer and tetramer staining***
- [0189] Primary T cells and Jurkat 76/CD4 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, a FITC-conjugated anti-NGFR mAb, and a PE-conjugated anti-Vβ22 mAb.
- [0190] ***ELISPOT assay***
- [0191] Cytokine ELISPOT assays were performed as previously reported (*see, e.g.,* Yamashita et al., *Nat. Commun.* 8:15244 (2017); and Anczurowski et al., *Sci. Rep.* 8:4804 (2018)).
- [0192] ***Immunoblotting***
- [0193] Immunoblot analysis was performed as previously reported (*see, e.g.,* Yamashita et al., *Nat. Commun.* 8:15244 (2017); and Anczurowski et al., *Sci. Rep.* 8:4804 (2018)).
- [0194] Protein modeling
- [0195] The HLA-DP4 and human CD4 complex model structures were predicted based on structures from PDB IDs 3S5L and 3T0E using Swiss-Model workspace for quaternary structure prediction.
- [0196] ***Statistical analysis***
- [0197] 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.
- [0198] ***Biolayer interferometry sensorgram***

[0199] The extracellular domain of human CD4 (residues 26-440 of NP_000607.1) followed by a GS linker and 10x histidine (His) tag was stably expressed in the human cell line A375 (SEQ ID NOs: 245-246; Table 4). Recombinant 10x His-tagged CD4 protein was purified from the supernatant with TALON metal affinity resin (Takara Bio, Shiga, Japan). The eluted protein was concentrated using an Amicon Ultra-15 spin column (MilliporeSigma, Burlington, MA) with a 10 kDa MWCO. Buffer was exchanged to HBS-EP (GE Healthcare Life Sciences, Marlborough, MA) using 10 kDa MWCO MINI Dialyzer (Thermo Fisher Scientific, Waltham, MA). The purity of the recombinant CD4 protein was consistently >90%, as confirmed by SDS-PAGE.

[0200] The recombinant DP4 protein consisted of extracellular domains of DPA1*01:03, and the wild-type DPB1*04:01 or L112W/V141M mutant. DPA1*01:03 was followed by an acid leucine zipper, a GS linker and a 10x histidine tag, while wild-type and mutant DPB1 was followed by a basic leucine zipper, a GS linker, and a biotinylation sequence (GLNDIFEAQKIEWHE; SEQ ID NO: 244). Both DPA and DPB genes were stably expressed in A375-BirA cells, which were transduced with the codon-optimized BirA gene encoding a leader sequence at the 5' end and an ER retention KDEL motif at the 3' end. Recombinant DP4 protein was purified from the supernatant with TALON metal affinity resin (Takara Bio, Shiga, Japan). Eluted protein was concentrated using Vivaspin 500 spin column (GE Healthcare Life Sciences, Marlborough, MA) with a 10 kDa MWCO, and reconstituted to working volume in PBS.

[0201] Binding for wild-type DP4 and DP4^{L112W/V141M} with CD4 was measured by the Octet Red system (ForteBio, Fremont, CA). Experiments were performed at 25°C using a 96-well OptiPlate (Perkin Elmer, Waltham, MA), with a 200-μl sample volume and constant shaking at 1,000 rpm. The biotinylated recombinant DP4 was loaded onto streptavidin-coated biosensors (ForteBio, Fremont, CA) until saturation, followed by baseline measurement in the HBS-EP buffer. Association was measured by incubating the loaded sensors for 400 sec with titrated concentrations of recombinant CD4 (0.8125 to 26 μM) before 300 sec dissociation in HBS-EP buffer alone. The steady-state analysis was fitted using a one-site specific binding model in GraphPad Prism 7.0

Table 4. Soluble 10x His-tagged CD4 Nucleic Acid Sequence

Fibroin L Signal Peptide; *CD4*; Gly/Ser Linker and His tag sequences (10X) (SEQ ID NO: 245)

ATGATGCGGCCCATCGTGCTGGTGCTGCTGTTTGCCACATCTGCCCTGGCCAAG
AAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAAGTACCTGTACAGCTTC
CCAGAAGAAGAGCATACAATTCCACTGGAAAACTCCAACCAGATAAAGATTC
TGGGAAATCAGGGCTCCTTCTTAATAAGGTCCATCCAAGCTGAATGATCGCG
CTGACTCAAGAAGAAGCCTTTGGGACCAAGGAACTTTCCCCTGATCATCAAG
AATCTTAAGATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAGAA
GGAGGAGGTGCAATTGCTAGTGTTTCGGATTGACTGCCAACTCTGACACCCACCT
GCTTCAGGGGCAGAGCCTGACCCTGACCTTGGAGAGCCCCCCTGGTAGTAGCC
CCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATACAGGGGGGGAAGAC
CCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACATGCACTGT
CTTGCAAGAACCAGAAGAAGGTGGAGTTCAAATAGACATCGTGGTGCTAGCTT
TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAGTTC
TCCTTCCCCTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGG
TGGCAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGATCACCTTTGACCTGAAG
AACAAGGAAGTGTCTGTAAAACGGGTACCCAGGACCCTAAGCTCCAGATGGG
CAAGAAGCTCCCGCTCCACCTCACCTGCCCCAGGCCTTGCCTCAGTATGCTGG
CTCTGGAAACCTCACCTGGCCCTTGAAGCGAAAACAGGAAAGTTGCATCAGG
AAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGAAAAATTTGACCTGT
GAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAACTGGAGAA
CAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTGCTGAACCCT
GAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTGGA
ATCCAACATCAAGGTTCTGCCACATGGGGCAGCCACCACCACCATCACCATCA
TCATCACCATTGA

Fibroin L Signal Peptide; *CD4*; Gly/Ser Linker and His tag sequences (10X) (SEQ ID NO: 246)

MMRPVLVLLFATSALAKKVVLGKKGDTVELTCTASQKKSIFHWKNSNQIKILGN
QGSFLTGPSKLNDRADSRRLWDQGNFPLIKNLKIEDSDTYICEVEDQKEEVQLL
VFGLTANS DTHLLQGQSLTLTLESPPGSSPSVQCRSPRGKNIQGGKTLVSQLELQD
SGTWTCTVLQNQKKVEFKIDIVLAFQKASSIVYKKEGEQVEFSFPLAFTVEKLTGS
GELWWQAERASSKSWITFDLKNKEVSVKRVTQDPKLQMGKKLPLHLTLPQALPQ

YAGSGNLTALAEAKTGKLTQEVNVLVVMRATQLQKNLTCEVWGPTSPKLMLSLKL
 ENKEAKVSKREKAVWVLNPEAGMWQCLLSDSGQVLLESNIKVLPTWGSHHHHHH
HHHH

***Example 2 – L112W/V141M substitutions of the DPβ chain
 enhance the binding of DP to CD4***

[0202] A cDNA expression library was generated of the DPB1*04:01 (DP4β) gene carrying random mutations at L112, V114, V141, L156, and M158, which corresponds to L114, V116, V143, L158, and M160 of the DR1β chain, respectively, and coexpressed the library along with the wild-type DPA1*01:03 (DPα) gene in class II-deficient K562 cells. After two rounds of screening using soluble CD4 protein (sCD4), cell populations with enhanced CD4 binding were isolated, from which a mutant DP4β gene carrying L112W, V114M, V141M, and M158I substitutions was molecularly cloned. When ectopically expressed in the K562 cells, the mutant DP4 molecules consisting of the wild-type DPα chain and cloned mutant DP4β chain carrying L112W, V114M, V141M, and M158I substitutions (DP4^{L112W/V114M/V141M/M158I}) indeed showed enhanced binding to sCD4 compared with the wild-type DP4 molecules, excluding the possibility that enhanced CD4 binding was an artifact of screening processes (FIGs. 1A-1F).

[0203] To determine which of the four mutations is critical for enhanced CD4 binding, a reversion mutagenesis study was conducted. All the possible reversion DP4 mutants were reconstituted on class II-negative K562 cells and stained with sCD4. Both the L112W and V141M but not V114M or M158I single substitutions individually enhanced the binding of DP4 to sCD4 (FIG. 1G). Importantly, the L112W/V141M double mutations (DP4^{L112W/V141M}) synergistically enhanced the DP4/CD4 binding (FIG. 1G). Interestingly, both the V114M and M158I single replacements appeared to have a negative effect on the enhanced binding enabled by the DP4^{L112W/V141M} mutations (FIG. 1G). Previous studies have estimated that the K_D value between CD4 and HLA class II is >2 mM. Using biolayer interferometry (BLI) binding assay, the affinity of DPR^{L112/V141M} for CD4 was measured. While no binding was detected between wild-type DP4 and CD4, DP4^{L112W/V141M} bound to CD4 with a K_D of 8.9 μM ± 1.1 (FIG. 1H and FIGs. 1X-1BK). This value represents an at least 200-fold improvement in the binding affinity. Further, the observed affinity between CD4 and

DP4^{L112W/V141M} is higher than that between human CD8 and HLA class I (~200 μ M) and is comparable to that between mouse CD8 and mouse MHC Class I (~10 μ M). To confirm that enhanced binding between DP4^{L112W/V141M} and CD4 leads to an enhanced CD4⁺ T cell response, a comparison was conducted of the immunostimulatory capacity of artificial APCs (aAPCs) expressing either wild-type DP4 or DP4^{L112W/V141M} as a single class II allele using DP4/WT1 TCR (clone 9)-transduced CD4⁻ and CD4⁺ Jurkat 76 T cells as responder cells. As expected, DP4^{L112W/V141M} carrying aAPCs demonstrated enhanced T cell stimulatory activity in a CD4-dependent manner (FIG. 1I).

[0204] Next other DP alleles to CD4 were analyzed to determine whether the L112W/V141M mutations also enhance binding. Although none of the wild-type DP2, DP5, or DP8 bound to CD4, all three molecules bound to CD4 strongly when the L112W/V141M double mutations were introduced in the DP β chains of these molecules (FIGs. 1J-1W). A structural model (FIGs. 2A-2D) constructed based on a previous report revealed that in the DP4^{L112W/V141M}-CD4 complex, the two L112W/V141M mutations apparently induced a hydrophobic effect at the positions, K35, Q40, and T45 of CD4. These results show that L112W/V141M mutations can enhance the CD4 binding of at least all 4 of the DP alleles tested.

***Example 3 – Affinity-matured DP4^{L112W/V141M} multimers
specifically stain cognate TCRs***

[0205] To determine the effect of the L112W/V141M double mutations of DP4 β on DP4 multimer staining, a soluble DP4^{L112W/V141M} monomer was produced, which was then dimerized with an anti-His tag mAb. Primary T cells were individually transduced with three different DP4-restricted TCRs specific for MAGE-A3 (clone R12C9), WT1 (clone 9), and NY-ESO-1 (clone 5B8) and then stained with cognate DP4^{L112W/V141M} dimers. As shown in FIGs. 3A-3P, each DP4^{L112W/V141M} dimer specifically stained CD4⁺ T cells expressing the cognate TCR. Costaining of R12C9- and clone 9-transduced T cells with an anti-V β 22 mAb and anti-NGFR mAb, respectively, along with the respective DP4^{L112W/V141M} dimer confirmed that virtually all TCR-transduced CD4⁺ T cells were successfully stained with the respective DP4^{L112W/V141M} dimers (FIGs. 4A-4H). Compared with conventional wild-type DP4 tetramers, our novel DP4^{L112W/V141M} dimers stained both DP4/WT1 and DP4/NY-ESO-1 T cells better than conventional wild-type DP4 tetramers (FIGs. 5A-5P). Notably, the conventional wild-

type DP4/NY-ESO-1 tetramer was unable to stain cognate T cells even at the highest concentration available (data not shown).

Example 4 – DP4^{L112W/V141M} dimer technology is robust and versatile

[0206] To demonstrate the robustness and versatility of the DP4^{L112W/V141M} multimer staining, a comprehensive screening was performed for the *in vitro* immunogenicity of potential DP4-restricted peptides derived from an array of tumor-associated antigens (Table 3). One hundred and ninety-six DP4-restricted and tumor-associated antigen-derived 20-mer peptides were predicted using a peptide prediction algorithm (NetMHC2 ver.2.2) and chemically synthesized (Table 3). The frequency of antigen-specific CD4⁺ T cells is generally very low in the periphery; therefore, primary CD4⁺ T cells isolated from six DP4⁺ melanoma patients were stimulated only once with DP4-aAPCs individually pulsed with the 196 peptides and stained with cognate DP4^{L112W/V141M} dimers. To avoid potential *in vitro* priming, weak stimulatory conditions were utilized. As shown in FIGs. 6A-6F, 103 predicted DP4 peptides were immunogenic, at least *in vitro*.

[0207] To validate the dimer staining results, we cloned seven DP4-restricted TCR genes specific for CCND1₂₁₉₋₂₃₈, HSD17B12₂₂₅₋₂₄₄, LGSN₂₉₆₋₃₁₅, MAGE-A2₁₀₈₋₁₂₇, and MUC5AC₄₉₂₂₋₄₉₄₁ (FIGs. 7A-7L and Table 5) from the dimer-positive T cells. When clonotypically reconstituted in human CD4⁺ TCR-deficient T cells, all these TCRs were successfully stained by the cognate DP4^{L112W/V141M} dimers (FIGs. 8A-8X) and were functional in a DP4-restricted and antigen-specific manner (FIGs. 9A-9G).

[0208] Among the four TCRs individually expressed in primary T cells, three TCRs, i.e., 03-CCND1₂₁₉₋₂₃₈, 06-MAGE-A2₁₀₈₋₁₂₇, and 05-MUC5AC₄₉₂₂₋₄₉₄₁, were able to recognize cognate peptides that were endogenously processed and presented by DP4 (FIGs. 10A-10Q and 11A-11E). Importantly, 06-MAGE-A2₁₀₈₋₁₂₇-transduced primary T cells were able to recognize melanoma cell lines in a DP4- and MAGE-A2-dependent manner (FIGs. 12A-12E).

Table 5: DP4-Restricted TCRs

No.	Peptide	TRAV	TRAJ	TCR-alpha CDR 3	TRB V	TRBJ	TCR-beta CDR 3
03	CCND1 ₂₁₉₋₂₃₈	2*01	21*01	CAVCTLYNFNKF YF (SEQ ID NO: 18)	6- 5*01	2-1*01	CASLTDNNEQFF (SEQ ID NO: 25)

05	HSD17B12 ₂ 25-244	22*01	18*01	CAVAPYDRGSTL GRLYF (SEQ ID NO: 19)	19*01	2-5*01	CASSTGQGLETQ YF (SEQ ID NO: 26)
09	HSD17B12 ₂ 25-244	27*01	33*01	CAGVKDSNYQLI W (SEQ ID NO: 20)	30*01	2-1*01	CAWSSYNEQFF (SEQ ID NO: 27)
05	LGSN ₂₉₆₋₃₁₅	9-2*03	32*01	CALSDLSYGGAT NKLIF (SEQ ID NO: 21)	27*01	1-5*01	CASSKGQGLGN QPQHF (SEQ ID NO: 28)
03	MAGE- A2 ₁₀₈₋₁₂₇	36/DV7 *04	40*01	CAVEVNSGTYK YIF(SEQ ID NO: 22)	2*01	1-1*01	CASRRDLAAFF (SEQ ID NO: 29)
06	MAGE- A2 ₁₀₈₋₁₂₇	19*01	40*01	CALSVGTYKYIF(SEQ ID NO: 23)	7- 9*01	2-5*01	CASSPGTGGRET QYF (SEQ ID NO: 30)
05	MUC5AC ₄₉₂ 2-4941	38-1*03	58*01	CAFMKRAETSGS RLTF(SEQ ID NO: 24)	6- 2*01	2-5*01	CASSYWPTRETQ YF (SEQ ID NO: 31)

[0209] In contrast to CD8, the role and function of CD4 as a coreceptor has yet to be fully elucidated. This lack of information exists mainly because the binding between CD4 and class II is exceptionally weak, which significantly limits research on the role of the association between CD4 and class II. In this study, an affinity-matured form of HLA-DP4, i.e., DP4^{L112W/V141M}, was isolated with enhanced CD4 binding, and a novel DP4^{L112W/V141M} dimer technology was developed, which introduces robustness and rigorousness in the detection of DP4-restricted antigen-specific CD4⁺ T cells.

[0210] Using this DP4^{L112W/V141M} dimer technology, DP4-restricted antitumor T cell responses were comprehensively studied *in vitro* and multiple DP4-restricted immunogenic peptides and cognate TCR genes were identified. HLA-DP4 is the most prevalent HLA allele in many ethnic groups and belongs to the DP^{84Gly} group. Unlike other class II molecules, DP^{84Gly} molecules such as DP4 constitutively present peptides derived from endogenous sources regardless of the invariant chain and HLA-DM expression. The improved

presentation of endogenous peptides via class II is correlated with improved survival of cancer patients. Notably, a first-in-human class II-restricted TCR gene therapy indeed targeted a DP4-restricted MAGE-A3 peptide (*see, e.g., Yao et al., J. Immunother. 39:191-201 (2016)*). The DP^{84Gly} genotype, such as in DP2 and DP4, acts as a risk allele for anti-neutrophil cytoplasmic autoantibody-associated vasculitis. DP4 molecules, which can constitutively present peptides derived from endogenous tumor-associated antigens, may induce more clinically relevant antitumor responses than other class II molecules, serving as a protective class II allele.

[0211] 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.

[0212] 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-DP alleles.

Example 5 – DP4^{L112W/V141M} Specificity and Binding

[0213] DP4 multimer staining of endogenous (untransduced) antigen specific CD4⁺ T cells was analyzed. The novel DP4^{L112W/V141M} dimers positively stained endogenous TRPC1₅₇₈₋₅₉₇-specific CD4⁺ T cells (FIGs. 14A-14B) more strongly than the conventional DP4 dextramer (FIGs. 14C-14D). The DP4^{L112W/V141M} dimers showed markedly improved staining of endogenous (untransduced) NY-ESO-1₁₅₇₋₁₇₀-specific CD4⁺ T cells (FIGs. 15A-15B; Table 6) compared with conventional tetramers (FIGs. 15C-15D) or dextramers (FIGs. 15E-15F).

Table 6: DP4-Restricted TCRs

Donor No.	TRAV	TRAJ	TCR-alpha CDR3	TRBV	TRBJ	TCR-beta CDR3
HD04	8-2*01	32*02	CVVSGGVNNGGAT NKLIF SEQ ID NO: 247	7-9*01	2-7*01	CASSLTGGVSYEQY F SEQ ID NO: 248
c6	13-1*01	18*01	CAASVRGSTLGRL	7-8*01	2-2*01	CASSLGTGGTGELF

			YF SEQ ID NO: 249			F SEQ ID NO: 250
c12	8-4*01	18*01	CAVSGGRGSTLG RLYF SEQ ID NO: 251	29*01	1-2*01	CSVQGGLDSNYGY TF SEQ ID NO: 252
c17	13-1*01	18*01	CAASVRGSTLGRL YF SEQ ID NO: 253	7-8*01	2-2*01	CASSLGTGGTGELF F SEQ ID NO: 254
c23	13-1*01	18*01	CAASVRGSTLGRL YF SEQ ID NO: 255	7-8*01	2-2*01	CASSLGTGGTGELF F SEQ ID NO: 256
c26	38-2/ DV8*01	21*01	CAYRSNNFNKFYF SEQ ID NO: 257	5-1*01	1-2*01	CASSLNTGAGYGY TF SEQ ID NO: 258
c31	13-1*01	18*01	CAASVRGSTLGRL YF SEQ ID NO: 259	7-8*01	2-2*01	CASSLGTGGTGELF F SEQ ID NO: 260
c37	13-1*01	18*01	CAASVRGSTLGRL YF SEQ ID NO: 261	7-8*01	2-2*01	CASSLGTGGTGELF F SEQ ID NO: 262
c39	2*01	9*01	CAVEERTGGFKTI F SEQ ID NO: 263	2*01	2-2*01	CASSLPSGGAPGTG ELFF SEQ ID NO: 264
c52	8-4*01	18*01	CAVSGGRGSTLG RLYF SEQ ID NO: 265	29*01	1-2*01	CSVQGGLDSNYGY TF SEQ ID NO: 266
c87	13-1*01	18*01	CAASVRGSTLGRL YF SEQ ID NO: 267	7-8*01	2-2*01	CASSLGTGGTGELF F SEQ ID NO: 268
c2	4*01	39*01	CLVGDLGANAGN MLTF SEQ ID NO: 269	19*01	2-2*01	CASSIATTNTGELFF SEQ ID NO: 270

c4	4*01	39*01	CLVGD LGANAGN MLTF SEQ ID NO: 271	11-3*01	2-7*01	CASSLETGTNYEQY F SEQ ID NO: 272
c6	8-3*01	32*02	CAVALYGGATNK LIF SEQ ID NO: 273	7-9*3	2-1*01	CASSLDIGNNEQFF SEQ ID NO: 274
c9	25*01	53*01	CAGRSGGSNYKL TF SEQ ID NO: 275	19*01	2-2*01	CASSIATTNTGELFF SEQ ID NO: 276
c29	25*01	53*01	CAGRSGGSNYKL TF SEQ ID NO: 277	19*01	2-2*01	CASSIATTNTGELFF SEQ ID NO: 278
c30	25*01	53*01	CAGRSGGSNYKL TF SEQ ID NO: 279	19*01	2-2*01	CASSIATTNTGELFF SEQ ID NO: 280
c32	25*01	53*01	CAGRSGGSNYKL TF SEQ ID NO: 281	19*01	2-2*01	CASSIATTNTGELFF SEQ ID NO: 282
c33	25*01	53*01	CAGRSGGSNYKL TF SEQ ID NO: 283	19*01	2-2*01	CASSIATTNTGELFF SEQ ID NO: 284

[0214] Next, *ex vivo* staining was performed of memory CD4⁺ T cells with DP4^{L112W/V141M} dimers specific to a series of pathogen-associated peptides without *in vitro* stimulation. A small subset of the CD4⁺ T cells were positively stained with DP4^{L112W/V141M} dimers for tetanus toxin₉₄₈₋₉₆₈ (TT₉₄₈₋₉₆₈), herpes simplex virus type-2-UL21₂₈₃₋₃₀₂ (HSV-2-UL21₂₈₃₋₃₀₂), and respiratory syncytial virus glycoprotein₁₆₂₋₁₇₅ (RSV-GP₁₆₂₋₁₇₅) (FIGs. 16A-16Y). Next, we established endogenous (untransduced) single-cell clones by limiting dilution from RSV-GP₁₆₂₋₁₇₅ (FIGs. 17A-17V) and TT₉₄₈₋₉₆₈ dimer⁺ CD4⁺ T cells (FIGs. 18A-18R). These T cell clones showed IL-2 production in an antigen-specific manner (FIGs. 17W and 18S). Multiple TCRαβ pairs, including one dominant pair, were isolated from both DP4^{L112W/V141M} RSV-GP and TT dimer⁺ single-cell clones (Table 6). In FIGs. 17A-17W and 18A-18S, single-cell

clones were established by limiting dilution from RSV-GP₁₆₂₋₁₇₅ and TT₉₄₈₋₉₆₈ dimer⁺ cells. When these RSV-GP and TT dimer⁺ single-cell clones were individually stained with three different DP4 multimers (DP4^{L112W/V141M} dimers, wild-type DP4 tetramers, or wild-type DP4 dextramers), the DP4^{L112W/V141M} dimers showed better staining of RSV-GP- (c12 and c39) and TT-specific clones (c2 and c9) than the conventional wild-type DP4 RSV-GP dextramers and wild-type DP4 TT tetramers and dextramers (FIGs. 19A-19NN).

[0215] *Methods*

[0216] *Cells*

[0217] 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)). HEK293T cells were grown in DMEM supplemented with 10% FBS and 50 µg/ml gentamicin.

[0218] *Peptides/Antibodies*

[0219] Synthetic peptides were dissolved at 50 mg/ml in DMSO. The following antibodies were used for flow cytometry analysis: APC-Cy7-conjugated anti-CD4 and PE-conjugated anti-His tag.

[0220] *Generation of the HLA class II monomer and dimer*

[0221] HEK293T cells were transfected with the α and β genes using the 293GPG cell-based retrovirus system (see 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., *Blood* **108**, 2662-2668 (2006)) and cultured in DMEM supplemented with 10% FBS and 50 µg/ml gentamicin. For DP4 dimer staining, HEK293T cells stably secreting soluble DP4^{L112W/V141M} protein were grown until confluent, and the medium was changed to serum-free 293 SFM II medium (Thermo Fisher Scientific, Waltham, MA). After forty-eight hours, the conditioned medium was harvested and concentrated using Amicon Ultra filters (molecular weight cut-off (MWCO) 10 kDa) (MilliporeSigma, Burlington, MA). The soluble HLA class II-containing supernatant was then mixed with 100 µg/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 a PE-conjugated anti-His mAb at a 2:1 molar ratio for 1.5 hours at 4°C for staining.

[0222] *Stimulation of DP4-restricted antigen-specific CD4⁺ T cells*

[0223] CD4⁺ T cells were purified and stimulated with DP4-expressing aAPCs pulsed with DP4-restricted peptides at 10 µg/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. After 2 weeks of stimulation, the T cells were subjected to DP4^{L112W/V141M} dimer staining.

[0224] *HLA class II dimer, tetramer and dextramer staining*

[0225] DP4 tetramers and dextramers were compared in multimer staining analysis. Primary 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 µg/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.

[0226] *Dimer staining of unstimulated CD4⁺ T cells from PBMCs from melanoma patients*

[0227] One million CD4⁺ T cells were purified and pretreated with 50 nM dasatinib for 30 min at 37°C. The cells were stained with 5-15 µg/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. The absolute counts of the dimer⁺ cells were determined by flow cytometry.

[0228] *Expansion of DP4 dimer⁺ T cells and establishment of single T cell clones.*

[0229] To expand DP4^{L112W/V141M} dimer⁺ T cells, CD4⁺ T cells were stimulated and stained with DP4^{L112W/V141M} dimers as described above. The dimer⁺ cells were sorted by using anti-PE magnetic beads and expanded by using artificial APC/mOKT3 irradiated at 200 Gy at an E:T ratio of 5-20:1 (*see* Butler, M.O. et al., *PLoS One* 7, e30229 (2012)). The culture medium supplemented with IL-2 (10 IU/ml) and IL-15 (10 ng/ml) was replenished every 2-3 days. Two to three weeks later, the T cells were subjected to DP4^{L112W/V141M} dimer staining. DP4^{L112W/V141M} dimer⁺ single-cell clones were generated by limiting dilution as previously described (*see* Su, L.F. et al., *Immunity* 38, 373-383 (2013)). Briefly, memory CD4⁺ T cells were purified and stained with DP4^{L112W/V141M} dimers without dasatinib pretreatment. The dimer⁺ cells were sorted and then stimulated with 5 µg/ml PHA-P and PBMCs from multiple allogeneic donors irradiated at 20 Gy in a 96-well plate. The culture medium was

supplemented and replenished after 1 week of stimulation with IL-2 (100 IU/ml) and IL-15 (10 ng/ml). Two weeks later, single-cell clones were stained with DP4^{L112W/V141M} dimers.

[0230] ***ELISPOT assay***

[0231] Cytokine ELISPOT assays were performed as previously reported (*see* Yamashita, Y. et al., *Nat Commun* 8, 15244 (2017); Anczurowski, M. et al., *Sci Rep* 8, 4804 (2018)).

Claims:

1. An HLA class II molecule comprising a DP beta chain, wherein the DP beta chain comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1.
2. An HLA class II molecule comprising a DP beta chain, wherein the DP beta chain comprises a substitution mutation at a position corresponding to amino acid residue 112 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 DP beta chain further comprises an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.
4. An HLA class II molecule comprising a DP beta chain, wherein the DP beta chain comprises an amino acid other than valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.
5. An HLA class II molecule comprising a DP beta chain, wherein the DP beta chain comprises a substitution mutation at a position corresponding to amino acid residue 141 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 DP beta chain further comprises an amino acid other than leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1.
7. The HLA class II molecule of any one of claims 1 to 6, wherein the DP 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.
8. The HLA class II molecule of any one of claims 1 to 3, 6, and 7, wherein the amino acid other than leucine comprises a hydrophobic side chain.
9. The HLA class II molecule of claim 8, wherein the amino acid other than leucine is selected from the group consisting of an alanine, a valine, an isoleucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan.

10. The HLA class II molecule of any one of claims 1 to 3 and 6 to 9, wherein the amino acid other than leucine is a tryptophan.
11. The HLA class II molecule of any one of claims 3 to 10, wherein the amino acid other than valine comprises a hydrophobic side chain.
12. The HLA class II molecule of claim 11, wherein the amino acid other than valine is selected from the group consisting of an alanine, an isoleucine, a leucine, a methionine, a phenylalanine, a tyrosine, and a tryptophan.
13. The HLA class II molecule of any one of claims 3 to 12, wherein the amino acid other than valine is a methionine.
14. The HLA of any one of claims 1 to 13, wherein the DP beta chain comprises a tryptophan at a position corresponding to amino acid residue 112 of SEQ ID NO: 1 and a methionine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.
15. The HLA class II molecule of any one of claims 1 to 14, wherein the DP beta chain comprises the amino acid sequence set forth in SEQ ID NO: 3.
16. The HLA class II molecule of any one of claims 1 to 15, wherein the DP beta chain comprises the amino acid sequence set forth in SEQ ID NO: 4.
17. The HLA class II molecule of any one of claims 1 to 16, wherein the DP beta chain is selected from DPB1*01, DPB1*02, DPB1*03, DPB1*04, DPB1*05, DPB1*06, DPB1*08, DPB1*09, DPB1*10, DPB1*100, DPB1*101, DPB1*102, DPB1*103, DPB1*104, DPB1*105, DPB1*106, DPB1*107, DPB1*108, DPB1*109, DPB1*11, DPB1*110, DPB1*111, DPB1*112, DPB1*113, DPB1*114, DPB1*115, DPB1*116, DPB1*117, DPB1*118, DPB1*119, DPB1*120, DPB1*121, DPB1*122, DPB1*123, DPB1*124, DPB1*125, DPB1*126, DPB1*127, DPB1*128, DPB1*129, DPB1*13, DPB1*130, DPB1*131, DPB1*132, DPB1*133, DPB1*134, DPB1*135, DPB1*136, DPB1*137, DPB1*138, DPB1*139, DPB1*14, DPB1*140, DPB1*141, DPB1*142, DPB1*143, DPB1*144, DPB1*145, DPB1*146, DPB1*147, DPB1*148, DPB1*149, DPB1*15, DPB1*150, DPB1*151, DPB1*152, DPB1*153, DPB1*154, DPB1*155, DPB1*156, DPB1*157, DPB1*158, DPB1*159, DPB1*16, DPB1*160, DPB1*161, DPB1*162,

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18. The HLA class II molecule of any one of claims 1 to 17, wherein the DP beta chain comprises the amino acid sequence set forth in SEQ ID NO: 1.
19. The HLA class II molecule of any one of claims 1 to 18, further comprising a DP alpha chain.
20. The HLA class II molecule of claim 19, wherein the DP alpha chain is selected from
 DPA1*01:03:01:01, DPA1*01:03:01:02, DPA1*01:03:01:03, DPA1*01:03:01:04,
 DPA1*01:03:01:05, DPA1*01:03:01:06, DPA1*01:03:01:07, DPA1*01:03:01:08,
 DPA1*01:03:01:09, DPA1*01:03:01:10, DPA1*01:03:01:11, DPA1*01:03:01:12,
 DPA1*01:03:01:13, DPA1*01:03:01:14, DPA1*01:03:01:15, DPA1*01:03:01:16,
 DPA1*01:03:01:17, DPA1*01:03:01:18Q, DPA1*01:03:01:19, DPA1*01:03:01:20,
 DPA1*01:03:01:21, DPA1*01:03:01:22, DPA1*01:03:01:23, DPA1*01:03:02,
 DPA1*01:03:03, DPA1*01:03:04, DPA1*01:03:05, DPA1*01:03:06, DPA1*01:03:07,
 DPA1*01:03:08, DPA1*01:03:09, DPA1*01:04, DPA1*01:05, DPA1*01:06:01,
 DPA1*01:06:02, DPA1*01:07, DPA1*01:08, DPA1*01:09, DPA1*01:10, DPA1*01:11,
 DPA1*01:12, DPA1*01:13, DPA1*01:14, DPA1*01:15, DPA1*01:16, DPA1*01:17,
 DPA1*01:18, DPA1*01:19, DPA1*02:01:01:01, DPA1*02:01:01:02, DPA1*02:01:01:03,
 DPA1*02:01:01:04, DPA1*02:01:01:05, DPA1*02:01:01:06, DPA1*02:01:01:07,
 DPA1*02:01:01:08, DPA1*02:01:01:09, DPA1*02:01:01:10, DPA1*02:01:01:11,
 DPA1*02:01:02:01, DPA1*02:01:02:02, DPA1*02:01:03, DPA1*02:01:04, DPA1*02:01:05,
 DPA1*02:01:06, DPA1*02:01:07, DPA1*02:01:08:01, DPA1*02:01:08:02,
 DPA1*02:02:02:01, DPA1*02:02:02:02, DPA1*02:02:02:03, DPA1*02:02:02:04,
 DPA1*02:02:02:05, DPA1*02:02:03, DPA1*02:02:04, DPA1*02:02:05, DPA1*02:02:06,
 DPA1*02:03, DPA1*02:04, DPA1*02:05, DPA1*02:06, DPA1*02:07:01:01,
 DPA1*02:07:01:02, DPA1*02:07:01:03, DPA1*02:08, DPA1*02:09, DPA1*02:10,
 DPA1*02:11, DPA1*02:12, DPA1*02:13N, DPA1*02:14, DPA1*02:15, DPA1*02:16,
 DPA1*03:01:01:01, DPA1*03:01:01:02, DPA1*03:01:01:03, DPA1*03:01:01:04,
 DPA1*03:01:01:05, DPA1*03:01:02, DPA1*03:02, DPA1*03:03, DPA1*03:04,
 DPA1*04:01:01:01, DPA1*04:01:01:02, and DPA1*04:01:01:03, DPA1*04:02.
21. The HLA class II molecule of claim 19 or 20, wherein the DP 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.

22. The HLA class II molecule of claim 21, wherein the DP alpha chain comprises the amino acid sequence set forth in SEQ ID NO: 6 or 8.
23. The HLA class II molecule of any one of claims 1 to 22, which is a DP1, DP2, DP3, DP4, DP5, DP6, DP8, or DP9 allele.
24. The HLA class II molecule of any one of claims 1 to 23, wherein the DP 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 DP beta chain comprising (i) a leucine at a position corresponding to amino acid residue 112 of SEQ ID NO: 1 and/or (ii) a valine at a position corresponding to amino acid residue 141 of SEQ ID NO: 1.
25. The HLA class II molecule of claim 24, 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.
26. The HLA class II molecule of any one of claims 1 to 25, wherein the DP beta chain is bound to a membrane of a cell.
27. The HLA class II molecule of any one of claims 1 to 25, wherein the DP beta chain is not bound to a membrane of a cell.
28. The HLA class II molecule of any one of claims 1 to 25, wherein the DP beta chain comprises an extracellular domain of a full length DP alpha chain.
29. The HLA class II molecule of claim 28, wherein the DP beta chain does not comprise a transmembrane domain of a full length DP beta chain.
30. The HLA class II molecule of any one of claims 19 to 29, wherein the DP alpha chain is bound to a membrane of a cell.

31. The HLA class II molecule of any one of claims 19 to 29, wherein the DP alpha chain is not bound to a membrane of a cell.
32. The HLA class II molecule of any one of claims 19 to 29, wherein the DP alpha chain comprises an extracellular domain of a full length DP alpha chain.
33. The HLA class II molecule of claim 32, wherein the DP alpha chain does not comprise a transmembrane domain of a full length DP alpha chain.
34. The HLA class II molecule of any one of claims 27 to 33, wherein the DP beta chain is linked to or associated with an inert particle.
35. The HLA class II molecule of claim 34, wherein the inert particle is a bead.
36. The HLA class II molecule of claim 35, wherein the inert particle is a nanoparticle.
37. The HLA class II molecule of claim 36, 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, polyethylenimine, block copolymer, polycaprolactone, SPIONS, USPIONS, Cd/Zn-selenide, or silica nanoparticle.
38. The HLA class II molecule of claim 36 or 37, wherein the nanoparticle is a pegylated iron oxide nanoparticle.
39. The HLA class II molecule of any one of claims 1 to 38, wherein the DP beta chain comprises a signal peptide.
40. The HLA class II molecule of any one of claims 19 to 39, wherein the DP alpha chain comprises a signal peptide.
41. The HLA class II molecule of claim 39 or 40, wherein the signal peptide comprises the amino acid sequence set forth in SEQ ID NO: 9.
42. A nucleic acid molecule encoding the DP beta chain of any one of claims 1 to 41.
43. The nucleic acid molecule of claim 42, further encoding the DP alpha chain of any of claims 19 to 41.

44. The nucleic acid molecule of claim 42 or 43, 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.
45. A vector comprising the nucleic acid molecule of any one of claims 42 to 44.
46. A cell comprising the HLA class II molecule of any one of claims 1 to 41, the nucleic acid molecule of any one of claims 42 to 44, or the vector of claim 45.
47. The cell of claim 46, which is a mammalian cell or an insect cell.
48. The cell of claim 46 or 47, 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.
49. The cell of any one of claims 46 to 48, which lacks endogenous MHC class II DP beta chain expression.
50. The cell of any one of claims 46 to 49, which lacks endogenous MHC class II DP alpha chain expression.
51. 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 46 to 50 with one or more peptide comprising the epitope, and stimulating one or more CD4⁺ T cell with the APC.
52. 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 41.
53. The method of claim 52, wherein the disease or condition is cancer or an infection.
54. The method of claim 53, 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.

55. The method of claim 53 or 54, wherein the cancer is relapsed or refractory.
56. The method of any one of claims 53 to 55, wherein the cancer is locally advanced.
57. The method of any one of claims 53 to 56, wherein the cancer is advanced.
58. The method of any one of claims 53 to 57, wherein the cancer is metastatic.
59. The HLA class II molecule of any one of claims 1 to 41, which binds CD4 with a K_D of less than about 10 μM .
60. The HLA class II molecule of any one of claims 1 to 1, which binds CD4 with a K_D of about 8.9 μM or less.
61. A complex comprising the HLA class II molecule of any one of claims 1 to 41, 59, and 60 and a peptide, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 32-237.

DP4

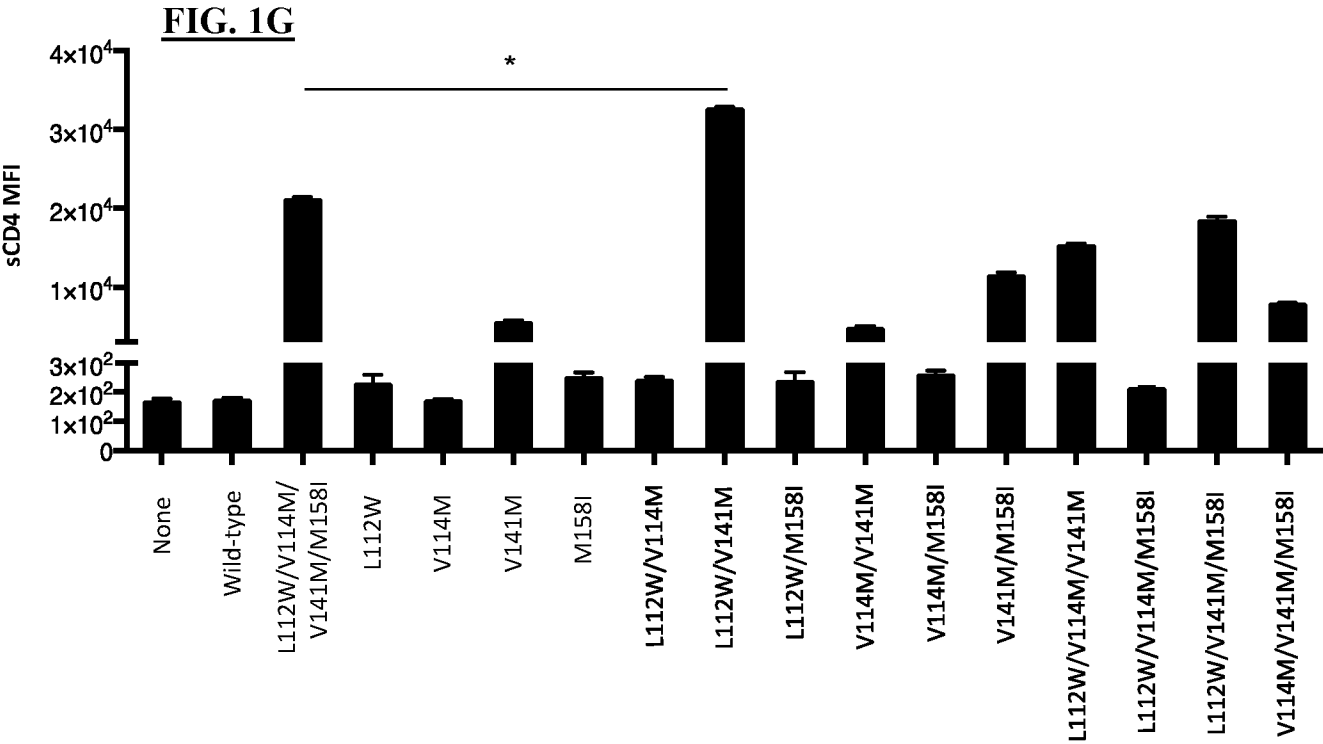
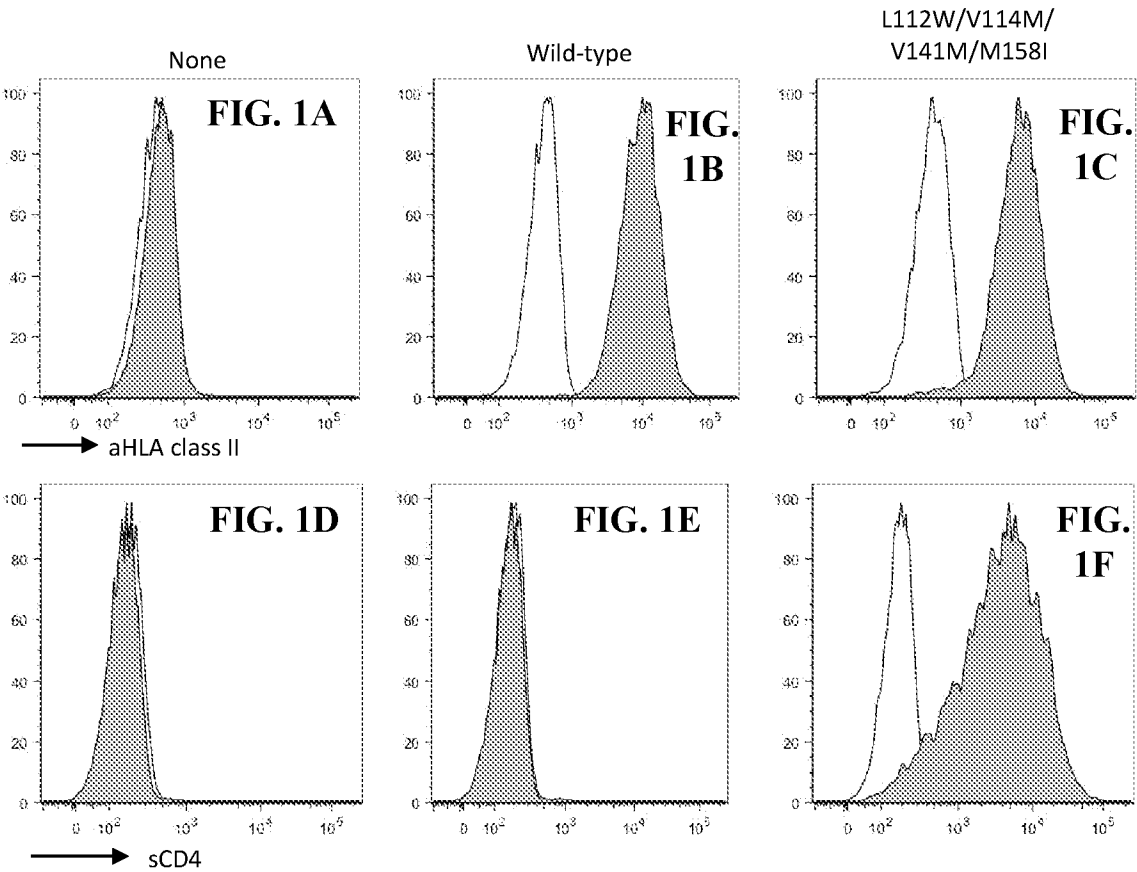


FIG. 1H

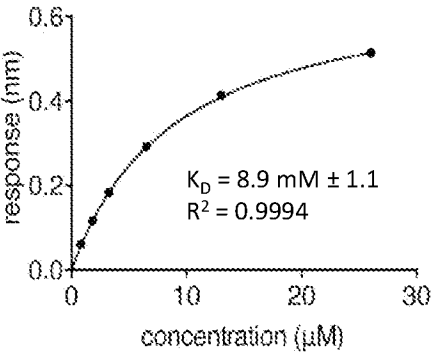


FIG. 1I

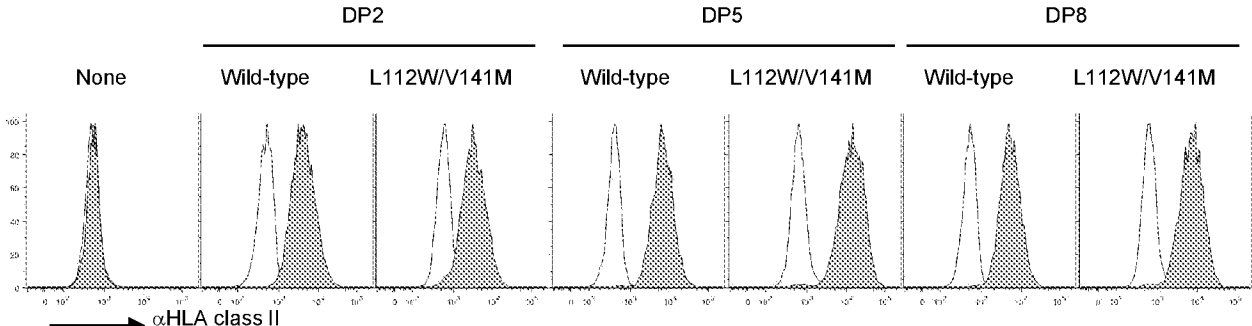
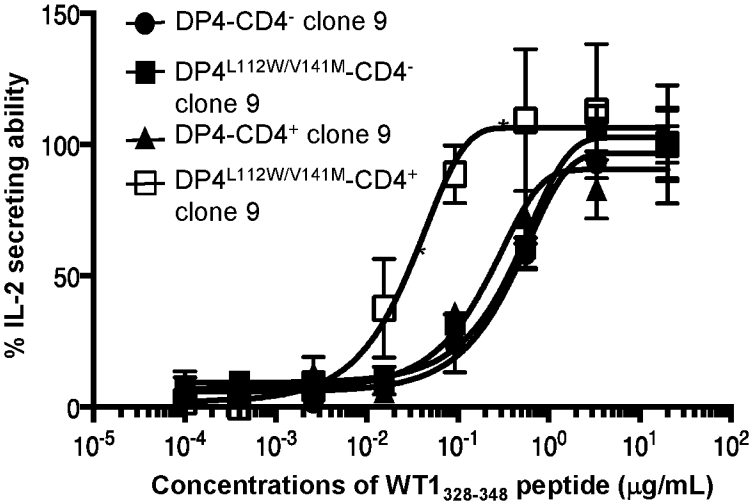


FIG. 1J **FIG. 1K** **FIG. 1L** **FIG. 1M** **FIG. 1N** **FIG. 1O** **FIG. 1P**

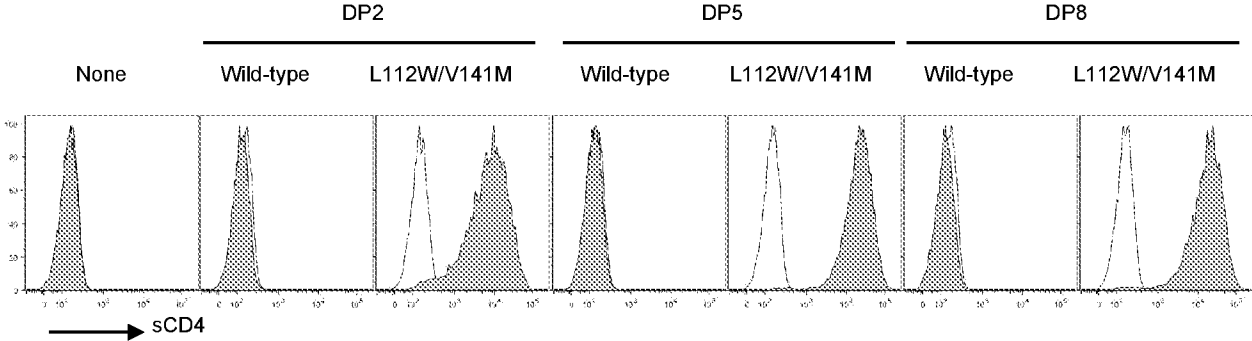
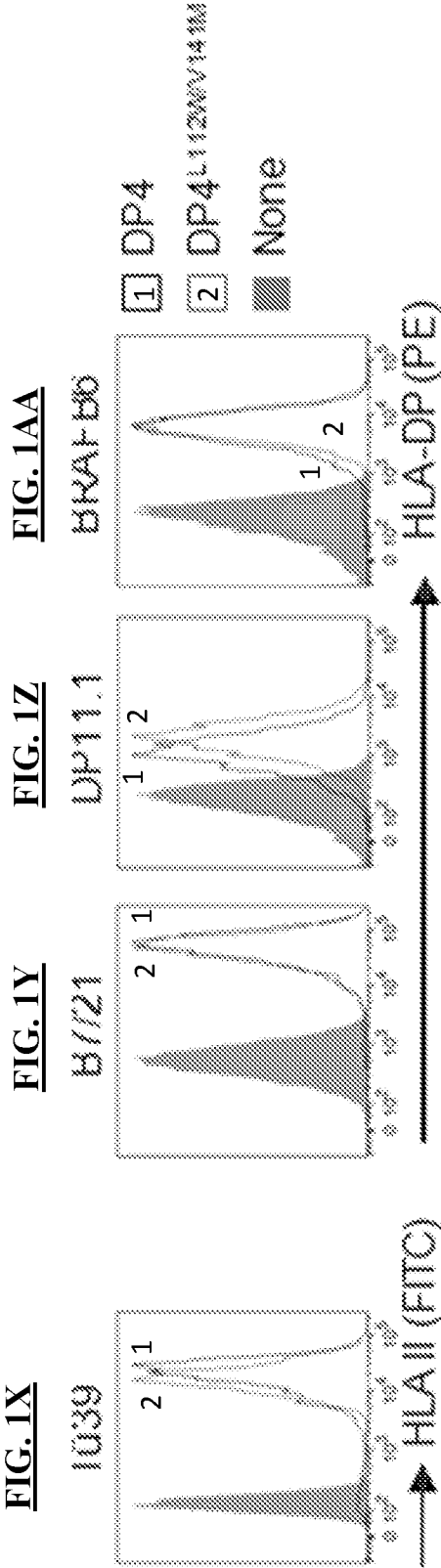


FIG. 1Q **FIG. 1R** **FIG. 1S** **FIG. 1T** **FIG. 1U** **FIG. 1V** **FIG. 1W**



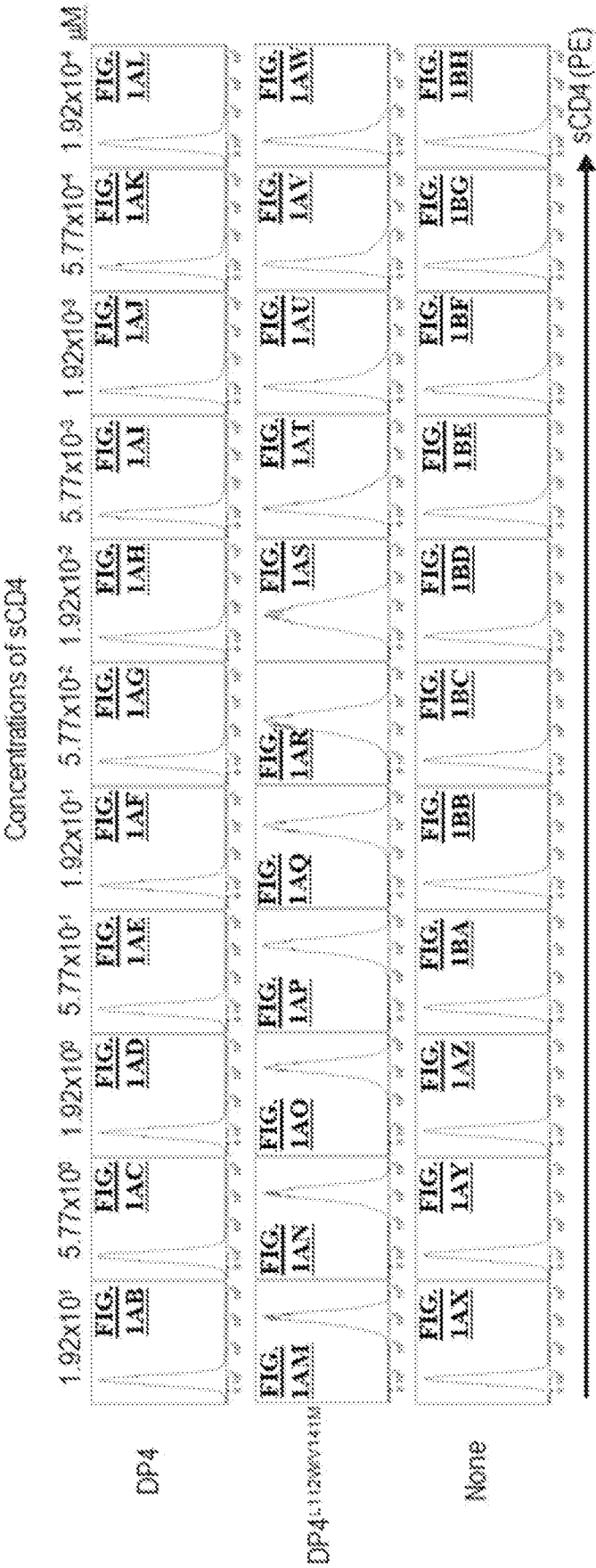


FIG. 1BI

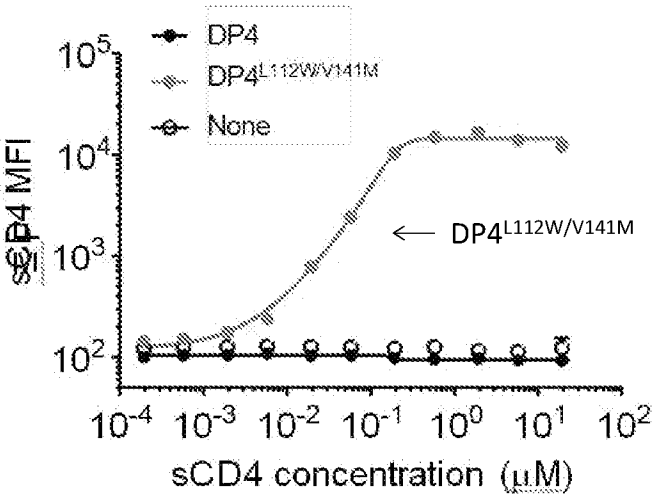


FIG. 1BJ

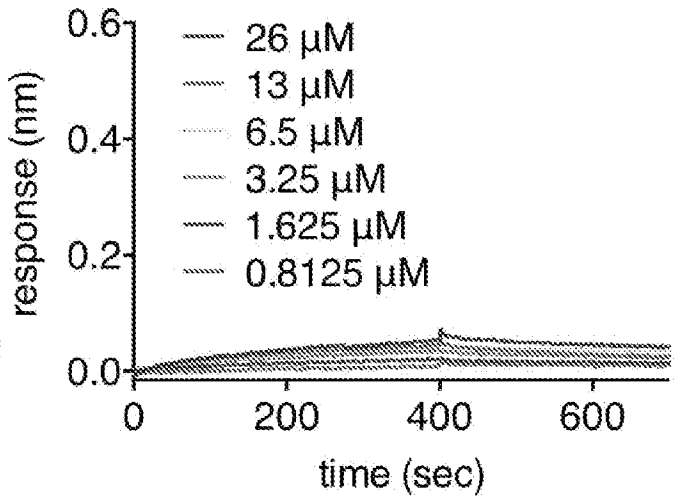
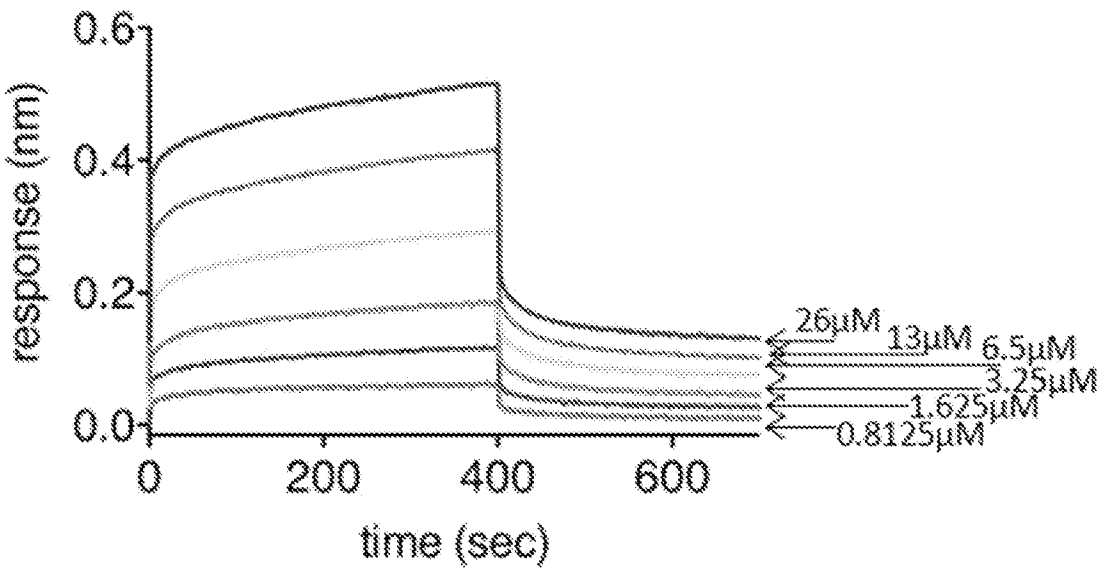
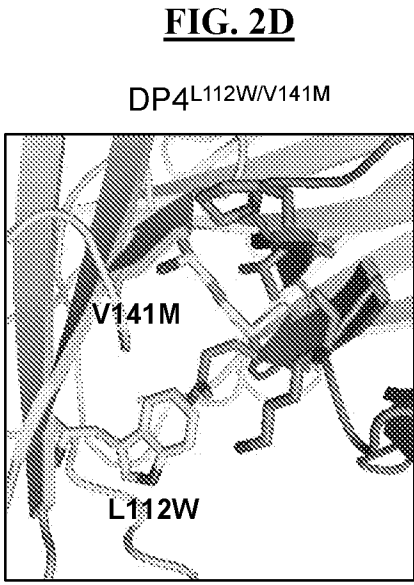
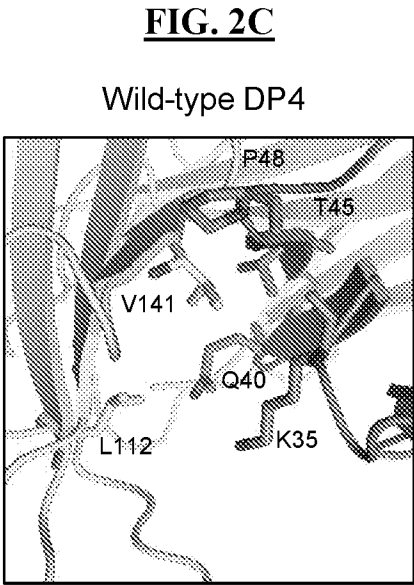
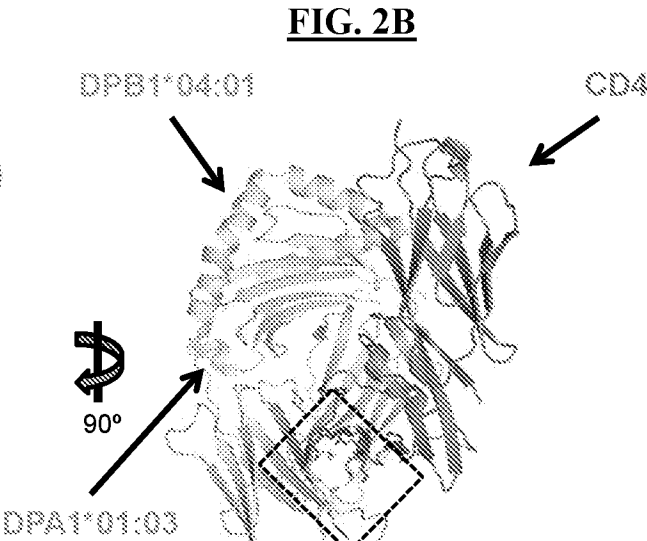
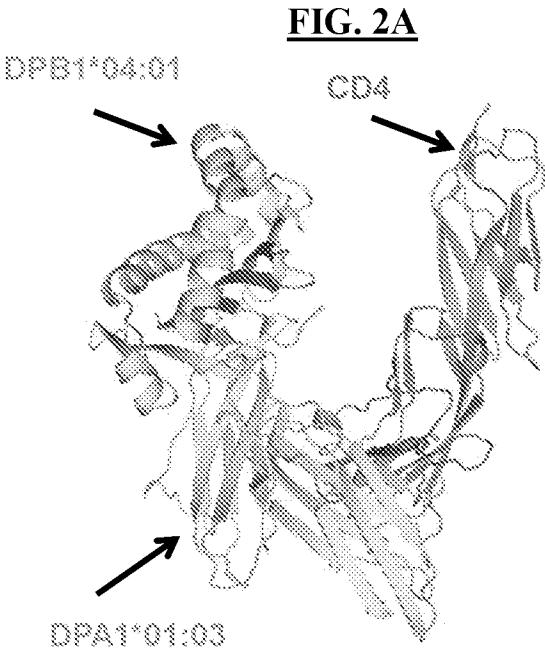
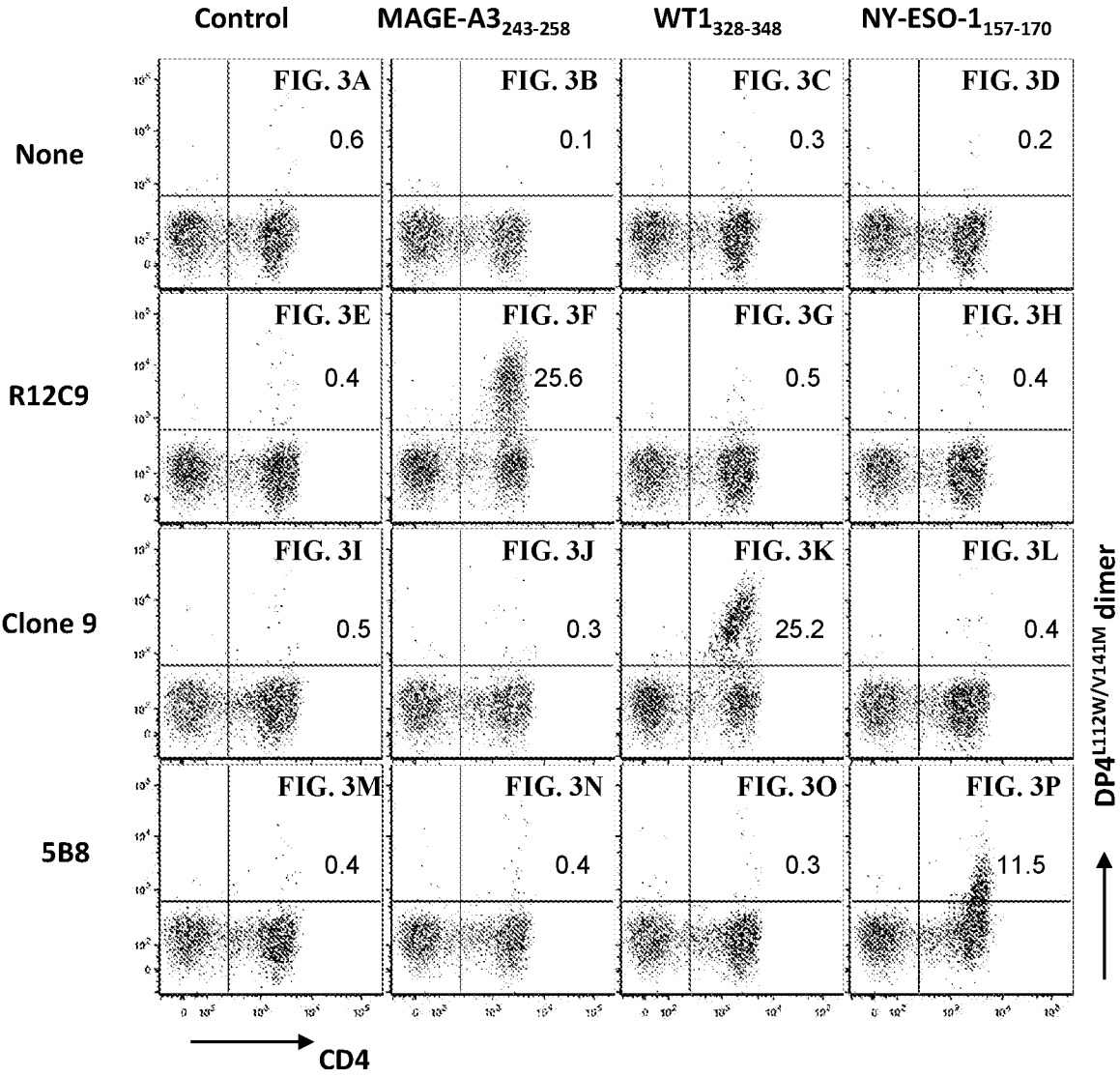


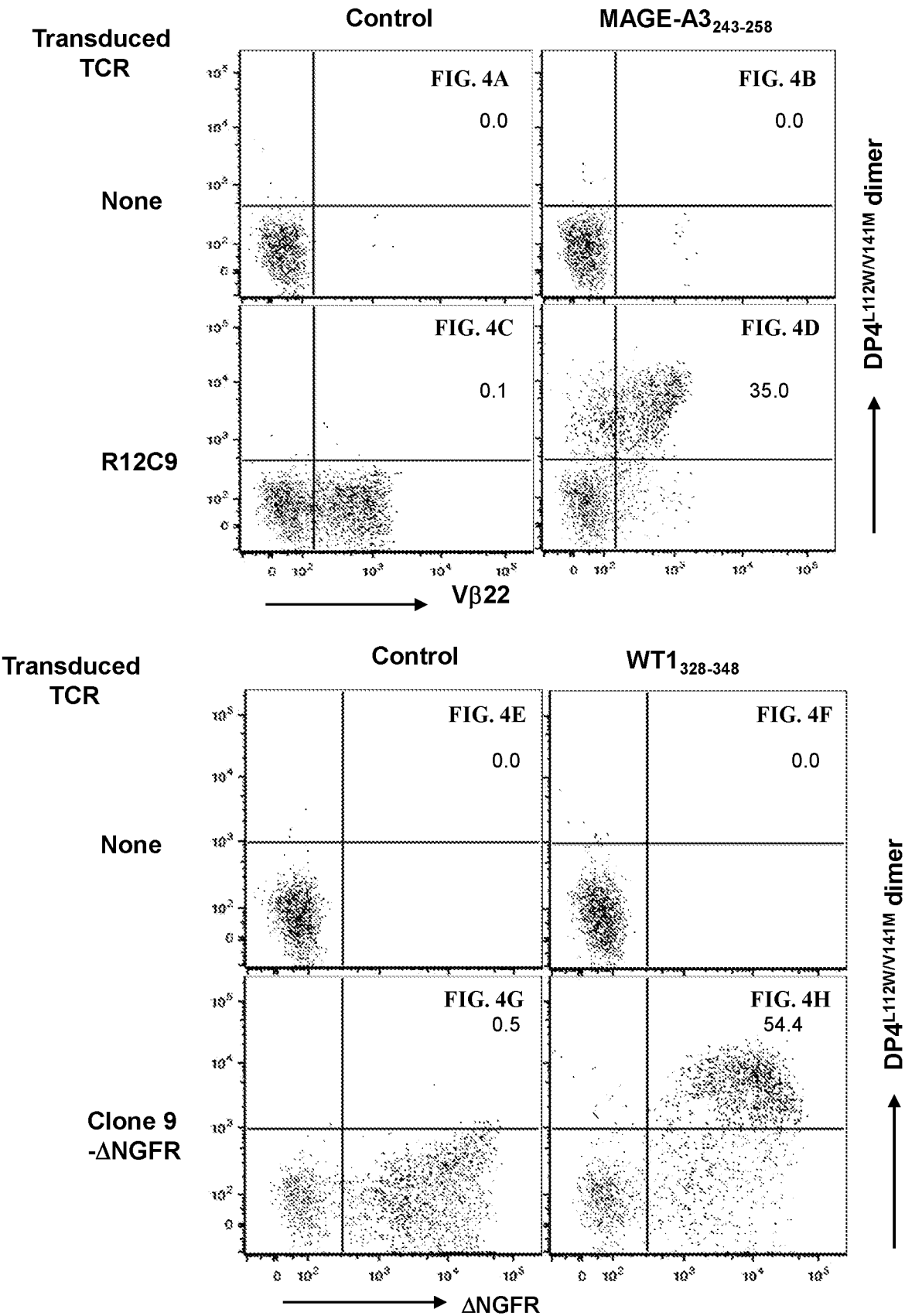
FIG. 1BK





Transduced
TCR





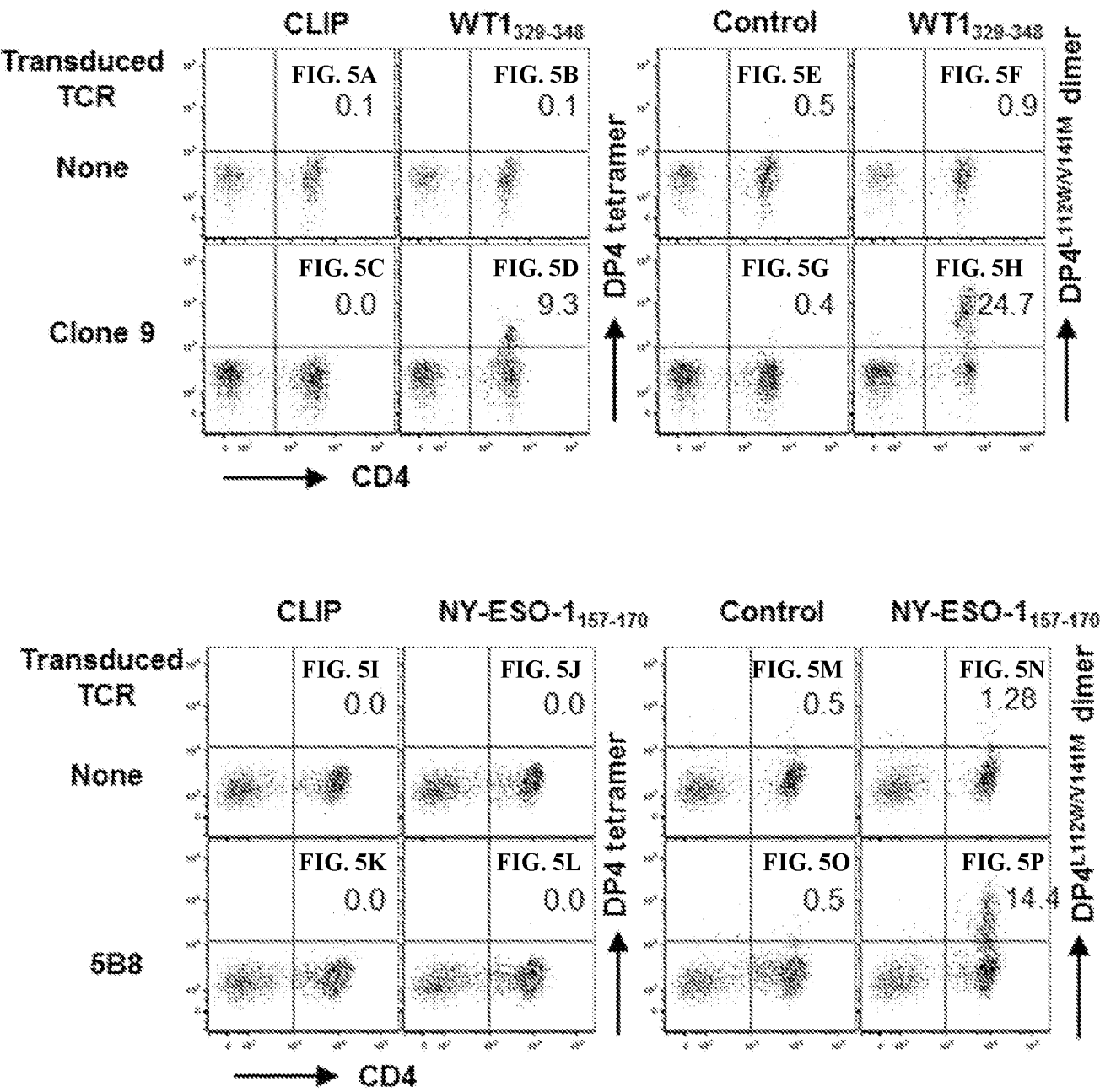


FIG. 6A

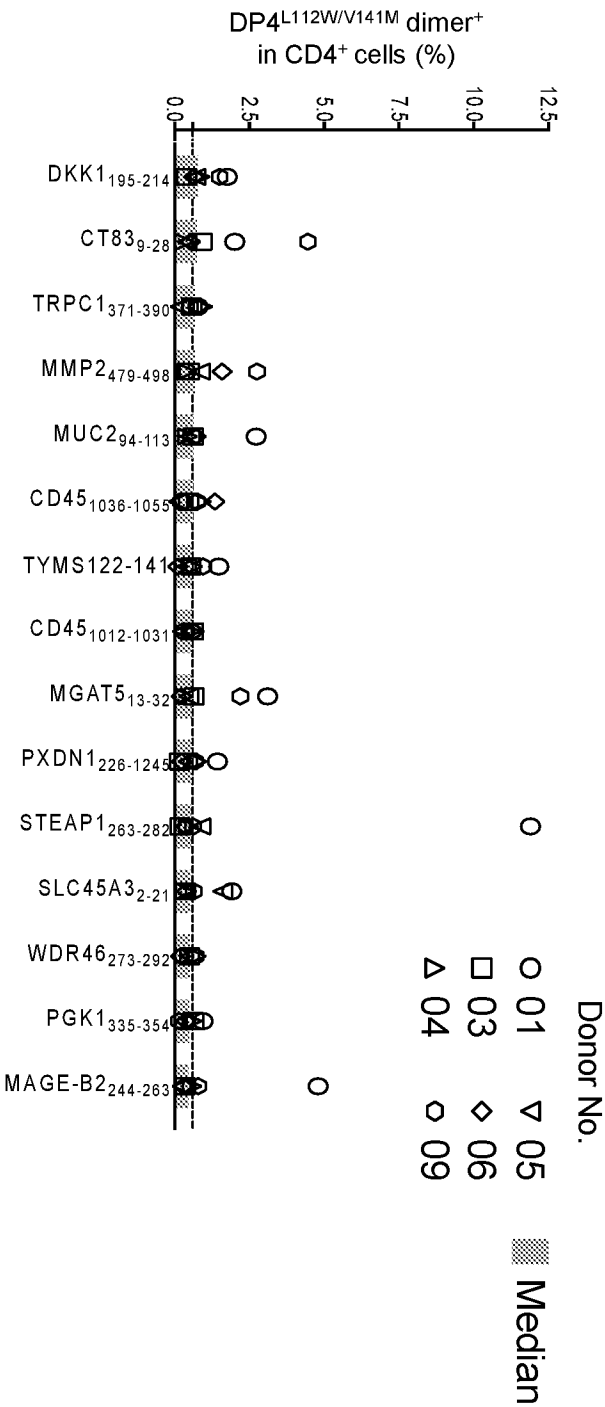


FIG. 6B

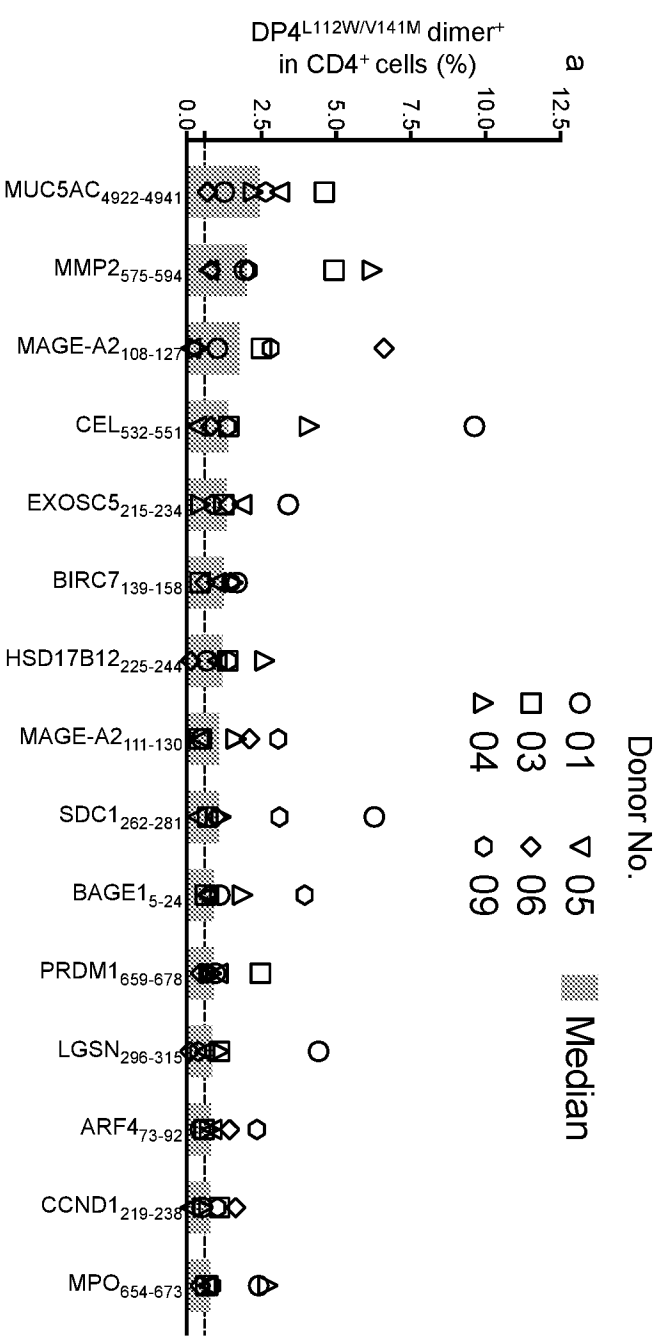


FIG. 6C

FIG. 6D

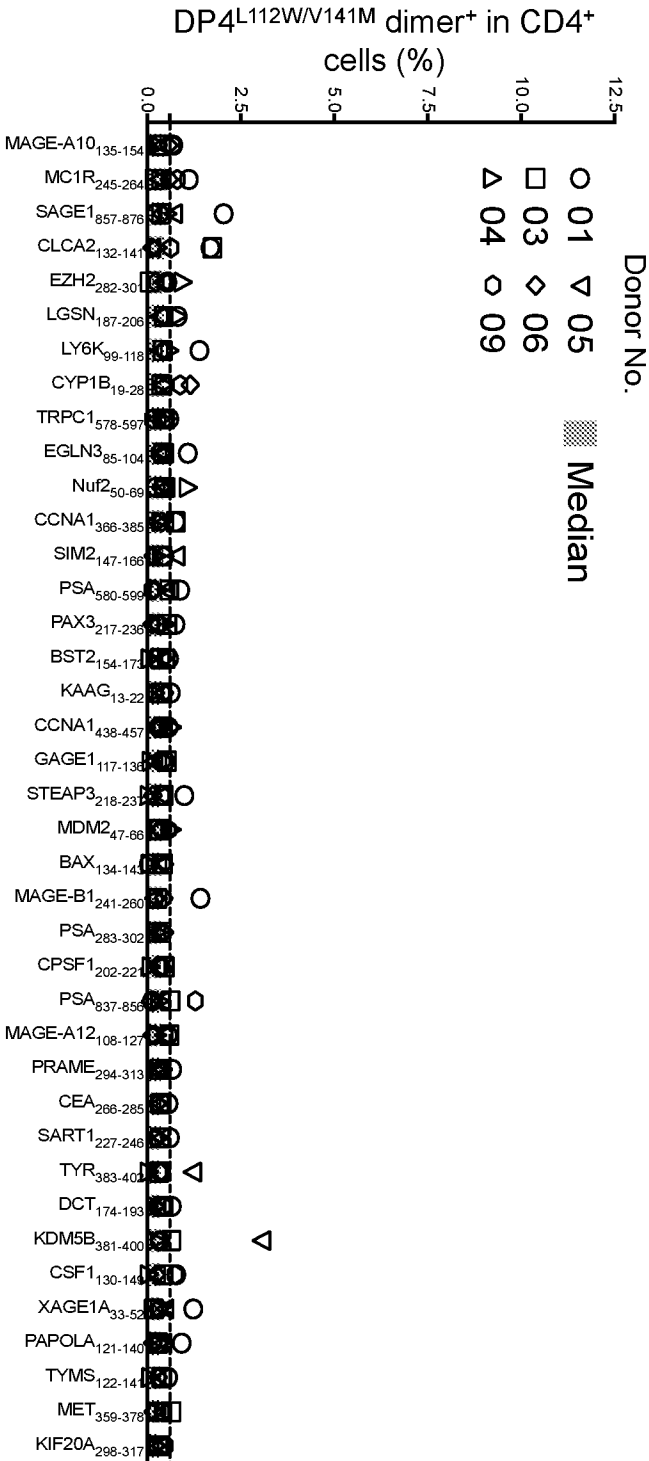
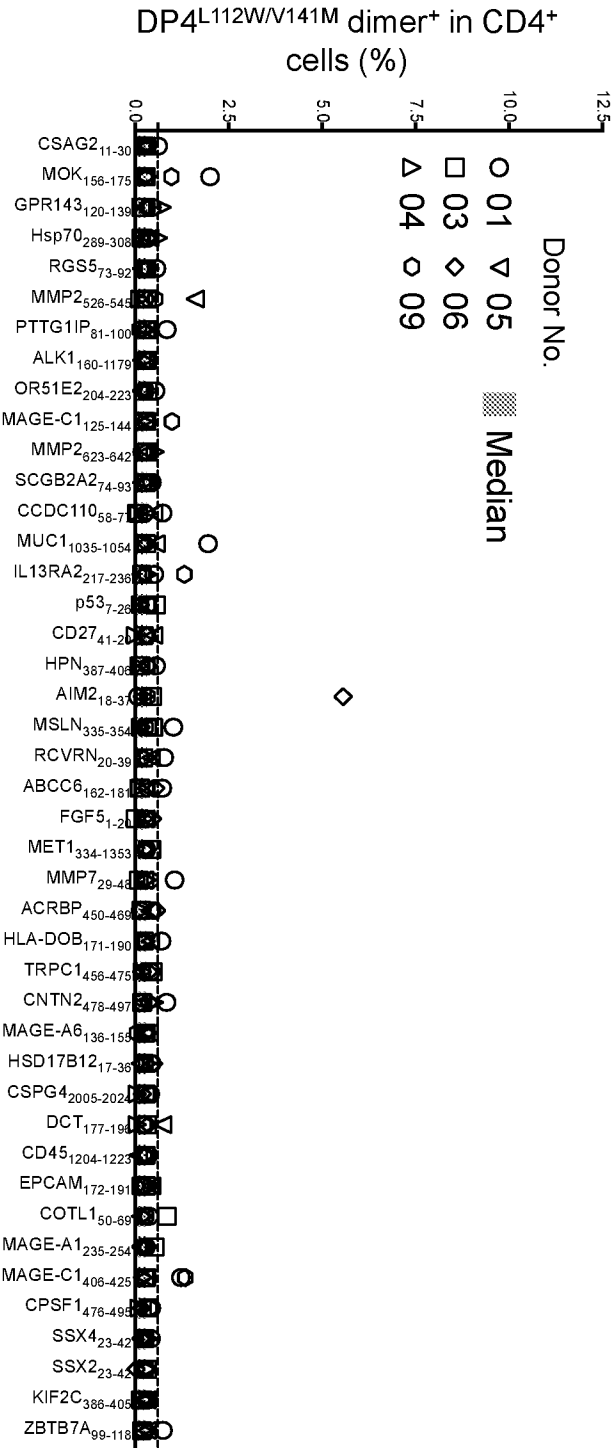
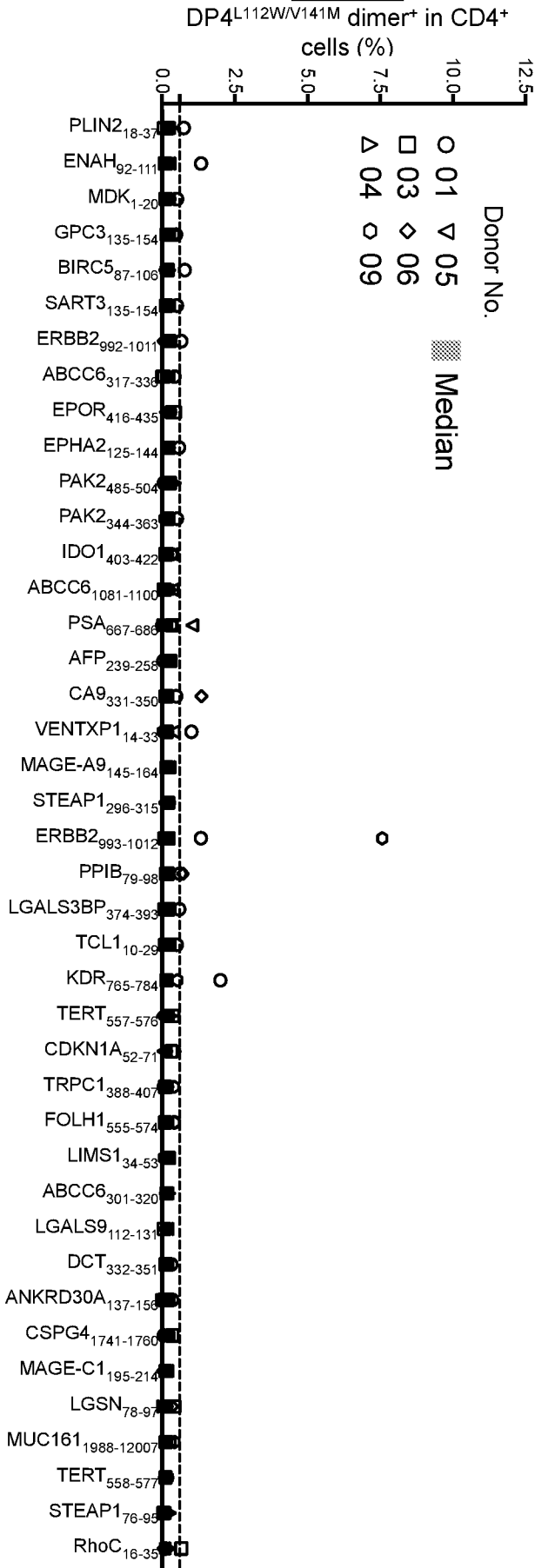
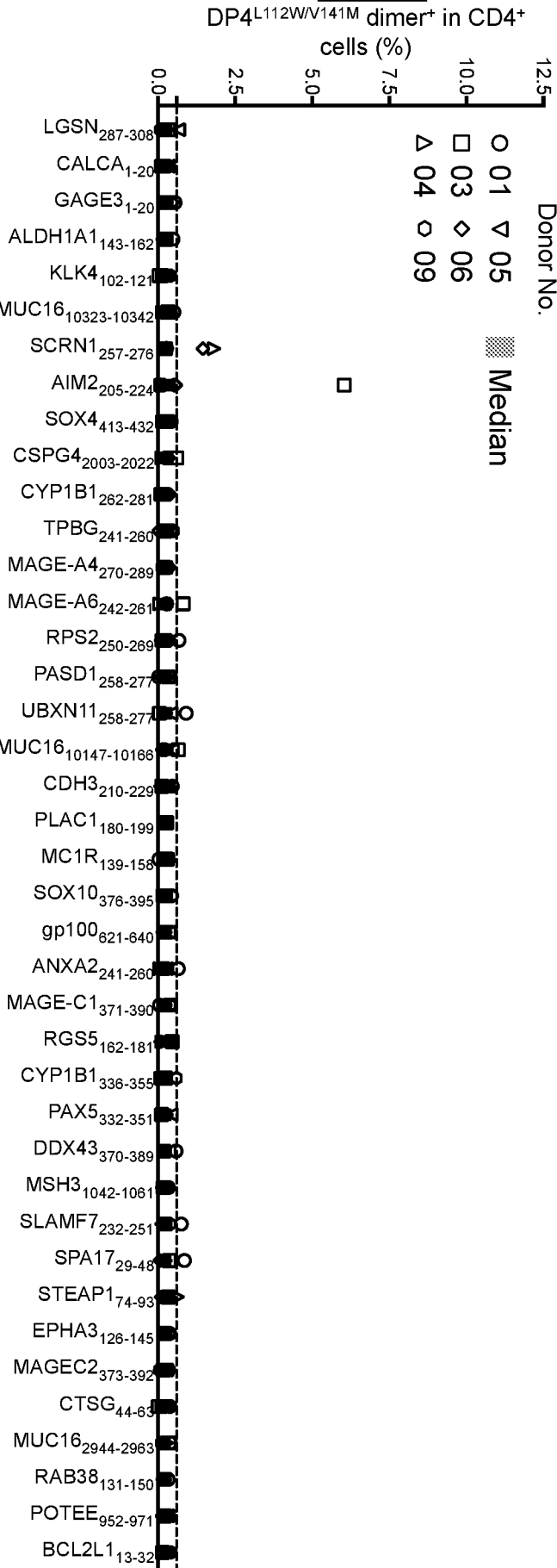


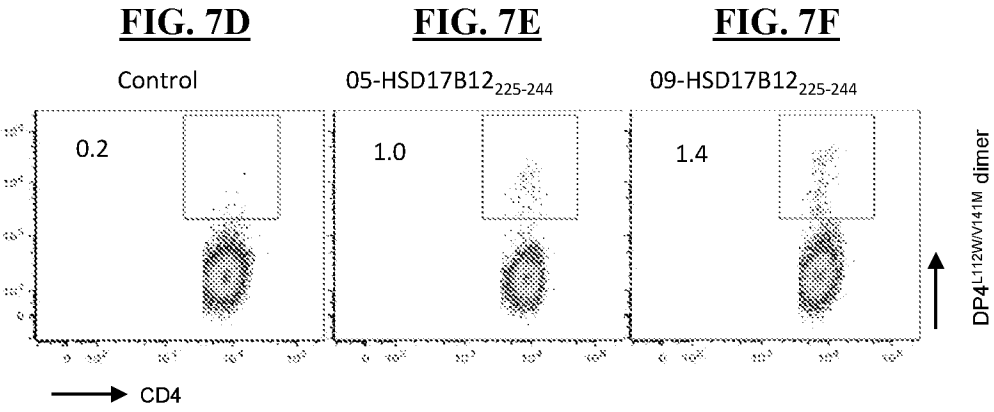
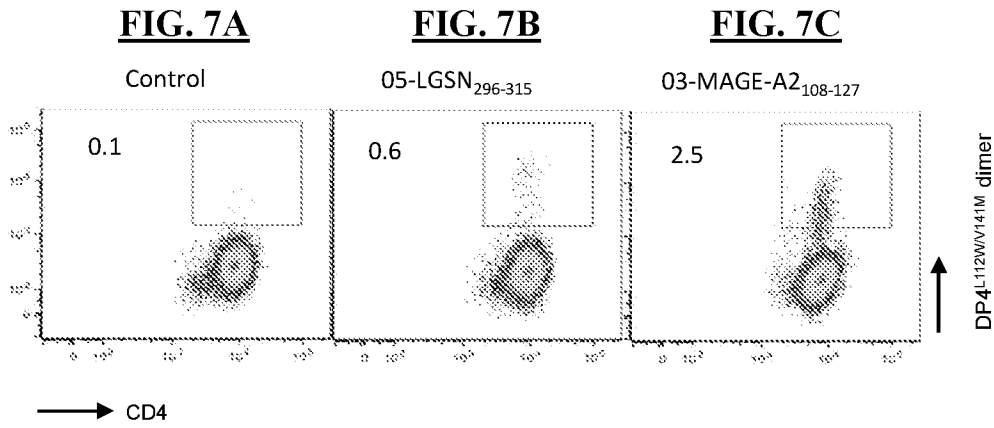
FIG. 6E

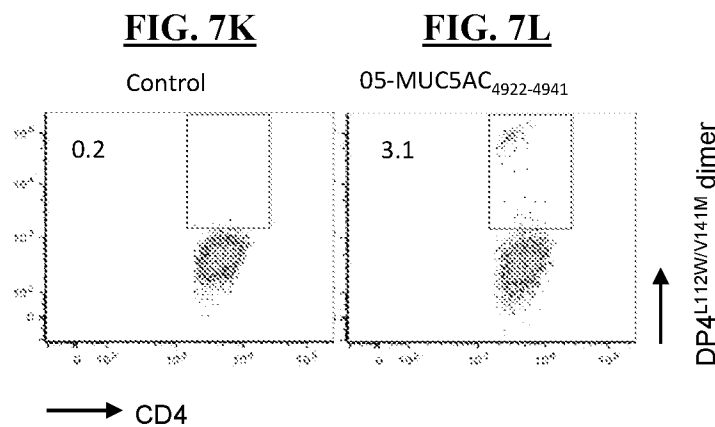
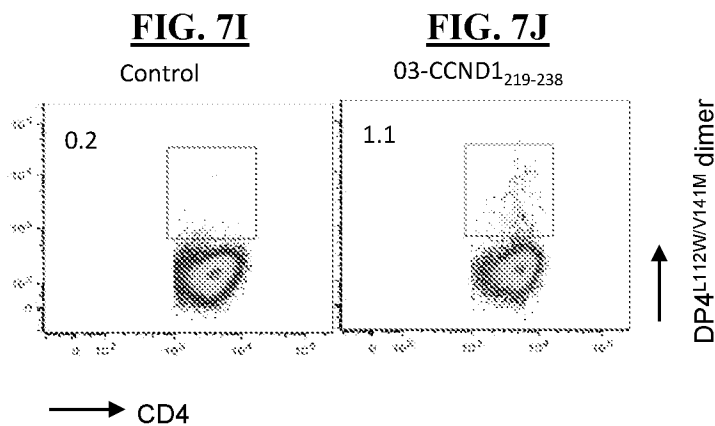
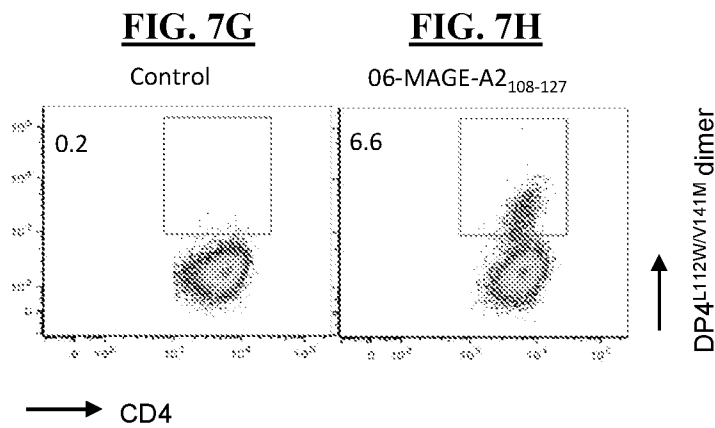


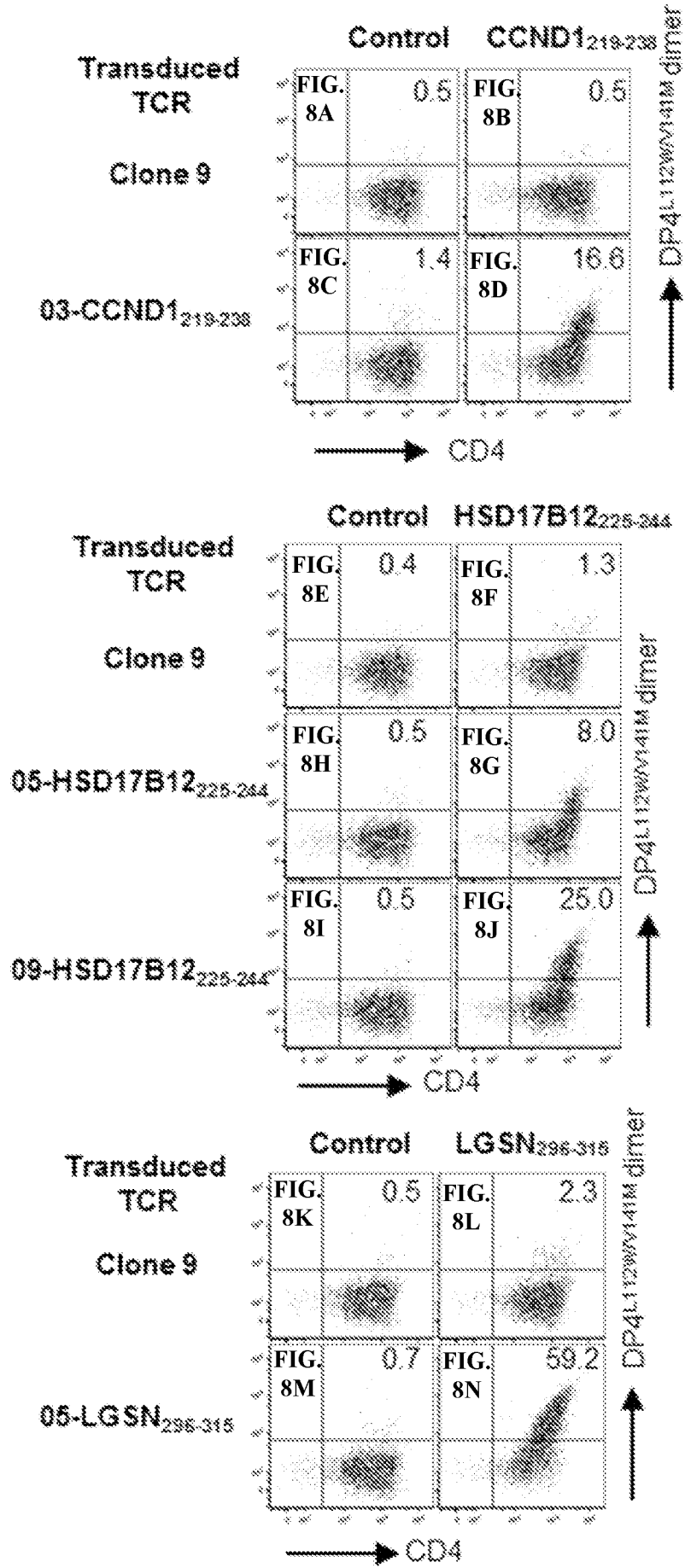
12/37

FIG. 6F









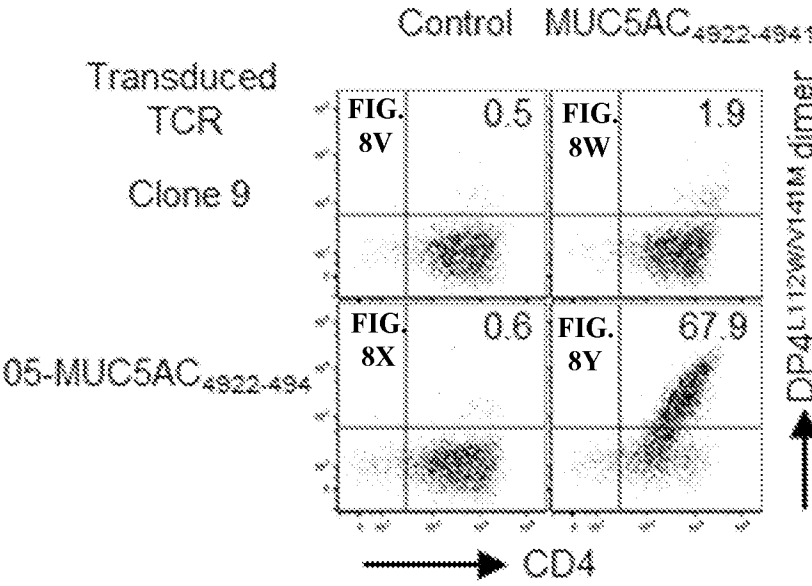
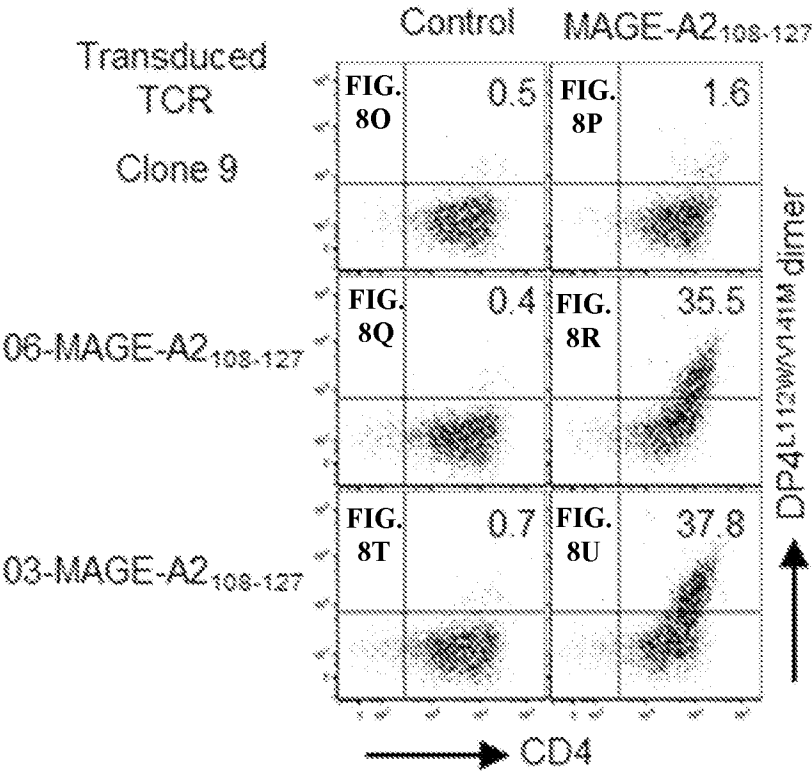


FIG. 9A

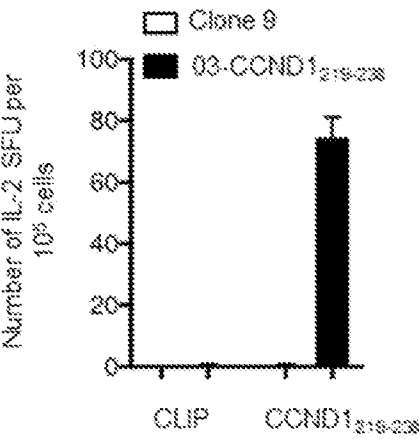


FIG. 9B

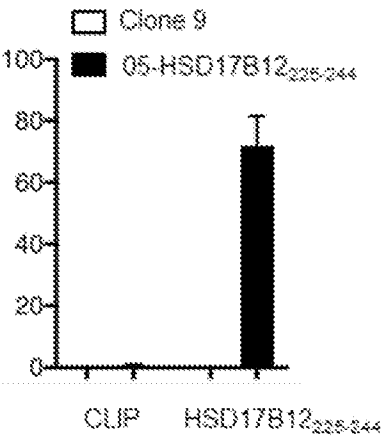


FIG. 9C

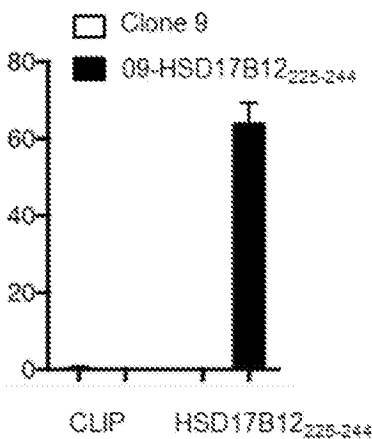


FIG. 9D

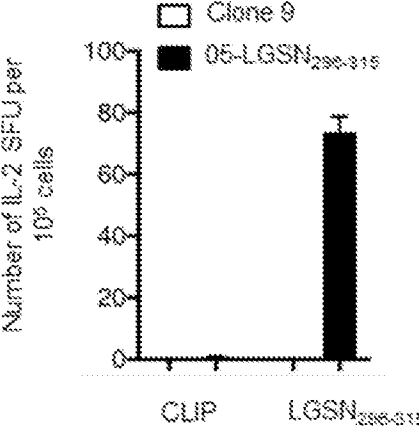


FIG. 9E

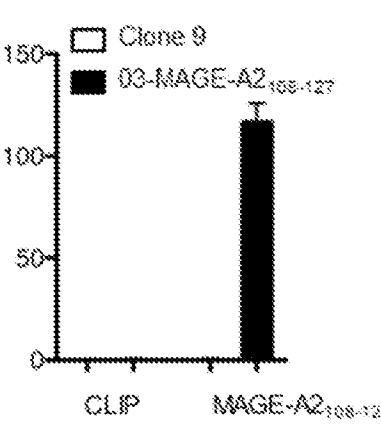


FIG. 9F

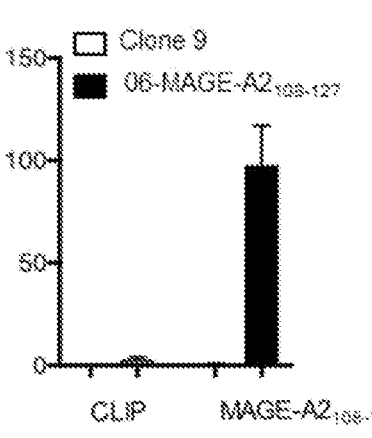
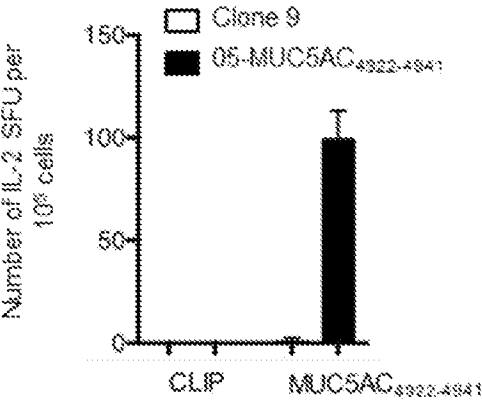
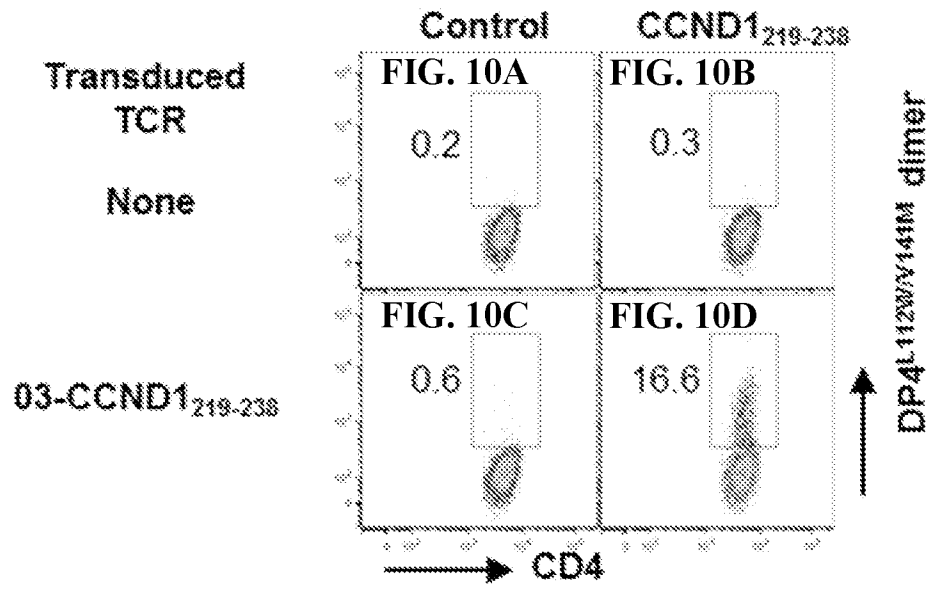
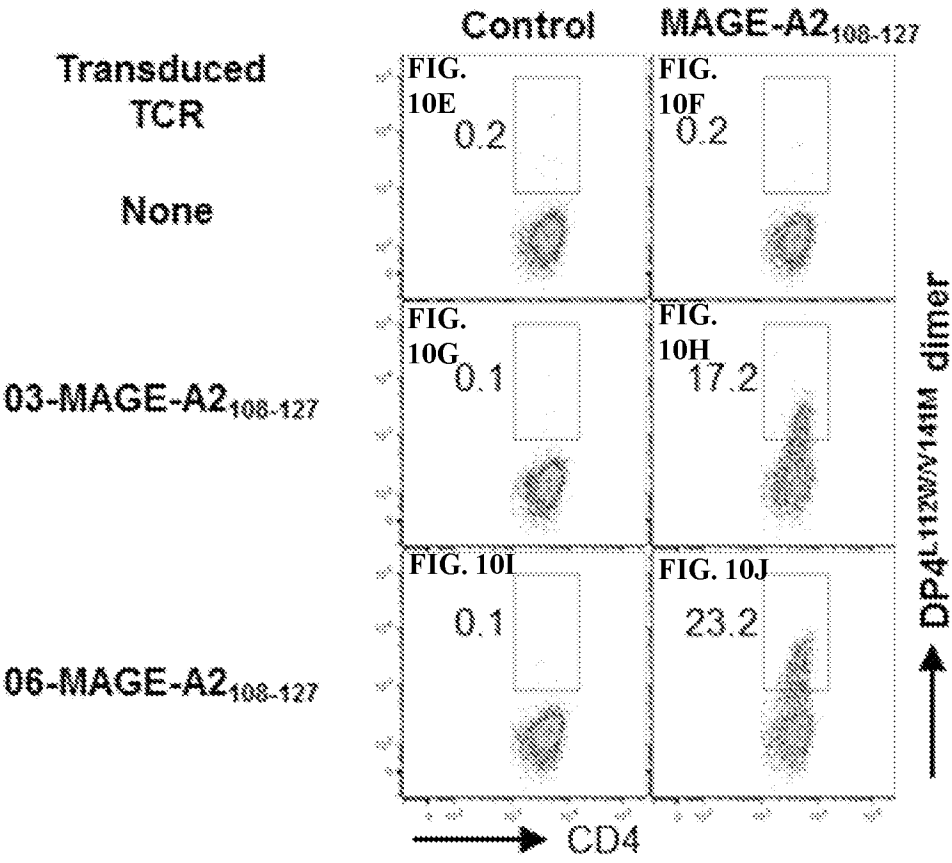


FIG. 9G







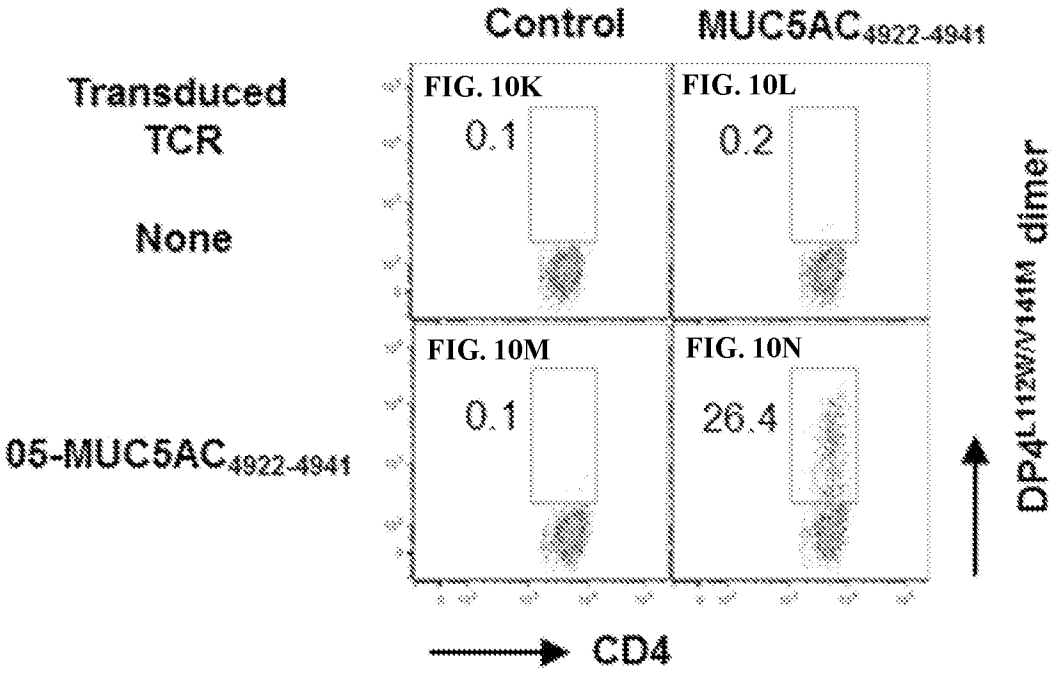


FIG. 10O

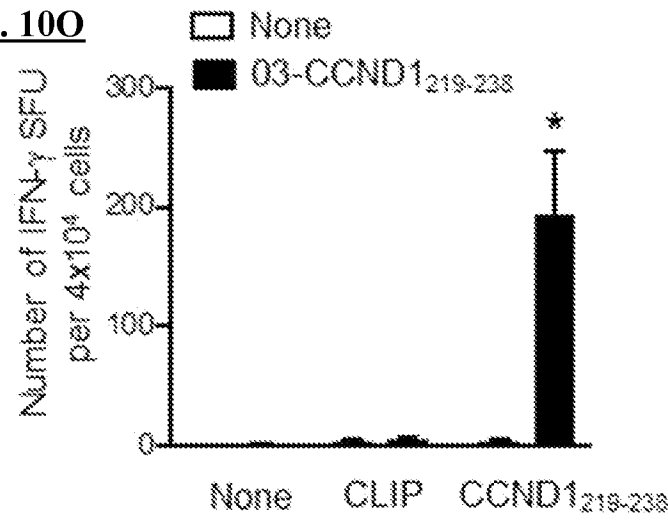


FIG. 10P

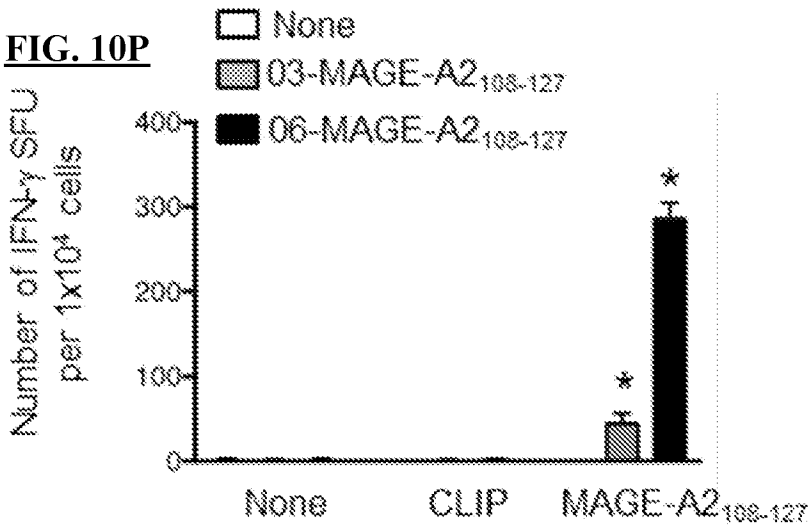


FIG. 10Q

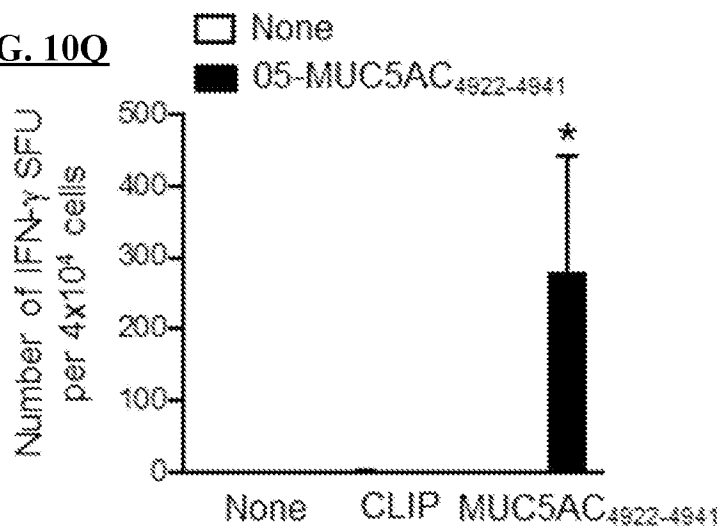


FIG. 11A

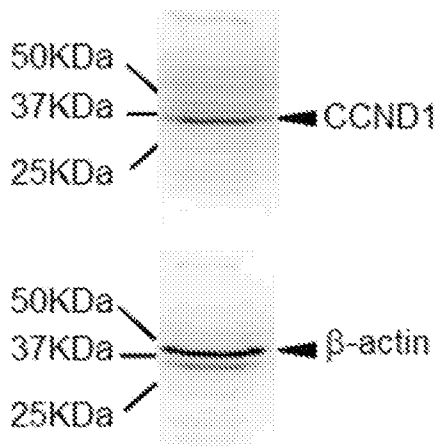


FIG. 11B

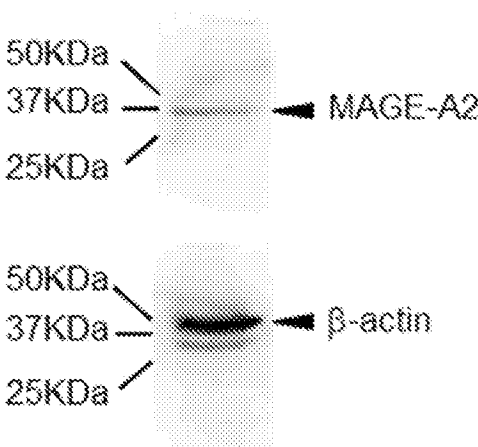


FIG. 11C

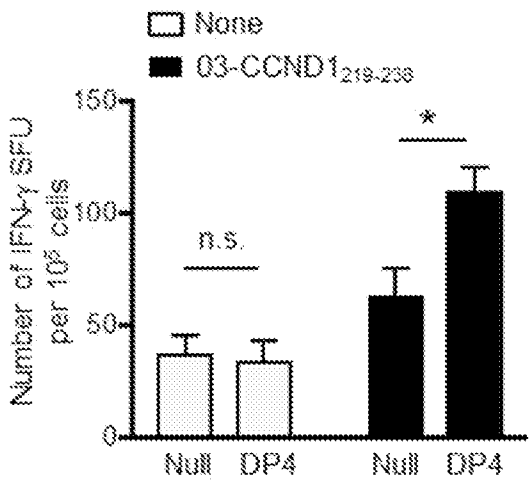


FIG. 11D

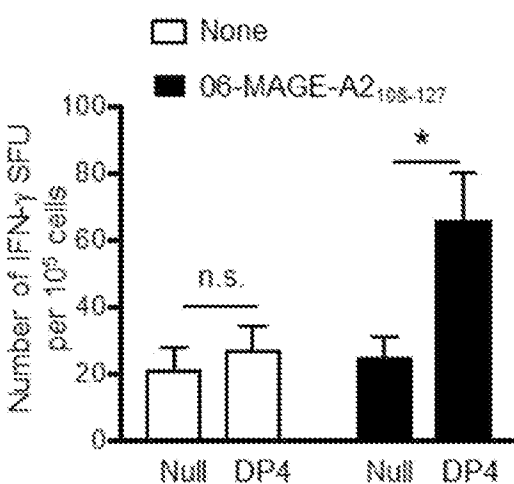
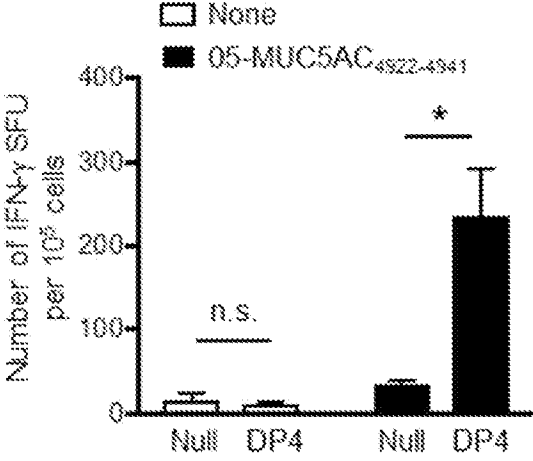
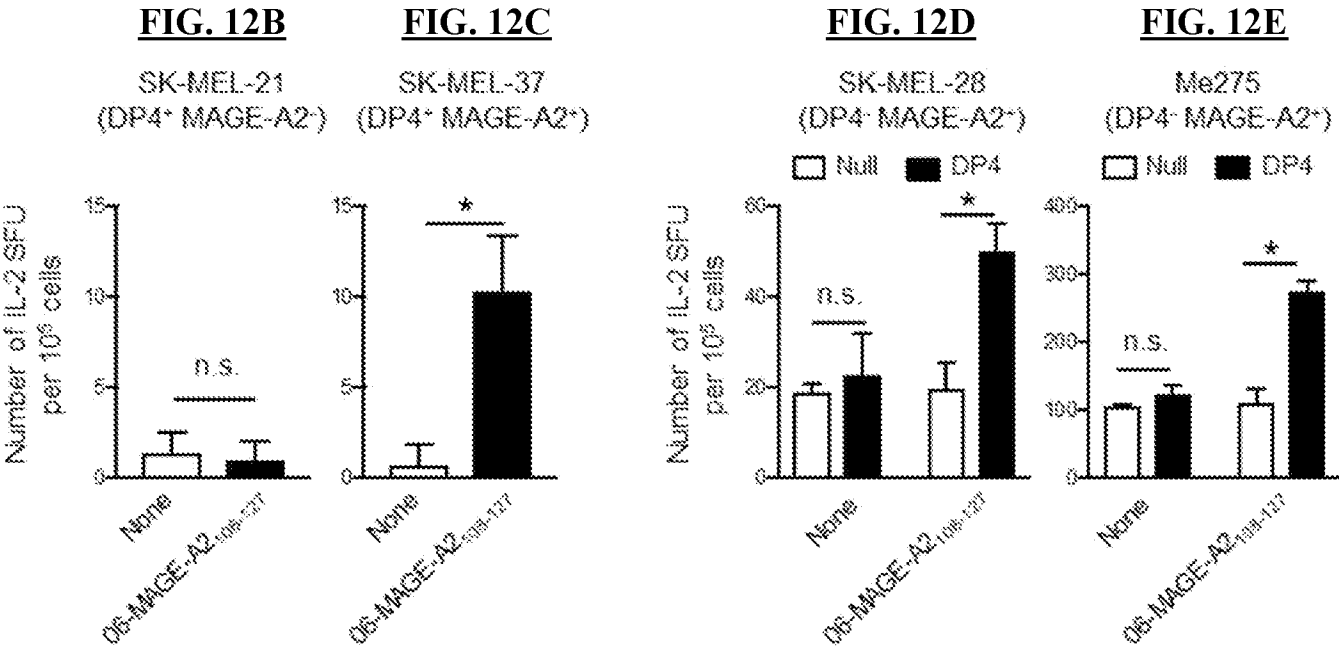
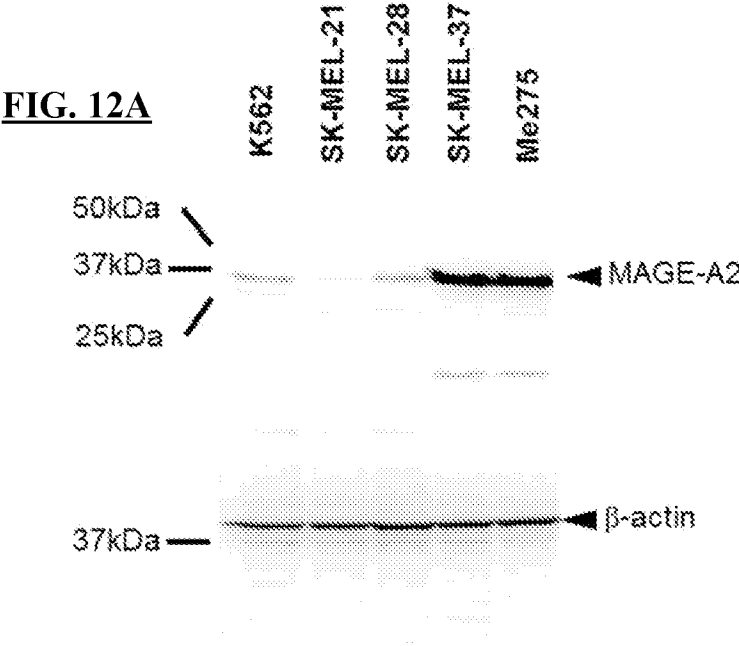
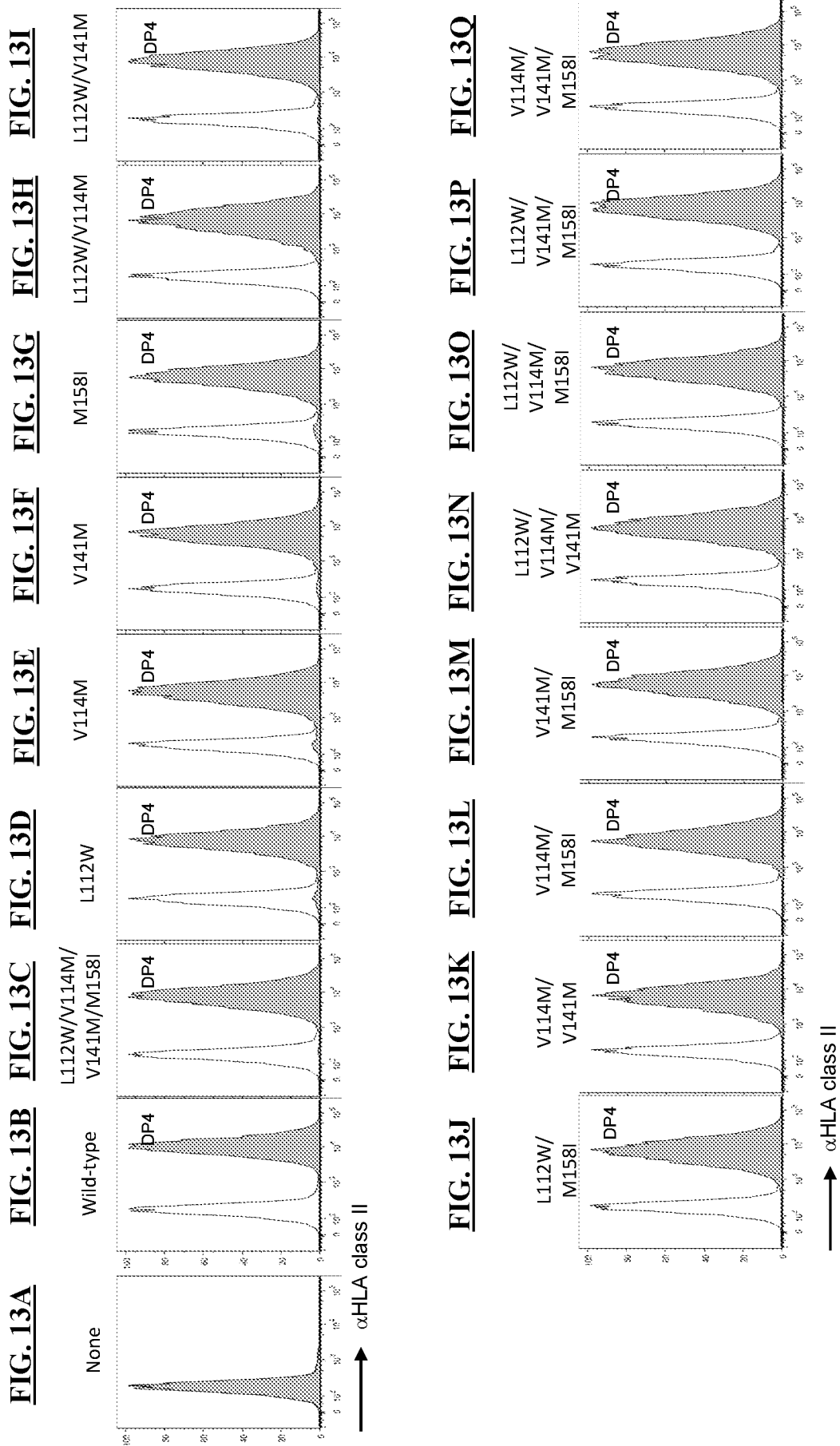
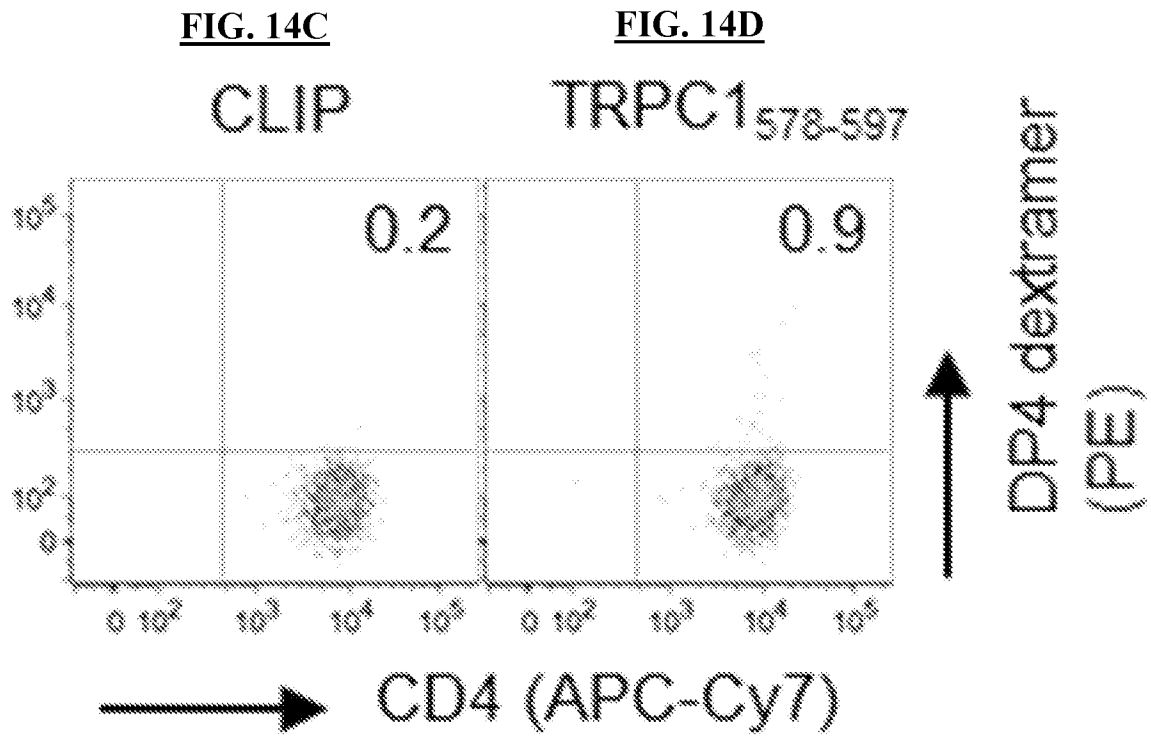
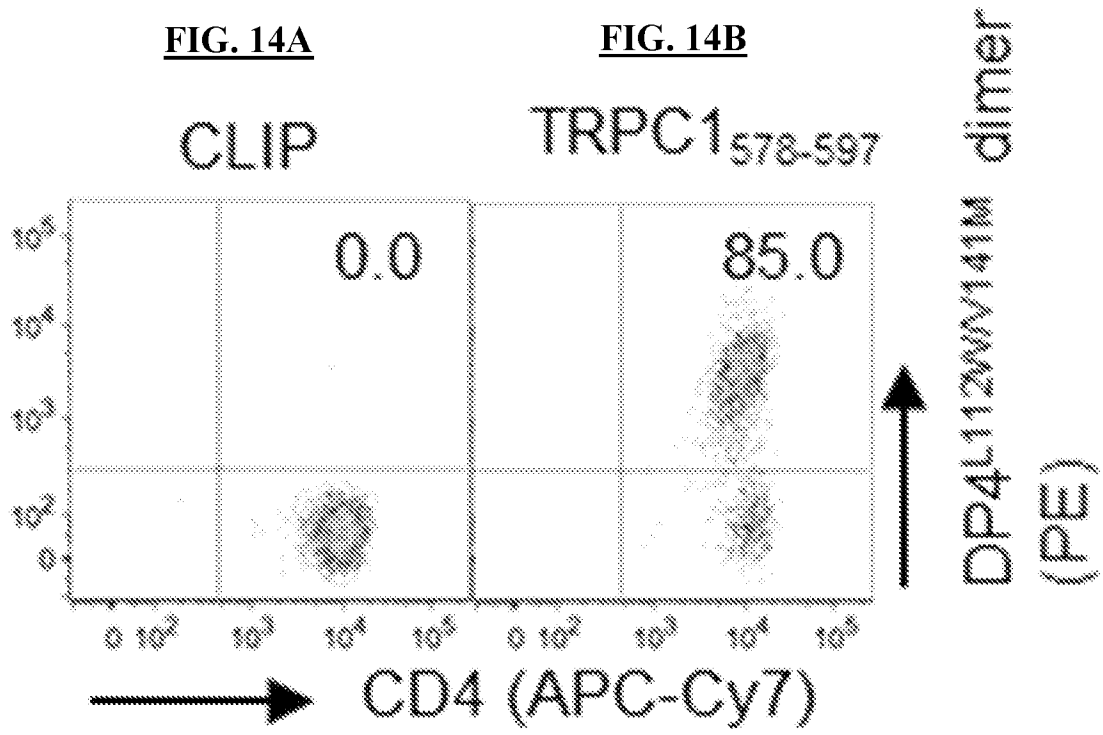


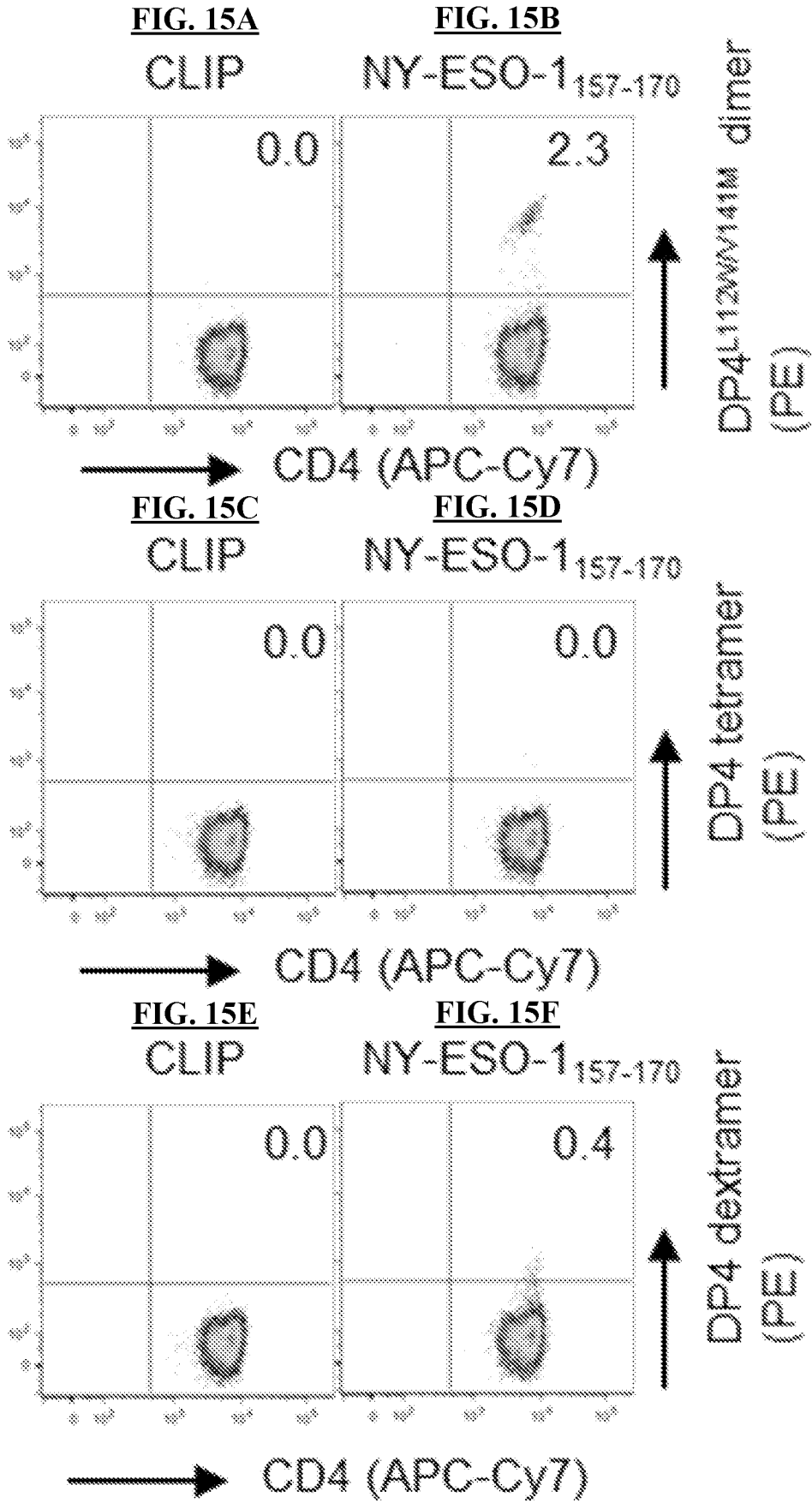
FIG. 11D

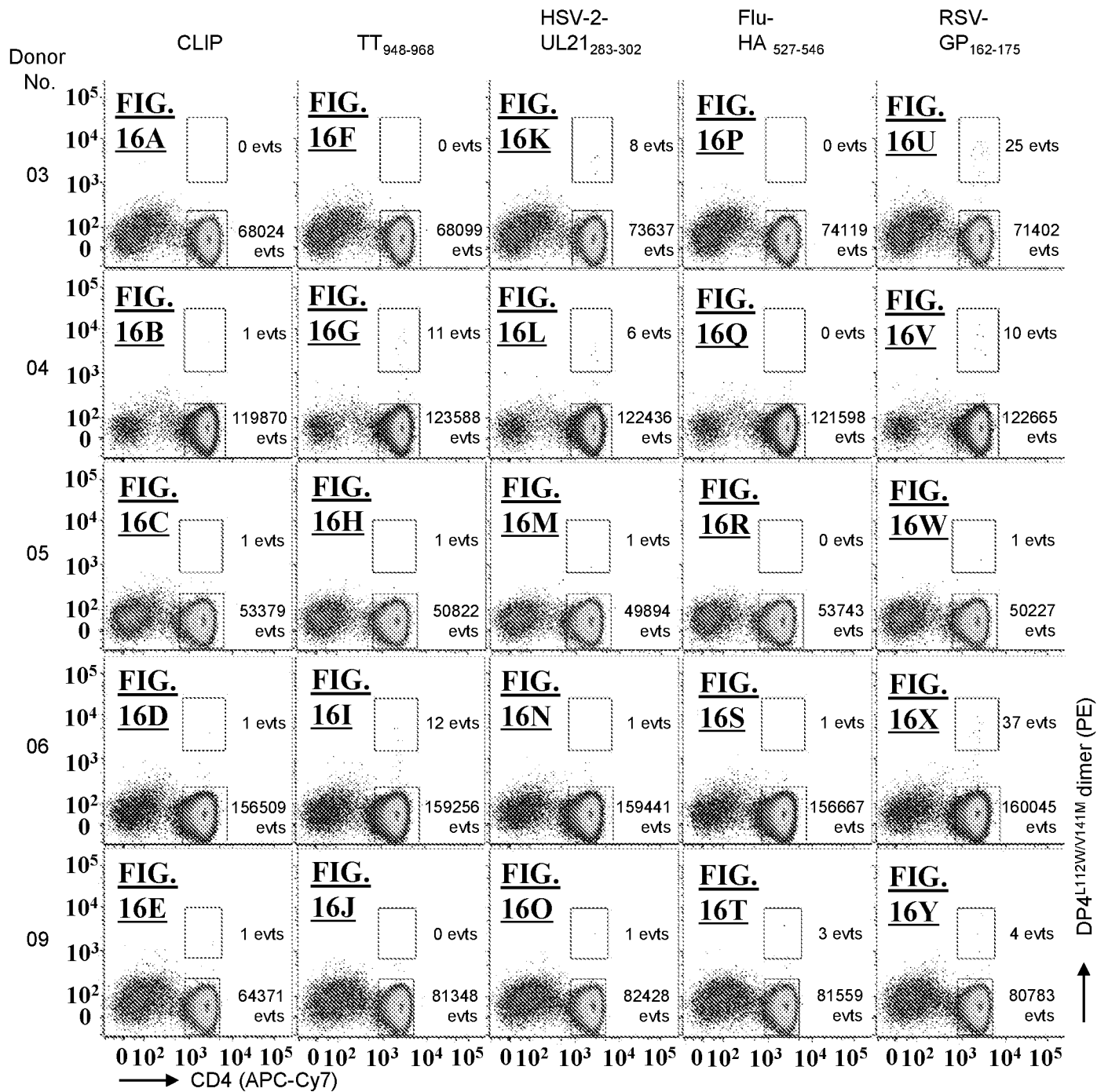












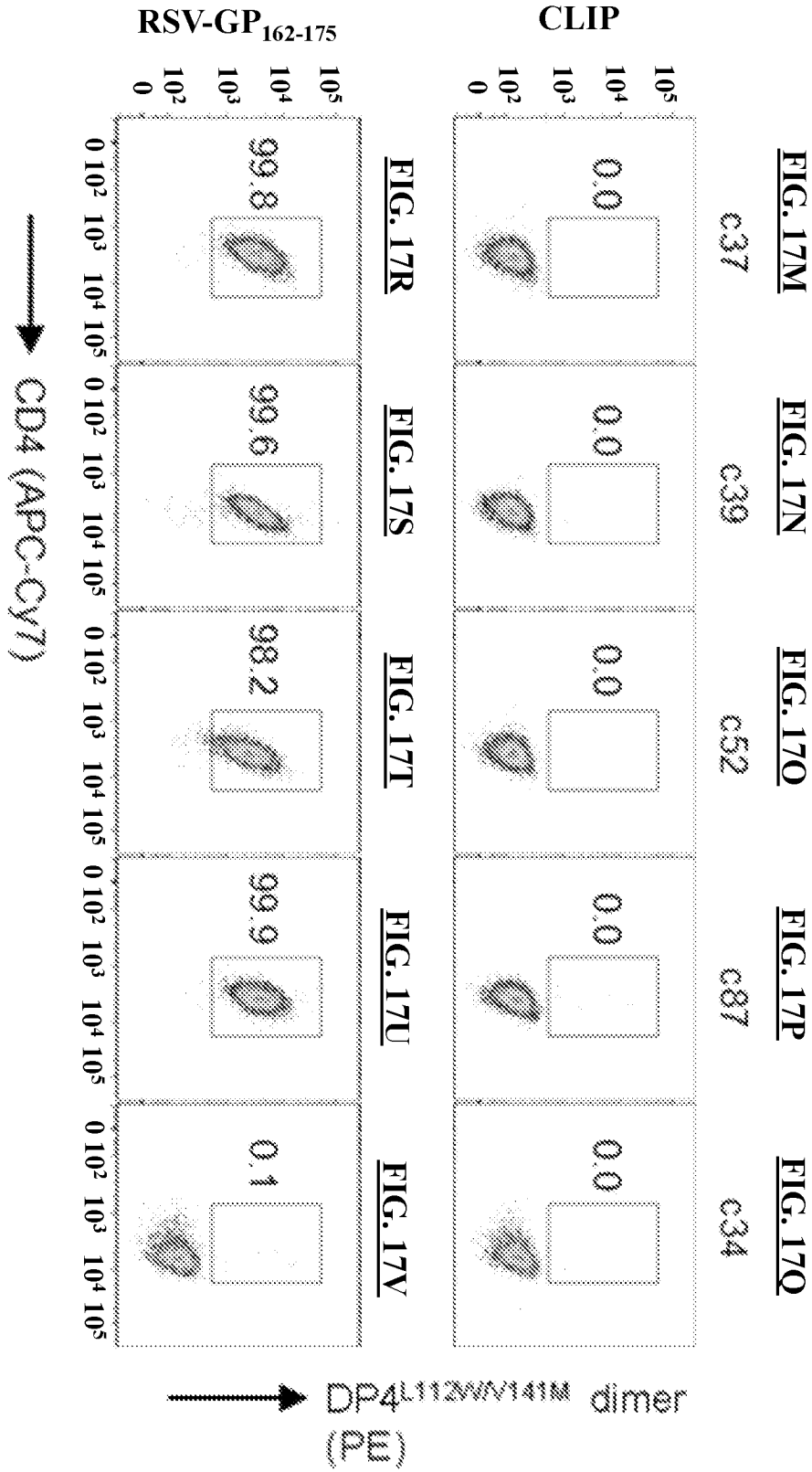
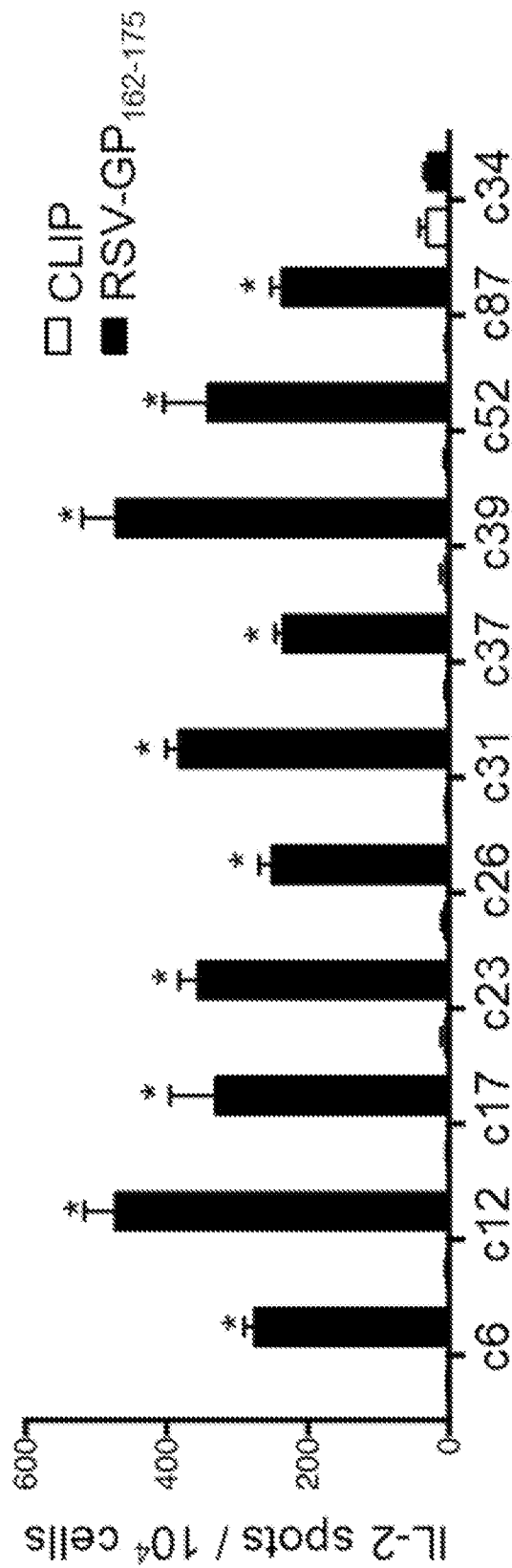
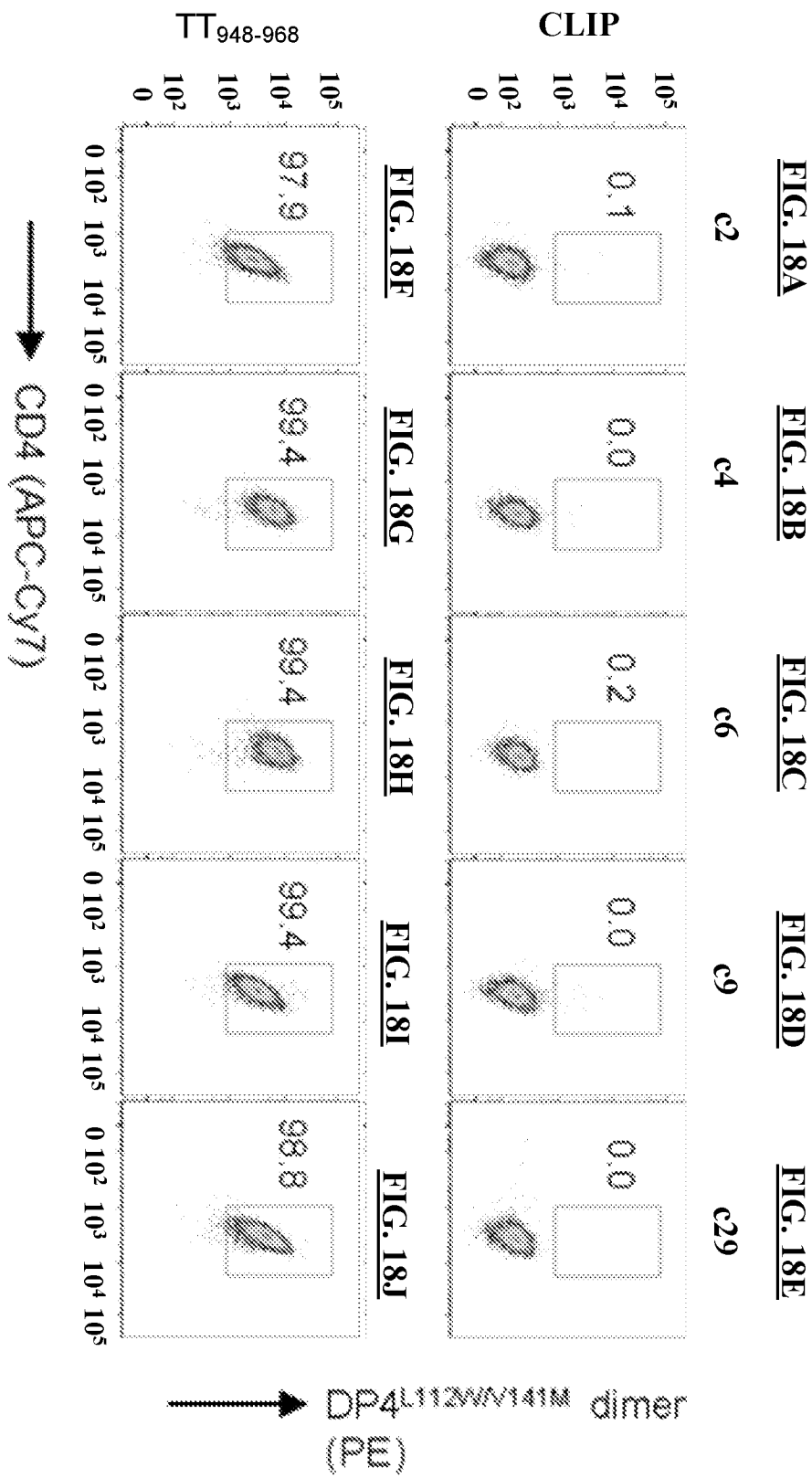


FIG. 17W





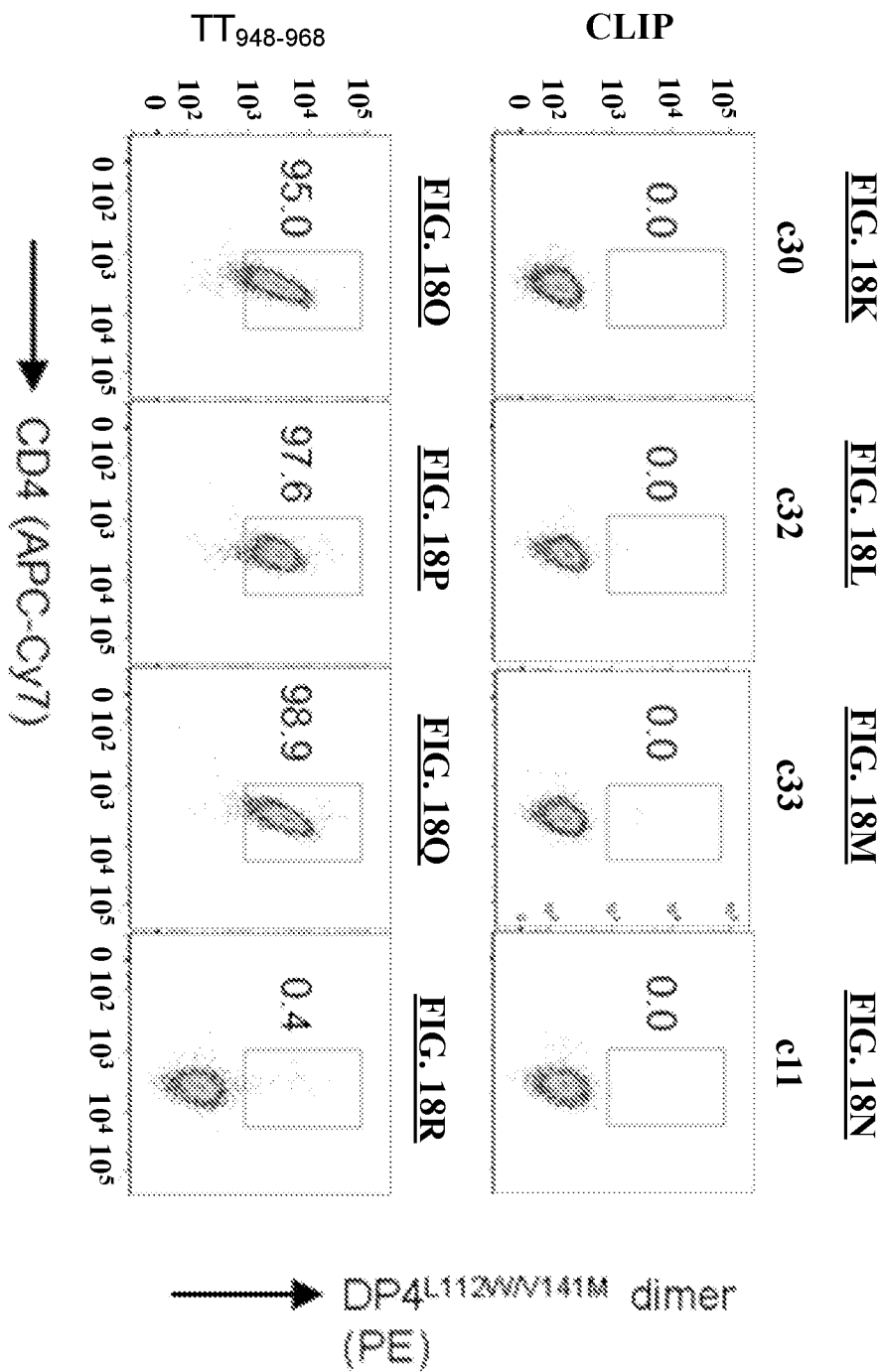
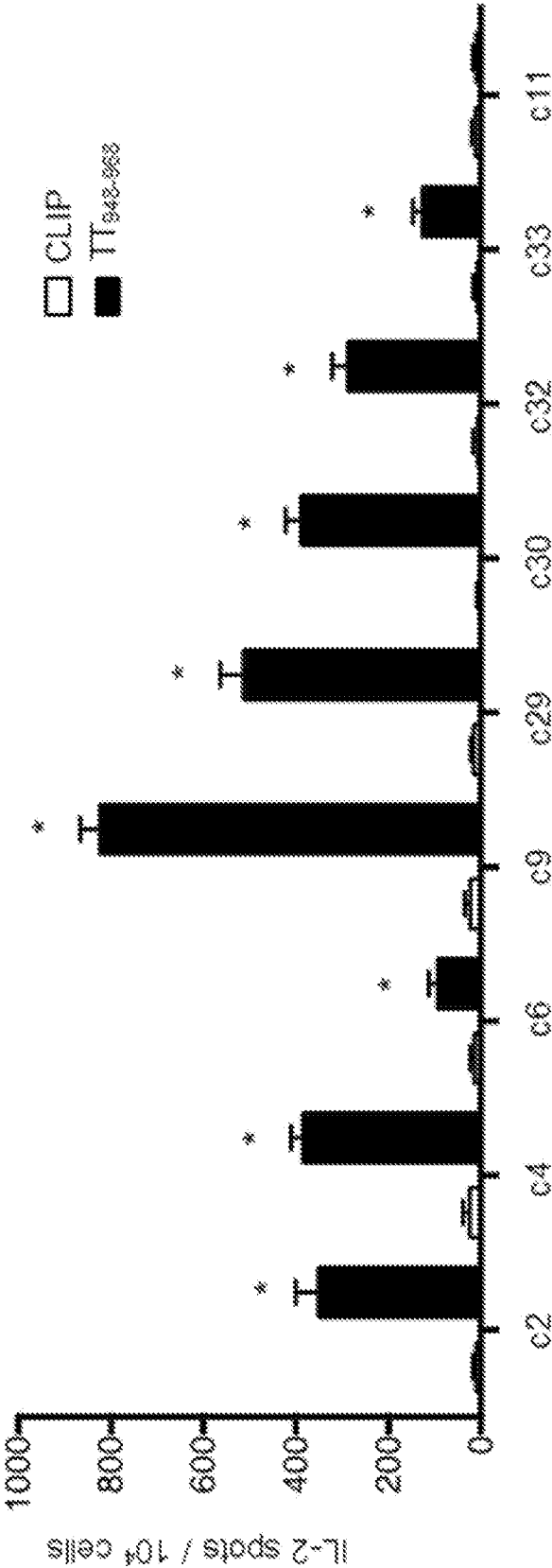
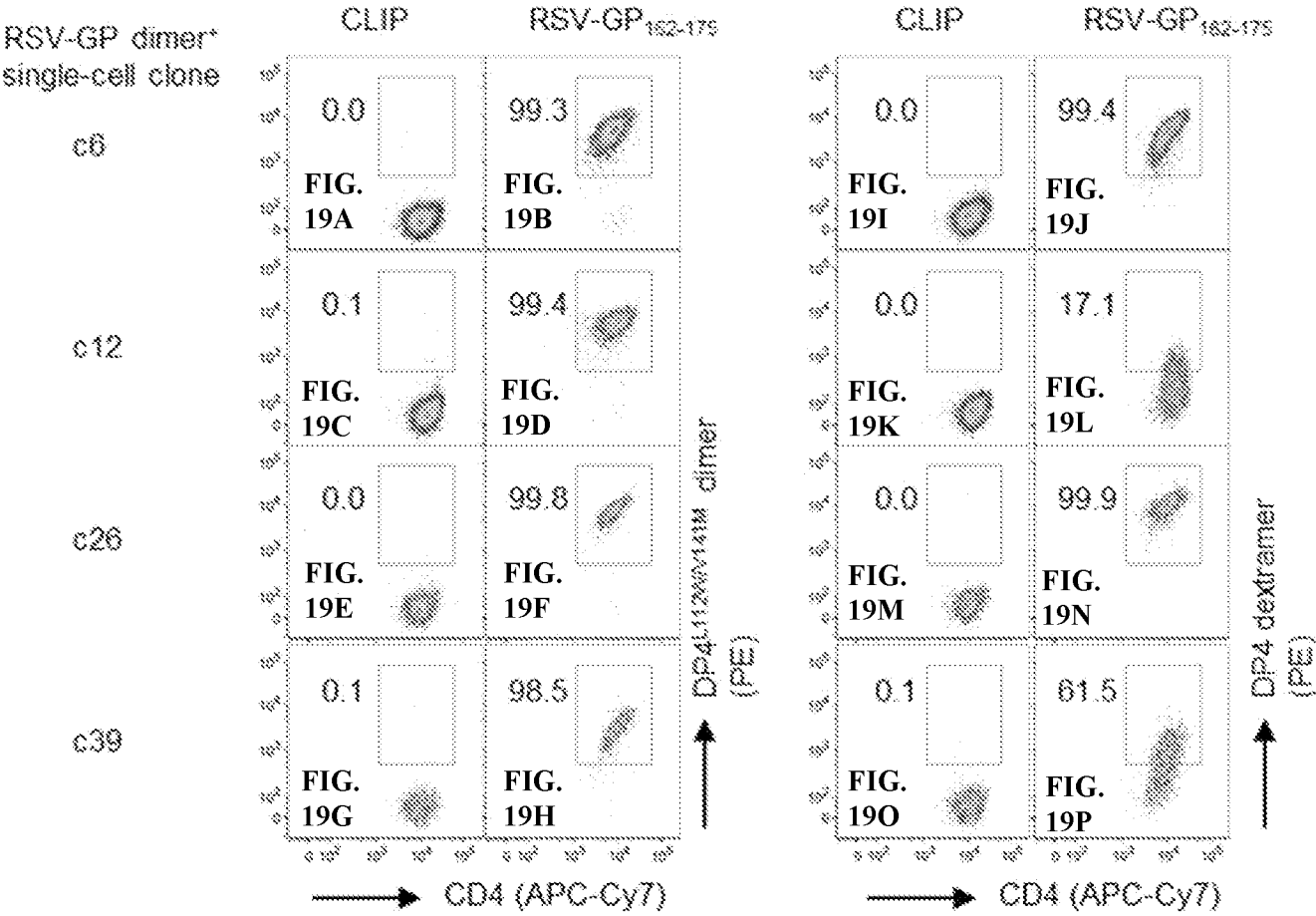
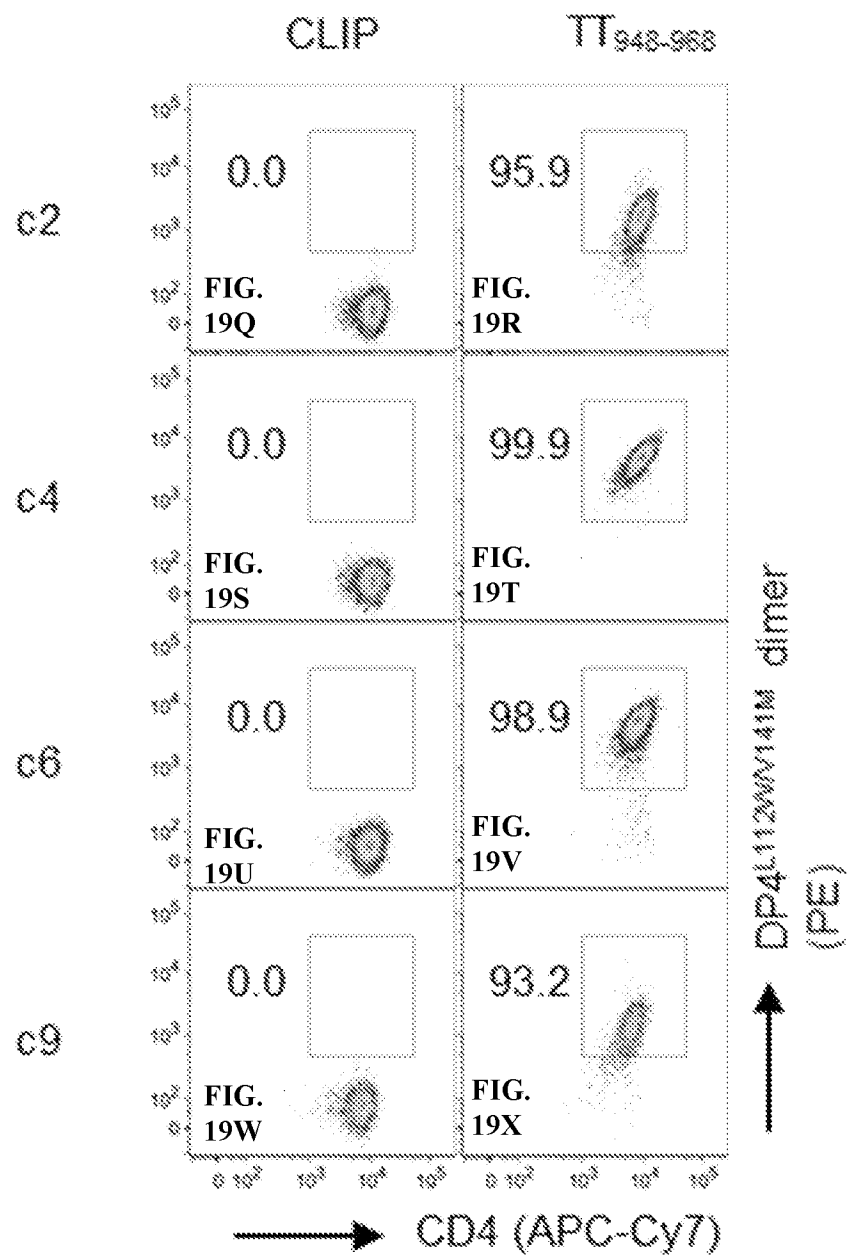
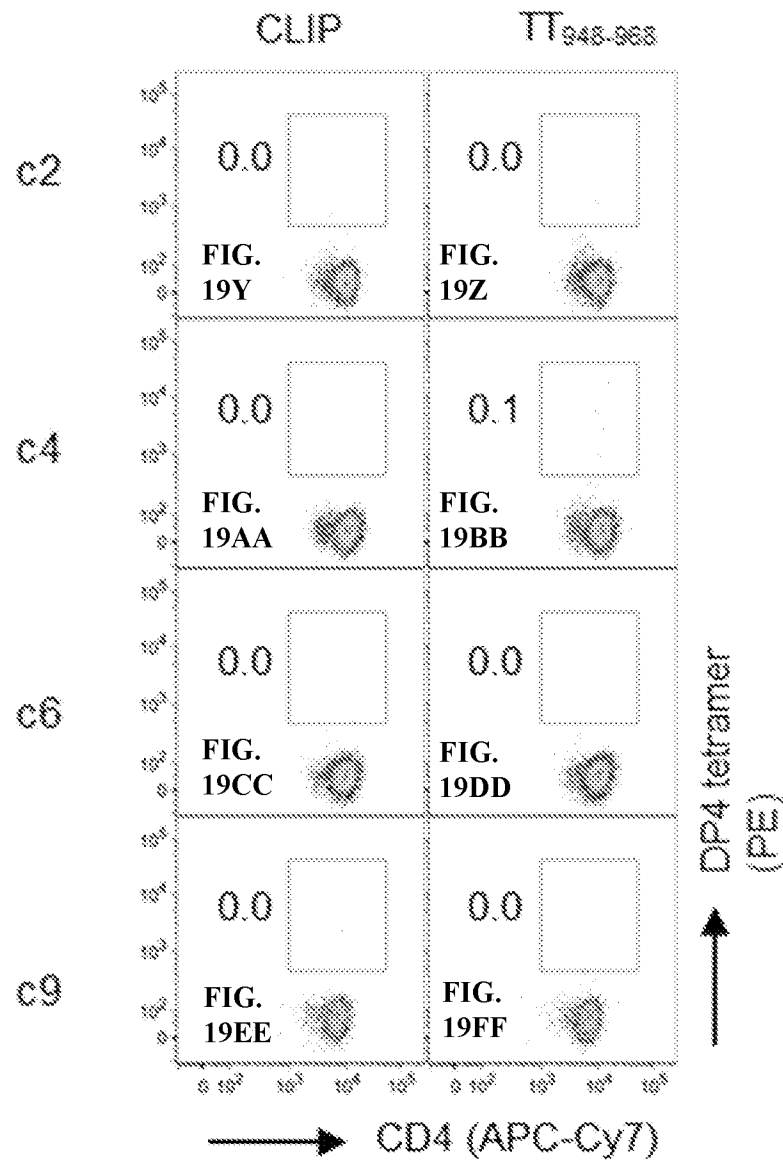


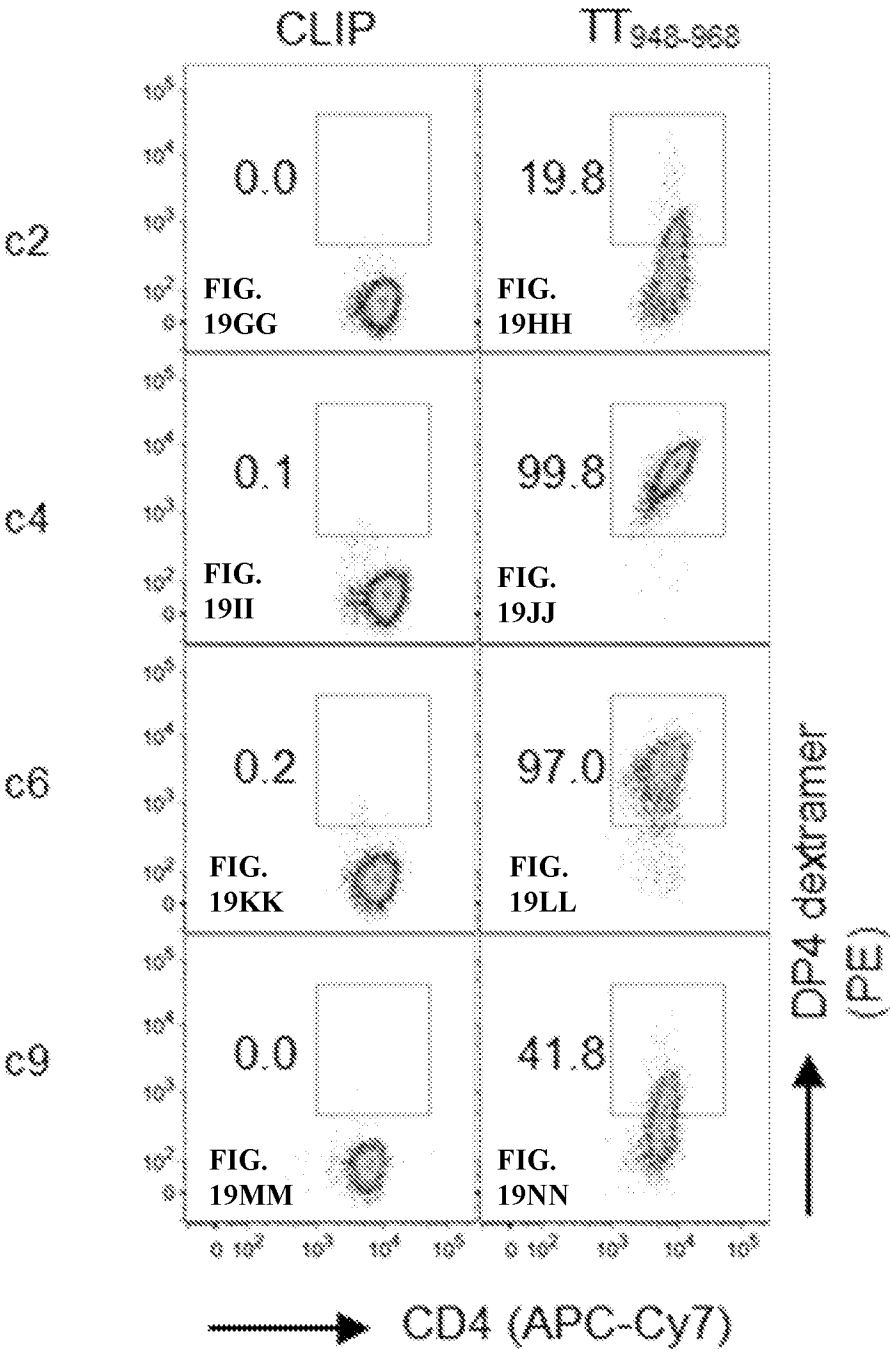
FIG. 18S











INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2020/057175

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C07K 14/74* (2006.01), *A61P 31/00* (2006.01), *A61P 35/00* (2006.01), *C12N 15/12* (2006.01),
C12N 5/10 (2006.01), *C12Q 1/02* (2006.01), *G01N 33/566* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Keywords used across the whole IPC

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: GenomeQuest, Canadian Patent Database, CAPlus, STN full text, PubMed, QuestelOrbit, Google.
 Keywords: HLA, DP beta, MHC Class II, CD4 binding, CD4+ binding, L112*, L141*, crystal structure, resol*
 structure (in various combinations)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2995838 A1 (ANDERSON, S. et al.) 23 February 2017 (23-02-2017)	1, 2, 7-9, 19-61 (all partially)
X	WANG, XX et al, Affinity Maturation of Human CD4 by Yeast Surface Display and Crystal Structure of a CD4-HLA-DR1 Complex. Proc Natl Acad Sci U S A. 2011, Vol.108, No.38, pages 15960-15965, ISSN:0027-8424 (Print).	1, 2, 7-9, 19-61 (all partially)
A	GenBank Accession No.: CQR91450 (MHC class II antigen [Homo sapiens]), 21 July 2017	1, 2, 7-9, 19-61 (all partially)

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"I" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
05 November 2020 (05-11-2020)

Date of mailing of the international search report
12 November 2020 (12-11-2020)

Name and mailing address of the ISA/CA
 Canadian Intellectual Property Office
 Place du Portage I, C114 - 1st Floor, Box PCT
 50 Victoria Street
 Gatineau, Quebec K1A 0C9
 Facsimile No.: 819-953-2476

Authorized officer

Riad Qanbar (819) 639-7618

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2020/057175**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The claims are directed to a plurality of inventive concepts as follows:

Group 1 - Claims 1, 2, 7-9, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 112 of said SEQ ID NO:1, wherein the leucine at position 112 is replaced with alanine;

Continued in supplemental box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:
1, 2, 7-9, 19-61 insofar as they relate to a L112A variant of SEQ ID NO:1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2020/057175

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GenBank Accession No.: XP_003473759 (HLA class II histocompatibility antigen, DP beta 1 chain [Cavia porcellus]), 19 January 2018	1, 2, 8, 9, 19-61 (all partially)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/IB2020/057175

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2995838A1	23 February 2017 (23-02-2017)	EP3337822A1 JP2018528191A US2018244759A1 WO2017031353A1	27 June 2018 (27-06-2018) 27 September 2018 (27-09-2018) 30 August 2018 (30-08-2018) 23 February 2017 (23-02-2017)

Continuation of Box No. III Observations where unity of invention is lacking

Groups 2 to 6 - Claims 1, 2, 7-9, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 112 of said SEQ ID NO:1, wherein the leucine at position 112 is replaced with valine, isoleucine, methionine, phenylalanine, or tyrosine, respectively;

Group 7 - Claims 1, 2, 7-10, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 112 of said SEQ ID NO:1, wherein the leucine at position 112 is replaced with tryptophan;

Groups 8 to 19 - Claims 1, 2, 7, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 112 of said SEQ ID NO:1, wherein the leucine at position 112 is replaced with an amino acid other than one of those in groups 1-7;

Group 20 - Claims 1, 3, 7-14, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that comprises an insertion at position 112 so that the amino acid at position 112 is not leucine;

Group 21 - Claims 1, 3, 7-14, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that comprises a deletion at position 112 so that the amino acid at position 112 is not leucine;

Groups 22 to 24 - Claims 4, 5, 7, 11, 12, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 141 of said SEQ ID NO:1, wherein the valine at position 141 is replaced with alanine, isoleucine, and leucine, respectively;

Group 25 - Claims 4, 5, 7, 11-13, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 141 of said SEQ ID NO:1, wherein the valine at position 141 is replaced with methionine;

Groups 26 to 28 - Claims 4, 5, 7, 11, 12, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 141 of said SEQ ID NO:1, wherein the valine at position 141 is replaced with phenylalanine, tyrosine, and tryptophan, respectively;

Groups 29 to 40 - Claims 4, 5, 7, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by a single amino acid located at position 141 of said SEQ ID NO:1, wherein the valine at position 141 is replaced with an amino acid other than one of those in groups 21-27;

Group 41 - Claims 4, 6-14, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that comprises an insertion at position 141 so that the amino acid at position 141 is not valine;

Group 42 - Claims 4, 6-14, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that comprises a deletion at position 141 so that the amino acid at position 141 is not valine;

Group 43 - Claims 1-14, 19-61 (partially) and claims 15, 16 (completely) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by two amino acids located at positions 112 and 141 of said SEQ ID NO:1, wherein the leucine at position 112 is replaced with tryptophan and the valine at position 141 is replaced with methionine; and

Groups 44-403 - Claims 1-14, 19-61 (all partially) featuring an HLA class II molecule comprising a beta DP chain that differs from that of SEQ ID NO:1 by two amino acids located at positions 112 and 141 of said SEQ ID NO:1, wherein each group corresponds to a unique combination of the two amino acids, excluding the combination L112W/V141M.

Claims 17 and 18 are not included with any group because the beta chain set forth in these claims is a native beta chain that does not comprise an amino acid substitution at either position 112 or position 141.

The subject matter of groups 1 to 403 relates to HLA class II molecule comprising a DP beta chain, wherein said DP beta chain is a variant of SEQ ID NO:1 in which the amino acid at position 112 is not leucine, the amino acid at position 141 is not valine, or the amino acids at positions 112 and 141 are not leucine and valine, respectively. When limited to amino acid substitutions at the identified positions, there are 399 unique variants. As variants of HLA class II DP beta chain comprising an amino acid substitution at position 112 (see, for example, CA2995838), position 141 (see, for example, GenBank Acc. No.: CQR91450), or both (see, for example, GenBank Accession No.: XP_003473759) were known in the art on the relevant date, each of the 403 unique variants is considered a separate alleged invention.

The claims must be limited to one inventive concept as set out in PCT Rule 13.