(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 May 2021 (14.05.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/092436 A1

(51) International Patent Classification:
C12Q 1/68 (2018.01) *A61K 38/05* (2006.01)
G16B 20/00 (2019.01)

(21) International Application Number:
PCT/US2020/059476

(22) International Filing Date:
06 November 2020 (06.11.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/932,751 08 November 2019 (08.11.2019) US
62/934,914 13 November 2019 (13.11.2019) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: IDENTIFICATION OF SPLICING-DERIVED ANTIGENS FOR TREATING CANCER

(57) Abstract: Methods and processes to identify neoplastic tissue antigens derived from alternative splicing (AS) are described, in accordance with various embodiments of the invention. Also described are novel tumor antigens that are useful as targets in various immunotherapeutic approaches to treating brain cancer as well as novel engineered T cell Receptors (TCRs) and chimeric antigen receptors (CARs) that target these antigenic peptides.

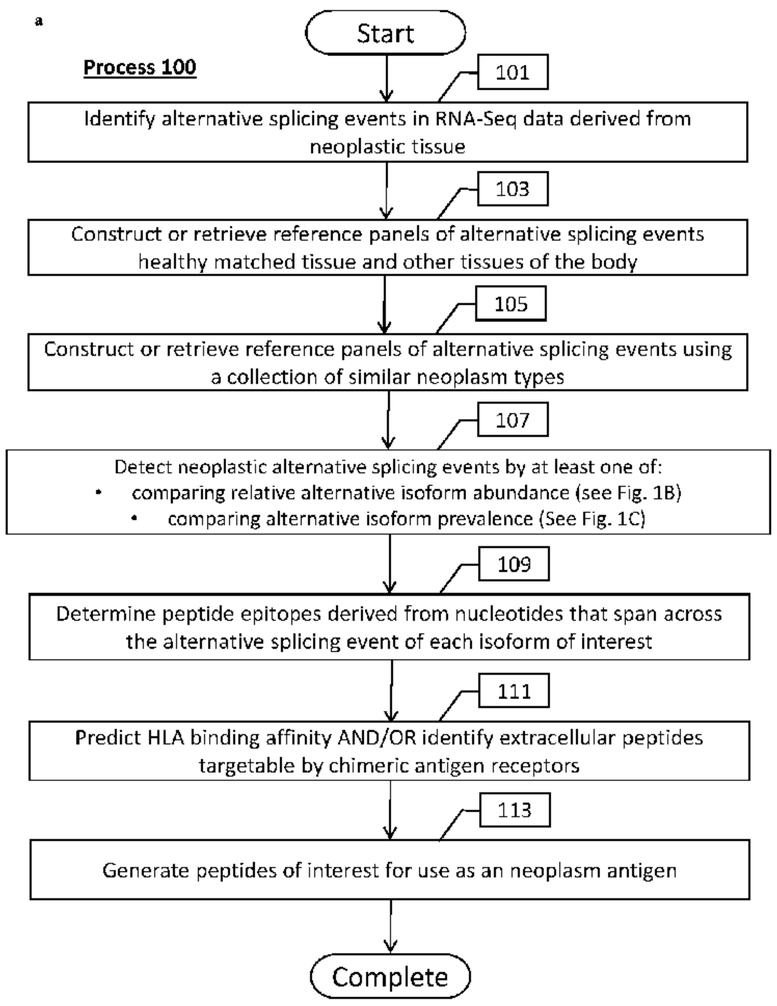

Fig. 1A

[Continued on next page]

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TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

IDENTIFICATION OF SPLICING-DERIVED ANTIGENS FOR TREATING CANCER

BACKGROUND OF THE INVENTION

[0001] This application claims benefit of priority of U.S. Provisional Patent Application No. 62/934,914, filed November 13, 2019 and U.S. Provisional Patent Application No. 62/932,751, filed November 8, 2019, both of which are hereby incorporated by reference in their entireties.

[0002] This invention was made with government support under Grant Numbers CA211015 and CA233074, awarded by the National Institutes of Health. The government has certain rights in the invention.

II. Field of the Invention

[0003] This invention relates to the field of cancer therapies.

III. Background

[0004] Cancer immunotherapy has gained tremendous momentum in the past decade. The clinical effectiveness of checkpoint inhibitors, such as neutralizing antibodies against PD-1 and CTLA-4, is thought to result from their ability to reactivate tumor-specific T cells. Meanwhile, adoptive cell therapies use genetically modified T-cell receptors (TCRs) or synthetic chimeric antigen receptor T cells (CAR-T) for tumor-specific antigen recognition. The finding that cancer cells express specific T-cell-reactive antigens has galvanized epitope discovery in recent years. Nevertheless, the identification of tumor antigens remains a major challenge. Although somatic mutation-derived antigens have been successfully targeted by cancer therapies, this approach remains largely ineffective for tumors with low or moderate mutation loads. Thus, there is a need in the art for the identification and characterization of novel tumor antigens that are useful targets in cancer immunotherapies.

SUMMARY OF THE INVENTION

[0005] Methods and processes to identify neoplastic tissue antigens derived from alternative splicing (AS) are described, in accordance with various embodiments. Also described are novel tumor antigens that are useful as targets in various immunotherapeutic approaches to treating

brain cancer as well as novel engineered T cell Receptors (TCRs) and chimeric antigen receptors (CARs) that target these antigenic peptides.

[0006] In several embodiments, RNA sequencing (RNA-seq) data derived from a neoplastic source (or a collection of neoplastic sources) are utilized to identify AS events. In a number of embodiments, neoplastic AS events are compared to AS events of non-neoplastic tissue such that AS events that are specific or increased in neoplastic tissue are identified. In some embodiments, neoplastic AS events are compared to AS events in similar neoplastic tissue such that recurrent AS events in neoplastic tissue are identified. Various processes to validate neoplastic AS events are performed, in accordance with some embodiments. Likewise, several embodiments utilize the identification of neoplastic AS events to synthesize peptides for use as an antigen of the neoplastic tissue.

[0007] Alternative splicing is a major cellular mechanism for generating expression complexity, especially in regulatory and functional aspects (e.g., two splice variants of the same gene can have different regulatory and functional properties). In addition, alternative splicing contributes to the diversity of phenotypes in eukaryotic cells of an organism, where each cell has the same DNA genotype. In neoplastic cells, alternative splicing mechanisms can be dysregulated, leading to aberrant expression of various isoforms and formation of neoplasm antigens. Various embodiments are directed towards identifying dysregulated isoforms and neoplasm antigens, which can be utilized in a number of applications. For instance, identified AS events can be utilized to develop peptides that are encoded by nucleotides that span the splice junction and these peptides may be utilized to develop various cancer treatments.

[0008] Aspects of the disclosure relate to an engineered T-cell Receptor (TCR) comprising: a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:30 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:31; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:32 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:33; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:34 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:35; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:36 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:37; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:38 and a TCR beta (TCR-b) CDR3

comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:39; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:40 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:41; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:42 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:43 or a TCR-a and TCR-b CDR3 comprising an amino acid sequence with at least 90% sequence identity to a TCR-a and TCR-b CDR3 pair from a clonotype listed in Table 6..

[0009] Further aspects relate to one or more nucleic acids encoding a TCR, CAR, or peptide of the disclosure. Certain aspects relate to a nucleic acid encoding a TCR-alpha and/or TCR-beta polypeptide. Also provided are nucleic acid vector(s) comprising the nucleic acid(s) of the disclosure. Further aspects relate to a cell, such as a therapeutic cell or a host cell, comprising a TCR, CAR, nucleic acid, or vector of the disclosure. Also provided are compositions comprising the cells, nucleic acids, or peptides of the disclosure. Yet further aspects relate to an in vitro isolated dendritic cell comprising a peptide, nucleic acid, or expression vector of the disclosure. Further aspects relate to an *in vitro* composition comprising a dendritic cell and a peptide of the disclosure. Further aspects relate to an engineered T-cell Receptor (TCR) or chimeric antigen receptor (CAR) that specifically recognizes a peptide of the disclosure. Aspects of the disclosure also relate to an antibody or antigen binding fragment thereof that specifically recognizes and binds to a peptide of the disclosure.

[0010] Further aspects of the disclosure relate to a method comprising transferring a nucleic acid of the disclosure into a cell. Further method aspects of the disclosure relate to a method for stimulating an immune response or for treating brain cancer comprising administering a composition, peptide, antibody, therapeutic cell, CAR, or TCR of the disclosure to a subject. Other method aspects of the disclosure relate to an *in vitro* method for making a dendritic cell vaccine comprising contacting a dendritic cell *in vitro* with a peptide of the disclosure. Other method aspects relate to a method of treating a subject for brain cancer comprising administering a peptide, composition, dendritic cell, antibody or antigen binding fragment, or cell of the disclosure.

[0011] Further aspects relate to: a peptide from the TRIM11 protein comprising at least 6 contiguous amino acids from the TRIM11 and comprising the amino acids QD, which correspond to the amino acids at positions 168-169 of SEQ ID NO:1; a peptide from the RCOR3 protein comprising at least 6 contiguous amino acids from the RCOR3 and comprising

the amino acids QG, which correspond to the amino acids at positions 358-359 of SEQ ID NO:2; a peptide from the FAM76B protein comprising at least 6 contiguous amino acids from the FAM76B and comprising the amino acids DS, which correspond to the amino acids at positions 230-231 of SEQ ID NO:3; a peptide from the SLMAP protein comprising at least 6 contiguous amino acids from the SLMAP and comprising the amino acids NP, which correspond to the amino acids at positions 332-333 of SEQ ID NO:4; a peptide from the TMEM62 protein comprising at least 6 contiguous amino acids from the TMEM62 and comprising the amino acids LG, which correspond to the amino acids at positions 495-496 of SEQ ID NO:5; and/or a peptide from the PLA2G6 protein comprising at least 6 contiguous amino acids from the PLA2G6 and comprising the amino acids RL, which correspond to the amino acids at positions 395-396 of SEQ ID NO:6. Further aspects relate to a peptide comprising at least 6 contiguous amino acids from a peptide of Table 1a, Table 1b, Table 1c, or 4, wherein the peptide comprises an alternative splice site junction. Yet further aspects relate to a peptide comprising at least 6 contiguous amino acids encoded by an alternatively spliced nucleic acid, wherein the at least 6 contiguous amino acids are encoded on a nucleic acid that comprises an alternative splice site junction, and wherein the alternative splice site junction is an AS event selected from an AS event in Table 3a or 3b.. An alternative splice site junction in a polypeptide refers to the amino acids that are encoded by the region of the mRNA that spans the alternative splice site. An alternative splice site in a nucleic acid refers to the nucleic acid residues that span the alternative splice site.

[0012] Also provided is a method of activating or expanding peptide-specific T cells comprising contacting a starting population of T cells from a mammalian subject and preferably from a blood sample from the mammalian subject cells ex vivo with the peptide of disclosure thereby activating, stimulating proliferation, and/or expanding peptide-specific T cells in the starting population. Further aspects relate to a peptide-specific T cell activated or expanded according to a method of the disclosure. Also provided are pharmaceutical compositions comprising the peptide-specific T cells activated or expanded according to a method of the disclosure.

[0013] In some embodiments, contacting is further defined as co-culturing the starting population of T cells with antigen presenting cells (APCs), wherein the APCs can present the peptide of the disclosure on their surface. In some embodiments, the APCs are dendritic cells. In some embodiments, the dendritic cells are autologous dendritic cells obtained from the mammalian subject. In some embodiments, contacting is further defined as co-culturing the starting population of T cells with artificial antigen presenting cells (aAPCs). In some

embodiments, the artificial antigen presenting cells (aAPCs) comprise or consist of poly(lactide-co-glycolide) (PLGA), K562 cells, paramagnetic beads coated with CD3 and CD28 agonist antibodies, beads or microparticles coupled with an HLA-dimer and anti-CD28, or nanosize-aAPCs (nano-aAPC) that are preferably less than 100 nm in diameter. In some embodiments, the T cells are CD8+ T cells or CD4+ T cells. In some embodiments, the T cells are cytotoxic T lymphocytes (CTLs). In some embodiments, the starting population of cells comprises or consists of peripheral blood mononuclear cells (PBMCs). In some embodiments, the method further comprises isolating or purifying the T cells from the peripheral blood mononuclear cells (PBMCs). In some embodiments, the mammalian subject is a human. In some embodiments, the method further comprises reinfusing or administering the activated or expanded peptide-specific T cells to the subject. Further aspects relate to a peptide-specific T cell activated or expanded according to a method of the disclosure. Also provided are pharmaceutical compositions comprising the peptide-specific T cells activated or expanded according to a method of the disclosure.

[0014] In some embodiments, the AS event is selected from an AS event in Table 3a. In some embodiments, the AS event is selected from an AS event in Table 3b. In some embodiments, the disclosure relates to a CAR that targets a peptide of the disclosure, wherein the peptide comprises an AS event from table 3b. In some embodiments, the disclosure relates to a TCR that targets a peptide of the disclosure, wherein the peptide comprises an AS event from table 3a.

[0015] In some embodiments, the TCR comprises: engineered T-cell Receptor (TCR) comprising: a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:30 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:31; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:32 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:33; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:34 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:35; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 70,

80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:36 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:37; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:38 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:39; a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:40 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:41; or a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:42 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:43.

[0016] In some embodiments, the TCR comprises: a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:44 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:45; a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:46 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:47; or a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:48 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:49.

[0017] In some embodiments, the TCR comprises: a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:44 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:45; a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:46 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID

NO:47; or a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:48 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 97, 98, 99, or 100% sequence identity to SEQ ID NO:49.

[0018] In some embodiments, the TCR comprises or consists of a bispecific TCR. The bispecific TCR may comprises an scFv that targets or selectively binds CD3. In some embodiments, the TCR is further defined as a single-chain TCR (scTCR), wherein the α chain and the β chain are covalently attached via a flexible linker.In some embodiments, the TCR comprises a modification or is chimeric. In some embodiments, the variable region of the TCR is fused to a TCR constant region that is different from the constant region of the cloned TCR that specifically binds to a peptide of the disclosure.

[0019] In some embodiments, the nucleic acid of the disclosure comprises a cDNA encoding the TCR. In some embodiments, the TCR alpha and beta genes are on the same nucleic acid and/or on the same vector.

[0020] In some embodiments, a cell of the disclosure comprises an immune cell. In some embodiments, a cell of the disclosure comprises stem cell, progenitor cell, T cell, NK cell, invariant NK cell, NKT cell, mesenchymal stem cell (MSC), induced pluripotent stem (iPS) cell, regulatory T cell, CD8+ T cell, CD4+ T cell, or γδ T cell. In some embodiments, the cell comprises a hematopoietic stem or progenitor cell, a T cell, or an induced pluripotent stem cell (iPSC). In some embodiments, the cell is isolated from a cancer patient. In some embodiments, is a HLA-A type. The cell of the disclosure may be autologous or allogeneic. In some embodiments, the cell is a HLA-A*03:01, HLA-A*01:01, or HLA-A*02:01 type. In some embodiments, the cell comprises at least one TCR and at least one CAR and wherein the TCR and CAR each recognize a different peptide. For example, embodiments of the disclosure relate to a cell that comprises a TCR that targets one peptide of the disclosure and a CAR that targets a different peptide of the disclosure.

[0021] In some embodiments, the composition of the disclosure has been determined to be serum-free, mycoplasma-free, endotoxin-free, and/or sterile.

[0022] In some embodiments, the method further comprises culturing the cell in media, incubating the cell at conditions that allow for the division of the cell, screening the cell, and/or freezing the cell. In some embodiments, the method further comprises isolating the expressed peptide or polypeptide from a cell of the disclosure.

[0023] In some embodiments, the brain cancer comprises glioblastoma or glioma. In some embodiments, the subject has previously been treated for the cancer. In some embodiments, the subject has been determined to be resistant to the previous treatment. In some embodiments, the method further comprises the administration of an additional therapy. In some embodiments, the additional therapy comprises an immunotherapy, chemotherapy, or an additional therapy described herein. In some embodiments, the cancer comprises stage I, II, III, or IV cancer. In some embodiments, the cancer comprises metastatic and/or recurrent cancer.

[0024] In some embodiments, a peptide of the disclosure comprises at least 6 contiguous amino acids from one of SEQ ID NOS:786 or 1364-1395. In some embodiments, a peptide of the disclosure has at least 70% sequence identity to a peptide of SEQ ID NO:786 or 1364-1395. In some embodiments, a peptide of the disclosure has at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to SEQ ID NOS:786 or 1364-1395.

[0025] In some embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO:7-9. In some embodiments, the peptide comprises an amino acid sequence with at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to SEQ ID NO:7-9. In some embodiments, the peptide comprises an amino acid sequence of SEQ ID NO:10. In some embodiments, the peptide comprises an amino acid sequence with at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to SEQ ID NO:10. In some embodiments, the peptide comprises an amino acid sequence of SEQ ID NO:11 or 12. In some embodiments, the peptide comprises an amino acid sequence with at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to SEQ ID NO:11 or 12. In some embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO:13-15. In some embodiments, the peptide comprises an amino acid sequence with at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to SEQ ID NO:13-15. In some embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO:16-22. In some embodiments, the peptide comprises an amino acid sequence with at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to SEQ ID NO:16-22. In some embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO:23-29. In some embodiments, the peptide comprises an amino acid sequence with at least 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity to SEQ ID NO:23-29. In some embodiments, the peptide comprises at least 10 amino acids. In some embodiments, the peptide comprises at least 6 contiguous amino acids of one of SEQ ID NO:7-29. In some embodiments, the peptide comprises at least 6, 7,

8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 (or any derivable range therein) contiguous amino acids of SEQ ID NOS:1-29. In some embodiments, the peptide consists of 10 amino acids. In some embodiments, the peptide consists of 8, 9, 10, 11, 12, 13, or 14 amino acids. In some embodiments, the peptide is less than 20 amino acids in length. In some embodiments, the peptide is less than 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, or 6 amino acids (or any derivable range therein) in length. In some embodiments, the peptide is modified. In some embodiments, the modification comprises conjugation to a molecule. In some embodiments, the molecule comprises an antibody, a lipid, an adjuvant, or a detection moiety.

[0026] In some embodiments, the compositions of the disclosure are formulated as a vaccine. In some embodiments, the compositions and methods of the disclosure provide for prophylactic therapies to prevent brain cancer. In some embodiments, the compositions and methods of the disclosure provide for therapeutic therapies to treat existing cancers, such as for the treatment of patients with a brain tumor. In some embodiments, the composition further comprises an adjuvant. Adjuvants are known in the art and include, for example, TLR agonists and aluminum salts. Other adjuvants include IL-1, IL-2, IL-4, IL-7, IL-12, -interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). Exemplary adjuvants may include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants, and/or aluminum hydroxide adjuvant. Further embodiments of adjuvants include amorphous aluminum hydroxyphosphate sulfate (AAHS), aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate, the combination of monophosphoryl lipid A (MPL) and aluminum salt, oil in water emulsion composed of squalene, a liposomal formulation of MPL and QS-21 (a natural compound extracted from the Chilean soapbark tree), and cytosine phosphoguanine (CpG), a synthetic form of DNA that mimics bacterial and viral genetic material.

[0027] In some embodiments, the dendritic cell comprises a mature dendritic cell. In some embodiments, the cell is a cell with an HLA type selected from HLA-A, HLA-B, or HLA-C. In some embodiments, the cell is a cell with an HLA type selected from HLA-A*02:01, HLA-A*03:01, HLA-A*23:01, HLA-A*68:02, HLA-B*07:05, HLA-B*18:01, HLA-B*40:01, HLA-C*03:03, HLA-C*14:02, or HLA-C*15:02.

[0028] In some embodiments the methods of the disclosure further comprise screening the dendritic cell for one or more cellular properties. In some embodiments, the method further comprises contacting the cell with one or more cytokines or growth factors. In some

embodiments, the one or more cytokines or growth factors comprises GM-CSF. In some embodiments, the cellular property comprises cell surface expression of one or more of CD86, HLA, and CD14. In some embodiments, the dendritic cell is derived from a CD34+ hematopoietic stem or progenitor cell.

[0029] In some embodiments, the dendritic cell is derived from a peripheral blood monocyte (PBMC). In some embodiments, the dendritic cells is isolated from PBMCs. In some embodiments, the dendritic cells are cells in which the DCs are derived from are isolated by leukaphereses.

[0030] In some embodiments, the composition further comprises one or more cytokines, growth factors, or adjuvants. In some embodiments, the composition comprises GM-CSF. In some embodiments, the peptide and GM-CSF are linked. In some embodiments, the composition is determined to be serum-free, mycoplasma-free, endotoxin-free, and sterile. In some embodiments, the peptide is on the surface of the dendritic cell. In some embodiments, the peptide is bound to a MHC molecule on the surface of the dendritic cell. In some embodiments, the composition is enriched for dendritic cells expressing CD86 on the surface of the cell. In some embodiments, the dendritic cell is derived from a CD34+ hematopoietic stem or progenitor cell. In some embodiments, the dendritic cell is derived from a peripheral blood monocyte (PBMC). In some embodiments, the dendritic cells or cells in which the DCs are derived are isolated by leukaphereses.

[0031] In some embodiments of the disclosure, the cell comprises a stem cell, a progenitor cell, or a T cell. In some embodiments, the cell comprises a hematopoietic stem or progenitor cell, a T cell, or an induced pluripotent stem cell (iPSC).

[0032] In some embodiments, the method comprises administering a cell or a composition comprising a cell and wherein the cell comprises an autologous cell. In some embodiments, the cell comprises a non-autologous cell.

[0033] Throughout this application, the term "about" is used according to its plain and ordinary meaning in the area of cell and molecular biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0034] The use of the word "a" or "an" when used in conjunction with the term "comprising" may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0035] As used herein, the terms "or" and "and/or" are utilized to describe multiple components in combination or exclusive of one another. For example, "x, y, and/or z" can refer to "x" alone, "y" alone, "z" alone, "x, y, and z," "(x and y) or z," "x or (y and z)," or "x or y or

z." It is specifically contemplated that x, y, or z may be specifically excluded from an embodiment.

[0036] The words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include"), "characterized by" (and any form of including, such as "characterized as"), or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0037] The compositions and methods for their use can "comprise," "consist essentially of," or "consist of" any of the ingredients or steps disclosed throughout the specification. The phrase "consisting of" excludes any element, step, or ingredient not specified. The phrase "consisting essentially of" limits the scope of described subject matter to the specified materials or steps and those that do not materially affect its basic and novel characteristics. It is contemplated that embodiments described in the context of the term "comprising" may also be implemented in the context of the term "consisting of" or "consisting essentially of."

[0038] It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention. Aspects of an embodiment set forth in the Examples are also embodiments that may be implemented in the context of embodiments discussed elsewhere in a different Example or elsewhere in the application, such as in the Summary of Invention, Detailed Description of the Embodiments, Claims, and description of Figure Legends.

[0039] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better

understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0041] FIG. 1A-C provides a process to generate antigenic peptides utilizing RNA-seq data derived from neoplastic tissue in accordance with an embodiment.

[0042] FIG. 2 provides a process to generate antigenic peptides utilizing RNA-seq data and mass spectrometry data derived from neoplastic tissue in accordance with an embodiment.

[0043] FIG. 3 provides an example of process Isoform peptides from RNA splicing for Immunotherapy target Screening (IRIS) that can be used to identify peptides for T cell receptor and chimeric antigen receptor therapies in accordance with an embodiment. Shown is the Workflow for IRIS, integrating computational modules, large-scale reference RNA-Seq panels, and dedicated statistical testing programs. IRIS has three main modules: RNA-Seq data processing (top), *in silico* screening (middle), and TCR/CAR-T target prediction (bottom). The prediction module includes an option for proteo-transcriptomics integration of RNA-Seq and MS data.

[0044] FIG. 4. IRIS: A big data-powered platform for discovering AS-derived cancer immunotherapy targets. Stepwise results of IRIS to identify AS-derived cancer immunotherapy targets from 22 GBM samples (top). Identified skipped-exon (SE) events from the IRIS data-processing module were screened against tissue-matched normal panel ('Normal Brain') to identify tumor-associated events ('Primary' set), followed by tumor panel and normal panel to identify tumor-recurrent and tumor-specific events, respectively ('Prioritized' set). After constructing splice-junction peptides of tumor isoforms, TCR/CAR-T targets were predicted. As an illustrative example, IRIS readouts for prioritized candidate TCR targets are shown (bottom). Violin plots (left) show PSI values of individual AS events across GBM ('GBM-input') versus three reference panels. Dots (middle) summarize screening results. Darker-colored dots indicate stronger tumor features (association/recurrence/specificity) versus each reference panel. FC is estimated fold change of tumor isoform's proportion in GBM versus tissue-matched normal panel ('Brain'). Predicted HLA-epitope binding (right) is output of prediction module. Preferred features for immunotherapy targets in this study are shown in blue. Amino acids at splice junctions in epitopes are underlined. 'Best HLA' is HLA type with best predicted affinity (median IC_{50}) for given splice-junction epitope. '#Pt. w/HLA' is number of patients with HLA type(s) predicted to bind to a given epitope. Three epitopes in *TMEM62* and *PLA2G6* (blue) were predicted to bind to common HLA types (HLA-A02:01 and HLA-A03:01) and were selected for experimental validation. Figure discloses SEQ ID NOS 7, 9, 10, 12, 11, 13, 15, 21, 22, 27, and 29, respectively, in order of appearance.

[0045] FIG. 5A-C. IRIS-predicted AS-derived TCR targets recognized by $CD3^+CD8^+$ T cells in tumors and peripheral blood from patients. **a,** Summary of dextramer-based validation of IRIS-predicted AS-derived epitopes. PBMCs and/or TILs from four HLA-A03 and two HLA-A02 patients were tested for recognition of IRIS-predicted epitopes. Within each HLA type, epitopes are listed by order of tumor specificity (high to low) versus normal panel (11 normal nonbrain tissues). Reactivity ('Positive', 'Marginal', or 'Negative') in assay was evaluated as percentage of dextramer-labeled cells among PBMCs/TILs (>0.1%, 0.01%-0.1%, or <0.01% of $CD3^+CD8^+$ cells, respectively) after subtracting negative control (nonhuman peptide). 'Dextramer assay summary' was determined by the mean percent reactivity of $CD3^+CD8^+$ cells across individual tests. **b,** Flow cytometric analysis showing that *ex vivo*-expanded TILs from one HLA-A03 patient (LB2867) contained T cells that recognized epitope KIGRLVTRK (SEQ ID NO:29). Rows correspond to cells that recognize APC- and PE-labeled dextramers (top), only PE-labeled dextramers (middle), or only APC-labeled dextramers (bottom). Percentages of epitope-specific cells are shown. **c,** Immune profiling results revealing immune repertoire composition of KIGRLVTRK (SEQ ID NO:29)-specific T cells from one patient (LB2867). The scRNA-Seq assay was performed on sorted KIGRLVTRK (SEQ ID NO:29)-specific T cells, whereas pairSEQ and immunoSEQ assays captured TCR clones from bulk TIL RNAs of same patient. Table (left) lists seven most abundant T-cell clones from scRNA-Seq, with percentages of matching CDR3 sequences from TCR β chains. *For pairSEQ and immunoSEQ, percentages are the best frequencies of matching TCR pair or β-chain clones. The 3D scatterplot (right) shows that these approaches converged on three dominant TCR clones. For comparison, the same epitope in the table and 3D scatterplot are identified by use of the same color for the sequence (table) and text box (plot). Figure discloses SEQ ID NOS 29, 557, 22, 556, 27, 227, 62, 30-43, 33, 31, and 35, respectively, in order of appearance.

[0046] FIG. 6A-C. RNA-Seq big-data reference panels in IRIS. **a,** Exon-based principal component analysis (PCA) of RNA-Seq data of 9,662 samples from 53 normal tissues from the GTEx consortium. Samples from the same histological site are grouped by color. Samples from different subregions of the same histological site are differentiated by different shapes. **b,** Summary of 53 normal tissues from the GTEx consortium. Data for all 53 tissues are available to IRIS users as a reference panel of normal tissues. In the present study, 11 selected vital tissues (heart, skin, blood, lung, liver, nerve, muscle, spleen, thyroid, kidney, and stomach) were used for the 'normal panel'. 'Events Selected' represent AS events with an average count ≥ 10 reads for the sum of all splice junctions across all samples in that tissue. **c,** Summary of

the tumor reference panel (TCGA tumor samples relevant to GBM). 'Events Selected' represent AS events with an average count ≥ 10 reads for the sum of all splice junctions across all samples in that tumor type.

[0047] FIG. 7A-B. Identification of AS events that are prone to measurement errors due to technical variances across big-data reference panels. a, Computational workflow to create a 'blacklist' of error-prone AS events. Normal 76-bp RNA-Seq reads were artificially trimmed to 48 bp. RNA-Seq files (76- and 48-bp) were aligned by using two different aligners (Tophat and STAR). AS events were quantified by rMATS-turbo. AS events with statistically significant differences in PSI values among RNA-Seq datasets with distinct technical conditions were identified and included in a blacklist. **b,** Scatter plots comparing PSI values of GTEx normal brain RNA-Seq data estimated under distinct technical conditions (read lengths: 48- and 76-bp, aligners: STAR and Tophat). 'Significantly different' AS events were defined as those with significantly different PSI values ($p < 0.05$, $abs(\Delta\psi) > 0.05$ from paired *t*-test).

[0048] FIG. 8A-B. CAR-T target prediction by IRIS. a, Computational workflow to annotate protein extracellular domain (ECD)-associated AS events for CAR-T target discovery. **b,** Five examples of IRIS-identified AS-derived CAR-T targets for 22 GBM samples. Position of the ECD in amino acid (aa) sequence was obtained from UniProtKB.

[0049] FIG. 9A-E. Proteo-transcriptomic analysis of HLA presentation of AS-derived epitopes in normal and tumor cell lines. a, Proteo-transcriptomics workflow adopted by IRIS to discover splice-junction peptides in MS datasets. IRIS inputs MS data (right), such as whole-cell proteomics, surfaceomics, or immunopeptidomics (HLA peptidomics) data. RNA-Seq-based custom proteome library is constructed and searched using MSGF+. **b,** Summary of HLA presentation of AS-derived epitopes in JeKo-1 (lymphoma) and B-LCL (normal) cell lines. Peptide-spectrum matches ('PSMs') and 'Unique peptides' are provided by MSGF+ with a target-decoy FDR of 5%. 'Predicted AS epitopes' are generated by the IRIS prediction module, which utilizes IEDB predictors. AS epitopes that are predicted by IRIS and detected in the MS data are considered 'MS-validated AS epitopes'. **c,** Percentage of IRIS-predicted AS-derived epitopes among all MS-detected peptides. Graph shows the percentage of all MS-detected peptides that are IRIS-predicted AS-derived epitopes (y-axis) as a function of the MSGF+ target-decoy FDR (x-axis). **d,** Preferential detection of high-affinity AS-derived peptides in MS data. Graph shows the number of AS-derived peptides detected in JeKo-1 MS data (y-axis) as a function of the MSGF+ target-decoy FDR (x-axis). Peptides with high (IC_{50} < 500 nM; Pred+, orange) and low (IC_{50} > 500 nM; Pred-, grey) predicted HLA binding affinities are shown. **e,** Heatmap depiction of distribution of AS-derived epitopes in JeKo-1

MS immunopeptidome, as a function of predicted HLA binding affinity and transcript expression level. AS-derived peptides are binned by the corresponding transcripts' expression levels and IEDB-predicted binding affinity scores. Heatmap is colored from red (high) to yellow (90^{th} percentile) to blue (low), reflecting the proportion of IRIS-predicted AS-derived epitopes that are MS-detected in each bin.

[0050] FIG. 10A-D. Consistent distributions of high-frequency TCR clones in one patient's TIL population revealed by multiple TCR sequencing approaches. a, Scatter plot comparing scRNA-Seq and bulk TIL pairSEQ for detection of high-frequency TCR clones. Graph shows frequency detected from bulk TIL samples using pairSEQ (y-axis) and scRNA-Seq on dextramer-positive sorted TIL samples (x-axis). As a complementary validation of scRNA-Seq, clonotypes from pairSEQ were matched to scRNA-Seq results by either CDR3 pairs or β chains, whichever matched best. The 10 most abundant TCR clones by scRNA-Seq that overlapped with clones detected by bulk TIL pairSEQ are circled. **b,** Table showing CDR3 amino acid sequences of the 10 most abundant TCR clones detected by scRNA-Seq and their corresponding detection frequencies by bulk TIL pairSEQ. As a complementary validation of scRNA-Seq, clonotypes from pairSEQ were matched to scRNA-Seq results by either CDR3 pairs or β chains, whichever matched best. **c,** Scatter plot comparing bulk TIL immunoSEQ and bulk TIL pairSEQ for detection of high-frequency TCR clones. Graph shows frequency detected from bulk TIL samples using immunoSEQ (y-axis) and pairSEQ (x-axis). Clonotypes from immunoSEQ were matched to pairSEQ results by the best CDR3 β chains. Four high-frequency overlapping clones from both methods are circled and color-coded, with β-chain CDR3 amino acid sequences and frequencies by each method shown in boxes. **d,** Scatter plot comparing scRNA-Seq and bulk TIL immunoSEQ for detection of high-frequency TCR clones. Graph shows frequency detected from bulk TIL samples using immunoSEQ (y-axis) and scRNA-Seq on dextramer-positive sorted TIL samples (x-axis). As a complementary validation of scRNA-Seq, clonotypes from immunoSEQ were matched to scRNA-Seq results by the best CDR3 β chains. Three high-frequency overlapping clones from both methods are circled and color-coded, with β-chain CDR3 amino acid sequences and frequencies by each method shown in boxes. Figure discloses SEQ ID NOS 30, 32, 34, 36, 38, 40, 42, 714, 715, 581, 746, 31, 33, 35, 37, 39, 41, 43, 572, 574, and 580 (in order of columns) and 558, 31, 33, 39, 33, 35, and 31, respectively, in order of appearance.

[0051] FIG. 11: **IRIS: A big data-powered platform for discovering AS-derived cancer immunotherapy targets.** Stepwise results of IRIS to identify AS-derived cancer immunotherapy targets from 22 GBM samples (top). Identified skipped-exon (SE) events from

the IRIS data-processing module were screened against tissue-matched normal panel ('Normal Brain') to identify tumor-associated events ('Primary' set), followed by tumor panel and normal panel to identify tumor-recurrent and tumor-specific events, respectively ('Prioritized' set). After constructing splice-junction peptides of tumor isoforms, TCR/CAR-T targets were predicted. As an illustrative example, IRIS readouts for prioritized candidate TCR targets are shown (bottom). Violin plots (left) show PSI values of individual AS events across GBM ('GBM-input') versus three reference panels. Dots (middle) summarize screening results. Darker-colored dots indicate stronger tumor features (association/recurrence/specificity) versus each reference panel. FC is estimated fold change of tumor isoform's proportion in GBM versus tissue-matched normal panel ('Brain'). Predicted HLA-epitope binding (right) is output of prediction module. Preferred features for immunotherapy targets in this study are shown in blue. Amino acids at splice junctions in epitopes are underlined. 'Best HLA' is HLA type with best predicted affinity (median IC_{50}) for given splice-junction epitope. '#Pt. w/HLA' is number of patients with HLA type(s) predicted to bind to a given epitope. Figure discloses SEQ ID NOS:1371, 1396, 1397, 1380, 1398, 1399, 1400, 1401, 1402, 21, and 22, respectively, in order of appearance.

DETAILED DESCRIPTION OF THE INVENTION

[0052] Aberrant alternative splicing (AS) is widespread in cancer, leading to an extensive but largely unexploited repertoire of potential immunotherapy targets. This disclosure describes computational platforms leveraging large-scale cancer and normal transcriptomics data to discover AS-derived tumor antigens for T-cell receptor (TCR) and chimeric antigen receptor T-cell (CAR-T) therapies. Applying AS identifying computational platforms to RNA-Seq data from 22 glioblastomas resected from patients, the inventors identified candidate epitopes and validated their recognition by patient T cells, demonstrating platforms' utility for expanding targeted cancer immunotherapy.

1. Identification and synthesis of neoplastic tissue antigens

[0053] An embodiment of a process to identify and synthesize neoplastic tissue antigens is illustrated in FIG. 1A. This embodiment is directed to utilizing RNA-seq data derived from neoplastic tissue to identify AS events, especially in neoplastic tissue, which in turn is utilized to identify antigens derived from the AS events. Various comparative and statistical methods are utilized to rank AS events and the antigens.

[0054] Process 100 can begin with identifying (101) AS event in RNA seq data derived from neoplastic tissue. AS events include (but are not limited to) exon skipping, an alternative 3' splice site, an alternative 5' splice site, and intron retention. For AS events identification applications described within, RNA sequencing provides a facile method to obtain sequence data, as it is typically abundant in the biological source, can be easily sequenced by known methods, readily available in numerous public and private databases, has intronic sequences already removed, and many exon reference databases exist for post-sequencing data analysis.

[0055] The source of RNA sequence data can be derived de novo (i.e., from biological tissue), or from a public or private database. Several methods can be utilized to derive RNA sequence data from biological tissue (or a collection of biological tissues). Generally, RNA molecules are extracted from tissue, prepped to be sequenced, and then run on a sequencer. For example, RNA can be extracted from a human tissue source, then prepped into a sequence library, and sequenced on a next-generation sequencing platform, such as those manufactured by Illumina, Inc. (San Diego, CA). Neoplastic tissue sources include (but are not limited to) tumor biopsy, nodal biopsy, surgical resection, and liquid/soft biopsies. Liquid and soft biopsies can be used to collect circulating neoplastic cells or cell-free nucleic acids, and include (but not limited to) blood, plasma, lymph, cerebral spinal fluid, urine, and stool. In many embodiments, biopsies are extracted from patients having been diagnosed with a particular neoplasm.

[0056] In some embodiments, RNA sequence data can be derived from an available database. For example, transcriptome data can be obtained from the National Center for Biotechnology Information (NCBI), Reference Sequence Database (RefSeq), Genotype-Tissue Expression Portal (GTEx), and The Cancer Genome Atlas Program (TCGA) databases. Sequence data could be in any appropriate sequence read format, including (but not limited to) single or paired-end reads.

[0057] Any appropriate neoplastic tissue can be analyzed, including (but not limited to) acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), anal cancer, astrocytomas, basal cell carcinoma, bile duct cancer, bladder cancer, breast cancer, Burkitt's lymphoma, cervical cancer, chronic lymphocytic leukemia (CLL) chronic myelogenous leukemia (CML), chronic myeloproliferative neoplasms, colorectal cancer, diffuse large B-cell lymphoma, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, Ewing sarcoma, fallopian tube cancer, follicular lymphoma, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, hairy cell leukemia, hepatocellular cancer, Hodgkin lymphoma, hypopharyngeal cancer, Kaposi sarcoma, Kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, Merkel cell

cancer, mesothelioma, mouth cancer, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic neuroendocrine tumors, pharyngeal cancer, pituitary tumor, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, skin cancer, small cell lung cancer, small intestine cancer, squamous neck cancer, T cell lymphoma, testicular cancer, thymoma, thyroid cancer, uterine cancer, vaginal cancer, and vascular tumors.

[0058] In many embodiments, RNA is processed before analysis. Any appropriate method can be used to process sequence data. For example, the sequence data can be trimmed with the publicly available TrimGalore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) or cutAdapt (https://cutadapt.readthedocs.io/en/stable/) methods, which remove adapter sequences and trim poor-quality bases. Mapping can be performed with any appropriate annotated genome, such as, for example, UCSC's hg19 (http://support.illumina.com/sequencing/sequencing_software/igenome.html) and alignment tool, such as, for example, Bowtie2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml), TopHat (https://ccb.jhu.edu/software/tophat/index.shtml), and STAR (https://github.com/alexdobin/STAR). Genes and their exons can be identified and their relative expression level determined. For instance, quantification of gene expression and AS events can be determined by GENCODE package (Harrow, J. et al. *Genome Res.* **22**, 1760–1774 (2012), the disclosure of which is incorporated herein by reference). Potential false-positive events can be removed by using a blacklist of AS events whose quantification across diverse RNA-Seq datasets is error-prone due to technical variances such as read length. Based on expression levels of exons, in several embodiments, splice-junction counts are determined by an appropriate method, such as the rMATS package (S. Shen, et al. *Proc. Natl. Acad. Sci.* **111**, E5593–E5601 (2014), the disclosure of which is incorporated herein by reference). Splice-junction counts can be utilized to find putative skipped exons, included exons, alternative 3' splice sites, alternative 5' splice sites, and/or retained introns in the sequencing result. In some embodiments, measurements of AS events, including splice junction count and percent-spliced-in (PSI) metric, are computed. Processing of the data will be dependent on the users' goal, and thus adaptable to the results desired. Although only a few methods of trimming, processing, and mapping sequence data are disclosed, it should be understood many more methods exist and are covered by various embodiments of the invention.

[0059] Returning back to FIG. 1, reference panels of AS events in healthy matched tissue and other tissues of the body are constructed or retrieved (103). In many embodiments, healthy

matched tissue is the same tissue origin as the neoplastic tissue, but has not transformed into a neoplasm. For instance, in some embodiments, healthy matched tissue of glioblastoma (GBM) is brain tissue. In some embodiments, other tissues of the body include any tissue that is not the source of the neoplasm. Tissues of single individual or tissue of collections of individuals can be analyzed. Analysis can be done on RNA-seq data derived from a single individual or a collection of individuals. For each non-neoplastic tissue to be analyzed, the RNA-seq data can be utilized to determine expression levels of exons, which can be utilized to determine splice-junction counts and putative skipped and/or included exons. These data can be stored to be utilized for comparisons with the neoplastic tissue of interest to be analyzed.

[0060] In a number of embodiments, utilizing the panel of AS events in healthy match tissue and the AS events of the neoplastic tissue, the relative abundance of AS events can be computed. The PSI is the percent of a particular isoform included in an AS event in the neoplastic tissue or the healthy matched or other heathy tissue and can be utilized for any type of AS event, including (but not limited to) exon skipping, an alternative 3' spice site, an alternative 5' spice site, and intron retention. Generally, a high PSI value in the neoplastic tissue, as compared to healthy match tissue, indicates including of the genetic material and a low PSI value in the neoplastic tissue indicates the neoplastic tissue spices out the genetic material. For example, in regards to exon-skipping, neoplastic tissue isoforms can be either an exon-skipped isoform (low PSI) or an exon-included isoform (high PSI), as compared to the tissue-matched normal panel.

[0061] In addition, a reference panel of AS events of a collection of similar neoplasm types is constructed or retrieved (105). Similar neoplasm types can be neoplasms having the same tissue origin. For instance, to construct a reference panel for GBM, other brain tumor sequencing data can be utilized, including (but not limited to) other samples of GBM and/or lower-grade glioma. The RNA-seq data of the collection of samples can be utilized to determine splice-junction counts and putative skipped exons, included exons, alternative 3' splice sites, alternative 5' splice sites, and/or retained introns. These data can be stored to be utilized for comparisons with the neoplastic tissue of interest to be analyzed.

[0062] Process 100 also detects (107) putative recurrent AS event candidates. In some embodiments, recurrent AS event candidates are determined comparing relative abundance of alternative isoforms (Fig. 1B). In some embodiments, putative recurrent AS event candidates are determined by comparing prevalence of alternative isoforms (Fig. 1C). In some embodiments, recurrent AS event candidates are determined comparing relative abundance and comparing prevalence of alternative isoforms.

[0063] As depicted in Fig. 1B, process 100B determines (107B) the relative abundance of the alternative isoforms by determining the relative expression of the alternative isoforms in the neoplastic tissue, as compared to the relative expression of the alternative isoforms in the panels of reference tissues (e.g., healthy matched tissue, other tissues, and similar neoplasm types). In many embodiments, statistical differential testing is utilized to determine the significance of a putative AS event candidate, as determined by the relative expression of an AS event. In some embodiments, a significant AS event is one that is a significant as determined by the resulting p-value of a statistical test comparing neoplastic tissue and a reference tissue. Statistical tests include (but are not limited to) parametric tests (e.g. two-sided/one sided t-test) and non-parametric tests (e.g. Mann-Whitney U test). In some embodiments, the difference of PSI values between comparing neoplastic tissue and reference tissue is utilized to identify significant AS events. In particular embodiments, a neoplastic AS event is significant when it satisfies the following: 1) a significant p-value from a statistical test (e.g., $p < 0.01$), and 2) a threshold of PSI value difference (e.g., $abs(\Delta\Psi) > 0.05$).

[0064] In some embodiments, significance testing (e.g., *t*-tests) and equivalence testing (e.g., two one-sided *t*-tests (TOSTs)) is used to identify neoplasm-associated, neoplasm-recurrent, and neoplasm-specific AS events in group comparisons. Specifically, AS events can be compared with a reference tissue (e.g., healthy matched tissue) to identify neoplastic tissue-associated AS events, with other tissue types to determine neoplastic tissue-specificity of AS events, and with similar neoplasm types to evaluate recurrence of AS events. In some embodiments, an AS event is considered significantly different when it meets two requirements: (1) a significant p-value from the statistical test (defaults: $p < 0.01$ for significance testing; $p < 0.05$ for equivalence testing), and (2) a threshold of PSI value difference (default: $abs(\Delta\psi)>0.05$ for significance testing; $abs(\Delta\psi)<0.05$ for equivalence testing).

[0065] In several embodiments, an AS event is defined as neoplasm-recurrent by comparing a panel of neoplastic tissue data with a panel of reference tissue (e.g., healthy matched tissue). For instance, in some embodiments, a neoplasm-recurrent AS event is identified when 1) a significant p-value from the statistical test in the same direction as the corresponding neoplasm-associated AS event (e.g., $p < 0.01$/number of neoplasm-associated events;), and 2) a threshold of PSI value difference (default: $abs(\Delta\Psi) > 0.05$). In some embodiments, a Bonferroni correction is applied wen determining p-value from the statistical test, which may be helpful due to large sample sizes in reference panels.

[0066] Additionally, in some embodiments, a threshold of the number of significant comparisons against groups in the normal or neoplasm reference panel is used to determine whether AS-derived antigens are neoplasm-specific or neoplasm-recurrent. In various embodiments, the neoplasm panel data and/or reference panel data includes multiple individual groups (e.g., tissue types) and a threshold of the number of significant comparisons against groups in the normal or tumor reference panel is used to determine whether AS-derived antigens are tumor-specific or tumor-recurrent. For each AS event, various embodiments utilize a definition that the 'neoplasm isoform' is the isoform that is more abundant in neoplastic tissue than in the tissue-matched normal panel. Optionally, in some embodiments, to rank or filter targets, the 'fold-change (FC) of neoplasm isoform' is estimated as the FC of the neoplasm isoform's proportion in neoplasms compared to the tissue-matched normal panel. Furthermore, in some embodiments, targets are screened for a specific patient sample through a 'personalized mode'. A personalized mode uses an outlier detection approach, combining a modified Tukey's rule and a threshold of PSI value difference of >5%.

[0067] As depicted in Fig. 1C, process 100C determines (107C) the prevalence of the alternative isoforms by determining the number of samples expressing the alternative isoform within a neoplastic tissue panel, as compared to the number of samples expressing the alternative isoform within the panels of reference tissues (e.g., healthy matched tissue, other tissues, and similar neoplasm types). In some of embodiments, a sample is considered to express a particular alternative isoform if the number of uniquely mapped junction read counts from RNA-seq data is greater than or equal to a junction count threshold.

[0068] Prevalence screening refers to the comparison the prevalence of a splice junction in a panel of neoplasm samples to one or more reference tissue samples. Specifically, in some embodiments, neoplasm samples of interest or related neoplasm samples, which can be selected from a neoplasm reference panel or other resource, are compared to reference tissue samples (e.g., tissue--matched normal samples or other normal tissue samples). In some embodiments, statistical tests (e.g., Fisher's exact test or chi-squared test) are employed to evaluate the difference of splice junction prevalence between the two groups in comparison. As a result, this allows identification of splice junctions prevalently expressed in neoplasm samples that are less observed in reference tissues. In some embodiments, to avoid false positive results by solely using read counts from RNA-seq, the same junction count information is used to calculate PSI-values and perform a relative abundance (PSI) based screening in parallel (see Fig. 1B). Employment of prevalence based methods has some advantages. For example, for annotated and unannotated splice junctions, this approach offers additional knowledge for

prioritization. Furthermore, this approach detects unannotated splice junctions derived from novel splice sites without the need to rebuild the splice graph. This allows for the evaluation of both junction prevalence and relative abundance for confident detection of neoplasm-specific splicing events.

[0069] Returning back to Fig. 1A,peptide epitopes derived from nucleotides that span across the AS event of each isoform of interest are determined (109). In a number of embodiments, to obtain protein sequences of AS-derived neoplasm isoforms, peptide sequences are generated by translating splice-junction sequences into amino-acid sequences. In some embodiments, splice-junction sequences are translated into amino-acid sequences using known ORFs from the UniProtKB database (www.uniprot.org). In some embodiments, splice-junction sequences are translated into amino-acid sequences for each potential open reading frame (i.e., the three open reading frames dependent on triple nucleotide codon window), which is useful for isoform junction derived from alternative and/or novel splice sites. Within each AS event, the splice-junction peptide sequence for the neoplasm isoform can be compared to that of the alternative normal isoform, to ensure that the neoplasm isoform splice junction produces a distinct peptide. It is noted that a single splice junction can give rise to multiple putative epitopes with distinct peptide sequences

[0070] Process 100 also predicts (111) HLA binding affinity and/or identifies targetable extracellular peptides by TCR and/or chimeric antigen receptors. For TCR target prediction, a computational package can be employed which uses RNA-Seq data to characterize HLA class I alleles for each tumor sample to identify putative epitopes. In some, embodiments, the seq2HLA is used for TCR epitope identification (Boegel, S. et al. *Genome Med.* **4**, 102 (2012), the disclosure of which is incorporated herein by reference). In addition, a computational package can predict the HLA binding affinities of candidate epitopes (e.g., the IEDB API from Vita, R. et al. *Nucleic Acids Res.* **43**, D405–D412 (2015), the disclosure of which is herein incorporated by reference). The IEDB 'recommended' mode runs several prediction tools to generate multiple predictions of binding affinity, which can be summarized by a median IC_{50} value. In some embodiments, a threshold of median(IC_{50}) < 500 nM denotes a positive prediction for an AS-derived TCR target, but any appropriate binding affinity can be utilized.

[0071] For CAR-T cell target prediction, AS-derived tumor isoforms can be mapped to known protein extracellular domains (ECDs) to identify potential candidates for CAR-T cell therapy. Protein cellular localization information can be retrieved from the UniProtKB database (www.uniprot.org). To retrieve ECD information from the UniProtKB database, a search for the term 'extracellular' in topological annotation fields can be performed, including

'TOPO_DOM', 'TRANSMEM', and 'REGION', in the flat file. In addition, BLAST (https://blast.ncbi.nlm.nih.gov/) can be used to map individual exons in the gene annotation to proteins with topological annotations. Furthermore, the BLAST result can be parsed to create annotations of the mapping between exons and ECDs in proteins. These pre-computed annotations can be queried to search for AS-derived peptides that can be mapped to protein ECDs as potential CAR-T cell targets.

[0072] Based on the results of HLA epitopes and CAR-T cell targets, peptides of interest can be generated (113) for use as a neoplasm antigen. Peptides can be synthesized directly (e.g., solid phase synthesis) or via molecular expression utilizing an expression vector and a host production cell.

[0073] While specific examples of identifying and synthesizing neoplastic tissue antigens are described above, one of ordinary skill in the art can appreciate that various steps of the process can be performed in different orders and that certain steps may be optional according to some embodiments of the invention. As such, it should be clear that the various steps of the process could be used as appropriate to the requirements of specific applications. Furthermore, any of a variety of processes for identifying and synthesizing neoplastic tissue antigens appropriate to the requirements of a given application can be utilized in accordance with various embodiments of the invention.

[0074] Provided in FIG. 2 is a process to identify and synthesize neoplastic tissue antigenic peptides integrating results of mass spectrometry data derived from a neoplastic tissue source. Process 200 can begin by identifying (201) alternative splicing events in RNA-Seq data derived from neoplastic tissue. In a manner similar to Process 100, RNA sequence data can be derived from a biological source or a database. In addition, RNA can be processed before analysis. Any appropriate method can be used to process sequence data as described herein. Potential false-positive events can be removed by using a blacklist of AS events whose quantification across diverse RNA-Seq datasets is error-prone due to technical variances such as read length. Based on expression levels of exons, in several embodiments, splice-junction counts are determined by an appropriate method, such as the rMATS package. Splice-junction counts can be utilized to find putative skipped exons, included exons, alternative 3' splice sites, alternative 5' splice sites, and/or retained introns in the sequencing result.

[0075] Peptide epitopes derived from nucleotides that span across the alternative splicing event of each isoform of interest is determined (203). In a number of embodiments, expression of the alternative isoforms in the neoplastic tissue compared to the panels of healthy matched tissue, other tissues, and similar neoplasm types. Generally, AS events can be compared with

refrence tissue (e.g., healthy matched tissue) to identify neoplastic tissue-associated AS events, with other tissue types to determine neoplastic tissue-specificity of AS events, and with similar neoplasm types to evaluate recurrence of AS events. In some embodiments, putative splice junction candidates are determined comparing relative abundance. In some embodiments, putative splice junction candidates are determined by comparing prevalence. In some embodiments, putative splice junction candidates are determined comparing relative abundance and comparing prevalence.

[0076] Process 200 also compares (205) the peptide sequences to mass spectrometry data derived from a collection of neoplasms to identify whether various isoforms are present. In some embodiments, proteo-transcriptomic data is integrated by incorporating various types of MS data, such as whole-cell proteomics, surfaceome, or immunopeptidomics data, to validate RNA-Seq based target discovery at the protein level. Specifically, sequences of AS-derived peptides are mapped to canonical and isoform sequences of the reference human proteome (downloaded from UniProtKB). For immunopeptidomics data, fragment MS spectra can be searched against the RNA-Seq based custom proteome library with no enzyme specificity. In some embodiments, the search length is limited to 7-15 amino acids. In some embodiments, the target-decoy approach is employed to control the false discovery rate (FDR) or 'QValue' at 5%.

[0077] Based on the results searching MS data for hits, peptides of interest can be generated (207) for use as a neoplasm antigen. Peptides can be synthesized directly (e.g., solid phase synthesis) or via biological translation utilizing an expression vector and a host production cell.

[0078] While specific examples of identifying and synthesizing neoplastic tissue antigens utilizing MS data are described above, one of ordinary skill in the art can appreciate that various steps of the process can be performed in different orders and that certain steps may be optional according to some embodiments of the invention. As such, it should be clear that the various steps of the process could be used as appropriate to the requirements of specific applications. Furthermore, any of a variety of processes for identifying and synthesizing neoplastic tissue antigens utilizing MS data appropriate to the requirements of a given application can be utilized in accordance with various embodiments of the invention.

II. Applications of antigenic peptides

[0079] Various embodiments are directed to development of and use of antigenic peptides that have been identified from neoplastic tissue. In many embodiments, antigenic peptides are

produced by chemical synthesis or by molecular expression in a host cell. Peptides can be purified and utilized in a variety of applications including (but not limited to) assays to determine peptide immunogenicity, assays to determine recognition by T cells, peptide vaccines for treatment of cancer, development of modified TCRs of T cells, development of antibodies, and development of CAR-T cells to recognize extracellular peptides.

[0080] Peptides can be synthesized chemically by a number of methods. One common method is to use solid-phase peptide synthesis (SPPS). Generally, SPPS is performed by repeating cycles of alternate N-terminal deprotection and coupling reactions, building peptides from the c-terminus to the n-terminus. The c-terminus of the first amino acid is coupled the resin, wherein then the amine is deprecated and then coupled with the free acid of the second amino acid. This cycle repeats until the peptide is synthesized.

[0081] Peptides can also be synthesized utilizing molecular tools and a host cell. Nucleic acid sequences corresponding with antigenic peptides can be synthesized. In some embodiments, synthetic nucleic acids synthesized in *in vitro* synthesizers (e.g., phosphoramidite synthesizer), bacterial recombination system, or other suitable methods. Furthermore, synthesized nucleic acids can be purified and lyophilized, or kept stored in a biological system (e.g., bacteria, yeast). For use in a biological system, synthetic nucleic acid molecules can be inserted into a plasmid vector, or similar. A plasmid vector can also be an expression vector, wherein a suitable promoter and a suitable 3'-polyA tail is combined with the transcript sequence.

[0082] Embodiments are also directed to expression vectors and expression systems that produce antigenic peptides or proteins. These expression systems can incorporate an expression vector to express transcripts and proteins in a suitable expression system. Typical expression systems include bacterial (e.g., *E. coli*), insect (e.g., SF9), yeast (e.g., *S. cerevisiae*), animal (e.g., CHO), or human (e.g., HEK 293) cell lines. RNA and/or protein molecules can be purified from these systems using standard biotechnology production procedures.

[0083] Assays to determine immunogenicity and/or TCR binding can be performed. One such as is the dextramer flow cytometery assay. Generally, custom-made HLA-matched MHC Class I dextramer:peptide (pMHC) complexes are developed or purchased (Immudex, Copenhagen, Denmark). T cells from peripheral blood mononuclear cells (PBMCs) or tumor-infiltrating lymphocytes (TILs) are incubated the pMHC complexes and stained, which are then run through a flow cytometer to determine if the peptide is capable of binding a TCR of a T cell.

III. Engineered T Cell Receptors

[0084] T-cell receptors comprise two different polypeptide chains, termed the T-cell receptor α (TCRα) and β (TCRβ) chains, linked by a disulfide bond. These α:β heterodimers are very similar in structure to the Fab fragment of an immunoglobulin molecule, and they account for antigen recognition by most T cells. A minority of T cells bear an alternative, but structurally similar, receptor made up of a different pair of polypeptide chains designated γ and δ. Both types of T-cell receptor differ from the membrane-bound immunoglobulin that serves as the B-cell receptor: a T-cell receptor has only one antigen-binding site, whereas a B-cell receptor has two, and T-cell receptors are never secreted, whereas immunoglobulin can be secreted as antibody.

[0085] Both chains of the T-cell receptor have an amino-terminal variable (V) region with homology to an immunoglobulin V domain, a constant (C) region with homology to an immunoglobulin C domain, and a short hinge region containing a cysteine residue that forms the interchain disulfide bond. Each chain spans the lipid bilayer by a hydrophobic transmembrane domain, and ends in a short cytoplasmic tail.

[0086] The three-dimensional structure of the T-cell receptor has been determined. The structure is indeed similar to that of an antibody Fab fragment, as was suspected from earlier studies on the genes that encoded it. The T-cell receptor chains fold in much the same way as those of a Fab fragment, although the final structure appears a little shorter and wider. There are, however, some distinct differences between T-cell receptors and Fab fragments. The most striking difference is in the Cα domain, where the fold is unlike that of any other immunoglobulin-like domain. The half of the domain that is juxtaposed with the Cβ domain forms a β sheet similar to that found in other immunoglobulin-like domains, but the other half of the domain is formed of loosely packed strands and a short segment of α helix. The intramolecular disulfide bond, which in immunoglobulin-like domains normally joins two β strands, in a Cα domain joins a β strand to this segment of α helix.

[0087] There are also differences in the way in which the domains interact. The interface between the V and C domains of both T-cell receptor chains is more extensive than in antibodies, which may make the hinge joint between the domains less flexible. And the interaction between the Cα and Cβ domains is distinctive in being assisted by carbohydrate, with a sugar group from the Cα domain making a number of hydrogen bonds to the Cβ domain. Finally, a comparison of the variable binding sites shows that, although the complementarity-determining region (CDR) loops align fairly closely with those of antibody molecules, there is

some displacement relative to those of the antibody molecule. This displacement is particularly marked in the Vα CDR2 loop, which is oriented at roughly right angles to the equivalent loop in antibody V domains, as a result of a shift in the β strand that anchors one end of the loop from one face of the domain to the other. A strand displacement also causes a change in the orientation of the Vβ CDR2 loop in two of the seven Vβ domains whose structures are known. As yet, the crystallographic structures of seven T-cell receptors have been solved to this level of resolution.

[0088] Embodiments of the disclosure relate to engineered T cell receptors. The term "engineered" refers to T cell receptors that have TCR variable regions grafted onto TCR constant regions to make a chimeric polypeptide that binds to peptides and antigens of the disclosure. In certain embodiments, the TCR comprises intervening sequences that are used for cloning, enhanced expression, detection, or for therapeutic control of the construct, but are not present in endogenous TCRs, such as multiple cloning sites, linker, hinge sequences, modified hinge sequences, modified transmembrane sequences, a detection polypeptide or molecule, or therapeutic controls that may allow for selection or screening of cells comprising the TCR.

[0089] In some embodiments, the TCR comprises non-TCR sequences. Accordingly, certain embodiments relate to TCRs with sequences that are not from a TCR gene. In some embodiments, the TCR is chimeric, in that it contains sequences normally found in a TCR gene, but contains sequences from at least two TCR genes that are not necessarily found together in nature.

[0090] In some embodiments the engineered TCRs of the disclosure comprise a variable as shown below:

Description	Sequence
TCR1-alpha – variable; TRAV8-6*01 (uppercase) TRAJ54*01 (lowercase)	MLLLLVPAFQVIFTLGGTRAQSVTQLDSQVPVFEEAPVE LRCNYSSSVSVYLFWYVQYPNQGLQLLLKYLSGSTLVE SINGFEAEFNKSQTSFHLRKPSVHISDTAEYFCAVHeiqga qklvfgqgtrltinpn (SEQ ID NO:44)
TCR1-alpha CDR3	CAVHEIQGAQKLVF (SEQ ID NO:30)
TCR1-beta – variable; TRBV7-6*01 (uppercase) TRBJ2-7*01 (lowercase)	MGTSLLCWVVLGFLGTDHTGAGVSQSPRYKVTKRGQD VALRCDPISGHVSLYWYRQALGQGPEFLTYFNYEAQQ DKSGLPNDRFSAERPEGSISTLTIQRTEQRDSAMYRCAS SFGVsyeqyfgpgtrltvt (SEQ ID NO:45)

TCR1-beta CDR3	CASSFGVSYEQYF (SEQ ID NO:31)
TCR2-alpha – variable; TRAV14/DV4*01 (uppercase) TRAJ4*01 (lowercase)	MSLSSLLKVVTASLWLGPGIAQKITQTQPGMFVQEKEA VTLDCTYDTSDPSYGLFWYKQPSSGEMIFLIYQGSYDQ QNATEGRYSLNFQKARKSANLVISASQLGDSAMYFCA MRPLggynklifgagtrlavhp (SEQ ID NO:46)
TCR2-alpha CDR3	CAMRPLGGYNKLIF (SEQ ID NO:32)
TCR2-beta – variable; TRBV4-1*01 (uppercase) TRBJ2-1*01 (lowercase)	MGCRLLCCAVLCLLGAVPIDTEVTQTPKHLVMGMTNK KSLKCEQHMGHRAMYWYKQKAKKPPELMFVYSYEKL SINESVPSRFSPECPNSSLLNLHLHALQPEDSALYLCASS QAAneqffgpgtrltvl (SEQ ID NO:47)
TCR2-beta CDR3	CASSQAANEQFF (SEQ ID NO:33)
TCR3-alpha – variable; TRAV5*01 (uppercase) TRAJ20*01 (lowercase)	MKTFAGFSFLFLWLQLDCMSRGEDVEQSLFLSVREGDS SVINCTYTDSSSTYLYWYKQEPGAGLQLLTYIFSNMDM KQDQRLTVLLNKKDKHLSLRIADTQTGDSAIYFCAEEgd rdyklsfgagttvtvran (SEQ ID NO:48)
TCR3-alpha CDR3	CAEEGDRDYKLSF (SEQ ID NO:34)
TCR3-beta – variable; TRBV7-8*01 (uppercase) TRBJ2-7*01 (lowercase)	MGTRLLCWVVLGFLGTDHTGAGVSQSPRYKVAKRGQ DVALRCDPISGHVSLFWYQQALGQGPEFLTYFQNEAQL DKSGLPSDRFFAERPEGSVSTLKIQRTQQEDSAVYLCAS TGRSGrseqyfgpgtrltvt (SEQ ID NO:49)
TCR3-beta CDR3	CASTGRSGRSEQYF (SEQ ID NO:35)
TCR1-alpha – complete gene	MLLLLVPAFQVIFTLGGTRAQSVTQLDSQVPVFEEAPVE LRCNYSSSVSVYLFWYVQYPNQGLQLLLKYLSGSTLVE SINGFEAEFNKSQTSFHLRKPSVHISDTAEYFCAVHEIQG AQKLVFGQGTRLTINPNIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS (SEQ ID NO:50)
TCR1-beta – complete gene	MGTSLLCWVVLGFLGTDHTGAGVSQSPRYKVTKRGQD VALRCDPISGHVSLYWYRQALGQGPEFLTYFNYEAQQ DKSGLPNDRFSAERPEGSISTLTIQRTEQRDSAMYRCAS SFGVSYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAE

	ISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVS TDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFR CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCG FTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMA MVKRKDSRG (SEQ ID NO:51)
TCR2-alpha – complete gene	MSLSSLLKVVTASLWLGPGIAQKITQTQPGMFVQEKEA VTLDCTYDTSDPSYGLFWYKQPSSGEMIFLIYQGSYDQ QNATEGRYSLNFQKARKSANLVISASQLGDSAMYFCA MRPLGGYNKLIFGAGTRLAVHPYIQNPDPAVYQLRDSK SSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRS MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPES SCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNL LMTLRLWSS (SEQ ID NO:52)
TCR2-beta – complete gene	MGCRLLCCAVLCLLGAVPIDTEVTQTPKHLVMGMTNK KSLKCEQHMGHRAMYWYKQKAKKPPELMFVYSYEKL SINESVPSRFSPECPNSSLLNLHLHALQPEDSALYLCASS QAANEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEIS HTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTD PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQ VQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT SESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMV KRKDSRG (SEQ ID NO:53)
TCR3-alpha – complete gene	MKTFAGFSFLFLWLQLDCMSRGEDVEQSLFLSVREGDS SVINCTYTDSSSTYLYWYKQEPGAGLQLLTYIFSNMDM KQDQRLTVLLNKKDKHLSLRIADTQTGDSAIYFCAEEG DRDYKLSFGAGTTVTVRANIQNPDPAVYQLRDSKSSDK SVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFK SNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV KLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTL RLWSS (SEQ ID NO:54)
TCR3-beta – complete gene	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPD HVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCL SSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDR

	AKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEIL LGKATLYAVLVSALVLMAMVKRKDSRG (SEQ ID NO:55)
TCR1-alpha constant	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSK DSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACAN AFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNL SVIGFRILLLKVAGFNLLMTLRLWSS (SEQ ID NO:56)
TCR1-beta constant (beta chain of NYESO TCR in lowercase)	DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDH VELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLss rlrvsatfwqnprnhfrcqvqfyglsendewtqdrakpvtqivsaeawgradcgfts esyqqgvlsatilyeillgkatlyavlvsalvlmamvkrkdsrg (SEQ ID NO:57)
TCR2-alpha constant	IQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDS DVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAF NNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVI GFRILLLKVAGFNLLMTLRLWSS (SEQ ID NO:58)
TCR2-beta constant	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPD HVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCL SSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDR AKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEIL LGKATLYAVLVSALVLMAMVKRKDSRG (SEQ ID NO:59)
TCR3-alpha constant	IQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDS DVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAF NNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVI GFRILLLKVAGFNLLMTLRLWSS (SEQ ID NO:60)
TCR3-beta constant	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPD HVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCL SSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDR AKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEIL LGKATLYAVLVSALVLMAMVKRKDSRG (SEQ ID NO:61)

IV. Antibodies

[0091] Aspects of the disclosure relate to antibodies that target the peptides of the disclosure, or fragments thereof. The term "antibody" refers to an intact immunoglobulin of any isotype, or a fragment thereof that can compete with the intact antibody for specific binding to the target antigen, and includes chimeric, humanized, fully human, and bispecific antibodies. As used herein, the terms "antibody" or "immunoglobulin" are used interchangeably and refer to any of several classes of structurally related proteins that function as part of the immune response of an animal, including IgG, IgD, IgE, IgA, IgM, and related proteins, as well as polypeptides comprising antibody CDR domains that retain antigen-binding activity.

[0092] The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody. An antigen may possess one or more epitopes that are capable of interacting with different antibodies.

[0093] The term "epitope" includes any region or portion of molecule capable eliciting an immune response by binding to an immunoglobulin or to a T-cell receptor. Epitope determinants may include chemically active surface groups such as amino acids, sugar side chains, phosphoryl or sulfonyl groups, and may have specific three-dimensional structural characteristics and/or specific charge characteristics. Generally, antibodies specific for a particular target antigen will preferentially recognize an epitope on the target antigen within a complex mixture.

[0094] The epitope regions of a given polypeptide can be identified using many different epitope mapping techniques are well known in the art, including: x-ray crystallography, nuclear magnetic resonance spectroscopy, site-directed mutagenesis mapping, protein display arrays, see, e.g., Epitope Mapping Protocols, (Johan Rockberg and Johan Nilvebrant, Ed., 2018) Humana Press, New York, N.Y. Such techniques are known in the art and described in, e.g., U.S. Pat. No. 4,708,871; Geysen et al. Proc. Natl. Acad. Sci. USA 81:3998-4002 (1984); Geysen et al. Proc. Natl. Acad. Sci. USA 82:178-182 (1985); Geysen et al. Molec. Immunol. 23:709-715 (1986). Additionally, antigenic regions of proteins can also be predicted and identified using standard antigenicity and hydropathy plots.

[0095] The term "immunogenic sequence" means a molecule that includes an amino acid sequence of at least one epitope such that the molecule is capable of stimulating the production of antibodies in an appropriate host. The term "immunogenic composition" means a composition that comprises at least one immunogenic molecule (e.g., an antigen or carbohydrate).

[0096] An intact antibody is generally composed of two full-length heavy chains and two full-length light chains, but in some instances may include fewer chains, such as antibodies naturally occurring in camelids that may comprise only heavy chains. Antibodies as disclosed herein may be derived solely from a single source or may be "chimeric," that is, different portions of the antibody may be derived from two different antibodies. For example, the variable or CDR regions may be derived from a rat or murine source, while the constant region is derived from a different animal source, such as a human. The antibodies or binding fragments may be produced in hybridomas, by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Unless otherwise indicated, the term "antibody" includes derivatives, variants, fragments, and muteins thereof, examples of which are described below (Sela-Culang et al., Front Immunol. 2013; 4: 302; 2013).

[0097] The term "light chain" includes a full-length light chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length light chain has a molecular weight of around 25,000 Daltons and includes a variable region domain (abbreviated herein as VL), and a constant region domain (abbreviated herein as CL). There are two classifications of light chains, identified as kappa (κ) and lambda (λ). The term "VL fragment" means a fragment of the light chain of a monoclonal antibody that includes all or part of the light chain variable region, including CDRs. A VL fragment can further include light chain constant region sequences. The variable region domain of the light chain is at the amino-terminus of the polypeptide.

[0098] The term "heavy chain" includes a full-length heavy chain and fragments thereof having sufficient variable region sequence to confer binding specificity. A full-length heavy chain has a molecular weight of around 50,000 Daltons and includes a variable region domain (abbreviated herein as VH), and three constant region domains (abbreviated herein as CH1, CH2, and CH3). The term "VH fragment" means a fragment of the heavy chain of a monoclonal antibody that includes all or part of the heavy chain variable region, including CDRs. A VH fragment can further include heavy chain constant region sequences. The number of heavy chain constant region domains will depend on the isotype. The VH domain is at the amino-terminus of the polypeptide, and the CH domains are at the carboxy-terminus, with the CH3 being closest to the —COOH end. The isotype of an antibody can be IgM, IgD, IgG, IgA, or IgE and is defined by the heavy chains present of which there are five classifications: mu (μ), delta (δ), gamma (γ), alpha (α), or epsilon (ε) chains, respectively. IgG has several subtypes, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. IgM subtypes include IgM1 and IgM2. IgA subtypes include IgA1 and IgA2.

1. Types of Antibodies

[0099] Antibodies can be whole immunoglobulins of any isotype or classification, chimeric antibodies, or hybrid antibodies with specificity to two or more antigens. They may also be fragments (e.g., F(ab′)2, Fab′, Fab, Fv, and the like), including hybrid fragments. An immunoglobulin also includes natural, synthetic, or genetically engineered proteins that act like an antibody by binding to specific antigens to form a complex. The term antibody includes genetically engineered or otherwise modified forms of immunoglobulins.

[0100] The term "monomer" means an antibody containing only one Ig unit. Monomers are the basic functional units of antibodies. The term "dimer" means an antibody containing two Ig units attached to one another via constant domains of the antibody heavy chains (the Fc, or fragment crystallizable, region). The complex may be stabilized by a joining (J) chain protein. The term "multimer" means an antibody containing more than two Ig units attached to one another via constant domains of the antibody heavy chains (the Fc region). The complex may be stabilized by a joining (J) chain protein.

[0101] The term "bivalent antibody" means an antibody that comprises two antigen-binding sites. The two binding sites may have the same antigen specificities or they may be bi-specific, meaning the two antigen-binding sites have different antigen specificities.

[0102] Bispecific antibodies are a class of antibodies that have two paratopes with different binding sites for two or more distinct epitopes. In some embodiments, bispecific antibodies can be biparatopic, wherein a bispecific antibody may specifically recognize a different epitope from the same antigen. In some embodiments, bispecific antibodies can be constructed from a pair of different single domain antibodies termed "nanobodies". Single domain antibodies are sourced and modified from cartilaginous fish and camelids. Nanobodies can be joined together by a linker using techniques typical to a person skilled in the art; such methods for selection and joining of nanobodies are described in PCT Publication No. WO2015044386A1, No. WO2010037838A2, and Bever et al., Anal Chem. 86:7875–7882 (2014), each of which are specifically incorporated herein by reference in their entirety.

[0103] Bispecific antibodies can be constructed as: a whole IgG, Fab′2, Fab′PEG, a diabody, or alternatively as scFv. Diabodies and scFvs can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction. Bispecific

antibodies may be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of Fab′ fragments. See, e.g., Songsivilai and Lachmann, Clin. Exp. Immunol. 79:315-321 (1990); Kostelny et al., J. Immunol. 148:1547-1553 (1992), each of which are specifically incorporated by reference in their entirety.

[0104] In certain aspects, the antigen-binding domain may be multispecific or heterospecific by multimerizing with VH and VL region pairs that bind a different antigen. For example, the antibody may bind to, or interact with, (a) a cell surface antigen, (b) an Fc receptor on the surface of an effector cell, or (c) at least one other component. Accordingly, aspects may include, but are not limited to, bispecific, trispecific, tetraspecific, and other multispecific antibodies or antigen-binding fragments thereof that are directed to epitopes and to other targets, such as Fc receptors on effector cells.

[0105] In some embodiments, multispecific antibodies can be used and directly linked via a short flexible polypeptide chain, using routine methods known in the art. One such example is diabodies that are bivalent, bispecific antibodies in which the VH and VL domains are expressed on a single polypeptide chain, and utilize a linker that is too short to allow for pairing between domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain creating two antigen binding sites. The linker functionality is applicable for embodiments of triabodies, tetrabodies, and higher order antibody multimers. (see, e.g., Hollinger et al., Proc Natl. Acad. Sci. USA 90:6444-6448 (1993); Polijak et al., Structure 2:1121-1123 (1994); Todorovska et al., J. Immunol. Methods 248:47-66 (2001)).

[0106] Bispecific diabodies, as opposed to bispecific whole antibodies, may also be advantageous because they can be readily constructed and expressed in E. coli. Diabodies (and other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is kept constant, for instance, with a specificity directed against a protein, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by alternative engineering methods as described in Ridgeway et al., (Protein Eng., 9:616-621, 1996) and Krah et al., (N Biotechnol. 39:167-173, 2017), each of which is hereby incorporated by reference in their entirety.

[0107] Heteroconjugate antibodies are composed of two covalently linked monoclonal antibodies with different specificities. See, e.g., U.S. Patent No. 6,010,902, incorporated herein by reference in its entirety.

[0108] The part of the Fv fragment of an antibody molecule that binds with high specificity to the epitope of the antigen is referred to herein as the “paratope.” The paratope consists of

the amino acid residues that make contact with the epitope of an antigen to facilitate antigen recognition. Each of the two Fv fragments of an antibody is composed of the two variable domains, VH and VL, in dimerized configuration. The primary structure of each of the variable domains includes three hypervariable loops separated by, and flanked by, Framework Regions (FR). The hypervariable loops are the regions of highest primary sequences variability among the antibody molecules from any mammal. The term hypervariable loop is sometimes used interchangeably with the term "Complementarity Determining Region (CDR)." The length of the hypervariable loops (or CDRs) varies between antibody molecules. The framework regions of all antibody molecules from a given mammal have high primary sequence similarity/consensus. The consensus of framework regions can be used by one skilled in the art to identify both the framework regions and the hypervariable loops (or CDRs) which are interspersed among the framework regions. The hypervariable loops are given identifying names which distinguish their position within the polypeptide, and on which domain they occur. CDRs in the VL domain are identified as L1, L2, and L3, with L1 occurring at the most distal end and L3 occurring closest to the CL domain. The CDRs may also be given the names CDR-1, CDR-2, and CDR-3. The L3 (CDR-3) is generally the region of highest variability among all antibody molecules produced by a given organism. The CDRs are regions of the polypeptide chain arranged linearly in the primary structure, and separated from each other by Framework Regions. The amino terminal (N-terminal) end of the VL chain is named FR1. The region identified as FR2 occurs between L1 and L2 hypervariable loops. FR3 occurs between L2 and L3 hypervariable loops, and the FR4 region is closest to the CL domain. This structure and nomenclature is repeated for the VH chain, which includes three CDRs identified as H1, H2 and H3. The majority of amino acid residues in the variable domains, or Fv fragments (VH and VL), are part of the framework regions (approximately 85%). The three dimensional, or tertiary, structure of an antibody molecule is such that the framework regions are more internal to the molecule and provide the majority of the structure, with the CDRs on the external surface of the molecule.

[0109] Several methods have been developed and can be used by one skilled in the art to identify the exact amino acids that constitute each of these regions. This can be done using any of a number of multiple sequence alignment methods and algorithms, which identify the conserved amino acid residues that make up the framework regions, therefore identifying the CDRs that may vary in length but are located between framework regions. Three commonly used methods have been developed for identification of the CDRs of antibodies: Kabat (as described in T. T. Wu and E. A. Kabat, "AN ANALYSIS OF THE SEQUENCES OF THE

VARIABLE REGIONS OF BENCE JONES PROTEINS AND MYELOMA LIGHT CHAINS AND THEIR IMPLICATIONS FOR ANTIBODY COMPLEMENTARITY," J Exp Med, vol. 132, no. 2, pp. 211–250, Aug. 1970); Chothia (as described in C. Chothia et al., "Conformations of immunoglobulin hypervariable regions," Nature, vol. 342, no. 6252, pp. 877–883, Dec. 1989); and IMGT (as described in M.-P. Lefranc et al., "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Developmental & Comparative Immunology, vol. 27, no. 1, pp. 55–77, Jan. 2003). These methods each include unique numbering systems for the identification of the amino acid residues that constitute the variable regions. In most antibody molecules, the amino acid residues that actually contact the epitope of the antigen occur in the CDRs, although in some cases, residues within the framework regions contribute to antigen binding.

[0110] One skilled in the art can use any of several methods to determine the paratope of an antibody. These methods include: 1) Computational predictions of the tertiary structure of the antibody/epitope binding interactions based on the chemical nature of the amino acid sequence of the antibody variable region and composition of the epitope. 2) Hydrogen-deuterium exchange and mass spectroscopy 3) Polypeptide fragmentation and peptide mapping approaches in which one generates multiple overlapping peptide fragments from the full length of the polypeptide and evaluates the binding affinity of these peptides for the epitope. 4) Antibody Phage Display Library analysis in which the antibody Fab fragment encoding genes of the mammal are expressed by bacteriophage in such a way as to be incorporated into the coat of the phage. This population of Fab expressing phage are then allowed to interact with the antigen which has been immobilized or may be expressed in by a different exogenous expression system. Non-binding Fab fragments are washed away, thereby leaving only the specific binding Fab fragments attached to the antigen. The binding Fab fragments can be readily isolated and the genes which encode them determined. This approach can also be used for smaller regions of the Fab fragment including Fv fragments or specific VH and VL domains as appropriate.

[0111] In certain aspects, affinity matured antibodies are enhanced with one or more modifications in one or more CDRs thereof that result in an improvement in the affinity of the antibody for a target antigen as compared to a parent antibody that does not possess those alteration(s). Certain affinity matured antibodies will have nanomolar or picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art, e.g., Marks et al., Bio/Technology 10:779 (1992) describes affinity maturation by VH and VL domain shuffling, random mutagenesis of CDR and/or framework residues employed in

phage display is described by Rajpal et al., PNAS. 24: 8466-8471 (2005) and Thie et al., Methods Mol Biol. 525:309-22 (2009) in conjugation with computation methods as demonstrated in Tiller et al., Front. Immunol. 8:986 (2017).

[0112] Chimeric immunoglobulins are the products of fused genes derived from different species; "humanized" chimeras generally have the framework region (FR) from human immunoglobulins and one or more CDRs are from a non-human source.

[0113] In certain aspects, portions of the heavy and/or light chain are identical or homologous to corresponding sequences from another particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity. U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851 (1984). For methods relating to chimeric antibodies, see, e.g., U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1985), each of which are specifically incorporated herein by reference in their entirety. CDR grafting is described, for example, in U.S. Pat. Nos. 6,180,370, 5,693,762, 5,693,761, 5,585,089, and 5,530,101, which are all hereby incorporated by reference for all purposes.

[0114] In some embodiments, minimizing the antibody polypeptide sequence from the non-human species optimizes chimeric antibody function and reduces immunogenicity. Specific amino acid residues from non-antigen recognizing regions of the non-human antibody are modified to be homologous to corresponding residues in a human antibody or isotype. One example is the "CDR-grafted" antibody, in which an antibody comprises one or more CDRs from a particular species or belonging to a specific antibody class or subclass, while the remainder of the antibody chain(s) is identical or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. For use in humans, the V region composed of CDR1, CDR2, and partial CDR3 for both the light and heavy chain variance region from a non-human immunoglobulin, are grafted with a human antibody framework region, replacing the naturally occurring antigen receptors of the human antibody with the non-human CDRs. In some instances, corresponding non-human residues replace framework region residues of the human immunoglobulin. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody to further refine performance. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. See, e.g., Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Presta,

Curr. Op. Struct. Biol. 2:593 (1992); Vaswani and Hamilton, Ann. Allergy, Asthma and Immunol. 1:105 (1998); Harris, Biochem. Soc. Transactions 23; 1035 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428 (1994); Verhoeyen et al., Science 239:1534-36 (1988).

[0115] Intrabodies are intracellularly localized immunoglobulins that bind to intracellular antigens as opposed to secreted antibodies, which bind antigens in the extracellular space.

[0116] Polyclonal antibody preparations typically include different antibodies against different determinants (epitopes). In order to produce polyclonal antibodies, a host, such as a rabbit or goat, is immunized with the antigen or antigen fragment, generally with an adjuvant and, if necessary, coupled to a carrier. Antibodies to the antigen are subsequently collected from the sera of the host. The polyclonal antibody can be affinity purified against the antigen rendering it monospecific.

[0117] Monoclonal antibodies or "mAb" refer to an antibody obtained from a population of homogeneous antibodies from an exclusive parental cell, e.g., the population is identical except for naturally occurring mutations that may be present in minor amounts. Each monoclonal antibody is directed against a single antigenic determinant.

B. Functional Antibody Fragments and Antigen-Binding Fragments

1. Antigen-Binding Fragments

[0118] Certain aspects relate to antibody fragments, such as antibody fragments that bind to a peptide of the disclosure. The term functional antibody fragment includes antigen-binding fragments of an antibody that retain the ability to specifically bind to an antigen. These fragments are constituted of various arrangements of the variable region heavy chain (VH) and/or light chain (VL); and in some embodiments, include constant region heavy chain 1 (CHl) and light chain (CL). In some embodiments, they lack the Fc region constituted of heavy chain 2 (CH2) and 3 (CH3) domains. Embodiments of antigen binding fragments and the modifications thereof may include: (i) the Fab fragment type constituted with the VL, VH, CL, and CHl domains; (ii) the Fd fragment type constituted with the VH and CHl domains; (iii) the Fv fragment type constituted with the VH and VL domains; (iv) the single domain fragment type, dAb, (Ward, 1989; McCafferty et al., 1990; Holt et al., 2003) constituted with a single VH or VL domain; (v) isolated complementarity determining region (CDR) regions. Such terms are described, for example, in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, NY (1989); Molec. Biology and Biotechnology: A Comprehensive Desk Reference (Myers, R. A. (ed.), New York: VCH Publisher, Inc.); Huston et al., Cell

Biophysics, 22:189-224 (1993); Pluckthun and Skerra, Meth. Enzymol., 178:497-515 (1989) and in Day, E. D., Advanced Immunochemistry, 2d ed., Wiley-Liss, Inc. New York, N.Y. (1990); Antibodies, 4:259-277 (2015), each of which are incorporated by reference.

[0119] Antigen-binding fragments also include fragments of an antibody that retain exactly, at least, or at most 1, 2, or 3 complementarity determining regions (CDRs) from a light chain variable region. Fusions of CDR-containing sequences to an Fc region (or a CH2 or CH3 region thereof) are included within the scope of this definition including, for example, scFv fused, directly or indirectly, to an Fc region are included herein.

[0120] The term Fab fragment means a monovalent antigen-binding fragment of an antibody containing the VL, VH, CL and CH1 domains. The term Fab′ fragment means a monovalent antigen-binding fragment of a monoclonal antibody that is larger than a Fab fragment. For example, a Fab′ fragment includes the VL, VH, CL and CH1 domains and all or part of the hinge region. The term F(ab′)2 fragment means a bivalent antigen-binding fragment of a monoclonal antibody comprising two Fab′ fragments linked by a disulfide bridge at the hinge region. An F(ab′)2 fragment includes, for example, all or part of the two VH and VL domains, and can further include all or part of the two CL and CH1 domains.

[0121] The term Fd fragment means a fragment of the heavy chain of a monoclonal antibody, which includes all or part of the VH, including the CDRs. An Fd fragment can further include CH1 region sequences.

[0122] The term Fv fragment means a monovalent antigen-binding fragment of a monoclonal antibody, including all or part of the VL and VH, and absent of the CL and CH1 domains. The VL and VH include, for example, the CDRs. Single-chain antibodies (sFv or scFv) are Fv molecules in which the VL and VH regions have been connected by a flexible linker to form a single polypeptide chain, which forms an antigen-binding fragment. Single chain antibodies are discussed in detail in International Patent Application Publication No. WO 88/01649 and U.S. Pat. Nos. 4,946,778 and 5,260,203, the disclosures of which are herein incorporated by reference. The term $(scFv)_2$ means bivalent or bispecific sFv polypeptide chains that include oligomerization domains at their C-termini, separated from the sFv by a hinge region (Pack et al. 1992). The oligomerization domain comprises self-associating a-helices, e.g., leucine zippers, which can be further stabilized by additional disulfide bonds. $(scFv)_2$ fragments are also known as “miniantibodies” or “minibodies.”

[0123] single domain antibody is an antigen-binding fragment containing only a VH or the VL domain. In some instances, two or more VH regions are covalently joined with a peptide

linker to create a bivalent domain antibody. The two VH regions of a bivalent domain antibody may target the same or different antigens.

2. Fragment Crystallizable Region, Fc

[0124] An Fc region contains two heavy chain fragments comprising the CH2 and CH3 domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the CH3 domains. The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are included.

C. Polypeptides with antibody CDRs & Scaffolding Domains that Display the CDRs

[0125] Antigen-binding peptide scaffolds, such as complementarity-determining regions (CDRs), are used to generate protein-binding molecules in accordance with the embodiments. Generally, a person skilled in the art can determine the type of protein scaffold on which to graft at least one of the CDRs. It is known that scaffolds, optimally, must meet a number of criteria such as: good phylogenetic conservation; known three-dimensional structure; small size; few or no post-transcriptional modifications; and/or be easy to produce, express, and purify. Skerra, J Mol Recognit, 13:167-87 (2000).

[0126] The protein scaffolds can be sourced from, but not limited to: fibronectin type III FN3 domain (known as "monobodies"), fibronectin type III domain 10, lipocalin, anticalin, Z-domain of protein A of Staphylococcus aureus, thioredoxin A or proteins with a repeated motif such as the "ankyrin repeat", the "armadillo repeat", the "leucine-rich repeat" and the "tetratricopeptide repeat". Such proteins are described in US Patent Publication Nos. 2010/0285564, 2006/0058510, 2006/0088908, 2005/0106660, and PCT Publication No. WO2006/056464, each of which are specifically incorporated herein by reference in their entirety. Scaffolds derived from toxins from scorpions, insects, plants, mollusks, etc., and the protein inhibiters of neuronal NO synthase (PIN) may also be used.

D. Antibody Binding

[0127] The term "selective binding agent" refers to a molecule that binds to an antigen. Non-limiting examples include antibodies, antigen-binding fragments, scFv, Fab, Fab′, F(ab′)2, single chain antibodies, peptides, peptide fragments and proteins.

[0128] The term "binding" refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. "Immunologically reactive" means that the selective binding agent or antibody of interest will bind with antigens present in a biological sample. The term "immune complex" refers the combination formed when an antibody or selective binding agent binds to an epitope on an antigen.

1. Affinity/Avidity

[0129] The term "affinity" refers the strength with which an antibody or selective binding agent binds an epitope. In antibody binding reactions, this is expressed as the affinity constant (Ka or ka sometimes referred to as the association constant) for any given antibody or selective binding agent. Affinity is measured as a comparison of the binding strength of the antibody to its antigen relative to the binding strength of the antibody to an unrelated amino acid sequence. Affinity can be expressed as, for example, 20- fold greater binding ability of the antibody to its antigen then to an unrelated amino acid sequence. As used herein, the term "avidity" refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms "immunoreactive" and "preferentially binds" are used interchangeably herein with respect to antibodies and/or selective binding agent.

[0130] There are several experimental methods that can be used by one skilled in the art to evaluate the binding affinity of any given antibody or selective binding agent for its antigen. This is generally done by measuring the equilibrium dissociation constant (KD or Kd), using the equation KD = koff / kon = [A][B]/[AB]. The term koff is the rate of dissociation between the antibody and antigen per unit time, and is related to the concentration of antibody and antigen present in solution in the unbound form at equilibrium. The term kon is the rate of antibody and antigen association per unit time, and is related to the concentration of the bound antigen-antibody complex at equilibrium. The units used for measuring the KD are mol/L (molarity, or M), or concentration. The Ka of an antibody is the opposite of the KD, and is determined by the equation Ka = 1/KD. Examples of some experimental methods that can be used to determine the KD value are: enzyme-linked immunosorbent assays (ELISA), isothermal titration calorimetry (ITC), fluorescence anisotropy, surface plasmon resonance

(SPR), and affinity capillary electrophoresis (ACE). The affinity constant (Ka) of an antibody is the opposite of the KD, and is determined by the equation Ka = 1/ KD.

[0131] Antibodies deemed useful in certain embodiments may have an affinity constant (Ka) of about, at least about, or at most about 10^6, 10^7, 10^8,10^9, or 10^{10} M or any range derivable therein. Similarly, in some embodiments, antibodies may have a dissociation constant of about, at least about or at most about 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M, or any range derivable therein. These values are reported for antibodies discussed herein and the same assay may be used to evaluate the binding properties of such antibodies. An antibody of the invention is said to "specifically bind" its target antigen when the dissociation constant (KD) is $\leqq 10^{-8}$ M. The antibody specifically binds antigen with "high affinity" when the KD is $\leqq 5\times 10^{-9}$ M, and with "very high affinity" when the KD is $\leqq 5\times 10^{-10}$ M.

2. Epitope Specificity

[0132] The epitope of an antigen is the specific region of the antigen for which an antibody has binding affinity. In the case of protein or polypeptide antigens, the epitope is the specific residues (or specified amino acids or protein segment) that the antibody binds with high affinity. An antibody does not necessarily contact every residue within the protein. Nor does every single amino acid substitution or deletion within a protein necessarily affect binding affinity. For purposes of this specification and the accompanying claims, the terms "epitope" and "antigenic determinant" are used interchangeably to refer to the site on an antigen to which B and/or T cells respond or recognize. Polypeptide epitopes can be formed from both contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a polypeptide. An epitope typically includes at least 3, and typically 5-10 amino acids in a unique spatial conformation.

[0133] Epitope specificity of an antibody can be determined in a variety of ways. One approach, for example, involves testing a collection of overlapping peptides of about 15 amino acids spanning the full sequence of the protein and differing in increments of a small number of amino acids (e.g., 3 to 30 amino acids). The peptides are immobilized in separate wells of a microtiter dish. Immobilization can be accomplished, for example, by biotinylating one terminus of the peptides. This process may affect the antibody affinity for the epitope, therefore different samples of the same peptide can be biotinylated at the N and C terminus and immobilized in separate wells for the purposes of comparison. This is useful for identifying end-specific antibodies. Optionally, additional peptides can be included terminating at a

particular amino acid of interest. This approach is useful for identifying end-specific antibodies to internal fragments. An antibody or antigen-binding fragment is screened for binding to each of the various peptides. The epitope is defined as a segment of amino acids that is common to all peptides to which the antibody shows high affinity binding.

3. Modification of Antibody Antigen-Binding Domains

[0134] It is understood that the antibodies of the present invention may be modified, such that they are substantially identical to the antibody polypeptide sequences, or fragments thereof, and still bind the epitopes of the present invention. Polypeptide sequences are "substantially identical" when optimally aligned using such programs as Clustal Omega, IGBLAST, GAP or BESTFIT using default gap weights, they share at least 80% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity or any range therein.

[0135] As discussed herein, minor variations in the amino acid sequences of antibodies or antigen-binding regions thereof are contemplated as being encompassed by the present invention, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% and most preferably at least 99% sequence identity. In particular, conservative amino acid replacements are contemplated.

[0136] Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families based on the chemical nature of the side chain; e.g., acidic (aspartate, glutamate), basic (lysine, arginine, histidine), nonpolar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine). For example, it is reasonable to expect that an isolated replacement of a leucine moiety with an isoleucine or valine moiety, or a similar replacement of an amino acid with a structurally related amino acid in the same family, will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Standard ELISA, Surface Plasmon Resonance (SPR), or other antibody binding assays can be performed by one skilled in the art to make a quantitative comparison of

antigen binging affinity between the unmodified antibody and any polypeptide derivatives with conservative substitutions generated through any of several methods available to one skilled in the art.

[0137] Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those skilled in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Standard methods to identify protein sequences that fold into a known three-dimensional structure are available to those skilled in the art; Dill and McCallum., Science 338:1042-1046 (2012). Several algorithms for predicting protein structures and the gene sequences that encode these have been developed, and many of these algorithms can be found at the National Center for Biotechnology Information (on the World Wide Web at ncbi.nlm.nih.gov/guide/proteins/) and at the Bioinformatics Resource Portal (on the World Wide Web at expasy.org/proteomics). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

[0138] Framework modifications can be made to antibodies to decrease immunogenicity, for example, by "backmutating" one or more framework residues to a corresponding germline sequence.

[0139] It is also contemplated that the antigen-binding domain may be multi-specific or multivalent by multimerizing the antigen-binding domain with VH and VL region pairs that bind either the same antigen (multi-valent) or a different antigen (multi-specific).

V. Proteinaceous Compositions

[0140] As used herein, a "protein" "peptide" or "polypeptide" refers to a molecule comprising at least five amino acid residues. As used herein, the term "wild-type" refers to the endogenous version of a molecule that occurs naturally in an organism. In some embodiments, wild-type versions of a protein or polypeptide are employed, however, in many embodiments of the disclosure, a modified protein or polypeptide is employed to generate an immune response. The terms described above may be used interchangeably. A "modified protein" or "modified polypeptide" or a "variant" refers to a protein or polypeptide whose chemical

structure, particularly its amino acid sequence, is altered with respect to the wild-type protein or polypeptide. In some embodiments, a modified/variant protein or polypeptide has at least one modified activity or function (recognizing that proteins or polypeptides may have multiple activities or functions). It is specifically contemplated that a modified/variant protein or polypeptide may be altered with respect to one activity or function yet retain a wild-type activity or function in other respects, such as immunogenicity.

[0141] Where a protein is specifically mentioned herein, it is in general a reference to a native (wild-type) or recombinant (modified) protein or, optionally, a protein in which any signal sequence has been removed. The protein may be isolated directly from the organism of which it is native, produced by recombinant DNA/exogenous expression methods, or produced by solid-phase peptide synthesis (SPPS) or other in vitro methods. In particular embodiments, there are isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a polypeptide (e.g., an antibody or fragment thereof). The term "recombinant" may be used in conjunction with a polypeptide or the name of a specific polypeptide, and this generally refers to a polypeptide produced from a nucleic acid molecule that has been manipulated in vitro or that is a replication product of such a molecule.

[0142] In certain embodiments the size of a protein or polypeptide (wild-type or modified) may comprise, but is not limited to, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1100, 1200, 1300, 1400, 1500, 1750, 2000, 2250, 2500 amino acid residues or greater, and any range derivable therein, or derivative of a corresponding amino sequence described or referenced herein. It is contemplated that polypeptides may be mutated by truncation, rendering them shorter than their corresponding wild-type form, also, they might be altered by fusing or conjugating a heterologous protein or polypeptide sequence with a particular function (e.g., for targeting or localization, for enhanced immunogenicity, for purification purposes, etc.).

[0143] The polypeptides, proteins, or polynucleotides encoding such polypeptides or proteins of the disclosure may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 (or any derivable range therein) or more variant amino acids or nucleic

acid substitutions or be at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (or any derivable range therein) similar, identical, or homologous with, with at least, or with at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids or nucleic acids, or any range derivable therein, of SEQ ID Nos:1-1403. In specific embodiments, the peptide or polypeptide is or is based on a human sequence. In certain embodiments, the peptide or polypeptide is not naturally occurring and/or is in a combination of peptides or polypeptides.

[0144] In some embodiments, a peptide or polypeptide described herein comprises, comprises at least, or comprises at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 substitutions (or any derivable range therein) at amino acid position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 (or any range derivable therein) of SEQ ID NOS:1-1403. In some embodiments, the amino acid at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111,

112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, or 130 of a peptide or polypeptide of SEQ ID NO:1-1403 is substituted with an alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine.

[0145] In some embodiments, the protein or polypeptide may comprise amino acids 1 to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559,

560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, or 1000, (or any derivable range therein) of SEQ ID NOs:1-1403.

[0146] In some embodiments, the protein, polypeptide, or nucleic acid may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199,

200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845,

846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, or 1000, (or any derivable range therein) contiguous amino acids of SEQ ID NOs:1-1403.

[0147] In some embodiments, the polypeptide, protein, or nucleic acid may comprise, comprise at least, comprises at most, or comprise about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452,

453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, or 1000 (or any derivable range therein) contiguous amino acids of SEQ ID Nos:1-1403 that are, are at least, are at most, are exactly, or are about 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (or

any derivable range therein) similar, identical, or homologous with one of SEQ ID NOS:1-1403.

[0148] In some aspects there is a nucleic acid molecule or polypeptide starting at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616,

617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, or 1000 of any of SEQ ID NOS:1-1403 and comprising, comprising at least, comprising at most, or comprising about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265,

266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911,

912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, or 1000 (or any derivable range therein) contiguous amino acids or nucleotides of any of SEQ ID NOS:1-1403.

[0149] The nucleotide as well as the protein, polypeptide, and peptide sequences for various genes have been previously disclosed, and may be found in the recognized computerized databases. Two commonly used databases are the National Center for Biotechnology Information's Genbank and GenPept databases (on the World Wide Web at ncbi.nlm.nih.gov/) and The Universal Protein Resource (UniProt; on the World Wide Web at uniprot.org). The coding regions for these genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art.

[0150] It is contemplated that in compositions of the disclosure, there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. The concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein).

[0151] The following is a discussion of changing the amino acid subunits of a protein to create an equivalent, or even improved, second-generation variant polypeptide or peptide. For example, certain amino acids may be substituted for other amino acids in a protein or polypeptide sequence with or without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's functional activity, certain amino acid substitutions can be made in a protein sequence and in its corresponding DNA coding sequence, and nevertheless produce a protein with similar or desirable properties. It is thus contemplated by the inventors that various changes may be made in the DNA sequences of genes which encode proteins without appreciable loss of their biological utility or activity.

[0152] The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six different codons for arginine. Also considered are "neutral substitutions" or "neutral mutations" which refers to a change in the codon or codons that encode biologically equivalent amino acids.

[0153] Amino acid sequence variants of the disclosure can be substitutional, insertional, or deletion variants. A variation in a polypeptide of the disclosure may affect 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more non-contiguous or contiguous amino acids of the protein or polypeptide, as compared to wild-type. A variant can comprise an amino acid sequence that is at least 50%, 60%, 70%, 80%, or 90%, including all values and ranges there between, identical to any sequence provided or referenced herein. A variant can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more substitute amino acids.

[0154] It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids, or 5′ or 3′ sequences, respectively, and yet still be essentially identical as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5′ or 3′ portions of the coding region.

[0155] Deletion variants typically lack one or more residues of the native or wild type protein. Individual residues can be deleted or a number of contiguous amino acids can be deleted. A stop codon may be introduced (by substitution or insertion) into an encoding nucleic acid sequence to generate a truncated protein.

[0156] Insertional mutants typically involve the addition of amino acid residues at a non-terminal point in the polypeptide. This may include the insertion of one or more amino acid residues. Terminal additions may also be generated and can include fusion proteins which are multimers or concatemers of one or more peptides or polypeptides described or referenced herein.

[0157] Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein or polypeptide, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar chemical properties. “Conservative amino acid substitutions” may involve exchange of a member of one amino acid class with another member of the same class. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to

asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics or other reversed or inverted forms of amino acid moieties.

[0158] Alternatively, substitutions may be "non-conservative", such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting an amino acid residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa. Non-conservative substitutions may involve the exchange of a member of one of the amino acid classes for a member from another class.

[0159] One skilled in the art can determine suitable variants of polypeptides as set forth herein using well-known techniques. One skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. The skilled artisan will also be able to identify amino acid residues and portions of the molecules that are conserved among similar proteins or polypeptides. In further embodiments, areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without significantly altering the biological activity or without adversely affecting the protein or polypeptide structure.

[0160] In making such changes, the hydropathy index of amino acids may be considered. The hydropathy profile of a protein is calculated by assigning each amino acid a numerical value ("hydropathy index") and then repetitively averaging these values along the peptide chain. Each amino acid has been assigned a value based on its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (−0.4); threonine (−0.7); serine (−0.8); tryptophan (−0.9); tyrosine (−1.3); proline (1.6); histidine (−3.2); glutamate (−3.5); glutamine (−3.5); aspartate (−3.5); asparagine (−3.5); lysine (−3.9); and arginine (−4.5). The importance of the hydropathy amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte et al., J. Mol. Biol. 157:105-131 (1982)). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein or polypeptide, which in turn defines the interaction of the protein or polypeptide with other molecules, for example, enzymes, substrates, receptors,

DNA, antibodies, antigens, and others. It is also known that certain amino acids may be substituted for other amino acids having a similar hydropathy index or score, and still retain a similar biological activity. In making changes based upon the hydropathy index, in certain embodiments, the substitution of amino acids whose hydropathy indices are within ±2 is included. In some aspects of the invention, those that are within ±1 are included, and in other aspects of the invention, those within ±0.5 are included.

[0161] It also is understood in the art that the substitution of like amino acids can be effectively made based on hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. In certain embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigen binding, that is, as a biological property of the protein. The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0±1); glutamate (+3.0±1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (−0.4); proline (−0.5±1); alanine (−0.5); histidine (−0.5); cysteine (−1.0); methionine (−1.3); valine (−1.5); leucine (−1.8); isoleucine (−1.8); tyrosine (−2.3); phenylalanine (−2.5); and tryptophan (−3.4). In making changes based upon similar hydrophilicity values, in certain embodiments, the substitution of amino acids whose hydrophilicity values are within ±2 are included, in other embodiments, those which are within ±1 are included, and in still other embodiments, those within ±0.5 are included. In some instances, one may also identify epitopes from primary amino acid sequences based on hydrophilicity. These regions are also referred to as "epitopic core regions." It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still produce a biologically equivalent and immunologically equivalent protein.

[0162] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides or proteins that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues important for activity or structure in similar proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[0163] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar proteins or polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an

antibody with respect to its three-dimensional structure. One skilled in the art may choose not to make changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. These variants can then be screened using standard assays for binding and/or activity, thus yielding information gathered from such routine experiments, which may allow one skilled in the art to determine the amino acid positions where further substitutions should be avoided either alone or in combination with other mutations. Various tools available to determine secondary structure can be found on the world wide web at expasy.org/proteomics/protein_structure.

[0164] In some embodiments of the invention, amino acid substitutions are made that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter ligand or antigen binding affinities, and/or (5) confer or modify other physicochemical or functional properties on such polypeptides. For example, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally occurring sequence. Substitutions can be made in that portion of the antibody that lies outside the domain(s) forming intermolecular contacts. In such embodiments, conservative amino acid substitutions can be used that do not substantially change the structural characteristics of the protein or polypeptide (e.g., one or more replacement amino acids that do not disrupt the secondary structure that characterizes the native antibody).

VI. Nucleic Acids

[0165] In certain embodiments, nucleic acid sequences can exist in a variety of instances such as: isolated segments and recombinant vectors of incorporated sequences or recombinant polynucleotides encoding one or both chains of an antibody, or a fragment, derivative, mutein, or variant thereof, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing described herein. Nucleic acids that encode the epitope to which certain of the antibodies provided herein are also provided. Nucleic acids encoding fusion proteins that include these peptides are also provided. The nucleic acids can

be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides and artificial variants thereof (e.g., peptide nucleic acids).

[0166] The term "polynucleotide" refers to a nucleic acid molecule that either is recombinant or has been isolated from total genomic nucleic acid. Included within the term "polynucleotide" are oligonucleotides (nucleic acids 100 residues or less in length), recombinant vectors, including, for example, plasmids, cosmids, phage, viruses, and the like. Polynucleotides include, in certain aspects, regulatory sequences, isolated substantially away from their naturally occurring genes or protein encoding sequences. Polynucleotides may be single-stranded (coding or antisense) or double- stranded, and may be RNA, DNA (genomic, cDNA or synthetic), analogs thereof, or a combination thereof. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide.

[0167] In this respect, the term "gene," "polynucleotide," or "nucleic acid" is used to refer to a nucleic acid that encodes a protein, polypeptide, or peptide (including any sequences required for proper transcription, post-translational modification, or localization). As will be understood by those in the art, this term encompasses genomic sequences, expression cassettes, cDNA sequences, and smaller engineered nucleic acid segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. A nucleic acid encoding all or part of a polypeptide may contain a contiguous nucleic acid sequence encoding all or a portion of such a polypeptide. It also is contemplated that a particular polypeptide may be encoded by nucleic acids containing variations having slightly different nucleic acid sequences but, nonetheless, encode the same or substantially similar protein.

[0168] In certain embodiments, there are polynucleotide variants having substantial sequence identity to the sequences disclosed herein; those comprising at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher sequence identity, including all values and ranges there between, compared to a polynucleotide sequence provided herein using the methods described herein (e.g., BLAST analysis using standard parameters). In certain aspects, the isolated polynucleotide will comprise a nucleotide sequence encoding a polypeptide that has at least 90%, preferably 95% and above, identity to an amino acid sequence described herein, over the entire length of the sequence; or a nucleotide sequence complementary to said isolated polynucleotide.

[0169] The nucleic acid segments, regardless of the length of the coding sequence itself, may be combined with other nucleic acid sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. The nucleic acids can be any length. They

can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1000, 1500, 3000, 5000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be a part of a larger nucleic acid, for example, a vector. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant nucleic acid protocol. In some cases, a nucleic acid sequence may encode a polypeptide sequence with additional heterologous coding sequences, for example to allow for purification of the polypeptide, transport, secretion, post-translational modification, or for therapeutic benefits such as targeting or efficacy. As discussed above, a tag or other heterologous polypeptide may be added to the modified polypeptide-encoding sequence, wherein "heterologous" refers to a polypeptide that is not the same as the modified polypeptide.

1. Hybridization

[0170] The nucleic acids that hybridize to other nucleic acids under particular hybridization conditions. Methods for hybridizing nucleic acids are well known in the art. See, e.g., Current Protocols in Molecular Biology, John Wiley and Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, a moderately stringent hybridization condition uses a prewashing solution containing 5× sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6×SSC, and a hybridization temperature of 55° C. (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42° C), and washing conditions of 60° C. in 0.5×SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6×SSC at 45° C., followed by one or more washes in 0.1×SSC, 0.2% SDS at 68° C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequence that are at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to each other typically remain hybridized to each other.

[0171] The parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11 (1989); Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley and Sons, Inc., sections 2.10 and 6.3-6.4 (1995), both of which are herein

incorporated by reference in their entirety for all purposes) and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA.

1. Mutation

[0172] Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (e.g., an antibody or antibody derivative) that it encodes. Mutations can be introduced using any technique known in the art. In one embodiment, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another embodiment, one or more randomly selected residues are changed using, for example, a random mutagenesis protocol. However it is made, a mutant polypeptide can be expressed and screened for a desired property.

[0173] Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-essential amino acid residues. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively changes the biological activity of a polypeptide that it encodes. See, eg., Romain Studer et al., Biochem. J. 449:581-594 (2013). For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative changes include altering the antigen specificity of an antibody.

2. Probes

[0174] In another aspect, nucleic acid molecules are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences. A nucleic acid molecule can comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion of a given polypeptide.

[0175] In another embodiment, the nucleic acid molecules may be used as probes or PCR primers for specific antibody sequences. For instance, a nucleic acid molecule probe may be used in diagnostic methods or a nucleic acid molecule PCR primer may be used to amplify regions of DNA that could be used, inter alia, to isolate nucleic acid sequences for use in producing variable domains of antibodies. See, eg., Gaily Kivi et al., BMC Biotechnol. 16:2

(2016). In a preferred embodiment, the nucleic acid molecules are oligonucleotides. In a more preferred embodiment, the oligonucleotides are from highly variable regions of the heavy and light chains of the antibody of interest. In an even more preferred embodiment, the oligonucleotides encode all or part of one or more of the CDRs.

[0176] Probes based on the desired sequence of a nucleic acid can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide of interest. The probe can comprise a label group, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide.

VII. Antibody Production

A. Antibody Production

[0177] Methods for preparing and characterizing antibodies for use in diagnostic and detection assays, for purification, and for use as therapeutics are well known in the art as disclosed in, for example, U.S. Pat. Nos. 4,011,308; 4,722,890; 4,016,043; 3,876,504; 3,770,380; and 4,372,745 (see, e.g., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; incorporated herein by reference). These antibodies may be polyclonal or monoclonal antibody preparations, monospecific antisera, human antibodies, hybrid or chimeric antibodies, such as humanized antibodies, altered antibodies, F(ab′)2 fragments, Fab fragments, Fv fragments, single-domain antibodies, dimeric or trimeric antibody fragment constructs, minibodies, or functional fragments thereof which bind to the antigen in question. In certain aspects, polypeptides, peptides, and proteins and immunogenic fragments thereof for use in various embodiments can also be synthesized in solution or on a solid support in accordance with conventional techniques. See, for example, Stewart and Young, (1984); Tarn et al, (1983); Merrifield, (1986); and Barany and Merrifield (1979), each incorporated herein by reference.

[0178] Briefly, a polyclonal antibody is prepared by immunizing an animal with an antigen or a portion thereof and collecting antisera from that immunized animal. The antigen may be altered compared to an antigen sequence found in nature. In some embodiments, a variant or altered antigenic peptide or polypeptide is employed to generate antibodies. Inocula are typically prepared by dispersing the antigenic composition in a physiologically tolerable diluent to form an aqueous composition. Antisera is subsequently collected by methods known in the arts, and the serum may be used as-is for various applications or else the desired antibody

fraction may be purified by well-known methods, such as affinity chromatography (Harlow and Lane, Antibodies: A Laboratory Manual 1988).

[0179] Methods of making monoclonal antibodies are also well known in the art (Kohler and Milstein, 1975; Harlow and Lane, 1988, U.S. Patent 4,196,265, herein incorporated by reference in its entirety for all purposes). Typically, this technique involves immunizing a suitable animal with a selected immunogenic composition, e.g., a purified or partially purified protein, polypeptide, peptide or domain. Resulting antibody-producing B-cells from the immunized animal, or all dissociated splenocytes, are then induced to fuse with cells from an immortalized cell line to form hybridomas. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing and have high fusion efficiency and enzyme deficiencies that render then incapable of growing in certain selective media that support the growth of only the desired fused cells (hybridomas). Typically, the fusion partner includes a property that allows selection of the resulting hybridomas using specific media. For example, fusion partners can be hypoxanthine/aminopterin/thymidine (HAT)-sensitive. Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Next, selection of hybridomas can be performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. Fusion procedures for making hybridomas, immunization protocols, and techniques for isolation of immunized splenocytes for fusion are known in the art.

[0180] Other techniques for producing monoclonal antibodies include the viral or oncogenic transformation of B-lymphocytes, a molecular cloning approach may be used to generate a nucleic acid or polypeptide, the selected lymphocyte antibody method (SLAM) (see, e.g., Babcook et al., Proc. Natl. Acad. Sci. USA 93:7843-7848 (1996), the preparation of combinatorial immunoglobulin phagemid libraries from RNA isolated from the spleen of the immunized animal and selection of phagemids expressing appropriate antibodies, or producing a cell expressing an antibody from a genomic sequence of the cell comprising a modified immunoglobulin locus using Cre-mediated site-specific recombination (see, e.g., U.S. 6,091,001).

[0181] Monoclonal antibodies may be further purified using filtration, centrifugation, and various chromatographic methods such as HPLC or affinity chromatography. Monoclonal antibodies may be further screened or optimized for properties relating to specificity, avidity,

half-life, immunogenicity, binding association, binding disassociation, or overall functional properties relative to being a treatment for infection. Thus, monoclonal antibodies may have alterations in the amino acid sequence of CDRs, including insertions, deletions, or substitutions with a conserved or non-conserved amino acid.

[0182] The immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Adjuvants that may be used in accordance with embodiments include, but are not limited to, IL-1, IL-2, IL-4, IL-7, IL-12, -interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). Exemplary adjuvants may include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund's adjuvants, and/or aluminum hydroxide adjuvant. In addition to adjuvants, it may be desirable to co-administer biologic response modifiers (BRM), such as but not limited to, Cimetidine (CIM; 1200 mg/d) (Smith/Kline, PA); low-dose Cyclophosphamide (CYP; 300 mg/m2) (Johnson/ Mead, NJ), cytokines such as β-interferon, IL-2, or IL-12, or genes encoding proteins involved in immune helper functions, such as B-7.A phage-display system can be used to expand antibody molecule populations in vitro. Saiki, et al., Nature 324:163 (1986); Scharf et al., Science 233:1076 (1986); U.S. Pat. Nos. 4,683,195 and 4,683,202; Yang et al., J Mol Biol. 254:392 (1995); Barbas, III et al., Methods: Comp. Meth Enzymol. (1995) 8:94; Barbas, III et al., Proc Natl Acad Sci USA 88:7978 (1991).

B. Fully Human Antibody Production

[0183] Methods are available for making fully human antibodies. Using fully human antibodies can minimize the immunogenic and allergic responses that may be caused by administering non-human monoclonal antibodies to humans as therapeutic agents. In one embodiment, human antibodies may be produced in a non-human transgenic animal, e.g., a transgenic mouse capable of producing multiple isotypes of human antibodies to protein (e.g., IgG, IgA, and/or IgE) by undergoing V-D-J recombination and isotype switching. Accordingly, this aspect applies to antibodies, antibody fragments, and pharmaceutical compositions thereof, but also non-human transgenic animals, B-cells, host cells, and hybridomas that produce monoclonal antibodies. Applications of humanized antibodies include, but are not limited to, detect a cell expressing an anticipated protein, either in vivo or in vitro, pharmaceutical

preparations containing the antibodies of the present invention, and methods of treating disorders by administering the antibodies.

[0184] Fully human antibodies can be produced by immunizing transgenic animals (usually mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. Antigens for this purpose typically have six or more contiguous amino acids, and optionally are conjugated to a carrier, such as a hapten. See, for example, Jakobovits et al., Proc. Natl. Acad. Sci. USA 90:2551-2555 (1993); Jakobovits et al., Nature 362:255-258 (1993); Bruggermann et al., Year in Immunol. 7:33 (1993). In one example, transgenic animals are produced by incapacitating the endogenous mouse immunoglobulin loci encoding the mouse heavy and light immunoglobulin chains therein, and inserting into the mouse genome large fragments of human genome DNA containing loci that encode human heavy and light chain proteins. Partially modified animals, which have less than the full complement of human immunoglobulin loci, are then crossbred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies that are immunospecific for the immunogen but have human rather than murine amino acid sequences, including the variable regions. For further details of such methods, see, for example, International Patent Application Publication Nos. WO 96/33735 and WO 94/02602, which are hereby incorporated by reference in their entirety. Additional methods relating to transgenic mice for making human antibodies are described in U.S. Pat. Nos. 5,545,807; 6,713,610; 6,673,986; 6,162,963; 6,300,129; 6,255,458; 5,877,397; 5,874,299 and 5,545,806; in International Patent Application Publication Nos. WO 91/10741 and WO 90/04036; and in European Patent Nos. EP 546073B1 and EP 546073A1, all of which are hereby incorporated by reference in their entirety for all purposes.

[0185] The transgenic mice described above, referred to herein as "HuMAb" mice, contain a human immunoglobulin gene minilocus that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg et al., Nature 368:856-859 (1994)). Accordingly, the mice exhibit reduced expression of mouse IgM or κ chains and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgG κ monoclonal antibodies (Lonberg et al., supra; Lonberg and Huszar, Intern. Ref. Immunol. 13:65-93 (1995); Harding and Lonberg, Ann. N.Y. Acad. Sci. 764:536-546 (1995)). The preparation of HuMAb mice is described in detail in Taylor et al., Nucl. Acids Res. 20:6287-6295 (1992); Chen et al., Int. Immunol. 5:647-656 (1993); Tuaillon et al., J. Immunol. 152:2912-2920 (1994); Lonberg et al., supra; Lonberg,

Handbook of Exp. Pharmacol. 113:49-101 (1994); Taylor et al., Int. Immunol. 6:579-591 (1994); Lonberg and Huszar, Intern. Ref. Immunol. 13:65-93 (1995); Harding and Lonberg, Ann. N.Y. Acad. Sci. 764:536-546 (1995); Fishwild et al., Nat. Biotechnol. 14:845-851 (1996); the foregoing references are herein incorporated by reference in their entirety for all purposes. See further, U.S. Pat. Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; 5,770,429; and 5,545,807; as well as International Patent Application Publication Nos. WO 93/1227; WO 92/22646; and WO 92/03918, the disclosures of all of which are hereby incorporated by reference in their entirety for all purposes. Technologies utilized for producing human antibodies in these transgenic mice are disclosed also in WO 98/24893, and Mendez et al., Nat. Genetics 15:146-156 (1997), which are herein incorporated by reference. For example, the HCo7 and HCo12 transgenic mice strains can be used to generate human antibodies.

[0186] Using hybridoma technology, antigen-specific humanized monoclonal antibodies with the desired specificity can be produced and selected from the transgenic mice such as those described above. Such antibodies may be cloned and expressed using a suitable vector and host cell, or the antibodies can be harvested from cultured hybridoma cells. Fully human antibodies can also be derived from phage-display libraries (as disclosed in Hoogenboom et al., J. Mol. Biol. 227:381 (1991); and Marks et al., J. Mol. Biol. 222:581 (1991)). One such technique is described in International Patent Application Publication No. WO 99/10494 (herein incorporated by reference), which describes the isolation of high affinity and functional agonistic antibodies for MPL- and msk-receptors using such an approach.

C. Antibody Fragments Production

[0187] Antibody fragments that retain the ability to recognize the antigen of interest will also find use herein. A number of antibody fragments are known in the art that comprise antigen-binding sites capable of exhibiting immunological binding properties of an intact antibody molecule and can be subsequently modified by methods known in the arts. Functional fragments, including only the variable regions of the heavy and light chains, can also be produced using standard techniques such as recombinant production or preferential proteolytic cleavage of immunoglobulin molecules. These fragments are known as Fv. See, e.g., Inbar et al., Proc. Nat. Acad. Sci. USA 69:2659-2662 (1972); Hochman et al., Biochem. 15:2706-2710 (1976); and Ehrlich et al., Biochem. 19:4091-4096 (1980).

[0188] Single-chain variable fragments (scFvs) may be prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (VL and VH). scFvs can form antigen-binding monomers, or they can form multimers (e.g., dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., Prot. Eng. 10:423 (1997); Kort et al., Biomol. Eng. 18:95-108 (2001)). By combining different VL- and VH-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum et al., Biomol. Eng. 18:31-40 (2001)). Antigen-binding fragments are typically produced by recombinant DNA methods known to those skilled in the art. Although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined using recombinant methods by a synthetic linker that enables them to be made as a single chain polypeptide (known as single chain Fv (sFv or scFv); see e.g., Bird et al., Science 242:423-426 (1988); and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988). Design criteria include determining the appropriate length to span the distance between the C-terminus of one chain and the N-terminus of the other, wherein the linker is generally formed from small hydrophilic amino acid residues that do not tend to coil or form secondary structures. Suitable linkers generally comprise polypeptide chains of alternating sets of glycine and serine residues, and may include glutamic acid and lysine residues inserted to enhance solubility. Antigen-binding fragments are screened for utility in the same manner as intact antibodies. Such fragments include those obtained by amino-terminal and/or carboxy-terminal deletions, where the remaining amino acid sequence is substantially identical to the corresponding positions in the naturally occurring sequence deduced, for example, from a full-length cDNA sequence.

[0189] Antibodies may also be generated using peptide analogs of the epitopic determinants disclosed herein, which may consist of non-peptide compounds having properties analogous to those of the template peptide. These types of non-peptide compound are termed “peptide mimetics” or “peptidomimetics”. Fauchere, J. Adv. Drug Res. 15:29 (1986); Veber and Freidinger TINS p. 392 (1985); and Evans et al., J. Med. Chem. 30:1229 (1987). Liu et al. (2003) also describe “antibody like binding peptidomimetics” (ABiPs), which are peptides that act as pared-down antibodies and have certain advantages of longer serum half-life as well as less cumbersome synthesis methods. These analogs can be peptides, non-peptides or combinations of peptide and non-peptide regions. Fauchere, Adv. Drug Res. 15:29 (1986); Veber and Freidiner, TINS p. 392 (1985); and Evans et al., J. Med. Chem. 30:1229 (1987), which are incorporated herein by reference in their entirety for any purpose. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce a similar

therapeutic or prophylactic effect. Such compounds are often developed with the aid of computerized molecular modeling. Generally, peptidomimetics of the invention are proteins that are structurally similar to an antibody displaying a desired biological activity, such as the ability to bind a protein, but have one or more peptide linkages optionally replaced by a linkage selected from: —CH2NH—, —CH2S—, —CH2—CH2—, —CH=CH— (cis and trans), —COCH2—, —CH(OH)CH2—, and —CH2SO— by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used in certain embodiments of the invention to generate more stable proteins. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch, Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference), for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0190] Once generated, a phage display library can be used to improve the immunological binding affinity of the Fab molecules using known techniques. See, e.g., Figini et al., J. Mol. Biol. 239:68 (1994). The coding sequences for the heavy and light chain portions of the Fab molecules selected from the phage display library can be isolated or synthesized and cloned into any suitable vector or replicon for expression. Any suitable expression system can be used.

VIII. Polypeptide Expression

[0191] In some aspects, there are nucleic acid molecule encoding polypeptides or peptides of the disclosure (e.g antibodies, TCR genes, and immunogenic peptides). These may be generated by methods known in the art, e.g., isolated from B cells of mice that have been immunized and isolated, phage display, expressed in any suitable recombinant expression system and allowed to assemble to form antibody molecules or by recombinant methods.

1. Expression

[0192] The nucleic acid molecules may be used to express large quantities of polypeptides. If the nucleic acid molecules are derived from a non-human, non-transgenic animal, the nucleic acid molecules may be used for humanization of the antibody or TCR genes.

2. Vectors

[0193] In some aspects, contemplated are expression vectors comprising a nucleic acid molecule encoding a polypeptide of the desired sequence or a portion thereof (e.g., a fragment containing one or more CDRs or one or more variable region domains). Expression vectors comprising the nucleic acid molecules may encode the heavy chain, light chain, or the antigen-binding portion thereof. In some aspects, expression vectors comprising nucleic acid molecules may encode fusion proteins, modified antibodies, antibody fragments, and probes thereof. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well.

[0194] To express the polypeptides or peptides of the disclosure, DNAs encoding the polypeptides or peptides are inserted into expression vectors such that the gene area is operatively linked to transcriptional and translational control sequences. In some aspects, a vector that encodes a functionally complete human CH or CL immunoglobulin sequence with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In some aspects, a vector that encodes a functionally complete human TCR alpha or TCR beta sequence with appropriate restriction sites engineered so that any variable sequence or CDR1, CDR2, and/or CDR3 can be easily inserted and expressed. Typically, expression vectors used in any of the host cells contain sequences for plasmid or virus maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" typically include one or more of the following operatively linked nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Such sequences and methods of using the same are well known in the art.

3. Expression Systems

[0195] Numerous expression systems exist that comprise at least a part or all of the expression vectors discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with an embodiment to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Commercially and widely available systems include in but are not limited to bacterial, mammalian, yeast, and insect cell systems. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins.

Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. Those skilled in the art are able to express a vector to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide using an appropriate expression system.

4. Methods of Gene Transfer

[0196] Suitable methods for nucleic acid delivery to effect expression of compositions are anticipated to include virtually any method by which a nucleic acid (e.g., DNA, including viral and nonviral vectors) can be introduced into a cell, a tissue or an organism, as described herein or as would be known to one of ordinary skill in the art. Such methods include, but are not limited to, direct delivery of DNA such as by injection (U.S. Patents 5,994,624,5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859, each incorporated herein by reference), including microinjection (Harland and Weintraub, 1985; U.S. Patent 5,789,215, incorporated herein by reference); by electroporation (U.S. Patent No. 5,384,253, incorporated herein by reference); by calcium phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe et al., 1990); by using DEAE dextran followed by polyethylene glycol (Gopal, 1985); by direct sonic loading (Fechheimer et al., 1987); by liposome mediated transfection (Nicolau and Sene, 1982; Fraley et al., 1979; Nicolau et al., 1987; Wong et al., 1980; Kaneda et al., 1989; Kato et al., 1991); by microprojectile bombardment (PCT Application Nos. WO 94/09699 and 95/06128; U.S. Patents 5,610,042; 5,322,783, 5,563,055, 5,550,318, 5,538,877 and 5,538,880, and each incorporated herein by reference); by agitation with silicon carbide fibers (Kaeppler et al., 1990; U.S. Patents 5,302,523 and 5,464,765, each incorporated herein by reference); by Agrobacterium mediated transformation (U.S. Patents 5,591,616 and 5,563,055, each incorporated herein by reference); or by PEG mediated transformation of protoplasts (Omirulleh et al., 1993; U.S. Patents 4,684,611 and 4,952,500, each incorporated herein by reference); by desiccation/inhibition mediated DNA uptake (Potrykus et al., 1985). Other methods include viral transduction, such as gene transfer by lentiviral or retroviral transduction.

5. Host Cells

[0197] In another aspect, contemplated are the use of host cells into which a recombinant expression vector has been introduced. Antibodies can be expressed in a variety of cell types. An expression construct encoding an antibody can be transfected into cells according to a

variety of methods known in the art. Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. Some vectors may employ control sequences that allow it to be replicated and/or expressed in both prokaryotic and eukaryotic cells. In certain aspects, the antibody expression construct can be placed under control of a promoter that is linked to T-cell activation, such as one that is controlled by NFAT-1 or NF-κB, both of which are transcription factors that can be activated upon T-cell activation. Control of antibody expression allows T cells, such as tumor- targeting T cells, to sense their surroundings and perform real-time modulation of cytokine signaling, both in the T cells themselves and in surrounding endogenous immune cells. One of skill in the art would understand the conditions under which to incubate host cells to maintain them and to permit replication of a vector. Also understood and known are techniques and conditions that would allow large-scale production of vectors, as well as production of the nucleic acids encoded by vectors and their cognate polypeptides, proteins, or peptides.

[0198] For stable transfection of mammalian cells, it is known, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods known in the arts.

B. Isolation

[0199] The nucleic acid molecule encoding either or both of the entire heavy and light chains of an antibody or the variable regions thereof may be obtained from any source that produces antibodies. Methods of isolating mRNA encoding an antibody are well known in the art. See e.g., Sambrook et al., supra. The sequences of human heavy and light chain constant region genes are also known in the art. See, e.g., Kabat et al., 1991, supra. Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed in a cell into which they have been introduced and the antibody isolated.

IX. Additional Therapies

A. Immunotherapy

[0200] In some embodiments, the methods comprise administration of an additional therapy. In some embodiments, the additional therapy comprises a cancer immunotherapy. Cancer immunotherapy (sometimes called immuno-oncology, abbreviated IO) is the use of the immune system to treat cancer. Immunotherapies can be categorized as active, passive or hybrid (active and passive). These approaches exploit the fact that cancer cells often have molecules on their surface that can be detected by the immune system, known as tumor-associated antigens (TAAs); they are often proteins or other macromolecules (e.g. carbohydrates). Active immunotherapy directs the immune system to attack tumor cells by targeting TAAs. Passive immunotherapies enhance existing anti-tumor responses and include the use of monoclonal antibodies, lymphocytes and cytokines. Immunotherapies are known in the art, and some are described below.

1. Checkpoint Inhibitors and Combination Treatment

[0201] Embodiments of the disclosure may include administration of immune checkpoint inhibitors, which are further described below.

1. PD-1, PDL1, and PDL2 inhibitors

[0202] PD-1 can act in the tumor microenvironment where T cells encounter an infection or tumor. Activated T cells upregulate PD-1 and continue to express it in the peripheral tissues. Cytokines such as IFN-gamma induce the expression of PDL1 on epithelial cells and tumor cells. PDL2 is expressed on macrophages and dendritic cells. The main role of PD-1 is to limit the activity of effector T cells in the periphery and prevent excessive damage to the tissues during an immune response. Inhibitors of the disclosure may block one or more functions of PD-1 and/or PDL1 activity.

[0203] Alternative names for "PD-1" include CD279 and SLEB2. Alternative names for "PDL1" include B7-H1, B7-4, CD274, and B7-H. Alternative names for "PDL2" include B7-DC, Btdc, and CD273. In some embodiments, PD-1, PDL1, and PDL2 are human PD-1, PDL1 and PDL2.

[0204] In some embodiments, the PD-1 inhibitor is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PDL1 and/or PDL2. In another embodiment, a PDL1 inhibitor is a molecule that inhibits the

binding of PDL1 to its binding partners. In a specific aspect, PDL1 binding partners are PD-1 and/or B7-1. In another embodiment, the PDL2 inhibitor is a molecule that inhibits the binding of PDL2 to its binding partners. In a specific aspect, a PDL2 binding partner is PD-1. The inhibitor may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. 8,735,553, 8,354,509, and 8,008,449, all incorporated herein by reference. Other PD-1 inhibitors for use in the methods and compositions provided herein are known in the art such as described in U.S. Patent Application Nos. US2014/0294898, US2014/022021, and US2011/0008369, all incorporated herein by reference.

[0205] In some embodiments, the PD-1 inhibitor is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and pidilizumab. In some embodiments, the PD-1 inhibitor is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PDL1 or PDL2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PDL1 inhibitor comprises AMP- 224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. Pidilizumab, also known as CT-011, hBAT, or hBAT-1, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PDL2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342. Additional PD-1 inhibitors include MEDI0680, also known as AMP-514, and REGN2810.

[0206] In some embodiments, the immune checkpoint inhibitor is a PDL1 inhibitor such as Durvalumab, also known as MEDI4736, atezolizumab, also known as MPDL3280A, avelumab, also known as MSB00010118C, MDX-1105, BMS-936559, or combinations thereof. In certain aspects, the immune checkpoint inhibitor is a PDL2 inhibitor such as rHIgM12B7.

[0207] In some embodiments, the inhibitor comprises the heavy and light chain CDRs or VRs of nivolumab, pembrolizumab, or pidilizumab. Accordingly, in one embodiment, the inhibitor comprises the CDR1, CDR2, and CDR3 domains of the VH region of nivolumab, pembrolizumab, or pidilizumab, and the CDR1, CDR2 and CDR3 domains of the VL region of nivolumab, pembrolizumab, or pidilizumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on PD-1, PDL1, or PDL2 as the above-

mentioned antibodies. In another embodiment, the antibody has at least about 70, 75, 80, 85, 90, 95, 97, or 99% (or any derivable range therein) variable region amino acid sequence identity with the above-mentioned antibodies.

b. CTLA-4, B7-1, and B7-2

[0208] Another immune checkpoint that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T cells and acts as an "off" switch when bound to B7-1 (CD80) or B7-2 (CD86) on the surface of antigen-presenting cells. CTLA4 is a member of the immunoglobulin superfamily that is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to B7-1 and B7-2 on antigen-presenting cells. CTLA-4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA-4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules. Inhibitors of the disclosure may block one or more functions of CTLA-4, B7-1, and/or B7-2 activity. In some embodiments, the inhibitor blocks the CTLA-4 and B7-1 interaction. In some embodiments, the inhibitor blocks the CTLA-4 and B7-2 interaction.

[0209] In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0210] Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in: US 8,119,129, WO 01/14424, WO 98/42752; WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab), U.S. Patent No. 6,207,156; Hurwitz et al., 1998; can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are hereby incorporated by reference. Antibodies that compete with any of these art-recognized antibodies for binding to CTLA-4 also can be used. For example, a humanized CTLA-4 antibody is described in International Patent Application No.

WO2001/014424, WO2000/037504, and U.S. Patent No. 8,017,114; all incorporated herein by reference.

[0211] A further anti-CTLA-4 antibody useful as a checkpoint inhibitor in the methods and compositions of the disclosure is ipilimumab (also known as 10D1, MDX- 010, MDX- 101, and Yervoy®) or antigen binding fragments and variants thereof (see, e.g., WO0 1/14424).

[0212] In some embodiments, the inhibitor comprises the heavy and light chain CDRs or VRs of tremelimumab or ipilimumab. Accordingly, in one embodiment, the inhibitor comprises the CDR1, CDR2, and CDR3 domains of the VH region of tremelimumab or ipilimumab, and the CDR1, CDR2 and CDR3 domains of the VL region of tremelimumab or ipilimumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on PD-1, B7-1, or B7-2 as the above- mentioned antibodies. In another embodiment, the antibody has at least about 70, 75, 80, 85, 90, 95, 97, or 99% (or any derivable range therein) variable region amino acid sequence identity with the above-mentioned antibodies.

2. Inhibition of co-stimulatory molecules

[0213] In some embodiments, the immunotherapy comprises an inhibitor of a co-stimulatory molecule. In some embodiments, the inhibitor comprises an inhibitor of B7-1 (CD80), B7-2 (CD86), CD28, ICOS, OX40 (TNFRSF4), 4-1BB (CD137; TNFRSF9), CD40L (CD40LG), GITR (TNFRSF18), and combinations thereof. Inhibitors include inhibitory antibodies, polypeptides, compounds, and nucleic acids.

2. Dendritic cell therapy

[0214] Dendritic cell therapy provokes anti-tumor responses by causing dendritic cells to present tumor antigens to lymphocytes, which activates them, priming them to kill other cells that present the antigen. Dendritic cells are antigen presenting cells (APCs) in the mammalian immune system. In cancer treatment they aid cancer antigen targeting. One example of cellular cancer therapy based on dendritic cells is sipuleucel-T.

[0215] One method of inducing dendritic cells to present tumor antigens is by vaccination with autologous tumor lysates or short peptides (small parts of protein that correspond to the protein antigens on cancer cells). These peptides are often given in combination with adjuvants (highly immunogenic substances) to increase the immune and anti-tumor responses. Other adjuvants include proteins or other chemicals that attract and/or activate dendritic cells, such as granulocyte macrophage colony-stimulating factor (GM-CSF).

[0216] Dendritic cells can also be activated in vivo by making tumor cells express GM-CSF. This can be achieved by either genetically engineering tumor cells to produce GM-CSF or by infecting tumor cells with an oncolytic virus that expresses GM-CSF.

[0217] Another strategy is to remove dendritic cells from the blood of a patient and activate them outside the body. The dendritic cells are activated in the presence of tumor antigens, which may be a single tumor-specific peptide/protein or a tumor cell lysate (a solution of broken down tumor cells). These cells (with optional adjuvants) are infused and provoke an immune response.

[0218] Dendritic cell therapies include the use of antibodies that bind to receptors on the surface of dendritic cells. Antigens can be added to the antibody and can induce the dendritic cells to mature and provide immunity to the tumor. Dendritic cell receptors such as TLR3, TLR7, TLR8 or CD40 have been used as antibody targets.

3. CAR-T cell therapy

[0219] Chimeric antigen receptors (CARs, also known as chimeric immunoreceptors, chimeric T cell receptors or artificial T cell receptors) are engineered receptors that combine a new specificity with an immune cell to target cancer cells. Typically, these receptors graft the specificity of a monoclonal antibody onto a T cell. The receptors are called chimeric because they are fused of parts from different sources. CAR-T cell therapy refers to a treatment that uses such transformed cells for cancer therapy.

[0220] The basic principle of CAR-T cell design involves recombinant receptors that combine antigen-binding and T-cell activating functions. The general premise of CAR-T cells is to artificially generate T-cells targeted to markers found on cancer cells. Scientists can remove T-cells from a person, genetically alter them, and put them back into the patient for them to attack the cancer cells. Once the T cell has been engineered to become a CAR-T cell, it acts as a “living drug”. CAR-T cells create a link between an extracellular ligand recognition domain to an intracellular signalling molecule which in turn activates T cells. The extracellular ligand recognition domain is usually a single-chain variable fragment (scFv). An important aspect of the safety of CAR-T cell therapy is how to ensure that only cancerous tumor cells are targeted, and not normal cells. The specificity of CAR-T cells is determined by the choice of molecule that is targeted.

[0221] Exemplary CAR-T therapies include Tisagenlecleucel (Kymriah) and Axicabtagene ciloleucel (Yescarta). In some embodiments, the CAR-T therapy targets CD19.

4. Cytokine therapy

[0222] Cytokines are proteins produced by many types of cells present within a tumor. They can modulate immune responses. The tumor often employs them to allow it to grow and reduce the immune response. These immune-modulating effects allow them to be used as drugs to provoke an immune response. Two commonly used cytokines are interferons and interleukins.

[0223] Interferons are produced by the immune system. They are usually involved in anti-viral response, but also have use for cancer. They fall in three groups: type I (IFNα and IFNβ), type II (IFNγ) and type III (IFNλ).

[0224] Interleukins have an array of immune system effects. IL-2 is an exemplary interleukin cytokine therapy.

5. Adoptive T-cell therapy

[0225] Adoptive T cell therapy is a form of passive immunization by the transfusion of T-cells (adoptive cell transfer). They are found in blood and tissue and usually activate when they find foreign pathogens. Specifically they activate when the T-cell's surface receptors encounter cells that display parts of foreign proteins on their surface antigens. These can be either infected cells, or antigen presenting cells (APCs). They are found in normal tissue and in tumor tissue, where they are known as tumor infiltrating lymphocytes (TILs). They are activated by the presence of APCs such as dendritic cells that present tumor antigens. Although these cells can attack the tumor, the environment within the tumor is highly immunosuppressive, preventing immune-mediated tumour death.[60]

[0226] Multiple ways of producing and obtaining tumour targeted T-cells have been developed. T-cells specific to a tumor antigen can be removed from a tumor sample (TILs) or filtered from blood. Subsequent activation and culturing is performed ex vivo, with the results reinfused. Activation can take place through gene therapy, or by exposing the T cells to tumor antigens.

B. Chemotherapies

[0227] In some embodiments, the additional therapy comprises a chemotherapy. Suitable classes of chemotherapeutic agents include (a) Alkylating Agents, such as nitrogen mustards (e.g., mechlorethamine, cylophosphamide, ifosfamide, melphalan, chlorambucil), ethylenimines and methylmelamines (e.g., hexamethylmelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomustine, chlorozoticin, streptozocin) and

triazines (e.g., dicarbazine), (b) Antimetabolites, such as folic acid analogs (e.g., methotrexate), pyrimidine analogs (e.g., 5-fluorouracil, floxuridine, cytarabine, azauridine) and purine analogs and related materials (e.g., 6-mercaptopurine, 6-thioguanine, pentostatin), (c) Natural Products, such as vinca alkaloids (e.g., vinblastine, vincristine), epipodophylotoxins (e.g., etoposide, teniposide), antibiotics (e.g., dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin and mitoxanthrone), enzymes (e.g., L-asparaginase), and biological response modifiers (e.g., Interferon-α), and (d) Miscellaneous Agents, such as platinum coordination complexes (e.g., cisplatin, carboplatin), substituted ureas (e.g., hydroxyurea), methylhydiazine derivatives (e.g., procarbazine), and adreocortical suppressants (e.g., taxol and mitotane). In some embodiments, cisplatin is a particularly suitable chemotherapeutic agent.

[0228] Cisplatin has been widely used to treat cancers such as, for example, metastatic testicular or ovarian carcinoma, advanced bladder cancer, head or neck cancer, cervical cancer, lung cancer or other tumors. Cisplatin is not absorbed orally and must therefore be delivered via other routes such as, for example, intravenous, subcutaneous, intratumoral or intraperitoneal injection. Cisplatin can be used alone or in combination with other agents, with efficacious doses used in clinical applications including about 15 mg/m^2 to about 20 mg/m^2 for 5 days every three weeks for a total of three courses being contemplated in certain embodiments. In some embodiments, the amount of cisplatin delivered to the cell and/or subject in conjunction with the construct comprising an Egr-1 promoter operably linked to a polynucleotide encoding the therapeutic polypeptide is less than the amount that would be delivered when using cisplatin alone.

[0229] Other suitable chemotherapeutic agents include antimicrotubule agents, e.g., Paclitaxel ("Taxol") and doxorubicin hydrochloride ("doxorubicin"). The combination of an Egr-1 promoter/TNFα construct delivered via an adenoviral vector and doxorubicin was determined to be effective in overcoming resistance to chemotherapy and/or TNF-α, which suggests that combination treatment with the construct and doxorubicin overcomes resistance to both doxorubicin and TNF-α.

[0230] Doxorubicin is absorbed poorly and is preferably administered intravenously. In certain embodiments, appropriate intravenous doses for an adult include about 60 mg/m^2 to about 75 mg/m^2 at about 21-day intervals or about 25 mg/m^2 to about 30 mg/m^2 on each of 2 or 3 successive days repeated at about 3 week to about 4 week intervals or about 20 mg/m^2 once a week. The lowest dose should be used in elderly patients, when there is prior bone-marrow depression caused by prior chemotherapy or neoplastic marrow invasion, or when the drug is combined with other myelopoietic suppressant drugs.

[0231] Nitrogen mustards are another suitable chemotherapeutic agent useful in the methods of the disclosure. A nitrogen mustard may include, but is not limited to, mechlorethamine (HN_2), cyclophosphamide and/or ifosfamide, melphalan (L-sarcolysin), and chlorambucil. Cyclophosphamide (CYTOXAN®) is available from Mead Johnson and NEOSTAR® is available from Adria), is another suitable chemotherapeutic agent. Suitable oral doses for adults include, for example, about 1 mg/kg/day to about 5 mg/kg/day, intravenous doses include, for example, initially about 40 mg/kg to about 50 mg/kg in divided doses over a period of about 2 days to about 5 days or about 10 mg/kg to about 15 mg/kg about every 7 days to about 10 days or about 3 mg/kg to about 5 mg/kg twice a week or about 1.5 mg/kg/day to about 3 mg/kg/day. Because of adverse gastrointestinal effects, the intravenous route is preferred. The drug also sometimes is administered intramuscularly, by infiltration or into body cavities.

[0232] Additional suitable chemotherapeutic agents include pyrimidine analogs, such as cytarabine (cytosine arabinoside), 5-fluorouracil (fluouracil; 5-FU) and floxuridine (fluorode-oxyuridine; FudR). 5-FU may be administered to a subject in a dosage of anywhere between about 7.5 to about 1000 mg/m2. Further, 5-FU dosing schedules may be for a variety of time periods, for example up to six weeks, or as determined by one of ordinary skill in the art to which this disclosure pertains.

[0233] Gemcitabine diphosphate (GEMZAR®, Eli Lilly & Co., "gemcitabine"), another suitable chemotherapeutic agent, is recommended for treatment of advanced and metastatic pancreatic cancer, and will therefore be useful in the present disclosure for these cancers as well.

[0234] The amount of the chemotherapeutic agent delivered to the patient may be variable. In one suitable embodiment, the chemotherapeutic agent may be administered in an amount effective to cause arrest or regression of the cancer in a host, when the chemotherapy is administered with the construct. In other embodiments, the chemotherapeutic agent may be administered in an amount that is anywhere between 2 to 10,000 fold less than the chemotherapeutic effective dose of the chemotherapeutic agent. For example, the chemotherapeutic agent may be administered in an amount that is about 20 fold less, about 500 fold less or even about 5000 fold less than the chemotherapeutic effective dose of the chemotherapeutic agent. The chemotherapeutics of the disclosure can be tested in vivo for the desired therapeutic activity in combination with the construct, as well as for determination of effective dosages. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including, but not limited to, rats, mice, chicken, cows,

monkeys, rabbits, etc. In vitro testing may also be used to determine suitable combinations and dosages, as described in the examples.

C. Radiotherapy

[0235] In some embodiments, the additional therapy or prior therapy comprises radiation, such as ionizing radiation. As used herein, "ionizing radiation" means radiation comprising particles or photons that have sufficient energy or can produce sufficient energy via nuclear interactions to produce ionization (gain or loss of electrons). An exemplary and preferred ionizing radiation is an x-radiation. Means for delivering x-radiation to a target tissue or cell are well known in the art.

D. Surgery

[0236] In some embodiments, the additional therapy comprises surgery. Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present embodiments, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[0237] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months (or any range derivable therein). These treatments may be of varying dosages as well.

X. Formulations and Culture of the Cells

[0238] In particular embodiments, the cells of the disclosure may be specifically formulated and/or they may be cultured in a particular medium. The cells may be formulated in such a manner as to be suitable for delivery to a recipient without deleterious effects.

[0239] The medium in certain aspects can be prepared using a medium used for culturing animal cells as their basal medium, such as any of AIM V, X-VIVO-15, NeuroBasal, EGM2, TeSR, BME, BGJb, CMRL 1066, Glasgow MEM, Improved MEM Zinc Option, IMDM, Medium 199, Eagle MEM, αMEM, DMEM, Ham, RPMI-1640, and Fischer's media, as well as any combinations thereof, but the medium may not be particularly limited thereto as far as it can be used for culturing animal cells. Particularly, the medium may be xeno-free or chemically defined.

[0240] The medium can be a serum-containing or serum-free medium, or xeno-free medium. From the aspect of preventing contamination with heterogeneous animal-derived components, serum can be derived from the same animal as that of the stem cell(s). The serum-free medium refers to medium with no unprocessed or unpurified serum and accordingly, can include medium with purified blood-derived components or animal tissue-derived components (such as growth factors).

[0241] The medium may contain or may not contain any alternatives to serum. The alternatives to serum can include materials which appropriately contain albumin (such as lipid-rich albumin, bovine albumin, albumin substitutes such as recombinant albumin or a humanized albumin, plant starch, dextrans and protein hydrolysates), transferrin (or other iron transporters), fatty acids, insulin, collagen precursors, trace elements, 2-mercaptoethanol, 3'-thiolgiycerol, or equivalents thereto. The alternatives to serum can be prepared by the method disclosed in International Publication No. 98/30679, for example (incorporated herein in its entirety). Alternatively, any commercially available materials can be used for more convenience. The commercially available materials include knockout Serum Replacement (KSR), Chemically-defined Lipid concentrated (Gibco), and Glutamax (Gibco).

[0242] In certain embodiments, the medium may comprise one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more of the following: Vitamins such as biotin; DL Alpha Tocopherol Acetate; DL Alpha-Tocopherol; Vitamin A (acetate); proteins such as BSA (bovine serum albumin) or human albumin, fatty acid free Fraction V; Catalase; Human Recombinant Insulin; Human Transferrin; Superoxide Dismutase; Other Components such as Corticosterone; D-Galactose; Ethanolamine HCl; Glutathione (reduced);

L-Carnitine HCl; Linoleic Acid; Linolenic Acid; Progesterone; Putrescine 2HCl; Sodium Selenite; and/or T3 (triodo-I-thyronine). . In specific embodiments, one or more of these may be explicitly excluded.

[0243] In some embodiments, the medium further comprises vitamins. In some embodiments, the medium comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 of the following (and any range derivable therein): biotin, DL alpha tocopherol acetate, DL alpha-tocopherol, vitamin A, choline chloride, calcium pantothenate, pantothenic acid, folic acid nicotinamide, pyridoxine, riboflavin, thiamine, inositol, vitamin B12, or the medium includes combinations thereof or salts thereof. In some embodiments, the medium comprises or consists essentially of biotin, DL alpha tocopherol acetate, DL alpha-tocopherol, vitamin A, choline chloride, calcium pantothenate, pantothenic acid, folic acid nicotinamide, pyridoxine, riboflavin, thiamine, inositol, and vitamin B12. In some embodiments, the vitamins include or consist essentially of biotin, DL alpha tocopherol acetate, DL alpha-tocopherol, vitamin A, or combinations or salts thereof. In some embodiments, the medium further comprises proteins. In some embodiments, the proteins comprise albumin or bovine serum albumin, a fraction of BSA, catalase, insulin, transferrin, superoxide dismutase, or combinations thereof. In some embodiments, the medium further comprises one or more of the following: corticosterone, D-Galactose, ethanolamine, glutathione, L-carnitine, linoleic acid, linolenic acid, progesterone, putrescine, sodium selenite, or triodo-I-thyronine, or combinations thereof. In some embodiments, the medium comprises one or more of the following: a B-27® supplement, xeno-free B-27® supplement, GS21™ supplement, or combinations thereof. In some embodiments, the medium comprises or futher comprises amino acids, monosaccharides, inorganic ions. In some embodiments, the amino acids comprise arginine, cystine, isoleucine, leucine, lysine, methionine, glutamine, phenylalanine, threonine, tryptophan, histidine, tyrosine, or valine, or combinations thereof. In some embodiments, the inorganic ions comprise sodium, potassium, calcium, magnesium, nitrogen, or phosphorus, or combinations or salts thereof. In some embodiments, the medium further comprises one or more of the following: molybdenum, vanadium, iron, zinc, selenium, copper, or manganese, or combinations thereof. In certain embodiments, the medium comprises or consists essentially of one or more vitamins discussed herein and/or one or more proteins discussed herein, and/or one or more of the following: corticosterone, D-Galactose, ethanolamine, glutathione, L-carnitine, linoleic acid, linolenic acid, progesterone, putrescine, sodium selenite, or triodo-I-thyronine, a B-27® supplement, xeno-free B-27® supplement, GS21™ supplement, an amino acid (such as arginine, cystine, isoleucine, leucine, lysine, methionine, glutamine, phenylalanine, threonine, tryptophan, histidine, tyrosine, or valine),

monosaccharide, inorganic ion (such as sodium, potassium, calcium, magnesium, nitrogen, and/or phosphorus) or salts thereof, and/or molybdenum, vanadium, iron, zinc, selenium, copper, or manganese. In specific embodiments, one or more of these may be explicitly excluded.

[0244] The medium can also contain one or more externally added fatty acids or lipids, amino acids (such as non-essential amino acids), vitamin(s), growth factors, cytokines, antioxidant substances, 2-mercaptoethanol, pyruvic acid, buffering agents, and/or inorganic salts. . In specific embodiments, one or more of these may be explicitly excluded.

[0245] One or more of the medium components may be added at a concentration of at least, at most, or about 0.1, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 180, 200, 250 ng/L, ng/ml, μg/ml, mg/ml, or any range derivable therein.

[0246] In specific embodiments, the cells of the disclosure are specifically formulated. They may or may not be formulated as a cell suspension. In specific cases they are formulated in a single dose form. They may be formulated for systemic or local administration. In some cases the cells are formulated for storage prior to use, and the cell formulation may comprise one or more cryopreservation agents, such as DMSO (for example, in 5% DMSO). The cell formulation may comprise albumin, including human albumin, with a specific formulation comprising 2.5% human albumin. The cells may be formulated specifically for intravenous administration; for example, they are formulated for intravenous administration over less than one hour. In particular embodiments the cells are in a formulated cell suspension that is stable at room temperature for 1, 2, 3, or 4 hours or more from time of thawing.

[0247] In some embodiments, the method further comprises priming the T cells. In some embodiments, the T cells are primed with antigen presenting cells. In some embodiments, the antigen presenting cells present tumor antigens or peptides, such as those disclosed herein.

[0248] In particular embodiments, the cells of the disclosure comprise an exogenous TCR, which may be of a defined antigen specificity. In some embodiments, the TCR can be selected based on absent or reduced alloreactivity to the intended recipient (examples include certain virus-specific TCRs, xeno-specific TCRs, or cancer-testis antigen-specific TCRs). In the example where the exogenous TCR is non-alloreactive, during T cell differentiation the exogenous TCR suppresses rearrangement and/or expression of endogenous TCR loci through a developmental process called allelic exclusion, resulting in T cells that express only the non-alloreactive exogenous TCR and are thus non-alloreactive. In some embodiments, the choice of exogenous TCR may not necessarily be defined based on lack of alloreactivity. In some embodiments, the endogenous TCR genes have been modified by genome editing so that they

do not express a protein. Methods of gene editing such as methods using the CRISPR/Cas9 system are known in the art and described herein.

[0249] In some embodiments, the cells of the disclosure further comprise one or more chimeric antigen receptors (CARs). Examples of tumor cell antigens to which a CAR may be directed include at least 5T4, 8H9, $\alpha_v\beta_6$ integrin, BCMA, B7-H3, B7-H6, CAIX, CA9, CD19, CD20, CD22, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD70, CD123, CD138, CD171, CEA, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EGP2, EGP40, ERBB3, ERBB4, ErbB3/4, EPCAM, EphA2, EpCAM, folate receptor-a, FAP, FBP, fetal AchR, FRα, GD2, G250/CAIX, GD3, Glypican-3 (GPC3), Her2, IL-13Rα2, Lambda, Lewis-Y, Kappa, KDR, MAGE, MCSP, Mesothelin, Muc1, Muc16, NCAM, NKG2D Ligands, NY-ESO-1, PRAME, PSC1, PSCA, PSMA, ROR1, SP17, Survivin, TAG72, TEMs, carcinoembryonic antigen, HMW-MAA, AFP, CA-125, ETA, Tyrosinase, MAGE, laminin receptor, HPV E6, E7, BING-4, Calcium-activated chloride channel 2, Cyclin-B1, 9D7, EphA3, Telomerase, SAP-1, BAGE family, CAGE family, GAGE family, MAGE family, SAGE family, XAGE family, NY-ESO-1/LAGE-1, PAME, SSX-2, Melan-A/MART-1, GP100/pmel17, TRP-1/-2, P. polypeptide, MC1R, Prostate-specific antigen, β-catenin, BRCA1/2, CML66, Fibronectin, MART-2, TGF-βRII, or VEGF receptors (*e.g.*, VEGFR2), for example. The CAR may be a first, second, third, or more generation CAR. The CAR may be bispecific for any two nonidentical antigens, or it may be specific for more than two nonidentical antigens.

XI. Administration of Therapeutic Compositions

[0250] The therapy provided herein may comprise administration of a combination of therapeutic agents, such as a first cancer therapy and a second cancer therapy. The therapies may be administered in any suitable manner known in the art. For example, the first and second cancer treatment may be administered sequentially (at different times) or concurrently (at the same time). In some embodiments, the first and second cancer treatments are administered in a separate composition. In some embodiments, the first and second cancer treatments are in the same composition.

[0251] Embodiments of the disclosure relate to compositions and methods comprising therapeutic compositions. The different therapies may be administered in one composition or in more than one composition, such as 2 compositions, 3 compositions, or 4 compositions. Various combinations of the agents may be employed.

[0252] The therapeutic agents of the disclosure may be administered by the same route of administration or by different routes of administration. In some embodiments, the cancer therapy is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the antibiotic is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. The appropriate dosage may be determined based on the type of disease to be treated, severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

[0253] The treatments may include various "unit doses." Unit dose is defined as containing a predetermined-quantity of the therapeutic composition. The quantity to be administered, and the particular route and formulation, is within the skill of determination of those in the clinical arts. A unit dose need not be administered as a single injection but may comprise continuous infusion over a set period of time. In some embodiments, a unit dose comprises a single administrable dose.

[0254] The quantity to be administered, both according to number of treatments and unit dose, depends on the treatment effect desired. An effective dose is understood to refer to an amount necessary to achieve a particular effect. In the practice in certain embodiments, it is contemplated that doses in the range from 10 mg/kg to 200 mg/kg can affect the protective capability of these agents. Thus, it is contemplated that doses include doses of about 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, and 200, 300, 400, 500, 1000 μg/kg, mg/kg, μg/day, or mg/day or any range derivable therein. Furthermore, such doses can be administered at multiple times during a day, and/or on multiple days, weeks, or months.

[0255] In certain embodiments, the effective dose of the pharmaceutical composition is one which can provide a blood level of about 1 μM to 150 μM. In another embodiment, the effective dose provides a blood level of about 4 μM to 100 μM.; or about 1 μM to 100 μM; or about 1 μM to 50 μM; or about 1 μM to 40 μM; or about 1 μM to 30 μM; or about 1 μM to 20 μM; or about 1 μM to 10 μM; or about 10 μM to 150 μM; or about 10 μM to 100 μM; or about 10 μM to 50 μM; or about 25 μM to 150 μM; or about 25 μM to 100 μM; or about 25 μM to 50 μM; or about 50 μM to 150 μM; or about 50 μM to 100 μM (or any range derivable therein).

In other embodiments, the dose can provide the following blood level of the agent that results from a therapeutic agent being administered to a subject: about, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 μM or any range derivable therein. In certain embodiments, the therapeutic agent that is administered to a subject is metabolized in the body to a metabolized therapeutic agent, in which case the blood levels may refer to the amount of that agent. Alternatively, to the extent the therapeutic agent is not metabolized by a subject, the blood levels discussed herein may refer to the unmetabolized therapeutic agent.

[0256] Precise amounts of the therapeutic composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the patient, the route of administration, the intended goal of treatment (alleviation of symptoms versus cure) and the potency, stability and toxicity of the particular therapeutic substance or other therapies a subject may be undergoing.

[0257] It will be understood by those skilled in the art and made aware that dosage units of μg/kg or mg/kg of body weight can be converted and expressed in comparable concentration units of μg/ml or mM (blood levels), such as 4 μM to 100 μM. It is also understood that uptake is species and organ/tissue dependent. The applicable conversion factors and physiological assumptions to be made concerning uptake and concentration measurement are well-known and would permit those of skill in the art to convert one concentration measurement to another and make reasonable comparisons and conclusions regarding the doses, efficacies and results described herein.

XII. Kits

[0258] Certain aspects of the present invention also concern kits containing compositions of the disclosure or compositions to implement methods of the invention. In some embodiments, kits can be used to evaluate one or more biomarkers or HLA types. In certain embodiments, a kit contains, contains at least or contains at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 100, 500, 1,000 or more probes, primers or primer sets, synthetic molecules or inhibitors, or any value or range and combination derivable therein.

[0259] Kits may comprise components, which may be individually packaged or placed in a container, such as a tube, bottle, vial, syringe, or other suitable container means.

[0260] Individual components may also be provided in a kit in concentrated amounts; in some embodiments, a component is provided individually in the same concentration as it would be in a solution with other components. Concentrations of components may be provided as 1x, 2x, 5x, 10x, or 20x or more.

[0261] In certain aspects, negative and/or positive control nucleic acids, probes, and inhibitors are included in some kit embodiments. In addition, a kit may include a sample that is a negative or positive control for methylation of one or more biomarkers.

[0262] It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein and that different embodiments may be combined. The claims originally filed are contemplated to cover claims that are multiply dependent on any filed claim or combination of filed claims.

XIII. Sequences

[0263] E3 ubiquitin-protein ligase TRIM11 isoform X2 [Homo sapiens]; NCBI Reference Sequence: XP_016857901.1:

MAAPDLSTNLQEEATCAICLDYFTDPVMTDCGHNFCRECIRRCWGQPEGPYACPEC
RELSPQRNLRPNRPLAKMAEMARRLHPPSPVPQGVCPAHREPLAAFCGDELRLLCAA
CERSGEHWAHRVRPLQDAAEDLKAKLEKSLEHLRKQMQDALLFQAQADETCVLW
QDIKDALRRVQDVKLQPPEVVPMELRTVCRVPGLVETLRRFRGDVTLDPDTANPELI
LSEDRRSVQRGDLRQALPDSPERFDPGPCVLGQERFTSGRHYWEVEVGDRTSWALG
VCRENVNRKEKGELSAGNGFWILVFLGSYYNSSERALAPLRDPPRRVGIFLDYEAGH
LSFYSATDGSLLFIFPEIPFSGTLRPLFSPLSSSPTPMTICRPKGGSGDTLAPQ (SEQ ID NO:1)

[0264] Peptide embodiments of the TRIM11 gene comprise: DETCVLWQD (SEQ ID NO:7), VLWQDIKDAL (SEQ ID NO:8), and LWQDIKDAL (SEQ ID NO:9)

[0265] RCOR3 protein [Homo sapiens]; GenBank: AAH31608.1:

MPGMMEKGPELLGKNRSANGSAKSPAGGGGSGASSTNGGLHYSEPESGCSSDDEHD
VGMRVGAEYQARIPEFDPGATKYTDKDNGGMLVWSPYHSIPDARLDEYIAIAKEKH
GYNVEQALGMLFWHKHNIEKSLADLPNFTPFPDEWTVEDKVLFEQAFSFHGKSFHRI
QQMLPDKTIASLVKYYYSWKKTRSRTSLMDRQARKLANRHNQGDSDDDVEETHPM
DGNDSDYDPKKEAKKEGNTEQPVQTSKIGLGRREYQSLQHRHHSQRSKCRPPKGMY

LTQEDVVAVSCSPNAANTILRQLDMELISLKRQVQNAKQVNSALKQKMEGGIEEFKP
PESNQKINARWTTEEQLLAVQGTDPTGSSDTGSITSCPIIHSNTNSPYCHSEPASTTSSS
NTACCPGSSPAASSTPAAGSVHPAPANFKSASTTSYSPC (SEQ ID NO:2)

[0266] Peptide embodiments of the RCOR3 gene comprise: LAVQGTDPT (SEQ ID NO:10).

[0267] Protein FAM76B isoform 1 [Homo sapiens]; NCBI Reference Sequence: NP_653265.3:

MAASALYACTKCTQRYPFEELSQGQQLCKECRIAHPIVKCTYCRSEFQQESKTNTICK
KCAQNVKQFGTPKPCQYCNIIAAFIGTKCQRCTNSEKKYGPPQTCEQCKQQCAFDRK
EEGRRKVDGKLLCWLCTLSYKRVLQKTKEQRKSLGSSHSNSSSSSLTEKDQHHPKH
HHHHHHHHHRHSSSHHKISNLSPEEEQGLWKQSHKSSATIQNETPKKKPKLESKPSN
GDSSSINQSADSGGTDNFVLISQLKEEVMSLKRLLQQRDQTILEKDKKLTELKADFQY
QESNLRTKMNSMEKAHKETVEQLQAKNRELLKQVAALSKGKKFDKSGSILTSP
(SEQ ID NO:3)

[0268] Peptide embodiments of the FAM76B gene comprise: PSNGDSSSI (SEQ ID NO:11) and KPSNGDSSSI (SEQ ID NO:12)

[0269] Sarcolemmal membrane-associated protein isoform f [Homo sapiens]; NCBI Reference Sequence: NP_001298107.1:

MDEQDLNEPLAKVSLLKDDLQGAQSEIEAKQEIQHLRKELIEAQELARTSKQKCFEL
QALLEEERKAYRNQVEESTKQIQVLQAQLQRLHIDTENLREEKDSEITSTRDELLSAR
DEILLLHQAAAKVASERDTDIASLQEELKKVRAELERWRKAASEYEKEITSLQNSFQL
RCQQCEDQQREEATRLQGELEKLRKEWNALETECHSLKRENVLLSSELQRQEKELH
NSQKQSLELTSDLSILQMSRKELENQVGSLKEQHLRDSADLKTLLSKAENQAKDVQK
EYEKTQTVLSELKLKFEMTEQEKQSITDELKQCKNNLKLLREKGNNPSILQPVPAVFI
GLFLAFLFWCFGPLW (SEQ ID NO:4)

[0270] Peptide embodiments of the SLMAP gene comprise: NNPSILQPV (SEQ ID NO:13), REKGNNPSI (SEQ ID NO:14), and REKGNNPSIL (SEQ ID NO:15)

[0271] Transmembrane protein 62 isoform a [Homo sapiens]; NCBI Reference Sequence: P_079232.3:

[0272] MAAVLALRVVAGLAAAAALVAMLLEHYGLAGQPSPLPRPAPPRRPHPAPGP
GDSNIFWGLQISDIHLSRFRDPGRAVDLEKFCSETIDIIQPALVLATGDLTDAKTKEQL
GSRQHEVEWQTYQGILKKTRVMEKTKWLDIKGNHDAFNIPSLDSIKNYYRKYSAVR
RDGSFHYVHSTPFGNYSFICVDATVNPGPKRPYNFFGILDKKKMEELLLLAKESSRSN
HTIWFGHFTTSTILSPSPGIRSIMSSAIAYLCGHLHTLGGLMPVLHTRHFQGTLELEVG
DWKDNRRYRIFAFDHDLFSFADLIFGKWPVVLITNPKSLLYSCGEHEPLERLLHSTHI

RVLAFSLSSITSVTVKIDGVHLGQAVHVSGPIFVLKWNPRNYSSGTHNIEVIVQDSAG
RSKSVHHIFSVQENNHLSFDPLASFILRTDHYIMARVLFVLIVLSQLTILIIFRYRGYPE
LKEPSGFINLTSFSLHVLSKINIFYYSVLLLTLYTVLGPWFFGEIIDGKFGCCFSFGIFVN
GHFLQGSITFIIGILQLAFFNIPLMAYMCWSLLQRCFGHNFRSHLHQRKYLKIMPVHL
LMLLLYIWQVYSCYFLYATYGTLAFLFSPLRTWLTLLTPVLIRYVWTLNSTKFGIFM
VQLKSHLSS (SEQ ID NO:5)

[0273] Peptide embodiments of the TMEM62 gene comprise: YTVLGPWFF (SEQ ID NO:16), TLYTVLGPW (SEQ ID NO:17), TLYTVLGPWF (SEQ ID NO:18), LYTVLGPWF (SEQ ID NO:19), LTLYTVLGPW (SEQ ID NO:20), LYTVLGPWFF (SEQ ID NO:21), and VLGPWFFGEI (SEQ ID NO:22).

[0274] PLA2G6 [Homo sapiens]; GenBank: CAG30429.1:
MQFFGRLVNTFSGVTNLFSNPFRVKEVAVADYTSSDRVREEGQLILFQNTPNRTWDC
VLVNPRNSQSGFRLFQLELEADALVNFHQYSSQLLPFYESSPQVLHTEVLQHLTDLIR
NHPSWSVAHLAVELGIRECFHHSRIISCANCAENEEGCTPLHLACRKGDGEILVELVQ
YCHTQMDVTDYKGETVFHYAVQGDNSQVLQLLGRNAVAGLNQVNNQGLTPLHLA
CQLGKQEMVRVLLLCNARCNIMGPNGYPIHSAMKFSQKGCAEMIISMDSSQIHSKDP
RYGASPLHWAKNAEMARMLLKRGCNVNSTSSAGNTALHVAVMRNRFDCAIVLLTH
GANADARGEHGNTPLHLAMSKDNVEMIKALIVFGAEVDTPNDFGETPTFLASKIGRL
VTRKAILTLLRTVGAEYCFPPIHGVPAEQGSAAPHHPFSLERAQPPPISLNNLELQDLM
HISRARKPAFILGSMRDEKRTHDHLLCLDGGGVKGLIIIQLLIAIEKASGVATKDLFD
WVAGTSTGGILALAILHSKSMAYMRGMYFRMKDEVFRGSRPYESGPLEEFLKREFG
EHTKMTDVRKPKVMLTGTLSDRQPAELHLFRNYDAPETVREPRFNQNVNLRPPAQP
SDQLVWRAARSSGAAPTYFRPNGRFLDGGLLANNPTLDAMTEIHEYNQDLIRKGQA
NKVKKLSIVVSLGTGRSPQVPVTCVDVFRPSNPWELAKTVFGAKELGKMVVDCCTD
PDGRAVDRARAWCEMVGIQYFRLNPQLGTDIMLDEVSDTVLVNALWETEVYIYEHR
EEFQKLIQLLLSP (SEQ ID NO:6)

[0275] Peptide embodiments of the TMEM62 gene comprise: TFLASKIGRLV (SEQ ID NO:23), RLVTRKAIL (SEQ ID NO:24), FLASKIGRL (SEQ ID NO:25), SKIGRLVTRK (SEQ ID NO:26), FLASKIGRLV (SEQ ID NO:27), LASKIGRLV (SEQ ID NO:28), and KIGRLVTRK (SEQ ID NO:29).

[0276] TRA and TRB CDR3 sequences include those listed below: TRA-1 CDR3: CAVHEIQGAQKLVF (SEQ ID NO:30); TRB-1 CDR3: CASSFGVSYEQYF (SEQ ID NO:31); TRA-2 CDR3: CAMRPLGGYNKLIF (SEQ ID NO:32); TRB-2 CDR3: CASSQAANEQFF (SEQ ID NO:33); TRA-3 CDR3: CAEEGDRDYKLSF (SEQ ID NO:34);

TRB-3 CDR3: CASTGRSGRSEQYF (SEQ ID NO:35); TRA-4 CDR3: CAFMKGRDDKIIF (SEQ ID NO:36); TRB-4 CDR3: CATTLPGDTEAFF (SEQ ID NO:37); TRA-5 CDR3: CATANNAGNMLTF(SEQ ID NO:38); TRB-5 CDR3: CASSLDRHQPQHF (SEQ ID NO:39); TRA-6 CDR3: CALWEGQGGSEKLVF (SEQ ID NO:40); TRB-6 CDR3: CASSLEARAPSGNTIYF (SEQ ID NO:41); and TRA-7 CDR3: CAVGAGTGTASKLTF (SEQ ID NO:42); TRB-7 CDR3: CASSLELAGGRDTQYF (SEQ ID NO:43).

[0277] Additional peptide embodiments include those below:

Peptide Sequence	HLA	SEQ ID NO:
NTEPVKDPY	HLA-A*01:01	1364
YTYQMHGEY	HLA-A*01:01	1365
LLKIDSFELLY	HLA-A*01:01	1366
KIDSFELLYY	HLA-A*01:01	1367
LKIDSFELLYY	HLA-A*01:01	1368
WLEAVYCGCY	HLA-A*01:01	1369
WSVFSTSLY	HLA-A*01:01	1370
NLLTTCSTV	HLA-A*02:01	1371
FTVTVTEPL	HLA-A*02:01	1372
YLEAKADLV	HLA-A*02:01	1373
YLDQLNHILA	HLA-A*02:01	1374
FLQEPLQVFNV	HLA-A*02:01	1375
FLPRGTPAL	HLA-A*02:01	1376
RMAEHHSFWV	HLA-A*02:01	1377
HLLRIFCTI	HLA-A*02:01	1378
SLSEVDIPSI	HLA-A*02:01	1379
LILKGIFCTI	HLA-A*02:01	1380
STWGGFDEL	HLA-A*02:01	1381
TVTEPLLVK	HLA-A*03:01	1382
GLQTRAFWK	HLA-A*03:01	1383
ILLYKNKRK	HLA-A*03:01	1384
KGFSIDSGK	HLA-A*03:01	1395
LLQGDTPVRK	HLA-A*03:01	1385
SLDWETPSK	HLA-A*03:01	1386
KEMRPARAK	HLA-A*03:01	1387

RAVTNHSVYY	HLA-A*03:01	1388
SVYYPSECSK	HLA-A*03:01	1389
QLFEGMKAFK	HLA-A*03:01	1390
ALGQLFEGMK	HLA-A*03:01	1391
KMFRKLHNSY	HLA-A*03:01	1392
RLQARPRLGR	HLA-A*03:01	1393
RIPYKVVARR	HLA-A*03:01	1394
ISWMKGVPGK	HLA-A*03:01	786
KIDSFELLYY	HLA-A*03:01	1367
TVASSCSSPT	HLA-A*68:01	1396
YEQHNGVDGL	HLA-B*40:01	1397
ILKGIFCTI	HLA-A*32:01	1398
SGKSRPLPV	HLA-B*08:01	1399
QPSGKSRPL	HLA-B*07:05	1400
TQAPPPPERK	HLA-A*11:01	1401
SSDCRVSQI	HLA-C*15:05	1402

XIV. Examples

[0278] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1: Tumor antigens arising from alternative splicing events may be targetable by Tumor infiltrating lymphocytes in glioblastomas

[0279] Alternative splicing, the cellular process that converts premature mRNA to mature mRNA and allows for a single gene to produce multiple protein products, is frequently dysregulated in many cancers, including glioblastoma. However, along with non-synonymous mutations in the DNA, altered splicing mechanisms in cancers may produce novel tumor

antigens that distinguish cancer cells from healthy cells and can thus be targeted by the immune system.

[0280] Provided in FIG. 3 is an exemplary computational pipeline to identify antigenic peptides from RNA data of neoplastic tissue. The computational pipeline, referred to Isoform peptides from RNA splicing for Immunotherapy target Screening (IRIS), is an integrated package. As can be seen, the process has three major modules: (1) processing of RNA-Seq data, (2) *in silico* screening of splice isoforms, and (3) integrated prediction of TCR/CAR-T targets.

[0281] The inventors used the IRIS (Isoform peptides from RNA splicing for Immunotherapy targets Screening) platform to take bulk RNA-sequencing data from 23 glioblastoma patient tumor samples and predict tumor antigens that may arise from alternative splicing events. Predicted tumor antigens that arose in HLA*A02:01 and HLA*A03:01 patients were prioritized and 8 potential tumor antigens were selected to generate peptide:MHC Class 1 dextramers. The inventors tested PBMCs and/or ex vivo expanded tumor infiltrating lymphocytes (TIL) from 6 glioblastoma patients against these dextramers, sorted for any tumor antigen-reactive T cells, and performed single-cell RNA sequencing on the sorted population to determine the TCR sequence.

[0282] Among the 8 predicted tumor antigens tested, 7 of the tumor antigens were recognized by at least 1 patient's T cells. 1 HLA*A03:01 epitope was recognized in 3 of the 4 HLA*A03:01 patients and this epitope was highly positive in one of those patients' expanded TIL population, representing 1.7% of all CD3+ CD8+ cells. When the inventors sorted for those tumor antigens reactive T cells from the expanded TIL population and performed single-cell RNA sequencing, they found 325 unique T cell clonotypes, but the top 10 clonotypes represented 83.6% of all clonotypes, with the most frequent clonotype representing 39.1% of all clonotypes and indicating clonal expansion of a select few TCR clones from within the tumor.

[0283] In total, the data indicates that tumor antigens arising from alternative splicing events may represent a potential target for immunotherapy in glioblastoma.

Example 2: IRIS: Big data-informed discovery of cancer immunotherapy targets arising from pre-mRNA alternative splicing

[0284] Cancer immunotherapy has gained tremendous momentum in the past decade. The clinical effectiveness of checkpoint inhibitors, such as neutralizing antibodies against PD-1 and

CTLA-4, is thought to result from their ability to reactivate tumor-specific T cells (1). Meanwhile, adoptive cell therapies use genetically modified T-cell receptors (TCRs) or synthetic chimeric antigen receptor T cells (CAR-T) for tumor-specific antigen recognition (2). The finding that cancer cells express specific T-cell-reactive antigens has galvanized epitope discovery in recent years (3-6). Nevertheless, the identification of tumor antigens remains a major challenge (7,8). Although somatic mutation-derived antigens have been successfully targeted by cancer therapies (9-12), this approach remains largely ineffective for tumors with low or moderate mutation loads (7-13).

[0285] Various types of dysregulation at the RNA level can generate immunogenic peptides in cancer cells (13-15). Notably, tumors harbor up to 30% more alternative splicing (AS) events than normal tissues, and the resulting peptides are predicted to be presented by human leukocyte antigen (HLA) (16). However, there are no integrated methods to systematically identify AS-derived tumor antigens. Therefore, the inventors leveraged tens of thousands of normal and tumor transcriptomes generated by large-scale consortium studies (e.g. GTEx, TCGA) (17,18) to build a versatile, big data-informed platform for discovering AS-derived immunotherapy targets. This *in silico* platform, named 'IRIS' (Isoform peptides from RNA splicing for Immunotherapy target Screening), incorporates three main components: processing of RNA-Seq data, *in silico* screening of tumor AS isoforms, and integrated prediction and prioritization of TCR and CAR-T targets (FIG. 3).

[0286] IRIS's RNA-Seq data-processing module uses standard input data to discover and quantify AS events in tumors using the ultra-fast rMATS-turbo software (19,20). Identified AS events are fed to the *in silico* screening module, which statistically compares AS events against any combination of events selected from large-scale (>10,000) reference RNA-Seq samples of normal and tumor tissues (FIG. 6) to identify AS events that are tumor-associated, tumor-recurrent, and potentially tumor-specific (Methods). Tumor specificity is a key metric for evaluating potential tissue toxicity, which is an important side effect of targeting lineage-specific antigens that are expressed by both tumor and normal cells (21). In addition to screening multiple patient samples simultaneously in the default 'group mode', IRIS can be performed in the 'personalized mode' to identify targets for a specific patient sample (Methods). Potential false-positive events are removed by using a blacklist of AS events whose quantification across diverse RNA-Seq datasets is error-prone due to technical variances such as read length (Methods and FIG. 7). IRIS's target prediction module first constructs splice-junction peptides of predicted tumor isoforms and then predicts AS-derived targets for TCR/CAR-T therapies (Methods). This module performs tumor HLA typing using RNA-Seq

data and then integrates multiple HLA-binding prediction algorithms for predicting TCR targets and/or peptide vaccines. In parallel, protein extracellular domain annotations are used for predicting CAR-T targets (FIG. 8). IRIS also includes the option to confirm predicted AS-derived targets using mass spectrometry (MS) data via proteo-transcriptomics data integration. This option provides an orthogonal approach for target discovery and validation by integrating RNA-Seq data with various types of MS data, such as whole-cell proteomics, surfaceomics, or immunopeptidomics data (Methods and FIG. 9A).

[0287] The inventors performed a proof-of-concept analysis and preliminary confirmation of AS-derived epitopes by applying IRIS to RNA-Seq and MS-based immunopeptidomics data of cancer and normal cell lines. The inventors identified hundreds of AS-derived epitopes that were supported by both RNA-Seq and MS data (FIG. 9B, Table 1). MS-supported epitopes were enriched for transcripts with high expression levels and peptides with strong predicted HLA-binding affinities (FIG. 9C-E), consistent with the expected pattern of HLA-epitope binding (22).

[0288] To explore IRIS's ability to discover AS-derived immunotherapy targets in clinical samples, the inventors generated RNA-Seq data from 22 resected glioblastomas (GBMs) and analyzed these data by IRIS. Candidate epitopes were then validated based on their recognition by patient T cells. FIG. 4 (top) summarizes the stepwise IRIS results. After uniform processing of RNA-Seq data by rMATS-turbo, IRIS discovered 190,232 putative skipped exon (SE) events from the 22 GBM samples. Using the *in silico* screening module, the inventors compared these AS events against reference normal and tumor panels to evaluate tumor association, recurrence, and specificity (Methods). Specifically, AS events were compared against: normal brain samples from GTEx (tissue-matched normal panel, for evaluating tumor association), two cohorts of brain tumor samples - GBM and lower-grade glioma (LGG) - from TCGA (tumor panel, for evaluating tumor recurrence), and 11 other selected normal (nonbrain) tissues from GTEx (normal panel, for evaluating tumor specificity). After initially screening against the tissue-matched normal panel and removing blacklisted events, IRIS identified 6,276 tumor-associated AS events in the 22 GBM samples ('Primary' set, FIG. 4). Of these, 1,738 events were identified as tumor-recurrent and tumor-specific based on comparison with the tumor panel and normal panel, respectively ('Prioritized' set, FIG. 4; Table 2).

[0289] Next, for each AS event, splice junctions of the tumor isoform (i.e. the isoform that was more abundant in the tumor samples than in the tissue-matched normal panel) were translated into peptides, followed by TCR/CAR-T target prediction (FIG. 4). For the GBM dataset, IRIS predicted 4,153 'primary' tumor-associated epitope-producing splice junctions.

Of these, 1,127 were tumor-recurrent and tumor-specific compared to the tumor panel and normal panel, respectively, and were predicted to be 'prioritized' TCR targets. In parallel, IRIS identified 416 'primary' tumor-associated extracellular peptide-producing splice junctions, of which 87 were predicted to be 'prioritized' CAR-T targets.

[0290] IRIS generates an integrative report for predicted immunotherapy targets (Table 3). Representative examples for six prioritized TCR targets are shown in the bottom panel of FIG. 4 (see FIG. 8B for CAR-T target examples). Violin plots depict exon inclusion levels across the 22 GBM samples ('GBM-input') and different sets of reference panels using the percent-spliced-in (PSI) metric (23). Tumor isoforms can be either the exon-skipped (low PSI) or the exon-included (high PSI) isoform compared to the tissue-matched normal panel. As illustrated by the darker dots in the 'Summary' column, all six epitope-producing splice junctions were tumor-associated compared to the tissue-matched normal panel ('Brain'), and tumor-recurrent compared to the tumor panel ('GBM' and 'LGG'). Two AS events (in *TRIM11* and *FAM76B*) consistently showed distinct PSI values in tumors compared to normal brain and nonbrain tissues, indicating high tumor specificity. For candidate splice junctions, IRIS also calculates the fold-change (FC) of tumor isoforms between tumor samples and the tissue-matched normal panel (Methods). For example, the tumor isoform in *TRIM11* had an average isoform proportion of 8.60% in the 22 GBM samples and 0.13% in normal brain samples, representing an FC of 65.6 in tumor samples versus the tissue-matched normal panel. The inventors note that, as shown under 'Predicted HLA-epitope binding', a single splice junction can give rise to multiple putative epitopes with distinct peptide sequences and HLA binding affinities.

[0291] Finally, the inventors sought to validate the immunogenicity and T-cell recognition of IRIS-identified candidate TCR targets using an MHC class I dextramer-based assay (12,24). The inventors focused on predicted AS-derived tumor epitopes with strong putative HLA-binding affinity to common HLA types found in at least five of the 22 patients. The inventors selected seven AS-derived tumor-associated epitopes (five HLA-A02:01 and two HLA-A03:01) for dextramer-based T-cell recognition testing (Table 4). All but one epitope (YAIVWVNGV (SEQ ID NO:62)) showed some degree of tumor specificity when evaluated in normal (nonbrain) tissues ('vs. Normal', see FIG. 5A). The inventors obtained customized HLA-matched, fluorescently labeled MHC class I dextramer:peptide (pMHC) complexes for each candidate epitope. The inventors conducted flow cytometry to detect $CD8^+$ T-cell binding with the pMHC complexes using available peripheral blood mononuclear cells (PBMCs) and/or *ex vivo*-expanded tumor-infiltrating lymphocytes (TILs). Based on the binding of each AS-derived tumor epitope to a patient's $CD3^+CD8^+$ T cells, the inventors classified epitope

reactivity as 'positive' (binding > 0.1% of cells), 'marginal' (binding 0.01-0.1% of cells), or 'negative' (binding < 0.01% of cells). Epitopes that showed at least marginal reactivity were considered to be 'recognized' by patient T cells. The inventors analyzed samples from two HLA-A02:01 and four HLA-A03:01 patients, as well as samples from three HLA-A02:01 and three HLA-A03:01 healthy donors (Table 5, Supplementary Data).

[0292] Both predicted HLA-A03:01 tumor epitopes were recognized by patient T cells. In particular, one epitope (KIGRLVTRK (SEQ ID NO:29), in *PLA2G6*) was recognized by T cells from all four tested patients but only one of the three tested healthy donors. In one patient (LB2867), recognition of tumor epitope KIGRLVTRK (SEQ ID NO:29) was marginal in PBMCs but positive in the expanded TIL population, with epitope-reactive T cells representing 0.03% of T cells in PBMCs and 1.69% of T cells in TILs. This patient had been previously treated with neoadjuvant anti-PD-1 and anti-CTLA-4 checkpoint blockade immunotherapy. These results suggest epitope KIGRLVTRK (SEQ ID NO:29) as a promising immunotherapy target in HLA-A03 patients from the GBM cohort. T cells from another patient (LB2907) showed positive reactivity to both tested HLA-A03:01 epitopes. All four predicted HLA-A02:01 epitopes were recognized by T cells from tested patients and healthy donors. The non-tumor-specific epitope (YAIVWVNGV (SEQ ID NO:62), bottom row in FIG. 5A) was tested in two patients and three healthy donors and was recognized by T cells in only one healthy donor (marginal reactivity, 0.013% of $CD3^+CD8^+$ T cells). Taken together, the dextramer-based assay results indicate that the AS-derived TCR targets predicted by IRIS can be recognized by tumor-infiltrating and peripheral $CD3^+CD8^+$ T cells.

[0293] Dextramer-positive T cells are expected to contain many clonotypes, only a few of which are dominant. To discover and quantify which TCR clonotypes comprise the epitope-reactive T cells, the inventors sorted the TILs from one patient (LB2867) for cells that reacted positively with the KIGRLVTRK (SEQ ID NO:29) pMHC complex (FIG. 5B), and performed V(D)J immune profiling using single-cell RNA-Seq (scRNA-Seq) on the sorted population (FIG. 5C). Of the 325 unique TCR clonotypes, the 10 most abundant TCRs represented 86.3% of all clonotypes (Table 6), with the most frequent clonotype comprising 38.9% of all epitope-reactive T cells. This result suggests that there was clonal expansion of a select few dominant TCR clones within the tumor that were able to recognize the AS-derived epitope. To further validate the inventors' findings using complementary approaches, the inventors analyzed bulk expanded TILs using immunoSEQ and pairSEQ assays (FIG. 5C, FIG. 10). The inventors confirmed that the top 10 reported clonotypes from scRNA-Seq were present in the bulk TIL population based on the TCR β-chain CDR3 region. In addition, the pairSEQ assay, which uses

statistical modeling to predict pairing of TCR α and β chains, found identically paired TCRs for seven of the top 10 TCRs from scRNA-Seq. Together, these data suggest that a select few TCR clones dominantly recognize the AS-derived epitope KIGRLVTRK (SEQ ID NO:29) in this patient.

[0294] In summary, the inventors have developed IRIS, a big data-powered platform for discovering AS-derived tumor antigens as an underexploited source of immunotherapy targets. Using IRIS followed by a dextramer-based assay, the inventors discovered and validated AS-derived tumor epitopes recognized by T cells in patients. These results provide experimental evidence for the immunogenicity of tumor antigens arising from AS and reveal novel potential targets for TCR and CAR-T therapies.

1. Methods

[0295] IRIS module for RNA-Seq data processing. IRIS accepts standard formats of raw RNA-Seq FASTQ files and/or tab-delimited files of quantified AS events (from rMATS-turbo) as input data (FIG. 3). For raw RNA-Seq data, IRIS provides a standalone pipeline that aligns RNA-Seq reads to the reference human genome hg19 using the STAR 2.5.3a (25) two-pass mode, followed by Cufflinks v2.2.1 (26) and rMATS v4.0.2 (rMATS-turbo) (19,20) for quantification of gene expression and AS events, respectively, based on the GENCODE (V26) (27) gene annotation. To quantify AS events, the inventors converted splice-junction counts in rMATS-turbo output into PSI (23) values. For each dataset, the inventors removed low-coverage AS events, defined as events with an average count of less than 10 reads for the sum of all splice junctions across all samples in that dataset (tissue/tumor type). The inventors applied this procedure to the 22 GBM samples from the UCLA cohort (BioProject: PRJNA577155), as well as to the normal and tumor samples of the reference panels used by IRIS. For the GTEx normal samples, aligned BAM files downloaded from the dbGAP repository were used directly for AS quantification.

[0296] Constructing big-data reference panels of AS events across normal human tissues and tumor samples. IRIS's big-data reference panels of normal and tumor samples are available as pre-processed, pre-indexed databases for fast retrieval by the IRIS program (FIG. 6). Specifically, 9,662 normal samples from the GTEx project (V7) (17) representing 53 tissue types of 30 histological sites were uniformly processed as described above. As shown in FIG. 6, exon-based quantification of AS events was able to distinguish samples by tissue type. Selected TCGA (16,28) tumor samples (FIG. 6C) were processed similarly to form the tumor

panel. Additionally, IRIS provides a stand-alone indexing function for users to include custom normal and tumor samples in their reference panels.

[0297] IRIS module for *in silico* screening of tumor AS events. IRIS performs *in silico* screening using two-sided and one-sided *t*-tests to identify tumor-associated, tumor-recurrent, and tumor-specific AS events in group comparisons. To define an AS event as significantly different from a reference group (i.e., to identify tumor-associated/tumor-specific events), IRIS sets two requirements: 1) a significant p-value from the two-sided *t*-test (default: $p < 0.01$), and 2) a threshold of PSI value difference (default: $abs(\Delta\psi) > 0.05$). With a slight modification, to define an AS event as recurrent in a reference group (tumor-recurrent events), IRIS compares a tumor reference group with the tissue-matched normal panel and requires: 1) a significant p-value from the one-sided *t*-test in the same direction as the corresponding 'tumor-associated' event (default: $p < 0.01$/number of 'tumor-associated' events [Bonferroni correction due to large sample sizes in reference panels]), and 2) a threshold of PSI value difference (default: $abs(\Delta\psi) > 0.05$). In addition, a threshold of the number of significant comparisons against groups in the normal or tumor reference panel is used to determine whether AS-derived antigens are tumor-specific or tumor-recurrent. For each AS event, IRIS defines the 'tumor isoform' as the isoform that is more abundant in tumors than in the tissue-matched normal panel. Optionally, to rank or filter targets, IRIS estimates the 'fold-change (FC) of tumor isoform' as the FC of the tumor isoform's proportion in tumors compared to the tissue-matched normal panel. In addition to the default 'group mode', IRIS can be used to screen targets for a specific patient sample through the 'personalized mode'. This mode uses an outlier detection approach, combining a modified Tukey's rule (29) and a user-defined threshold of PSI value difference.

[0298] Identification of AS events that are prone to measurement errors due to technical variances across big-data reference panels. IRIS's big-data reference panels were constructed by integrating various large-scale datasets with distinct technical conditions, such as RNA-Seq read length (30). Such technical variances across datasets could introduce discrepancies in the quantification of AS events (30). To identify error-prone AS events, the inventors employed a data-based heuristic strategy to assess the effects of RNA-Seq read length (48 bp vs. 76 bp) and aligner (STAR vs. Tophat) on AS quantification (PSI value) (FIG. 7A). For a given tissue type (in this study, brain tissue), 10 randomly selected 76-bp RNA-Seq files from GTEx were artificially trimmed to 48 bp, and both 76- and 48-bp RNA-Seq files were aligned with STAR2.5.3a. Corresponding Tophat (v.1.4.1)-aligned 76-bp BAM files were directly downloaded from GTEx. AS events were quantified by rMATS-turbo. Events with

significantly different PSI values ($p < 0.05$, $abs(\Delta\psi) > 0.05$ from paired *t*-test) among RNA-Seq datasets with distinct technical conditions were included in a blacklist. Results of this analysis for GTEx normal brain samples are shown in FIG. 7B.

[0299] IRIS module for predicting AS-derived TCR and CAR-T targets. To obtain protein sequences of AS-derived tumor isoforms, IRIS generates peptides by translating splice-junction sequences into amino-acid sequences using known ORFs from the UniProtKB (31) database. Within each AS event, the splice-junction peptide sequence for the tumor isoform is compared to that of the alternative normal isoform, to ensure that the tumor isoform splice junction produces a distinct peptide.

[0300] For TCR target prediction, IRIS employs seq2HLA (32), which uses RNA-Seq data to characterize HLA class I alleles for each tumor sample. IRIS then uses IEDB API (33) predictors to obtain the putative HLA binding affinities of candidate epitopes. The IEDB 'recommended' mode runs several prediction tools to generate multiple predictions of binding affinity, which IRIS summarizes as a median IC_{50} value. By default, a threshold of median(IC_{50}) < 500 nM denotes a positive prediction for an AS-derived TCR target.

[0301] For CAR-T target prediction, IRIS maps AS-derived tumor isoforms to known protein extracellular domains (ECDs), as potential candidates for CAR-T therapy (FIG. 8A). Specifically, IRIS generates pre-computed annotations of protein ECDs. First, protein cellular localization information was retrieved from the UniProtKB (31) database (flat file downloaded in April 2018). ECD information was retrieved by searching for the term 'extracellular' in topological annotation fields, including 'TOPO_DOM', 'TRANSMEM', and 'REGION', in the flat file. Second, BLAST (34) was used to map individual exons in the gene annotation (GENCODE V26) to proteins with topological annotations. Third, the BLAST result was parsed to create annotations of the mapping between exons and ECDs in proteins. These pre-computed annotations are queried to search for AS-derived peptides that can be mapped to protein ECDs as potential CAR-T targets.

[0302] Proteo-transcriptomics data integration for MS validation. IRIS includes an optional proteo-transcriptomics data integration function that incorporates various types of MS data, such as whole-cell proteomics, surfaceomics, or immunopeptidomics data, to validate RNA-Seq-based target discovery at the protein level (FIG. 9A). Specifically, sequences of AS-derived peptides are added to canonical and isoform sequences of the reference human proteome (downloaded from UniProtKB in September 2018). For immunopeptidomics data, fragment MS spectra are searched against the RNA-Seq-based custom proteome library with no enzyme specificity using MSGF+[35]. The search length is limited to 7-15 amino acids. The

target-decoy approach is employed to control the false discovery rate (FDR) or 'QValue' at 5%.

[0303] IRIS analysis of immunopeptidomics data. Published matching RNA-Seq and MS immunopeptidomics data of B-LCL-S1 and B-LCL-S2 cell lines (B lymphoblastoid cell lines from two individual donors) were retrieved from Laumont *et al.* (36) (GEO: GSM1641206, GSM1641207, and PRIDE: PXD001898). Raw RNA-Seq data of the JeKo-1 lymphoma cell line were obtained from the Cancer Cell Line Encyclopedia via the NCI Genomic Data Commons (available online at portal.gdc.cancer.gov/legacy-archive/). Corresponding immunopeptidomics MS data of JeKo-1 were retrieved from Khodadoust *et al.*[37] (PRIDE: PXD004746).

[0304] RNA-Seq data of the normal (B-LCL-S1, B-LCL-S2) and cancer (JeKo-1) cell lines were analyzed by IRIS as described above, with minor modifications. Specifically, AS events identified by the IRIS RNA-Seq data processing module were not subjected to the *in silico* screening module, but instead were directly used for the MS search. For MSGF+, FDR was set at 5%, which had the best concordance with predicted binding affinities (FIG. 9C-D). For comparison of predicted HLA binding and nonbinding peptides (FIG. 9D), a set of nonbinding peptides was created by randomly selecting peptides with median(IC_{50}) > 500 nM to the same number of binding peptides (median(IC_{50}) < 500 nM).

[0305] IRIS discovery of candidate TCR and CAR-T targets from 22 GBM samples. RNA-Seq samples were processed by IRIS. Detected skipped exon (SE) events were analyzed by using the IRIS screening and target prediction modules with the aforementioned default parameters. For reference panels, the 'tissue-matched normal panel' comprised normal brain tissue samples from GTEx; the 'normal panel' comprised other normal (nonbrain) tissue samples of 11 selected vital tissues (heart, skin, blood, lung, liver, nerve, muscle, spleen, thyroid, kidney and stomach) from GTEx; and the 'tumor panel' comprised two cohorts of brain tumor samples (GBM and LGG) from TCGA. The blacklist of AS events created for brain was applied before *in silico* screening by IRIS to eliminate error-prone AS events (FIG. 7).

[0306] In screening for the 'Primary' set of AS events, the inventors considered an event to be 'tumor-associated' if it was significantly different from the tissue-matched normal panel, using the default criteria described in 'IRIS module for *in silico* screening of tumor AS events'. In screening for the 'Prioritized' set, the inventors prioritized an AS event if it was both 'tumor-recurrent' (significantly similar to at least 1 of 2 groups in the GBM/LGG tumor panel) and 'tumor-specific' (significantly different from multiple of 11 groups in the normal panel in the

same direction as the tissue-matched normal panel. Here, the inventors used at least 2 groups to allow detection of AS events distinct from multiple groups in the normal panel).

[0307] When selecting potential TCR targets for dextramer validation, the inventors applied three additional criteria: 1) predicted median(IC_{50}) $\leq$ 300 nM; 2) predicted binding to common HLA types, including HLA-A02:01 and HLA-A03:01; and 3) predicted binding to at least five patients in the GBM cohort. After excluding targets with low gene expression (average FPKM < 5), the inventors selected seven epitopes to test for T-cell recognition by dextramer assays.

[0308] **Patients.** Tumor specimens were collected from 22 consenting patients with GBM who underwent surgical resection for tumor removal at the University of California, Los Angeles (UCLA; Los Angeles, CA). From these patients, the inventors also obtained PBMCs and TILs from two HLA-A02:01+ and four HLA-A03:01+ patients. All patients provided written informed consent, and this study was conducted in accordance with established Institutional Review Board-approved protocols.

[0309] **PBMC collection.** Peripheral blood was drawn from patients before surgery and diluted 1:1 in RPMI media (Thermo Fisher Scientific, cat. no. MT10041CV). PBMCs, extracted by Ficoll gradient (Thermo Fisher Scientific, cat. no. 45-001-750), were washed twice in RPMI media. Collected PBMCs were frozen in 90% human AB serum (Thermo Fisher Scientific, cat. no. MT35060CI) and 10% DMSO (Sigma, cat. no. C6295-50ML) and stored in liquid nitrogen. In parallel, PBMCs from healthy HLA-A02:01 and HLA-A03:01 donors were purchased from Bloodworks Northwest (Seattle, WA) or Astarte Biologics (Bothell, WA).

[0310] **TIL collection.** Surgically resected tumor samples were digested with a brain tumor dissociation kit (Miltenyi Biotec, cat. no. 130-095-42) and gentle MACS dissociator (cat. no. 130-093-235). After digestion and myelin depletion, collected cells were labeled with CD45 microbeads (cat. no. 130-045-801) and separated on Miltenyi LS columns (cat. no. 130-042-401) and MidiMACS Separator (cat no. 130-042-302). Collected $CD45^+$ cells were cultured at 1×10^6 cells/mL in X-VIVO 15 Media (Fisher Scientific, cat. no. BW04-418Q) containing 2% human AB serum with 50 ng/mL anti-CD3 antibody (BioLegend, cat. no. 317304), 1 μg/mL anti-CD28 antibody (BD Biosciences, cat. no. 555725), 1 μg/mL anti-CD49d antibody (BD Biosciences, cat. no. 555501), 300 IU/mL IL-2 (NIH, cat. no. 11697), and 10 ng/mL IL-15 (BioLegend, cat. no. 570302). Cells were expanded for 3-4 weeks and replenished with fresh media and cytokines every 2-3 days. Before freezing, expanded cells were placed in media containing 50 IU/mL IL-2 for 1-2 days and then frozen in the same freezing media as PBMCs.

[0311] **Collection of tumor RNAs and RNA sequencing.** RNA from freshly collected or flash-frozen tumor specimens was extracted by using the RNeasy Mini Kit (Qiagen, cat. no.

74014). Paired-end RNA-Seq was performed at the UCLA Clinical Microarray Core using an Illumina HiSeq 3000 at a read length of 2×100 bp or 2×150 bp.

[0312] Dextramer flow-cytometric analysis of PBMCs and TILs. For each AS-derived peptide selected for validation, custom-made HLA-matched MHC Class I dextramer:peptide (pMHC) complexes were purchased from Immudex (Copenhagen, Denmark). Immudex also provided pMHC complexes for common cytomegalovirus (CMV) epitopes (cat. nos. WB2132 and WC2197) and for a nonhuman epitope (NI3233) as a negative control. Each pMHC complex was purchased with two separate tags for APC or PE fluorescence labeling, to increase specificity to targeted T cells with dual labeling.

[0313] To facilitate proper gating of $CD8^+$ T cells from PBMC and TIL populations, the following panel of antibodies (from BioLegend) was set up: CD3 BV605 (cat. no. 300460), CD8 FITC (cat. no. 344704), CD4 BV421 (cat. no. 317434), CD19 BV421 (cat. no. 302234), CD56 BV421 (cat. no. 362552), and CD14 BV421 (cat. no. 301828). For single-color compensation controls, OneComp eBeads were used (Thermo Fisher Scientific, cat. no. 01-1111-41).

[0314] For each set of pMHC complexes, at least 3×10^6 cells were stained according to manufacturer's guidelines. Briefly, cells were thawed in a 37°C water bath and washed with RPMI and D-PBS (Fisher Scientific, cat. no. MT21031CV) before staining for cell viability with the Zombie Violet Viability Kit (BioLegend, cat. no. 423113). Next, the appropriate amount of each pMHC complex in a staining buffer of D-PBS with 5% fetal bovine serum (Fisher Scientific, cat. no. MT35016CV) was added to each sample. After 10 min, the aforementioned antibody cocktail was added. After a 30-min incubation period, cells were washed twice in the same staining buffer. All samples were tested in a BD LSRII flow cytometer, and data were analyzed with FlowJo (Treestar). For gating, the lymphocyte population was first selected using forward and side scatter, and then the BV421-negative population was gated out (i.e. excluding dead cells and the CD14, CD19, CD56, and CD4 populations) before selecting the $CD3^+CD8^+$ population. To set for proper gating of dextramer-positive cells, the inventors used cells that were stained with the full antibody panel but no pMHC complexes, and cells that were given the nonhuman pMHC complex.

[0315] TCR sequencing using scRNA-Seq. Cells were stained by following the dextramer procedure with PE-conjugated pMHC complexes only. Cells were sorted by using the BD FACSAria flow cytometer, and PE^+ cells were collected. V(D)J immune profiling of sorted cells was done with scRNA-Seq, using the 10X Genomics Chromium Single Cell Immune Profiling Workflow at the UCLA Clinical Microarray Core. Each T cell was encapsulated in

an oil emulsion droplet with a barcoded gel bead, and reverse transcription was performed to create a barcoded cDNA library. The V(D)J-enriched and gene expression libraries were sequenced using the 10X Genomics Chromium Controller. After sequencing, the Cell Ranger pipeline was used to align reads, filter, count barcodes and assign unique molecular identifiers.

[0316] Next-generation immune repertoire sequencing using the immunoSEQ platform. To assess the T-lymphocyte repertoire of bulk expanded TIL populations, the inventors used the immunoSEQ assay (Adaptive Biotechnologies). This multiplex PCR system uses a mixture of primers that target the rearranged V and J segments of the CDR3 region to assess TCR diversity within a given sample. Genomic DNA from each sample was extracted by using the QIAamp DNA Blood Midi Kit (Qiagen, cat. no. 51185). The inventors provided at least 1 μg of DNA (~60,000 cells) from each sample to Adaptive Biotechnologies for sequencing at a deep resolution. Resulting sequencing data were analyzed with the immunoSEQ Analyzer Platform (Adaptive Biotechnologies).

[0317] High-throughput αβ TCR pairing using the pairSEQ platform. The inventors provided Adaptive Biotechnologies with frozen bulk expanded TIL samples for their pairSEQ assay, to predict which α and β chains may pair to form a functional TCR. Briefly, T cells were randomly distributed into wells of a 96-well plate. The mRNA was extracted, converted to cDNA, and amplified by using TCR-specific primers. The cDNA of T cells from each well was given a specific barcode, and all wells were pooled together for sequencing. Each TCR sequence was mapped back to the original well through computational demultiplexing. Putative TCR pairs were identified by examining whether a sequenced TCR α chain was frequently seen to share the same well with a specific sequenced TCR β chain, above statistical noise.

[0318] The 22 UCLA GBM RNA-Seq data generated for this study were uploaded to BioProject database (BioProject: PRJNA577155). For the IRIS proteo-transcriptomics analysis, matching RNA-Seq data and MS immunopeptidomics data of B-LCL-S1 and B-LCL-S2 cell lines were retrieved from Laumont *et al.* (GEO: GSM1641206, GSM1641207 and PRIDE: PXD001898). Raw RNA-Seq data of the JeKo-1 lymphoma cell line were obtained from the Cancer Cell Line Encyclopedia via the NCI Genomic Data Commons. Corresponding MS immunopeptidomics MS data of JeKo-1 were retrieved from Khodadoust *et al.* (PRIDE: PXD004746).

B. Tables

Table 1a. IRIS MS analysis of AS-derived epitopes in cell line immunopeptidomics datasets. a. JeKo-1 cancer cell line with FDR = 5%.

Peptide sequence	SEQ ID NO:	HLA type| Predicted binding affinity	Peptide sequence	SEQ ID NO:	HLA type| Predicted binding affinity
AAYGRSPMV	63	HLA-A*02:06|128.43	LAPTDVKEL	178	HLA-C*14:02|268.49
AFYDPTHAW	64	HLA-C*14:02|13.15	LILDPIFKV	179	HLA-A*02:06|7.64
AGAEGFVRV	65	HLA-A*02:06|115.24	LLDEEISRV	180	HLA-A*02:06|28.52
ALIEKLVEL	66	HLA-A*02:06|16.03	LLFEGEKITI	181	HLA-A*02:06|444.16
ALVTPGSLGL	67	HLA-A*02:06|328.12	LPFEPVPVI	182	HLA-B*51:01|68.94
AQAEDMNVKL	68	HLA-A*02:06|397.46	LPKEPQGII	183	HLA-B*51:01|292.28
AQIDHYLGL	69	HLA-A*02:06|8.03	LPLLEQKQV	184	HLA-B*51:01|351.84
AQLELISRL	70	HLA-A*02:06|14.29	LPPNLYPTV	185	HLA-B*51:01|231.32
AQVHHFMEL	71	HLA-A*02:06|4.13	LPRAEVSRI	186	HLA-B*51:01|326.62
ATDDSLHQL	72	HLA-A*02:06|80.41	LQTSIREEI	187	HLA-A*02:06|248.7
ATFKGIVRA	73	HLA-A*02:06|457.03	LYATVHQSY	188	HLA-C*14:02|28.57
ATLGNFAKA	74	HLA-A*02:06|141.06	LYQKEVSHL	189	HLA-C*14:02|20.07
ATYVFLHTV	75	HLA-A*02:06|26.49	MPDDHARKI	190	HLA-B*51:01|396.6
AVDFGNYHL	76	HLA-A*02:06|225.66	MPLVKSVTV	191	HLA-B*51:01|136.16
AVDLTLPKV	77	HLA-A*02:06|48.09	MPPLNTQRI	192	HLA-B*51:01|304.28
AVFKELVKL	78	HLA-A*02:06|214.12	MQAERIFSEV	193	HLA-A*02:06|14.75
AVIKGLTYL	79	HLA-A*02:06|25.92	NALRELETV	194	HLA-A*02:06|151.22
AVLDRMHSL	80	HLA-C*14:02|244.74;HLA-A*02:06|21.32	NAMPVRLQV	195	HLA-A*02:06|204.71
AVQEKVFEL	81	HLA-A*02:06|79.22	NFQAIRVTL	196	HLA-C*14:02|319.17
AVVTQVNKV	82	HLA-A*02:06|237.37	NFSHGTHEY	197	HLA-C*14:02|189.66
AYQYTIRAH	83	HLA-C*14:02|27.78	NMVDIIHSV	198	HLA-A*02:06|5.27
AYYVSNEEL	84	HLA-C*14:02|5.95	NQADTTHPL	199	HLA-C*14:02|240.93;HLA-A*02:06|13.23
DYQALRTSI	85	HLA-C*14:02|120.55	NQFDVVIRL	200	HLA-A*02:06|29.44
ELFTGFIKV	86	HLA-A*02:06|124.99	NQIGAKFWEV	201	HLA-A*02:06|5.41
EQIAEFKEA	87	HLA-A*02:06|497.03	NTFTKLITV	202	HLA-A*02:06|62.48
EQIGQTHRV	88	HLA-A*02:06|387.98	NVIERLQEA	203	HLA-A*02:06|37.56
EQIKDIFYI	89	HLA-A*02:06|32.39	NVPDSFNEV	204	HLA-A*02:06|12.21
EVIEKFDYV	90	HLA-A*02:06|26.5	QIGAKFWEV	205	HLA-A*02:06|19.21
EVPEYLNHV	91	HLA-A*02:06|56.03	QMISRIEYI	206	HLA-A*02:06|80.58
EYVKANDAY	92	HLA-C*14:02|152.99	QMYAKYVKM	207	HLA-C*14:02|64.5
FAMEIDPSL	93	HLA-A*02:06|11.29;HLA-C*14:02|27.45	QQIGEKIFA	208	HLA-A*02:06|130.42

FASHVSPEV	94	HLA-A*02:06|14.32	QTPQRLLEV	209	HLA-A*02:06|143.75
FILEKIEYL	95	HLA-C*14:02|257.45;HLA-A*02:06|6.77	QVIEELFKA	210	HLA-A*02:06|29.2
FISEFEHRV	96	HLA-A*02:06|16.97	QVLEDFPTI	211	HLA-A*02:06|12.94
FLFNKVVNL	97	HLA-A*02:06|67.94;HLA-C*14:02|220.68	QVMDEVIKV	212	HLA-A*02:06|25.16
FLKNELDNV	98	HLA-A*02:06|48.0	QYYNGKIHAY	213	HLA-C*14:02|191.5
FLLEKGYEV	99	HLA-A*02:06|2.98	RIPIAVSGV	214	HLA-A*02:06|194.8
FLMDFIHQV	100	HLA-C*14:02|234.83;HLA-A*02:06|1.47	RLLDEEISRV	215	HLA-A*02:06|98.68
FPHHLQEYI	101	HLA-B*51:01|166.57	RQDLTTLDV	216	HLA-A*02:06|112.52
FPRHIEPEI	102	HLA-B*51:01|325.13	RVIGTLEEV	217	HLA-A*02:06|9.31
FPYMTHPSV	103	HLA-B*51:01|99.46;HLA-C*14:02|35.5	SALKEFEQV	218	HLA-A*02:06|105.18
FQDGTKATF	104	HLA-A*02:06|94.03	SFLPSGSEM	219	HLA-C*14:02|32.2
FSLEELRVA	105	HLA-A*02:06|154.73	SFQDFVSHF	220	HLA-C*14:02|166.62
FSSPHLVQV	106	HLA-A*02:06|10.39	SFSEQTRSL	221	HLA-C*14:02|60.86
FTVPRVVAV	107	HLA-C*14:02|132.29;HLA-A*02:06|5.4	SIIGRLLEV	222	HLA-A*02:06|14.85
FVLDKVPFL	108	HLA-C*14:02|193.09;HLA-A*02:06|5.07	SIIQRLLEV	223	HLA-A*02:06|24.47
FVLPVTPPR	109	HLA-C*14:02|427.61;HLA-A*02:06|74.57	SLITRLLEV	224	HLA-A*02:06|17.33
FVPIKVEQI	110	HLA-A*02:06|404.87	SLYKSFLQL	225	HLA-C*14:02|161.22;HLA-A*02:06|125.49
FYYDGKVMKL	111	HLA-C*14:02|152.44	SQADKFLSL	226	HLA-A*02:06|8.23
GIMDRFLQV	112	HLA-A*02:06|7.34	SQDHFHVFV	227	HLA-A*02:06|7.6
GLDVDGIYRV	113	HLA-A*02:06|185.97	SQDPGTHVV	228	HLA-A*02:06|16.47
GMPDFLEKL	114	HLA-A*02:06|128.63	SQFDEELYKV	229	HLA-A*02:06|11.75
GMYIFLHTV	115	HLA-A*02:06|31.92	SQFNKEILL	230	HLA-A*02:06|207.15
GQDVGRYQV	116	HLA-A*02:06|115.17	SQSTVKVLI	231	HLA-A*02:06|465.48
GQIDSMEQL	117	HLA-A*02:06|27.18	STVKVLIRL	232	HLA-A*02:06|278.88
GQLDKVVEL	118	HLA-A*02:06|18.67	SVAEKLSKL	233	HLA-A*02:06|208.31
GQLISVEKV	119	HLA-A*02:06|74.55	SVASSVKGV	234	HLA-A*02:06|317.63
GQLTKLHQL	120	HLA-A*02:06|38.56	SVIKIFPDL	235	HLA-A*02:06|116.13
GQTGKLMYV	121	HLA-A*02:06|6.99	SVLKELLQV	236	HLA-A*02:06|36.58
GQTPVFSKA	122	HLA-A*02:06|67.48	SVVDHFNRI	237	HLA-A*02:06|33.17
GVAESIHLWEV	123	HLA-A*02:06|321.61	SYIIGTSSV	238	HLA-C*14:02|11.01
GVIAEILRGV	124	HLA-A*02:06|79.48	SYKKPGIHL	239	HLA-C*14:02|100.75
GVIKRTENV	125	HLA-A*02:06|356.54	TAISLFYEL	240	HLA-A*02:06|71.77

GVYGDVHRV	126	HLA-A*02:06|68.5	TILTEIYRI	241	HLA-A*02:06|311.69
GVYGDVQRV	127	HLA-A*02:06|121.64	TIQEEFYRV	242	HLA-A*02:06|37.61
HAAELIKTV	128	HLA-A*02:06|333.73	TLFEDSFQQI	243	HLA-A*02:06|313.51
HAHPFFFTI	129	HLA-A*02:06|144.13	TLPDLVPQL	244	HLA-C*14:02|191.38;HLA-A*02:06|166.52
HAIPSMSHV	130	HLA-A*02:06|109.34	TPYWMAPEV	245	HLA-B*51:01|365.74;HLA-C*14:02|421.48
HFFDGKTAL	131	HLA-C*14:02|59.87	TQIDHYVGI	246	HLA-A*02:06|11.21
HLIGDFSKV	132	HLA-A*02:06|31.06	TQVTALIKL	247	HLA-A*02:06|35.19
HLIHEVTKV	133	HLA-A*02:06|129.7	TVADHFDHI	248	HLA-A*02:06|25.13
HQLENLSNFI	134	HLA-A*02:06|78.73	TVAEHLWKL	249	HLA-A*02:06|17.34
HSIPVTVEV	135	HLA-A*02:06|83.0	TVAKELQTL	250	HLA-A*02:06|335.02
HVADMLVKV	136	HLA-A*02:06|29.05	TVLDKTLSV	251	HLA-C*14:02|340.43;HLA-A*02:06|25.83
HVFDHPWETV	137	HLA-A*02:06|203.65	TVVERLVKV	252	HLA-A*02:06|17.66
IIDQHTFEV	138	HLA-A*02:06|13.41	TVVMYLHHV	253	HLA-A*02:06|8.17
IPPIQVTKV	139	HLA-B*51:01|439.22	TYGERVVAF	254	HLA-C*14:02|18.96
IQDDMHLVI	140	HLA-A*02:06|48.79	TYSEFAREL	255	HLA-C*14:02|19.85
IQKESTLHL	141	HLA-A*02:06|111.69	VIAEILRGV	256	HLA-A*02:06|26.05
IQVRETQAL	142	HLA-C*14:02|469.61;HLA-A*02:06|78.17	VILKILPTL	257	HLA-A*02:06|58.44
IQVTKVTQV	143	HLA-A*02:06|20.57	VLLGKVYVV	258	HLA-A*02:06|60.33
ISFTEFVKV	144	HLA-A*02:06|94.4	VLVDQTTGL	259	HLA-A*02:06|77.94
ITFEDFLKI	145	HLA-A*02:06|45.9	VPAQVQSEI	260	HLA-B*51:01|275.89
IYQIHKEYL	146	HLA-C*14:02|93.3	VQIEEVRQV	261	HLA-A*02:06|24.17
KIAEIEAEM	147	HLA-A*02:06|249.54	VQPEAQAKV	262	HLA-A*02:06|59.11
KILPTLEAV	148	HLA-C*14:02|382.13;HLA-A*02:06|5.73	VSPVSFHQV	263	HLA-A*02:06|377.78
KILQELPSV	149	HLA-A*02:06|8.43	VTASSLVKI	264	HLA-A*02:06|368.82
KLIDRTESL	150	HLA-A*02:06|45.33;HLA-C*14:02|135.86	VTLEKVLGI	265	HLA-A*02:06|103.08
KLILLITQV	151	HLA-A*02:06|20.28	VVDEKLPGL	266	HLA-A*02:06|28.5
KLISDINKA	152	HLA-A*02:06|144.48	VVLEEIPKL	267	HLA-A*02:06|50.62
KLISELQKL	153	HLA-A*02:06|90.63	VVLPKNVNL	268	HLA-A*02:06|357.18
KLLDPEDISV	154	HLA-A*02:06|40.14	VYIPSKTDL	269	HLA-C*14:02|19.62
KLPEKWESV	155	HLA-A*02:06|17.24	VYSPHVLNL	270	HLA-C*14:02|19.66
KLVGEFLEV	156	HLA-A*02:06|4.29	WPYGNRQEI	271	HLA-C*14:02|245.67
KMPEINAKV	157	HLA-A*02:06|43.0;HLA-C*14:02|190.52	YAIEKIERI	272	HLA-A*02:06|114.76

KPYAHILSV	158	HLA-C*14:02|237.83;HLA-A*02:06|477.12	YAVELFEGL	273	HLA-A*02:06|12.68
KQADPFASL	159	HLA-A*02:06|4.55	YFAERVTSL	274	HLA-A*02:06|365.87;HLA-C*14:02|24.24
KQAEIVKRL	160	HLA-A*02:06|124.54	YFIPATGHSL	275	HLA-C*14:02|104.3
KQFEKQVEL	161	HLA-A*02:06|24.62	YFIVTRQDY	276	HLA-C*14:02|28.23
KQLEGIWHT	162	HLA-A*02:06|3.78	YIPTELDQV	277	HLA-A*02:06|35.28
KQMEDGHTL	163	HLA-A*02:06|13.18;HLA-C*14:02|176.62	YISEENFRV	278	HLA-A*02:06|12.77
KQTDLEITV	164	HLA-A*02:06|10.47	YLTHDSPSV	279	HLA-C*14:02|45.26;HLA-A*02:06|12.4
KTLERSYLL	165	HLA-A*02:06|35.53	YPESNSRAI	280	HLA-C*14:02|439.65
KTVEGEFTGV	166	HLA-A*02:06|21.86	YQDPQGRHV	281	HLA-A*02:06|54.04
KVIDEIYRV	167	HLA-A*02:06|7.55	YQLSKEYTL	282	HLA-A*02:06|25.3
KVIDGLETL	168	HLA-A*02:06|12.83	YVMDTRPKL	283	HLA-C*14:02|16.68;HLA-A*02:06|51.31
KVLAAVYKA	169	HLA-A*02:06|67.12	YVTEKVLAA	284	HLA-A*02:06|55.36
KVLDILQHI	170	HLA-A*02:06|8.87	YYFDYKEQL	285	HLA-C*14:02|5.4
KVLGIVVGV	171	HLA-A*02:06|8.47	YYNGKIHAY	286	HLA-C*14:02|4.1
KVLGQGKWNL	172	HLA-A*02:06|353.0	YYQTAPKAGY	287	HLA-C*14:02|499.54
KVLMEWINA	173	HLA-A*02:06|78.63	YYRYPTGESY	288	HLA-C*14:02|25.16
KVTNEFVHI	174	HLA-A*02:06|44.48	YYYDGDIGNY	289	HLA-C*14:02|236.87
KYFPSRVSI	175	HLA-C*14:02|10.98	YYYDGDIGNYY	290	HLA-C*14:02|177.13
KYHVSEKPY	176	HLA-C*14:02|7.47	YYYNGKAVY	291	HLA-C*14:02|2.51
KYMEHRQTY	177	HLA-C*14:02|5.94	YYYTKEEQF	292	HLA-C*14:02|4.51

Table 1b. IRIS MS analysis of AS-derived epitopes in cell line immunopeptidomics datasets. b. B-LCL-S1 normal cell line with FDR = 5%

Peptide sequence	SEQ ID NO:	**HLA type| Predicted binding affinity**	**Peptide sequence**	SEQ ID NO:	**HLA type| Predicted binding affinity**
AASTARHLY	293	HLA-A*29:02|166.82	KMADIEYRL	382	HLA-A*02:01|14.24
AEAYLGKTV	294	HLA-B*44:03|193.36	KMMNGGHYTY	383	HLA-A*29:02|66.76
AEDELFNRY	295	HLA-B*44:03|155.93	KSMETKVQF	384	HLA-B*57:01|87.71
AEDYAKERY	296	HLA-B*44:03|471.05	KVIDEIYRV	385	HLA-A*02:01|20.19
AEEESFRKL	297	HLA-B*44:03|257.67	KVQEQVHKV	386	HLA-A*02:01|290.23
AEEKQTKSF	298	HLA-B*44:03|312.1	LEELYTKKLW	387	HLA-B*44:03|416.33

AEELERQGY	299	HLA-B*44:03|272.87	LEVDLKNGY	388	HLA-B*44:03|211.92;HLA-B*18:01|103.26
AEHDVTVKY	300	HLA-B*18:01|259.15;HLA-B*44:03|45.29	LLANKVPAA	389	HLA-A*02:01|67.1
AEHDVTVKYY	301	HLA-B*44:03|56.92	LLDEEISRV	390	HLA-A*02:01|18.44
AEHIESRTL	302	HLA-B*44:03|428.34	METHIMHTF	391	HLA-B*44:03|49.55;HLA-B*18:01|17.92
AEHPNPGVRY	303	HLA-B*44:03|108.35	MEVNDIKKAL	392	HLA-B*44:03|444.95
AEITPKGRAL	304	HLA-B*44:03|179.98	MVPPEWHRW	393	HLA-B*57:01|50.04
AEKELHEKF	305	HLA-B*44:03|361.67;HLA-B*18:01|456.63	NEDLIKHGW	394	HLA-B*18:01|203.21;HLA-B*44:03|221.84
AEKLITQTF	306	HLA-B*18:01|405.64;HLA-B*44:03|260.03	NEGDKYKFF	395	HLA-B*44:03|406.79
AELESSKEQF	307	HLA-B*44:03|43.07	NEHDYAKAAF	396	HLA-B*18:01|309.96;HLA-B*44:03|129.21
AELPAELTGV	308	HLA-A*02:01|230.71	NEIEDTFRQF	397	HLA-B*44:03|151.52
AELTPHQTF	309	HLA-B*44:03|82.6;HLA-B*18:01|329.07	NELDAVQREY	398	HLA-B*44:03|148.75
AELYINEHDY	310	HLA-B*44:03|71.6	NEQPRQLFW	399	HLA-B*44:03|251.11
AEMQWKRDY	311	HLA-B*44:03|13.67	NEQTLQRSW	400	HLA-B*18:01|166.25;HLA-B*44:03|66.12
AENLGKEHF	312	HLA-B*44:03|278.07	QEAGIKTAF	401	HLA-B*44:03|155.79;HLA-B*18:01|82.32
AETGNLPHSY	313	HLA-B*44:03|53.31	QEAVHSAKEY	402	HLA-B*44:03|50.09
AEVDKVTGRF	314	HLA-B*44:03|102.83	QEDEVMRAY	403	HLA-B*44:03|300.68;HLA-B*18:01|69.68
AEVEPGVHL	315	HLA-B*44:03|388.73	QEDLRTFSW	404	HLA-B*44:03|211.11
AEVNSSGKTI	316	HLA-B*44:03|95.59	QEDRPGLHAY	405	HLA-B*44:03|317.35
AEYNSDLHQF	317	HLA-B*44:03|40.71	QEFEKHEVHQY	406	HLA-B*44:03|235.39
AFQERLNSY	318	HLA-A*29:02|401.1	QEFLKPSDSF	407	HLA-B*44:03|165.25
DEINHVQKTY	319	HLA-B*44:03|385.15	QEGDTTRKY	408	HLA-B*44:03|107.5
DEVQVVRGHY	320	HLA-B*18:01|74.61	QEHVKSFSW	409	HLA-B*44:03|57.24
EEAERPLHF	321	HLA-B*44:03|408.17	QENNTKQLRF	410	HLA-B*44:03|109.42
EEAQFETKKL	322	HLA-B*44:03|441.01	QESAPVHNSF	411	HLA-B*44:03|138.72
EEAQFETKKY	323	HLA-B*44:03|50.96	QEVDFFQRY	412	HLA-B*44:03|22.38;HLA-B*18:01|260.22

EEAQYQREY	324	HLA-B*18:01|131.77;HLA-B*44:03|62.0	QEYQKALQEY	413	HLA-B*44:03|64.52
EEAYNAVVRY	325	HLA-B*18:01|167.45;HLA-B*44:03|125.09	QLAEDLKKV	414	HLA-A*02:01|153.55
EEDAALFKAW	326	HLA-B*44:03|37.01	QMISRIEYV	415	HLA-A*02:01|23.06
EEEKGGKVY	327	HLA-B*18:01|173.76;HLA-B*44:03|395.88	QTFRLYREY	416	HLA-A*29:02|62.71
EEEPLNIHSF	328	HLA-B*18:01|295.01;HLA-B*44:03|336.37	QTLQRSWAY	417	HLA-A*29:02|68.72
EEEQAVRPKY	329	HLA-B*44:03|222.6;HLA-B*18:01|330.28	RGAEYVSAREW	418	HLA-B*57:01|455.56
EEEQENNKSF	330	HLA-B*44:03|483.1	RLLDEEISRV	419	HLA-A*02:01|32.24
EEFLNGRAVY	331	HLA-B*18:01|26.51;HLA-B*44:03|123.15	RVADRLYGV	420	HLA-A*02:01|35.57
EEGLVNNKY	332	HLA-B*18:01|254.5;HLA-B*44:03|174.08	SEAQEGLQKF	421	HLA-B*44:03|94.37
EEGQLRPKTY	333	HLA-B*44:03|142.04	SEEEKGGKVY	422	HLA-B*44:03|349.8
EEIKKNYRY	334	HLA-B*44:03|108.29	SEEGHPFKAY	423	HLA-B*44:03|46.86
EEIVYPQKAY	335	HLA-B*44:03|28.48	SEFPGAQHY	424	HLA-B*44:03|41.92;HLA-B*18:01|158.39;HLA-A*29:02|220.33
EEIVYPQKAYY	336	HLA-A*29:02|323.08	SEGDITQKGY	425	HLA-B*44:03|110.59
EELYTKKLW	337	HLA-B*44:03|40.4	SEGFSHSRW	426	HLA-B*44:03|44.67
EEPTVIKKY	338	HLA-B*44:03|391.84;HLA-B*18:01|444.83	SEGKITGRRY	427	HLA-B*44:03|119.06
EEQAVRPKY	339	HLA-B*44:03|197.29;HLA-B*18:01|41.75	SEGYPGKRY	428	HLA-B*44:03|89.81
EETELDRMW	340	HLA-B*44:03|27.4	SEGYPGKRYY	429	HLA-B*44:03|120.48
EETNIPKRLY	341	HLA-B*44:03|105.42	SEGYPGQRY	430	HLA-B*44:03|91.57
EEVEAKFKRY	342	HLA-B*44:03|50.3	SEHAVNKQL	431	HLA-B*44:03|250.45
EEVPVYENKY	343	HLA-B*44:03|131.56	SELQSQVRY	432	HLA-B*44:03|57.33
EEVRQGLKAY	344	HLA-B*44:03|120.67	SENELKKAY	433	HLA-B*18:01|52.19;HLA-B*44:03|20.08
FEYNENTRKY	345	HLA-B*18:01|476.74;HLA-B*44:03|454.75	SENELKKAYR	434	HLA-B*44:03|62.66
FFPDKPITQY	346	HLA-A*29:02|162.61	SEVDLTRSF	435	HLA-B*18:01|93.83;HLA-B*44:03|30.32

FISEFEHRV	347	HLA-A*02:01|19.64	SEVPFSKRY	436	HLA-B*44:03|33.57
FLTTLKPRL	348	HLA-A*02:01|79.04	SEVPFSKRYL	437	HLA-B*44:03|85.38
FLYAGHIFL	349	HLA-B*39:03|258.0;HLA-A*02:01|5.15	SEVTASSRHY	438	HLA-B*44:03|55.69
GEITHPKNNY	350	HLA-B*44:03|115.59	SFYEKGPLTF	439	HLA-A*29:02|179.58
GELLPTKKF	351	HLA-B*44:03|186.91	SLDGRQIRV	440	HLA-A*02:01|73.55
GENGTGKTTF	352	HLA-B*44:03|214.66	SSAPTARVF	441	HLA-B*57:01|412.36
GEWTPTLQHY	353	HLA-B*44:03|300.28	SSTPHNVSW	442	HLA-B*57:01|41.12
GTSSVARAW	354	HLA-B*57:01|75.54	STYEKALGY	443	HLA-A*01:01|225.0;HLA-A*29:02|22.3
HEQEAILHNF	355	HLA-B*18:01|220.29;HLA-B*44:03|223.67	STYPRPHEY	444	HLA-A*29:02|20.75;HLA-A*01:01|376.0
HESDINSVRY	356	HLA-B*18:01|471.09;HLA-B*44:03|385.63	SVRPPKKSW	445	HLA-B*57:01|80.91
HLAVVAPRL	357	HLA-A*02:01|254.14	SVTGVHRLY	446	HLA-A*29:02|38.85
HLIDTNKIQL	358	HLA-A*02:01|333.45	TEAEKLKSY	447	HLA-B*44:03|347.67;HLA-B*18:01|125.39
HSNEQTLQRSW	359	HLA-B*57:01|145.55	TEDEAIHKY	448	HLA-B*44:03|431.75;HLA-B*18:01|37.21
IEFARKNVY	360	HLA-B*18:01|63.25	TEFDSTVTH	449	HLA-B*18:01|324.24
IENPALLRW	361	HLA-B*44:03|23.61	TELDQVRKW	450	HLA-B*44:03|64.38
IENQNSRQY	362	HLA-B*44:03|72.49;HLA-B*18:01|161.85	TELEDARQKF	451	HLA-B*44:03|383.33
IGTSSVARAW	363	HLA-B*57:01|376.97	TENQRPKTGF	452	HLA-B*44:03|467.76
ILDAGGHNV	364	HLA-A*02:01|47.47	TEQSQIKGY	453	HLA-B*44:03|312.89;HLA-B*18:01|209.84
ILDKKVEKV	365	HLA-A*02:01|106.92	TEQTAPKIF	454	HLA-B*18:01|141.55
ILQEREYRL	366	HLA-A*02:01|92.79	TESPLVKAI	455	HLA-B*18:01|450.01;HLA-B*44:03|340.82
ITAPTVRQY	367	HLA-A*29:02|85.68;HLA-B*57:01|210.03	TEVEENQKYF	456	HLA-B*44:03|374.93
ITQGIPRSY	368	HLA-A*29:02|181.07	TIIDRSFRY	457	HLA-A*29:02|26.91
KAASLIQGY	369	HLA-B*57:01|494.36	TLWPEVQKL	458	HLA-A*02:01|94.68
KAWNDIKKVY	370	HLA-B*57:01|381.84	TTHNQSRKW	459	HLA-B*57:01|35.43
KEAETRAEF	371	HLA-B*18:01|104.66	VEISNEVRKF	460	HLA-B*44:03|308.79
KEIDMLLKEY	372	HLA-B*44:03|76.67	VIAEILRGV	461	HLA-A*02:01|97.32

KEIGTKQSF	373	HLA-B*44:03|197.15;HLA-B*18:01|371.35	VSHSTHRTF	462	HLA-B*57:01|361.84
KELELAHRY	374	HLA-B*44:03|34.41;HLA-B*18:01|99.19	VTDETGGKY	463	HLA-A*01:01|37.26
KELESIKETY	375	HLA-B*44:03|122.0	VTNPHSSQW	464	HLA-B*57:01|39.73
KESIAQRSY	376	HLA-B*44:03|198.81;HLA-B*18:01|405.05	VTRHPINEYY	465	HLA-A*01:01|417.26
KESTLHLVL	377	HLA-B*18:01|449.97	YLDQTLPRA	466	HLA-A*02:01|26.94
KEYDLAKKY	378	HLA-B*18:01|119.96;HLA-B*44:03|28.69	YLFERIKEL	467	HLA-B*39:03|160.7;HLA-A*02:01|10.65
KEYEKALKY	379	HLA-B*44:03|63.93;HLA-B*18:01|157.9	YLLPAIVHI	468	HLA-A*02:01|7.06
KLFEKVKEV	380	HLA-A*02:01|13.07	YLLQEPPRTV	469	HLA-A*02:01|17.26
KLLTEVHAA	381	HLA-A*02:01|45.76	YSKDRIYTY	470	HLA-A*29:02|100.26

Table 1c. IRIS MS analysis of AS-derived epitopes in cell line immunopeptidomics datasets. c. B-LCL-S2 normal cell line with FDR = 5%.

Peptide sequence	SEQ ID NO:	**HLA type| Predicted binding affinity**	**Peptide sequence**	SEQ ID NO:	**HLA type| Predicted binding affinity**
ASEFPGAQHY	471	HLA-A*01:01|415.11	LLDEEISRV	514	HLA-A*02:01|18.44
AVDAARLHY	472	HLA-A*01:01|181.58;HLA-A*29:02|168.19	LTDADAMKY	515	HLA-A*29:02|178.31;HLA-A*01:01|22.68
AVDPRFLAY	473	HLA-A*29:02|65.54;HLA-A*01:01|83.07	LTDFIREEY	516	HLA-A*01:01|17.55;HLA-A*29:02|212.51
DEEGKGAVY	474	HLA-B*18:01|19.96	LTDSEKGNSY	517	HLA-A*01:01|82.92
DEEGLKLTF	475	HLA-B*18:01|5.86	MLGAGLIKI	518	HLA-A*02:01|45.16
DEFEFLEKA	476	HLA-B*18:01|57.67	MLTEKQHLL	519	HLA-A*02:01|38.07
DEFPLLTTK	477	HLA-B*18:01|23.11	NEHDYAKAAF	520	HLA-B*18:01|309.96;HLA-B*44:03|129.21
DEKLNNAKY	478	HLA-B*18:01|29.58	NENPLRHQF	521	HLA-B*18:01|22.25;HLA-B*44:03|95.49
DEVDMAHRF	479	HLA-B*44:03|90.53;HLA-B*18:01|15.86	NEQTLQRSWAY	522	HLA-B*18:01|434.4
DEVQVVRGH	480	HLA-B*18:01|134.66	NHNKITQSL	523	HLA-B*39:03|47.7
DEVQVVRGHY	481	HLA-B*18:01|74.61	NTDRNIDKY	524	HLA-A*01:01|36.44
DTDHYFLRY	482	HLA-A*29:02|85.82;HLA-A*01:01|9.87	PTDPKVVVY	525	HLA-A*01:01|39.69
EEVEAKFKRY	483	HLA-B*44:03|50.3	PTDTNVIRY	526	HLA-A*01:01|18.44

EEYLLRQVF	484	HLA-B*44:03|115.35;HLA-B*18:01|26.19	QLAEDLKKV	527	HLA-A*02:01|153.55
EHFPNHQQL	485	HLA-B*39:03|266.4	QRQQVRPVI	528	HLA-B*39:03|296.2
ESDINSVRY	486	HLA-A*01:01|40.69	RLQEDPPAGV	529	HLA-A*02:01|109.11
ETDSSEEISRY	487	HLA-A*01:01|167.05	SEFPGAQHY	530	HLA-B*44:03|41.92;HLA-B*18:01|158.39;HLA-A*29:02|220.33
ETELDGLRY	488	HLA-A*01:01|79.21	SENELKKAY	531	HLA-B*18:01|52.19;HLA-B*44:03|20.08
FISEFEHRV	489	HLA-A*02:01|19.64	SEVDLTRSF	532	HLA-B*18:01|93.83;HLA-B*44:03|30.32
FSDSKIQKY	490	HLA-A*01:01|20.48	SHWVKRTTL	533	HLA-B*39:03|44.2
FTDKSAEDY	491	HLA-A*01:01|17.58;HLA-A*29:02|481.09	TEFDSTVTH	534	HLA-B*18:01|324.24
GLAPPQHLIRV	492	HLA-A*02:01|370.43	THISRVGQAM	535	HLA-B*39:03|459.4
GSDRIKDGY	493	HLA-A*01:01|101.76	TLDTKPGLY	536	HLA-A*29:02|138.68;HLA-A*01:01|112.34
HTELPRGDHY	494	HLA-A*01:01|155.4	VETYQVHEY	537	HLA-B*18:01|76.83;HLA-B*44:03|174.83
IEFARKNVY	495	HLA-B*18:01|63.25	VLDPYLLKY	538	HLA-A*29:02|49.32;HLA-A*01:01|64.73
IEFEYNKSKY	496	HLA-B*44:03|195.79;HLA-B*18:01|102.07	VQDDIKESV	539	HLA-A*02:01|439.63
IENPALLRW	497	HLA-B*44:03|23.61	VSDFGLSRY	540	HLA-A*01:01|24.76
ILDAGGHNV	498	HLA-A*02:01|47.47	VTDETGGKY	541	HLA-A*01:01|37.26
ILDKKVEKV	499	HLA-A*02:01|106.92	VTEIDQDKY	542	HLA-A*01:01|178.05
ILQDRLNQV	500	HLA-A*02:01|37.73	WSDYSDNLKY	543	HLA-A*29:02|282.19;HLA-A*01:01|99.47
ILQEREYRL	501	HLA-A*02:01|92.79	YELPWVEKY	544	HLA-B*44:03|90.0;HLA-A*29:02|151.75;HLA-B*18:01|30.19
ILQTKLYTL	502	HLA-A*02:01|35.48	YLDQTLPRA	545	HLA-A*02:01|26.94
ISEAGKDLLY	503	HLA-A*29:02|322.51;HLA-A*01:01|127.05	YLFERIKEL	546	HLA-B*39:03|160.7;HLA-A*02:01|10.65
ITDPDPDIRY	504	HLA-A*01:01|231.59	YLLPAIVHI	547	HLA-A*02:01|7.06
ITQGTPLKY	505	HLA-A*01:01|257.17;HLA-A*29:02|26.7	YMYILPYKEF	548	HLA-A*02:01|499.24;HLA-A*29:02|110.75
KLIDRTESL	506	HLA-A*02:01|33.22	YSDDIPHAL	549	HLA-A*01:01|121.33;HLA-A*02:01|245.89;HLA-B*39:03|160.5
KLPEKWESV	507	HLA-A*02:01|47.88	YSDKYGLGY	550	HLA-A*29:02|72.01;HLA-A*01:01|5.92

KLSEVNKRL	508	HLA-A*02:01|235.49	YSEGYPGQRY	551	HLA-A*01:01|184.48;HLA-A*29:02|151.26
KMADIEYRL	509	HLA-A*02:01|14.24	YSFDNLPEKY	552	HLA-A*01:01|161.79;HLA-A*29:02|45.23
KVIDEIYRV	510	HLA-A*02:01|20.19	YTDFDGTRVY	553	HLA-A*29:02|193.03;HLA-A*01:01|131.98
KVQEQVHKV	511	HLA-A*02:01|290.23	YTFDPVTKV	554	HLA-A*02:01|44.78
LHKSIQNSL	512	HLA-B*39:03|365.8	YVEGVHQQY	555	HLA-A*29:02|63.25
LIDQDARDLY	513	HLA-A*01:01|215.04			

Table 2a. IRIS screening results of tumor AS events in 22 GBM samples. a. IRIS identified 6,276 tumor-associated AS events (Primary set)

AS_event	AS_event	AS_event
ENSG00000117410:ATP6V0B:chr1:+:44441336:44441520:44440779:44441761	ENSG00000197599:CCDC154:chr16:-:1487817:1487955:1486812:1488050	ENSG00000132781:MUTYH:chr1:-:45798062:45798160:45797982:45798434
ENSG00000028203:VEZT:chr12:+:95650925:95651015:95645847:95656681	ENSG00000197498:RPF2:chr6:+:111318385:111318467:111312989:111329240	ENSG00000124831:LRRFIP1:chr2:+:238636518:238636578:238629465:238647874
ENSG00000043143:JADE2:chr5:+:133912457:133912728:133909452:133914186	ENSG00000219545:UMAD1:chr7:+:7907960:7908164:7841374:7916911	ENSG00000124074:ENKD1:chr16:-:67697559:67697723:67697459:67699973
ENSG00000104936:DMPK:chr19:-:46274228:46274318:46273898:46274825	ENSG00000008517:IL32:chr16:+:3117984:3118011:3117416:3118180	ENSG00000109452:INPP4B:chr4:-:143081510:143081714:143067149:143094784
ENSG00000127603:MACF1:chr1:+:39844132:39844195:39838268:39844874	ENSG00000079841:RIMS1:chr6:+:72975662:72975752:72970470:72993749	ENSG00000081377:CDC14B:chr9:-:99277930:99278074:99266071:99284787
ENSG00000124067:SLC12A4:chr16:-:67984556:67984614:67984396:67985042	ENSG00000149177:PTPRJ:chr11:+:48168427:48168515:48166676:48170998	ENSG00000130706:ADRM1:chr20:+:60883423:60883526:60883234:60883710
ENSG00000117600:PLPPR4:chr1:+:99767279:99767453:99766522:99771240	ENSG00000166532:RIMKLB:chr12:+:8852380:8853210:8850893:8866406	ENSG00000271447:MMP28:chr17:-:34093215:34093913:34083438:34094770
ENSG00000120697:ALG5:chr13:-:37569125:37569172:37567809:37569561	ENSG00000111271:ACAD10:chr12:+:112191575:112191719:112187149:112193471	ENSG00000005379:TSPOAP1:chr17:-:56381698:56381760:56379808:56382421
ENSG00000151789:ZNF385D:chr3:-:21552352:21552515:21478695:21606065	ENSG00000214435:AS3MT:chr10:+:104629840:104629968:104629350:104632204	ENSG00000047249:ATP6V1H:chr8:-:54723723:54723777:54714456:54727221
ENSG00000005194:CIAPIN1:chr16:-:57474683:57474895:57473207:57481253	ENSG00000140157:NIPA2:chr15:-:23033277:23033413:23027922:23034146	ENSG00000138430:OLA1:chr2:-:175111442:175111543:175094179:175113179
ENSG00000183317:EPHA10:chr1:-:38186454:38186516:38186226:38187331	ENSG00000196353:CPNE4:chr3:-:131624107:131624288:131442469:131753410	ENSG00000185988:PLK5:chr19:+:1528896:1528973:1528427:1529404
ENSG00000158882:TOMM40L:chr1:+:161197425:161197527:161196394:161197673	ENSG00000146574:CCZ1B:chr7:-:6852606:6852668:6851694:6854394	ENSG00000116584:ARHGEF2:chr1:-:155935421:155935551:155935203:155936206
ENSG00000100379:KCTD17:chr22:+:37457143:37457215:37455478:37457578	ENSG00000186166:CCDC84:chr11:+:118883941:118883972:118881993:118885703	ENSG00000104866:PPP1R37:chr19:+:45643493:45643539:45596785:45643763
ENSG00000197223:C1D:chr2:-:68280192:68280418:68274451:68290076	ENSG00000072518:MARK2:chr11:+:63671457:63671619:63670630:63672257	ENSG00000107077:KDM4C:chr9:+:7105401:7105500:7103870:7128065

ENSG00000144134:RABL2A:chr2:+:114398932:114399027:114392707:114399357

ENSG00000095637:SORBS1:chr10:-:97110965:97111133:97106209:97114638

ENSG00000006283:CACNA1G:chr17:+:48684260:48684350:48681642:48685187

ENSG00000138757:G3BP2:chr4:-:76649239:76649360:76587233:76649459

ENSG00000162929:KIAA1841:chr2:+:61324834:61324987:61319722:61330987

ENSG00000178035:IMPDH2:chr3:-:49065631:49065755:49065349:49065863

ENSG00000162910:MRPL55:chr1:-:228296655:228296722:228296019:228296849

ENSG00000137877:SPTBN5:chr15:-:42145779:42145985:42145645:42146247

ENSG00000204278:TMEM235:chr17:+:76230673:76230811:76228294:76235104

ENSG00000188674:C2orf80:chr2:-:209049674:209049756:209047771:209054676

ENSG00000089159:PXN:chr12:-:120653732:120653893:120653464:120654075

ENSG00000105483:CARD8:chr19:-:48726975:48727125:48725112:48733694

ENSG00000129159:KCNC1:chr11:+:17803216:17803448:17801191:17875089

ENSG00000127663:KDM4B:chr19:+:5119128:5119230:5110829:5119663

ENSG00000100568:VTI1B:chr14:-:68129745:68129907:68129252:68141091

ENSG00000139116:KIF21A:chr12:-:39711874:39712003:39709772:39716469

ENSG00000172995:ARPP21:chr3:+:35750460:35750562:35732497:35756930

ENSG00000119487:MAPKAP1:chr9:-:128434594:128434922:128432186:128469249

ENSG00000144504:ANKMY1:chr2:-:241468453:241468926:241465266:241492330

ENSG00000101333:PLCB4:chr20:+:9353693:9353751:9353050:9360700

ENSG00000073605:GSDMB:chr17:-:38065210:38065295:38062524:38066008

ENSG00000135597:REPS1:chr6:-:139247537:139247615:139242261:139251113

ENSG00000152154:TMEM178A:chr2:+:39931220:39931334:39893514:39944149

ENSG00000143207:RFWD2:chr1:-:176145045:176145143:176118210:176153768

ENSG00000154134:ROBO3:chr11:+:124747832:124748027:124747554:124748193

ENSG00000205937:RNPS1:chr16:-:2317175:2317219:2314761:2318055

ENSG00000137177:KIF13A:chr6:-:17771344:17771449:17765177:17772138

ENSG00000139624:CERS5:chr12:-:50535835:50535893:50532400:50536856

ENSG00000117114:ADGRL2:chr1:+:82452584:82452713:82451039:82456074

ENSG00000196535:MYO18A:chr17:-:27412621:27412666:27409456:27413455

ENSG00000196204:RNF216P1:chr7:+:5035105:5035213:5024706:5036240

ENSG00000144115:THNSL2:chr2:+:88472769:88472892:88469975:88474157

ENSG00000124357:NAGK:chr2:+:71302684:71302740:71300724:71303733

ENSG00000080823:MOK:chr14:-:102744942:102745031:102718332:102749814

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ENSG00000164329:PAPD4:chr5:+:78938666:78938733:78937019:78940945

ENSG00000177106:EPS8L2:chr11:+:722090:722165:721691:722400

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ENSG00000198909:MAP3K3:chr17:+:61712068:61712161:61710162:61723393

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ENSG00000103319:EEF2K:chr16:+:22268963:22269091:22268706:22269814

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ENSG00000011638:TMEM159:chr16:+:21185330:21185469:21181926:21190795

ENSG00000172508:CARNS1:chr11:+:67185901:67185991:67183234:67186226

ENSG00000141469:SLC14A1:chr18:+:43310264:43310436:43304978:43310979

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ENSG00000137312:FLOT1:chr6:-:30709568:30709644:30709110:30709923
ENSG00000176731:C8orf59:chr8:-:86131464:86131592:86129731:86132534

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ENSG00000162066:AMDHD2:chr16:+:2577573:2577616:2571124:2577773

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ENSG00000213983:AP1G2:chr14:-:24031170:24031275:24030844:24031496

ENSG00000165949:IFI27:chr14:+:94582126:94582131:94581226:94582131

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ENSG00000091129:NRCAM:chr7:-:107880402:107880614:107875132:107953108
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ENSG00000085871:MGST2:chr4:+:140599696:140599796:140587231:140616350
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ENSG00000185985:SLITRK2:chrX:+:144903522:144903671:144903402:144903900

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ENSG00000164828:SUN1:chr7:+:889156:889240:883157:889559

ENSG00000007541:PIGQ:chr16:+:631198:631341:630972:632247

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ENSG00000148482:SLC39A12:chr10:+:18282112:18282220:18280232:18284584
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ENSG00000168314:MOBP:chr3:+:39540957:39541023:39521614:39554873
ENSG00000134262:AP4B1:chr1:-:114440461:114440565:114438660:114441339
ENSG00000148634:HERC4:chr10:-:69718869:69718893:69716733:69726439
ENSG00000213599:SLX1A-SULT1A3:chr16:+:30212516:30212614:30212427:30214010
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ENSG00000169255:B3GALNT1:chr3:-:160807747:160807850:160804576:160818926
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ENSG00000015153:YAF2:chr12:-:42592937:42593037:42555567:42631400
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ENSG00000112651:MRPL2:chr6:-:43023282:43023356:43022224:43025802

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ENSG00000112983:BRD8:chr5:-:137497484:137497553:137496757:137498818
ENSG00000141644:MBD1:chr18:-:47801739:47801814:47801615:47801969
ENSG00000135083:CCNJL:chr5:-:159707531:159707745:159686778:159738864

ENSG00000197122:SRC:chr20:+:36011021:36011189:35993680:36012552
ENSG00000073921:PICALM:chr11:-:85689112:85689136:85687725:85692171
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ENSG00000115760:BIRC6:chr2:+:32815872:32816045:32800433:32818981

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ENSG00000055483:USP36:chr17:-:76793867:76793961:76783740:76794481
ENSG00000066117:SMARCD1:chr12:+:50481145:50481268:50480661:50483666
ENSG00000050405:LIMA1:chr12:-:50579172:50579231:50575820:50589612

ENSG00000006125:AP2B1:chr17:+:33997875:33997917:33984810:33998772

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ENSG00000160753:RUSC1:chr1:+:155295391:155295463:155295281:155295654

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ENSG00000197558:SSPO:chr7:+:149522888:149523027:149522480:149523163
ENSG00000187118:CMC1:chr3:+:28304781:28304871:28283392:28357823

ENSG00000197971:MBP:chr18:-:74700450:74700483:74697166:74700832
ENSG00000163517:HDAC11:chr3:+:13538235:13538352:13525064:13543370
ENSG00000074054:CLASP1:chr2:-:122176197:122176305:122168545:122182714
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ENSG00000144504:ANKMY1:chr2:-:241421599:241421678:241420514:241439374
ENSG00000149131:SERPING1:chr11:+:57367351:57367850:57365794:57369507
ENSG00000070476:ZXDC:chr3:-:126160607:126160789:126158570:126178495
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ENSG00000117280:RAB29:chr1:-:205741623:205741695:205739982:205743960
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ENSG00000166979:EVA1C:chr21:+:33829904:33830028:33785321:33840003
ENSG00000151690:MFSD6:chr2:+:191364557:191364582:191362445:191364740
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ENSG00000156958:GALK2:chr15:+:49574183:49574282:49531564:49584523
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ENSG00000166377:ATP9B:chr18:+:76973961:76974038:76967012:77013380
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ENSG00000171606:ZNF274:chr19:+:58697063:58697205:58695365:58698313
ENSG00000159063:ALG8:chr11:-:77838403:77838485:77835260:77850539

ENSG00000092108:SCFD1:chr14:+:31097414:31097485:31091605:31099682

ENSG00000197302:ZNF720:chr16:+:31734578:31734674:31724756:31765086
ENSG00000125962:ARMCX5:chrX:+:101855847:101855960:101854775:101856391

ENSG00000081760:AACS:chr12:+:125603186:125603311:125599103:125618548

ENSG00000143252:SDHC:chr1:+:161293403:161293460:161284215:161310383
ENSG00000114988:LMAN2L:chr2:-:97378843:97378876:97377762:97399255
ENSG00000214078:CPNE1:chr20:-:34246851:34246936:34220845:34252681

ENSG00000128739:SNRPN:chr15:+:25219788:25220104:25219603:25221451
ENSG00000197226:TBC1D9B:chr5:-:179294444:179294495:179292888:179294777
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ENSG00000123562:MORF4L2:chrX:-:102939608:102939657:102931979:102940098
ENSG00000006071:ABCC8:chr11:-:17452360:17452506:17450217:17453750
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ENSG00000215039:CD27-AS1:chr12:-:6560058:6560146:6557903:6560634

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ENSG00000082805:ERC1:chr12:+:1221380:1221464:1219513:1225031

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ENSG00000151090:THRB:chr3:-:24270428:24270492:24231825:24378790
ENSG00000060237:WNK1:chr12:+:1013618:1013660:1006847:1017012
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ENSG00000181045:SLC26A11:chr17:+:78222373:78222473:78222056:78225127
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ENSG00000106348:IMPDH1:chr7:-:128040885:128040945:128040593:128041068
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ENSG00000126106:TMEM53:chr1:-:45120611:45120881:45111136:45125845

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ENSG00000125337:KIF25:chr6:+:168442648:168442831:168440896:168445506
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ENSG00000005243:COPZ2:chr17:-:46105041:46105155:46103841:46106490
ENSG00000167114:SLC27A4:chr9:+:131112590:131112660:131110982:131114916
ENSG00000106868:SUSD1:chr9:-:114814678:114814718:114804240:114820707
ENSG00000102606:ARHGEF7:chr13:+:111862218:111862349:111806338:111870025
ENSG00000188566:NDOR1:chr9:+:140108418:140108520:140108330:140108655
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ENSG00000110321:EIF4G2:chr11:-:10823207:10823321:10822634:10823595
ENSG00000135090:TAOK3:chr12:-:118673599:118674498:118673476:118675877
ENSG00000070061:IKBKAP:chr9:-:111681090:111681181:111679950:111685121
ENSG00000198208:RPS6KL1:chr14:-:75385215:75385308:75378083:75386547
ENSG00000276234:TADA2A:chr17:+:35818625:35818689:35804870:35825534
ENSG00000168970:JMJD7-PLA2G4B:chr15:+:42134751:42134789:42134474:42136668
ENSG00000163482:STK36:chr2:+:219553113:219553216:219549951:219553419

ENSG00000214548:MEG3:chr14:+:101302075:101302216:101297871:101302503
ENSG00000126768:TIMM17B:chrX:-:48754041:48754141:48751508:48755006
ENSG00000141837:CACNA1A:chr19:-:13338244:13338341:13335586:13342523
ENSG00000163608:NEPRO:chr3:-:112729489:112729551:112727277:112729891
ENSG00000028203:VEZT:chr12:+:95650325:95650398:95645847:95650925
ENSG00000103174:NAGPA:chr16:-:5077278:5077380:5077199:5077846

ENSG00000167107:ACSF2:chr17:+:48549788:48549940:48548496:48551025
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ENSG00000198369:SPRED2:chr2:-:65561738:65561907:65559185:65571852
ENSG00000101639:CEP192:chr18:+:13103873:13103966:13103587:13104982

ENSG00000137033:IL33:chr9:+:6251139:6251265:6241785:6252865
ENSG00000118473:SGIP1:chr1:+:67147551:67148052:67145435:67154830
ENSG00000143537:ADAM15:chr1:+:155033893:155033965:155033308:155034379
ENSG00000108064:TFAM:chr10:+:60150524:60150620:60148579:60154117
ENSG00000171634:BPTF:chr17:+:65959448:65959622:65955991:65960327
ENSG00000153113:CAST:chr5:+:96064856:96064913:96063234:96065315

ENSG00000125676:THOC2:chrX:-:122757494:122757560:122757134:122757637
ENSG00000077522:ACTN2:chr1:+:236897732:236897818:236894614:236900421
ENSG00000145526:CDH18:chr5:-:19878140:19878199:19839351:19981168
ENSG00000091129:NRCAM:chr7:-:107866088:107866145:107864280:107866651
ENSG00000176454:LPCAT4:chr15:-:34653600:34653733:34652410:34654396

ENSG00000186868:MAPT:chr17:+:44049224:44049311:44039836:44064405

ENSG00000081377:CDC14B:chr9:-:99277930:99278074:99272071:99284787
ENSG00000171817:ZNF540:chr19:+:38091907:38092006:38090653:38102413
ENSG00000147041:SYTL5:chrX:+:37984550:37984759:37981468:37985840

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ENSG00000116641:DOCK7:chr1:-:63010617:63010710:63009416:63018402
ENSG00000169762:TAPT1:chr4:-:16204084:16204203:16193146:16227881

ENSG00000005884:ITGA3:chr17:+:48165588:48165730:48165233:48166473

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ENSG00000179915:NRXN1:chr2:-:50172250:50172344:50170929:50201105

ENSG00000168958:MFF:chr2:+:228195310:228195562:228190143:228197134

ENSG00000163618:CADPS:chr3:-:62479319:62479340:62478121:62484836
ENSG00000258461:RP11-164J13.1:chr15:+:42700408:42700522:42695975:42701500
ENSG00000156973:PDE6D:chr2:-:232601896:232602002:232597743:232602722
ENSG00000214548:MEG3:chr14:+:101300968:101301088:101297871:101302503

ENSG00000153707:PTPRD:chr9:-:8523512:8523524:8521546:8524924
ENSG00000149182:ARFGAP2:chr11:-:47197401:47197474:47193351:47198066

ENSG00000182272:B4GALNT4:chr11:+:380670:380951:380445:381668
ENSG00000198157:HMGN5:chrX:-:80374228:80374258:80373984:80377033

ENSG00000124275:MTRR:chr5:+:7873485:7873625:7871036:7875370

ENSG00000075151:EIF4G3:chr1:-:21324093:21324126:21307720:21329205
ENSG00000214941:ZSWIM7:chr17:-:15881113:15881227:15880406:15881357
ENSG00000183576:SETD3:chr14:-:99880212:99880271:99872927:99924615

ENSG00000119231:SENP5:chr3:+:196654666:196654750:196650422:196656503
ENSG00000162813:BPNT1:chr1:-:220253068:220253196:220247413:220263027

ENSG00000111011:RSRC2:chr12:-:123005050:123005128:123003576:123005931
ENSG00000176293:ZNF135:chr19:+:58571336:58571403:58570678:58572947

ENSG00000149743:TRPT1:chr11:-:63991571:63991634:63991439:63991756

ENSG00000108848:LUC7L3:chr17:+:48826579:48826705:48825777:48827861
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ENSG00000077522:ACTN2:chr1:+:236890977:236891056:236889320:236894532

ENSG00000159596:TMEM69:chr1:+:46156645:46156782:46153947:46158875
ENSG00000123636:BAZ2B:chr2:-:160193452:160193588:160189197:160194077
ENSG00000079156:OSBPL6:chr2:+:179236852:179236960:179226542:179238616
ENSG00000145868:FBXO38:chr5:+:147806775:147807285:147805264:147812986

ENSG00000119403:PHF19:chr9:-:123624195:123624283:123623452:123624865
ENSG00000115109:EPB41L5:chr2:+:120925455:120925583:120925083:120932416
ENSG00000006634:DBF4:chr7:+:87533606:87533731:87530193:87536502
ENSG00000179314:WSCD1:chr17:+:6014090:6014255:5998543:6021307
ENSG00000226174:TEX22:chr14:+:105911754:105911848:105905076:105916394
ENSG00000095637:SORBS1:chr10:-:97131082:97131184:97127456:97141441
ENSG00000074657:ZNF532:chr18:+:56598877:56598987:56587865:56601664
ENSG00000139624:CERS5:chr12:-:50529215:50529435:50528485:50529514
ENSG00000062598:ELMO2:chr20:-:45027335:45027410:45017859:45035186
ENSG00000160741:CRTC2:chr1:-:153923735:153924142:153921860:153924493

ENSG00000163618:CADPS:chr3:-:62516328:62516487:62503925:62518545
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ENSG00000074370:ATP2A3:chr17:-:3831520:3831621:3828735:3831956

ENSG00000119048:UBE2B:chr5:+:133712359:133712385:133710154:133725915
ENSG00000145016:RUBCN:chr3:-:197426057:197426102:197423920:197427483
ENSG00000068831:RASGRP2:chr11:-:64507839:64507921:64507635:64508419
ENSG00000136828:RALGPS1:chr9:+:129796709:129796793:129740024:129815125
ENSG00000165055:METTL2B:chr7:+:128118002:128118046:128117227:128119211
ENSG00000122786:CALD1:chr7:+:134647570:134647685:134645379:134650056
ENSG00000270249:RP11-514P8.7:chr7:-:102207028:102207183:102182109:102207443
ENSG00000159761:C16orf86:chr16:+:67701129:67701428:67700975:67701780
ENSG00000126777:KTN1:chr14:+:56068474:56068598:56047072:56078736
ENSG00000011347:SYT7:chr11:-:61314648:61314780:61300596:61318855
ENSG00000205571:SMN2:chr5:+:69372347:69372401:69366578:69372845

ENSG00000145819:ARHGAP26:chr5:+:142586762:142586873:142526946:142593561
ENSG00000065559:MAP2K4:chr17:+:11984672:11984847:11958308:11998891
ENSG00000066084:DIP2B:chr12:+:51086731:51086796:51080465:51089049

ENSG00000198909:MAP3K3:chr17:+:61769601:61769779:61769222:61770908
ENSG00000105426:PTPRS:chr19:-:5231320:5231626:5222231:5238929

ENSG00000060971:ACAA1:chr3:-:38167652:38167832:38167372:38168000

ENSG00000161395:PGAP3:chr17:-:37830869:37830932:37829119:37840849

ENSG00000104852:SNRNP70:chr19:+:49605370:49605430:49604728:49607890

ENSG00000266967:AARSD1:chr17:-:41105740:41105795:41103911:41107174

ENSG00000156958:GALK2:chr15:+:49575762:49575915:49531564:49584523

ENSG00000124222:STX16:chr20:+:57246219:57246353:57245659:57248686

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ENSG00000107147:KCNT1:chr9:+:138661792:138661901:138660783:138662702

ENSG00000138814:PPP3CA:chr4:-:101950322:101950352:101947218:101953423

ENSG00000174099:MSRB3:chr12:+:65702308:65702435:65672645:65720605

ENSG00000169891:REPS2:chrX:+:17092236:17092282:17088116:17121840

ENSG00000133226:SRRM1:chr1:+:24973569:24973699:24973280:24975349

ENSG00000148660:CAMK2G:chr10:-:75587846:75587879:75581488:75597225

ENSG00000106133:NSUN5P2:chr7:-:72422690:72422834:72420735:72423864

ENSG00000101294:HM13:chr20:+:30155880:30156083:30154098:30156922

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ENSG00000070814:TCOF1:chr5:+:149771106:149771358:149769586:149771519

ENSG00000117616:RSRP1:chr1:-:25570944:25570989:25570124:25571640

ENSG00000184271:POU6F1:chr12:-:51585378:51585547:51584375:51586112

ENSG00000169783:LINGO1:chr15:-:78027333:78027395:77983243:78088280

ENSG00000169045:HNRNPH1:chr5:-:179045145:179045324:179044912:179047892

ENSG00000174483:BBS1:chr11:+:66278675:66278710:66278560:66281876

ENSG00000232560:LINC01549:chr21:+:18816400:18816518:18811315:18821182

ENSG00000145362:ANK2:chr4:+:114253127:114253226:114251626:114254209

ENSG00000205531:NAP1L4:chr11:-:2970456:2970494:2966876:2972488

ENSG00000185008:ROBO2:chr3:+:77681624:77681807:77671583:77684020

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ENSG00000073536:NLE1:chr17:-:33466838:33467085:33466333:33468997

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ENSG00000167528:ZNF641:chr12:-:48739127:48739257:48738495:48741034

ENSG00000169896:ITGAM:chr16:+:31332561:31332692:31309275:31332784

ENSG00000142675:CNKSR1:chr1:+:26510561:26510632:26510321:26510704

ENSG00000166734:CASC4:chr15:+:44620882:44620985:44615217:44671887

ENSG00000080573:COL5A3:chr19:-:10116471:10116661:10116390:10116748

ENSG00000138031:ADCY3:chr2:-:25043252:25043320:25042983:25043592

ENSG00000072163:LIMS2:chr2:-:128431251:128431317:128415136:128432587

ENSG00000127481:UBR4:chr1:-:19476256:19476289:19475120:19477070

ENSG00000180098:TRNAU1AP:chr1:+:28901100:28901230:28898412:28904011

ENSG00000069275:NUCKS1:chr1:-:205698699:205698749:205693109:205719084

ENSG00000079337:RAPGEF3:chr12:-:48134424:48134606:48134178:48134697

ENSG00000175182:FAM131A:chr3:+:184056174:184056317:184055159:184059511

ENSG00000196776:CD47:chr3:-:107768465:107768498:107766139:107776323

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ENSG00000105771:SMG9:chr19:-:44248923:44249036:44244370:44251593

ENSG00000077063:CTTNBP2:chr7:-:117385884:117385984:117375475:117396608

ENSG00000107282:APBA1:chr9:-:72073071:72073104:72072055:72082738

ENSG00000188385:JAKMIP3:chr10:+:133980486:133980559:133978239:133981456

ENSG00000223705:NSUN5P1:chr7:+:75040937:75041042:75039743:75042066

ENSG00000234420:ZNF37BP:chr10:-:43020987:43021108:43019186:43046004

ENSG00000075413:MARK3:chr14:+:103957288:103957444:103946827:103958113

ENSG00000102271:KLHL4:chrX:+:86919763:86919935:86890775:86924328

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ENSG00000075413:MARK3:chr14:+:103964838:103964865:103958371:103966492

ENSG00000134717:BTF3L4:chr1:+:52525506:52525573:52522051:52530496

ENSG00000085382:HACE1:chr6:-:105259185:105259268:105244900:105280916
ENSG00000101203:COL20A1:chr20:+:61957447:61957501:61952451:61958103
ENSG00000196295:AC005154.6:chr7:-:30618621:30618744:30617707:30618846
ENSG00000133612:AGAP3:chr7:+:150817606:150817832:150817232:150819811

ENSG00000188338:SLC38A3:chr3:+:50254669:50254752:50253253:50255107
ENSG00000198276:UCKL1:chr20:-:62575005:62575022:62573845:62575749
ENSG00000214783:POLR2J4:chr7:-:44026199:44026276:44012922:44053191
ENSG00000011347:SYT7:chr11:-:61313502:61313727:61300596:61314648

ENSG00000082458:DLG3:chrX:+:69715257:69715303:69713325:69718369
ENSG00000159082:SYNJ1:chr21:-:34017277:34017316:34014276:34017955
ENSG00000181929:PRKAG1:chr12:-:49399525:49399664:49399326:49406844
ENSG00000122482:ZNF644:chr1:-:91403041:91403647:91383711:91447866
ENSG00000168175:MAPK1IP1L:chr14:+:55528397:55528419:55518521:55529335
ENSG00000241231:RP11-275H4.1:chr3:-:181157764:181157822:181156487:181160161
ENSG00000089022:MAPKAPK5:chr12:+:112306556:112306665:112304035:112308074

ENSG00000172366:MCRIP2:chr16:+:696471:696608:692043:697416
ENSG00000143093:STRIP1:chr1:+:110584157:110584264:110583325:110585709
ENSG00000113712:CSNK1A1:chr5:-:148897356:148897440:148892772:148899852

ENSG00000283886:RP11-204M4.3:chr9:-:41975030:41975128:41963942:42018927
ENSG00000070718:AP3M2:chr8:+:42011995:42012054:42010623:42012133

ENSG00000081803:CADPS2:chr7:-:122120905:122120914:122114571:122130134
ENSG00000068024:HDAC4:chr2:-:240066278:240066410:240061492:240078347
ENSG00000136699:SMPD4:chr2:-:130918758:130918845:130914969:130921946
ENSG00000141994:DUS3L:chr19:-:5786759:5786856:5786553:5787071

ENSG00000067225:PKM:chr15:-:72495362:72495529:72492996:72499068
ENSG00000122481:RWDD3:chr1:+:95712097:95712213:95699871:95712311

ENSG00000068078:FGFR3:chr4:+:1804640:1804791:1803752:1805418
ENSG00000137502:RAB30:chr11:-:82705080:82705148:82698812:82708265
ENSG00000136059:VILL:chr3:+:38042412:38042491:38040930:38042946
ENSG00000132470:ITGB4:chr17:+:73751135:73751294:73750896:73751781
ENSG00000184182:UBE2F:chr2:+:238903385:238903451:238881867:238933982
ENSG00000091136:LAMB1:chr7:-:107599693:107599925:107596075:107600135
ENSG00000125347:IRF1:chr5:-:131823617:131823717:131822822:131826236
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ENSG00000175061:LRRC75A-AS1:chr17:+:16342894:16343017:16342728:16343498
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ENSG00000122490:PQLC1:chr18:-:77679183:77679400:77664183:77693968
ENSG00000101849:TBL1X:chrX:+:9621584:9621729:9608400:9622254

ENSG00000178538:CA8:chr8:-:61144842:61144938:61102544:61178483
ENSG00000198929:NOS1AP:chr1:+:162330601:162330714:162326926:162335193
ENSG00000235398:LINC00623:chr1:-:144340525:144340623:144301536:144341669
ENSG00000061938:TNK2:chr3:-:195596367:195596412:195595580:195596984
ENSG00000078674:PCM1:chr8:+:17793097:17793215:17780697:17794642
ENSG00000115257:PCSK4:chr19:-:1487601:1487690:1487312:1487783
ENSG00000110888:CAPRIN2:chr12:-:30872012:30872159:30869610:30876192
ENSG00000168661:ZNF30:chr19:+:35433497:35433577:35424627:35434126
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ENSG00000144840:RABL3:chr3:-:120412999:120413071:120409334:120417269
ENSG00000084693:AGBL5:chr2:+:27291915:27291962:27291612:27292440
ENSG00000166839:ANKDD1A:chr15:+:65219099:65219198:65218369:65223019
ENSG00000114956:DGUOK:chr2:+:74177753:74177859:74174033:74185272
ENSG00000154358:OBSCN:chr1:+:228480223:228480487:228479862:228481053
ENSG00000196586:MYO6:chr6:+:76604947:76604977:76602407:76608089
ENSG00000167524:SGK494:chr17:-:26940095:26940164:26939705:26941104

ENSG00000164828:SUN1:chr7:+:888054:888252:883157:889559

ENSG00000047849:MAP4:chr3:-:47917174:47917390:47913590:47918906
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ENSG00000166295:ANAPC16:chr10:+:73983645:73983814:73980137:73990123

ENSG00000125388:GRK4:chr4:+:29904
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ENSG00000196151:WDSUB1:chr2:-
:160114268:160114375:160112886:1601
36271

ENSG00000183597:TANGO2:chr22:+:2
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ENSG00000197989:SNHG12:chr1:-
:28907605:28907741:28907158:2890810
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ENSG00000137955:RABGGTB:chr1:+:
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3

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:131823617:131823744:131822822:1318
26236
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4

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116:67161176:67154958:67184976

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ENSG00000188766:SPRED3:chr19:+:38
885282:38885426:38882928:38886119

ENSG00000187118:CMC1:chr3:+:2836
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ENSG00000104774:MAN2B1:chr1
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ENSG00000181027:FKRP:chr19:+:
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:34856669:34856799:34855004:348
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ENSG00000120896:SORBS3:chr8:
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ENSG00000127483:HP1BP3:chr1:-:21106842:21107033:21106404:21113687

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ENSG00000148660:CAMK2G:chr10:-:75587846:75587879:75583842:75597225
ENSG00000145349:CAMK2D:chr4:-:114376881:114376977:114375671:114378490

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ENSG00000273590:SMIM11B:chr21:+:35772008:35772197:35757942:35774388

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ENSG00000095794:CREM:chr10:+:35437295:35437419:35416121:35467816

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ENSG00000143224:PPOX:chr1:+:161137160:161137276:161137024:161140409
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ENSG00000088451:TGDS:chr13:-:95243106:95243197:95235490:95244494
ENSG00000183762:KREMEN1:chr22:+:29517344:29517469:29494941:29521250
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ENSG00000068878:PSME4:chr2:-:54127017:54127154:54125491:54128486
ENSG00000184156:KCNQ3:chr8:-:133184844:133184940:133182675:133186485
ENSG00000134222:PSRC1:chr1:-:109825301:109825358:109823668:109825684

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ENSG00000257621:PSMA3-AS1:chr14:-:58759783:58759856:58758425:58764672
ENSG00000125814:NAPB:chr20:-:23375775:23375822:23375636:23383629

ENSG00000159761:C16orf86:chr16:+:67701198:67701428:67700975:67701780

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ENSG00000151150:ANK3:chr10:-:61867945:61868044:61865817:61868587
ENSG00000106070:GRB10:chr7:-:50772836:50773020:50771605:50823583
ENSG00000146112:PPP1R18:chr6:-:30652184:30653823:30647166:30654890

ENSG00000114062:UBE3A:chr15:-:25652213:25652409:25650649:25654234
ENSG00000132872:SYT4:chr18:-:40853544:40854305:40851797:40857212

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ENSG00000149633:KIAA1755:chr20:-:36854066:36854183:36852038:36859603
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ENSG00000203805:PLPP4:chr10:+:122280482:122280607:122263438:122334642
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ENSG00000136059:VILL:chr3:+:38044658:38044804:38044066:38045745
ENSG00000176406:RIMS2:chr8:+:105010412:105010478:105001649:105026733
ENSG00000135541:AHI1:chr6:-:135644299:135644462:135639754:135679269
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ENSG00000176155:CCDC57:chr17:-:80136349:80136481:80130736:80141649

ENSG00000163607:GTPBP8:chr3:+:112711872:112711971:112710182:112713981

ENSG00000235954:TTC28-AS1:chr22:+:28318005:28318166:28315533:28331138

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ENSG00000115137:DNAJC27:chr2:-:25174262:25174423:25170617:25179911

ENSG00000165233:CARD19:chr9:+:95874160:95874220:95870109:95874499

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ENSG00000166199:ALKBH3:chr11:+:43913590:43913679:43911378:43923065

ENSG00000175395:ZNF25:chr10:-:38256844:38256867:38246474:38260635

ENSG00000244187:TMEM141:chr9:+:139686162:139686229:139685876:139686398

ENSG00000106976:DNM1:chr9:+:131015379:131015416:131013219:131016284

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ENSG00000119383:PTPA:chr9:+:131890242:131890347:131885417:131891263

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ENSG00000181038:METTL23:chr17:+:74725771:74725876:74722961:74729059

ENSG00000111412:C12orf49:chr12:-:117157567:117157681:117155698:117175594

ENSG00000072803:FBXW11:chr5:-:171384600:171384702:171337801:171433461

ENSG00000174796:THAP6:chr4:+:76465068:76465169:76447071:76467618

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ENSG00000150779:TIMM8B:chr11:-:111956701:111957034:111956186:111957363

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ENSG00000177200:CHD9:chr16:+:53355074:53355129:53352252:53355437

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ENSG00000100211:CBY1:chr22:+:39061550:39061690:39052755:39064021

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ENSG00000072657:TRHDE:chr12:+:73046795:73046936:73046269:73050706

ENSG00000105173:CCNE1:chr19:+:30312902:30313037:30312724:30313146

ENSG00000136444:RSAD1:chr17:+:48557240:48557445:48556380:48559451

ENSG00000196295:AC005154.6:chr7:-

ENSG00000196132:MYT1:chr20:+:62854643:62854712:62854526:62858767

ENSG00000177303:CASKIN2:chr17:-:73500892:73501154:73500561:73501637

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ENSG00000134313:KIDINS220:chr2:-:8888016:8888130:8877129:8890241

ENSG00000107147:KCNT1:chr9:+:138679945:138680008:138678367:138683642

ENSG00000005448:WDR54:chr2:+:74649246:74649502:74648943:74649996

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ENSG00000134278:SPIRE1:chr18:-:12485957:12485999:12479870:12493070

ENSG00000148840:PPRC1:chr10:+:103904006:103904064:103902855:103906428

ENSG00000164751:PEX2:chr8:-:77900542:77900574:77896431:77912225

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ENSG00000116698:SMG7:chr1:+:183516237:183516387:183515472:183518898

ENSG00000130382:MLLT1:chr19:-:6226987:6227113:6222695:6230580

ENSG00000119866:BCL11A:chr2:-:60695866:60695968:60679801:60773105

ENSG00000143367:TUFT1:chr1:+:151535060:151535162:151512902:151536379

ENSG00000197119:SLC25A29:chr14:-:100761962:100762330:100759714:100765178

ENSG00000279765:RP11-437B10.1:chr15:+:93472259:93472321:93470560:93482807

ENSG00000168306:ACOX2:chr3:-:58516192:58516365:58514683:58516977

ENSG00000152348:ATG10:chr5:+:81354313:81354421:81283497:81474308

ENSG00000145214:DGKQ:chr4:-:957860:957881:957078:958963

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ENSG00000137842:TMEM62:chr15:+:43470804:43470909:43461875:43473378

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ENSG00000163618:CADPS:chr3:-:62483820:62483829:62478121:62484836
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ENSG00000107679:PLEKHA1:chr10:+:124187791:124187832:124186547:124189139
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ENSG00000124126:PREX1:chr20:-:47364345:47364417:47361684:474441778
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ENSG00000132591:ERAL1:chr17:+:27184956:27185003:27183600:27185391
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ENSG00000136280:CCM2:chr7:+:45109424:45109560:45104245:45113058

ENSG00000175497:DPP10:chr2:+:116257085:116257180:116101488:116283473

ENSG00000110675:ELMOD1:chr11:+:107496122:107496258:107488932:107501142

ENSG00000079819:EPB41L2:chr6:-:131197813:131197915:131191266:131199243

ENSG00000161647:MPP3:chr17:-:41903123:41903209:41901373:41905118

ENSG00000171735:CAMTA1:chr1:+:6946299:6946406:6931900:6947713

ENSG00000169592:INO80E:chr16:+:30015888:30015978:30012361:30016541

ENSG00000100296:THOC5:chr22:-:29927066:29927099:29925228:29927819

ENSG00000136891:TEX10:chr9:-:103091421:103091557:103090244:103102538

ENSG00000118705:RPN2:chr20:+:35866804:35866852:35862498:35869705

ENSG00000214706:IFRD2:chr3:-:50326877:50327193:50326368:50327359

ENSG00000153391:INO80C:chr18:-:33060416:33060527:33048706:33077682

ENSG00000152117:AC093838.4:chr2:+:132273174:132273320:132266181:132273886

ENSG00000112320:SOBP:chr6:+:107908283:107908379:107854814:107979410

ENSG00000006194:ZNF263:chr16:+:3335680:3335754:3335239:3336022

ENSG00000132694:ARHGEF11:chr1:-:156941488:156941608:156939835:156947995

ENSG00000131626:PPFIA1:chr11:+:70197099:70197129:70194526:70200406

ENSG00000198039:ZNF273:chr7:+:64377407:64377496:64363797:64377958

ENSG00000060656:PTPRU:chr1:+:29633640:29633658:29631940:29637255

ENSG00000228315:GUSBP11:chr22:-:24042912:24043032:24002187:24047615

ENSG00000249853:HS3ST5:chr6:-:114549748:114549942:114489626:114663358

ENSG00000154065:ANKRD29:chr18:-:21189187:21189255:21181273:21192055

ENSG00000188735:TMEM120B:chr12:+:122209393:122209455:122199644:122211326

ENSG00000162086:ZNF75A:chr16:+:3362676:3362768:3361948:3367189

ENSG00000137411:VARS2:chr6:+:30889701:30889772:30889468:30889892

ENSG00000117425:PTCH2:chr1:-:45295580:45295702:45295433:45296519

ENSG00000186654:PRR5:chr22:+:45121123:45121176:45110551:45122456

ENSG00000180229:HERC2P3:chr15:-:20671447:20671623:20668254:20688655

ENSG00000188130:MAPK12:chr22:-:50687259:50687319:50686901:50687531

ENSG00000168502:MTCL1:chr18:+:8783527:8784841:8777890:8796229

ENSG00000227232:WASH7P:chr1:-:17232:17368:17055:17605

ENSG00000149761:NUDT22:chr11:+:63995039:63995138:63994604:63996718

ENSG00000111879:FAM184A:chr6:-:119295592:119295739:119288117:119296188

ENSG00000168255:POLR2J3:chr7:-:102208456:102208631:102182109:102210281

ENSG00000117597:DIEXF:chr1:+:210016795:210017041:210015792:210024548

ENSG00000114956:DGUOK:chr2:+:74184251:74184367:74177859:74185272

ENSG00000143786:CNIH3:chr1:+:224872497:224872545:224868728:224918163

ENSG00000204227:RING1:chr6:+:33178934:33179324:33177563:33179505

ENSG00000169398:PTK2:chr8:-:141679825:141679834:141678523:141684396

ENSG00000099957:P2RX6:chr22:+:21380299:21380365:21380187:21380708

ENSG00000121067:SPOP:chr17:-:47745388:47745440:47714171:47755294

ENSG00000159082:SYNJ1:chr21:-:34007170:34007194:34004111:34011217

ENSG00000167323:STIM1:chr11:+:4109931:4109968:4107773:4112511

ENSG00000165487:MICU2:chr13:-:22070190:22070299:22069457:22077064

ENSG00000110888:CAPRIN2:chr12:-:30872012:30872159:30869610:30873744

ENSG00000163995:ABLIM2:chr4:-:7986565:7986620:7985281:8009785

ENSG00000114867:EIF4G1:chr3:+:184033919:184034006:184032462:184035108

ENSG00000164924:YWHAZ:chr8:-:101964156:101964353:101961128:101965077

ENSG00000124608:AARS2:chr6:-:44274985:44275131:44274768:44278730

ENSG00000254995:STX16-NPEPL1:chr20:+:57266089:57266283:57248767:57266779

ENSG00000204628:RACK1:chr5:-:180665098:180665239:180664042:180666486

ENSG00000115525:ST3GAL5:chr2:-:86097194:86097381:86090608:86115946

ENSG00000084444:FAM234B:chr12:+:13232722:13232943:13229077:13235936

ENSG00000143702:CEP170:chr1:-:243349080:243349374:243336148:243349560

ENSG00000136141:LRCH1:chr13:+:47289694:47289799:47286732:47297355

ENSG00000143622:RIT1:chr1:-:155874393:155874401:155874293:155874521

ENSG00000006283:CACNA1G:chr17:+:48695219:48695269:48694932:48695408

ENSG00000100241:SBF1:chr22:-:50895462:50895540:50895102:50897683

ENSG00000171097:KYAT1:chr9:-:131608958:131609138:131607690:131644175

ENSG00000143850:PLEKHA6:chr1:-

ENSG00000123636:BAZ2B:chr2:-:160284451:160284553:160269056:160284821 ENSG00000145428:RNF175:chr4:-:154669796:154669979:154649513:154672589 ENSG00000133226:SRRM1:chr1:+:24980796:24980834:24979523:24981345 ENSG00000266338:NBPF15:chr1:+:148577071:148577251:148568877:148577357 ENSG00000136280:CCM2:chr7:+:45077851:45078025:45039456:45103516 ENSG00000102181:CD99L2:chrX:-:149983334:149983409:149963959:149999703 ENSG00000142856:ITGB3BP:chr1:-:63955753:63955889:63944504:63974198 ENSG00000022840:RNF10:chr12:+:120977170:120977271:120972771:120984207 ENSG00000168824:NSG1:chr4:+:4388845:4388900:4388370:4389330 ENSG00000198952:SMG5:chr1:-:156236331:156236469:156236171:156237260 ENSG00000170231:FABP6:chr5:+:159655479:159655699:159640826:159656552 ENSG00000170954:ZNF415:chr19:-:53626753:53626908:53625996:53636108 ENSG00000106070:GRB10:chr7:-:50674033:50674111:50660795:50680437 ENSG00000143207:RFWD2:chr1:-:176105623:176105683:176104222:176118141 ENSG00000108788:MLX:chr17:+:40720487:40720577:40719673:40720854 ENSG00000140463:BBS4:chr15:+:73015129:73015188:73009191:73016868 ENSG00000120438:TCP1:chr6:-:160209089:160209175:160208903:160210436 ENSG00000006283:CACNA1G:chr17:+:48695219:48695290:48694932:48695457 ENSG00000219626:FAM228B:chr2:+:24362239:24362320:24358057:24392224	ENSG00000140538:NTRK3:chr15:-:88524456:88524591:88522694:88576087 ENSG00000067840:PDZD4:chrX:-:153072733:153072824:153072295:153095693 ENSG00000132716:DCAF8:chr1:-:160231074:160231140:160213824:160232238 ENSG00000179912:R3HDM2:chr12:-:57682639:57682693:57677839:57682791 ENSG00000028203:VEZT:chr12:+:95650925:95651015:95650398:95656681 ENSG00000181555:SETD2:chr3:-:47142947:47143045:47139571:47155365 ENSG00000121578:B4GALT4:chr3:-:118953991:118954074:118949091:118955699 ENSG00000163513:TGFBR2:chr3:+:30664690:30664765:30648469:30686238 ENSG00000165695:AK8:chr9:-:135668020:135668162:135602921:135690024 ENSG00000184056:VPS33B:chr15:-:91560192:91560254:91557663:91565383 ENSG00000149182:ARFGAP2:chr11:-:47193831:47193884:47193351:47198066 ENSG00000140859:KIFC3:chr16:-:57793036:57793065:57792821:57794637 ENSG00000245573:BDNF-AS:chr11:+:27679787:27680009:27661552:27680717 ENSG00000090661:CERS4:chr19:+:8275589:8275746:8274378:8306224 ENSG00000162004:CCDC78:chr16:-:775412:775580:775293:775793 ENSG00000168477:TNXB:chr6:-:32011783:32011906:32011669:32012157 ENSG00000184162:NR2C2AP:chr19:-:19313278:19313384:19313207:19313599 ENSG00000009950:MLXIPL:chr7:-:73010968:73011119:73010809:73011194 ENSG00000142046:TMEM91:chr19:+:41889465:41889537:41888826:41889767	:204224753:204224807:204220739:204225929 ENSG00000104093:DMXL2:chr15:-:51751794:51751857:51750992:51755575 ENSG00000078747:ITCH:chr20:+:32981575:32981687:32957276:32996456 ENSG00000151276:MAGI1:chr3:-:65361419:65361620:65350621:65364935 ENSG00000166169:POLL:chr10:-:103342519:103342648:103340173:103347002 ENSG00000076685:NT5C2:chr10:-:104865114:104865138:104861083:104865462 ENSG00000161395:PGAP3:chr17:-:37832866:37833008:37830932:37840849 ENSG00000138326:RPS24:chr10:+:79799958:79799983:79797062:79800372 ENSG00000007376:RPUSD1:chr16:-:837555:837645:837477:838255 ENSG00000120963:ZNF706:chr8:-:102214560:102214675:102213971:102216877 ENSG00000105327:BBC3:chr19:-:47729820:47730011:47725175:47734185 ENSG00000112290:WASF1:chr6:-:110499800:110499945:110448832:110501043 ENSG00000185046:ANKS1B:chr12:-:99201621:99201696:99194903:99446934 ENSG00000141696:P3H4:chr17:-:39959538:39959683:39959231:39963047 ENSG00000131966:ACTR10:chr14:+:58680348:58680416:58675825:58681922 ENSG00000144040:SFXN5:chr2:-:73195576:73195662:73188377:73215386 ENSG00000164176:EDIL3:chr5:-:83525672:83525702:83476339:83549901 ENSG00000230606:AC159540.1:chr2:-:98088031:98088249:98082014:98088547 ENSG00000128739:SNRPN:chr15:+:25219544:25219603:25213229:25221451 ENSG00000021762:OSBPL5:chr11:-:3141773:3141854:3140861:3143226

ENSG00000076555:ACACB:chr12:+:109646969:109647158:109644661:109650640

ENSG00000029363:BCLAF1:chr6:-:136590278:136590441:136589477:136590574
ENSG00000160813:PPP1R35:chr7:-:100033253:100033390:100033156:100033470
ENSG00000178252:WDR6:chr3:+:49050723:49051549:49044964:49051642

ENSG00000132466:ANKRD17:chr4:-:74005247:74006000:74000979:74007457
ENSG00000203805:PLPP4:chr10:+:122334642:122334813:122280607:122348814
ENSG00000204843:DCTN1:chr2:-:74601449:74601467:74598855:74604558

ENSG00000170004:CHD3:chr17:+:7810918:7811020:7810806:7811211

ENSG00000173681:CXorf23:chrX:-:19953926:19954016:19948058:19955622
ENSG00000149403:GRIK4:chr11:+:120732667:120732829:120707553:120744774
ENSG00000113851:CRBN:chr3:-:3216846:3216950:3215945:3221304

ENSG00000174485:DENND4A:chr15:-:65992881:65993010:65989721:65993388
ENSG00000135164:DMTF1:chr7:+:86783705:86783844:86781871:86792810

ENSG00000204653:ASPDH:chr19:-:51016183:51016268:51015030:51017734

ENSG00000106133:NSUN5P2:chr7:-:72422690:72422834:72420735:72424897
ENSG00000143537:ADAM15:chr1:+:155034051:155034122:155033965:155034720

ENSG00000134138:MEIS2:chr15:-:37388489:37388608:37387815:37390167

ENSG00000081189:MEF2C:chr5:-:88026027:88026051:88025164:88027545
ENSG00000141294:LRRC46:chr17:+:45913031:45913141:45912765:45913398
ENSG00000073060:SCARB1:chr12:-:125267228:125267357:125263132:125270986
ENSG00000101972:STAG2:chrX:+:123155216:123155281:123094716:123156380
ENSG00000147439:BIN3:chr8:-:22494037:22494099:22488091:22494434
ENSG00000113163:COL4A3BP:chr5:-:74695134:74695212:74685512:74696029
ENSG00000146215:CRIP3:chr6:-:43273804:43273862:43273613:43273956

ENSG00000245573:BDNF-AS:chr11:+:27561566:27561663:27528445:27661391
ENSG00000176444:CLK2:chr1:-:155238498:155238586:155238150:155239278
ENSG00000179889:PDXDC1:chr16:+:15095632:15095744:15092262:15098043
ENSG00000060237:WNK1:chr12:+:1010726:1010819:1009836:1013618
ENSG00000091136:LAMB1:chr7:-:107576723:107576818:107576101:107577537
ENSG00000031823:RANBP3:chr19:-:5941631:5941718:5933490:5957928
ENSG00000168958:MFF:chr2:+:228211941:228212100:228205096:228217229
ENSG00000089847:ANKRD24:chr19:+:4202022:4202087:4200168:4207238

ENSG00000159147:DONSON:chr21:-:34955793:34955972:34954552:34956895
ENSG00000154277:UCHL1:chr4:+:41259131:41259143:41259026:41262663
ENSG00000010803:SCMH1:chr1:-:41540890:41541123:41514562:41578954
ENSG00000155329:ZCCHC10:chr5:-:132358532:132358598:132342612:132362188
ENSG00000186575:NF2:chr22:+:30079008:30079053:30074312:30090740
ENSG00000138867:GUCD1:chr22:-:24944884:24944969:24944041:24951582
ENSG00000128512:DOCK4:chr7:-:111424152:111424179:111424008:111428717

ENSG00000135049:AGTPBP1:chr9:-:88293249:88293313:88292497:88296182
ENSG00000180488:MIGA1:chr1:+:78325724:78325811:78325071:78326908
ENSG00000004534:RBM6:chr3:+:50036872:50036946:50012825:50085677
ENSG00000175267:VWA3A:chr16:+:22161107:22161252:22159627:22163831
ENSG00000106070:GRB10:chr7:-:50771517:50771605:50742355:50860920
ENSG00000163618:CADPS:chr3:-:62499312:62499381:62484943:62501733
ENSG00000158106:RHPN1:chr8:+:144461998:144462345:144461678:144462758

ENSG00000010270:STARD3NL:chr7:+:38256625:38256679:38254706:38256788
ENSG00000151466:SCLT1:chr4:-:129864150:129864343:129812292:129867161

Table 2b. IRIS screening results of tumor AS events in 22 GBM samples. b. IRIS identified 1,738 tumor-recurrent AS events with high tumor-specificity in GBM samples (Prioritized set)

AS_event	AS_event	AS_event
ENSG00000127603:MACF1:chr1:+:39844132:39844195:39838268:39844874	ENSG00000150712:MTMR12:chr5:-:32233878:32234040:32230453:32239106	ENSG00000081377:CDC14B:chr9:-:99277930:99278074:99266071:99284787
ENSG00000197223:C1D:chr2:-:68280192:68280418:68274451:68290076	ENSG00000226210:ABC7-42389800N19.1:chr12:+:87880:88017:87703:88569	ENSG00000271447:MMP28:chr17:-:34093215:34093913:34083438:34094770
ENSG00000095637:SORBS1:chr10:-:97110965:97111133:97106209:97114638	ENSG00000139746:RBM26:chr13:-:79927287:79927359:79918929:79928573	ENSG00000005379:TSPOAP1:chr17:-:56381698:56381760:56379808:56382421
ENSG00000105483:CARD8:chr19:-:48726975:48727125:48725112:48733694	ENSG00000138326:RPS24:chr10:+:79797722:79797740:79797062:79799961	ENSG00000106077:ABHD11:chr7:-:73151258:73151440:73151021:73151891
ENSG00000127663:KDM4B:chr19:+:5119128:5119230:5110829:5119663	ENSG00000110514:MADD:chr11:+:47310518:47310578:47308085:47310941	ENSG00000134909:ARHGAP32:chr11:-:128910822:128910904:128868363:128932174
ENSG00000061273:HDAC7:chr12:-:48189688:48189799:48189550:48189989	ENSG00000214174:AMZ2P1:chr17:-:62969359:62969635:62969045:62970672	ENSG00000075391:RASAL2:chr1:+:178439827:178439858:178436556:178442209
ENSG00000124214:STAU1:chr20:-:47782533:47782822:47770608:47790731	ENSG00000153391:INO80C:chr18:-:33058245:33058313:33048706:33077682	ENSG00000079819:EPB41L2:chr6:-:131191467:131191521:131191266:131206235
ENSG00000148057:IDNK:chr9:+:86243787:86243874:86238136:86256462	ENSG00000198838:RYR3:chr15:+:34148084:34148252:34147113:34149980	ENSG00000274769:RP11-493E12.3:chr2:+:61344491:61344520:61343205:61344601
ENSG00000068724:TTC7A:chr2:+:47178740:47178872:47177665:47183977	ENSG00000173264:GPR137:chr11:+:64055536:64055686:64055418:64055811	ENSG00000033627:ATP6V0A1:chr17:+:40666306:40666478:40665996:40673044
ENSG00000007545:CRAMP1:chr16:+:1716073:1716178:1715139:1716422	ENSG00000122786:CALD1:chr7:+:134620438:134620516:134618141:134625842	ENSG00000198925:ATG9A:chr2:-:220093155:220093204:220092775:220094256
ENSG00000166167:BTRC:chr10:+:103190101:103190209:103113985:103221737	ENSG00000082898:XPO1:chr2:-:61761692:61761813:61761038:61764696	ENSG00000196652:ZKSCAN5:chr7:+:99117449:99117532:99110214:99123435
ENSG00000138614:INTS14:chr15:-:65899496:65899780:65897574:65903435	ENSG00000138162:TACC2:chr10:+:123988860:123989001:123987523:123996909	ENSG00000137814:HAUS2:chr15:+:42852979:42853068:42851606:42853467
ENSG00000134444:KIAA1468:chr18:+:59947592:59947675:59947089:59947878	ENSG00000075151:EIF4G3:chr1:-:21329205:21329301:21307720:21377358	ENSG00000136044:APPL2:chr12:-:105570653:105570794:105569860:105571003
ENSG00000008382:MPND:chr19:+:4354945:4355018:4354417:4357249	ENSG00000205581:HMGN1:chr21:-:40717755:40719218:40717200:40719304	ENSG00000050130:JKAMP:chr14:+:59953430:59953522:59951311:59954391
ENSG00000165801:ARHGEF40:chr14:+:21555478:21555622:21554025:21556126	ENSG00000078403:MLLT10:chr10:+:21906040:21906136:21903853:21940601	ENSG00000227036:LINC00511:chr17:-:70416840:70417105:70400957:70424376
ENSG00000107036:RIC1:chr9:+:5753535:5753646:5753238:5754840	ENSG00000146707:POMZP3:chr7:-:76240770:76240908:76239532:76247499	ENSG00000163466:ARPC2:chr2:+:219103386:219103573:219093573:219110142
ENSG00000127054:INTS11:chr1:-:1258560:1258667:1256473:1259960	ENSG00000115310:RTN4:chr2:-:55255299:55255356:55214834:55276880	ENSG00000073921:PICALM:chr11:-:85671717:85671820:85670103:85685750
ENSG00000196295:AC005154.6:chr7:-:30590933:30591095:30590397:30603346	ENSG00000245910:SNHG6:chr8:-:67834558:67834627:67834349:67834848	ENSG00000090097:PCBP4:chr3:-:51996825:51996908:51995320:52001341

ENSG00000167766:ZNF83:chr19:-:53119970:53120128:53118050:53122188
ENSG00000119844:AFTPH:chr2:+:64806619:64808407:64800202:64812555
ENSG00000197381:ADARB1:chr21:+:46548316:46548488:46494708:46591524
ENSG00000160201:U2AF1:chr21:-:44521475:44521542:44520629:44524424
ENSG00000150712:MTMR12:chr5:-:32235067:32235235:32230453:32239106
ENSG00000072110:ACTN1:chr14:-:69345705:69345786:69345240:69346678
ENSG00000114209:PDCD10:chr3:-:167443188:167443261:167438061:167452001
ENSG00000129353:SLC44A2:chr19:+:10753572:10753697:10753127:10753954
ENSG00000169062:UPF3A:chr13:+:115051776:115051875:115047602:115051993
ENSG00000127527:EPS15L1:chr19:-:16487932:16488065:16472795:16495939
ENSG00000148399:DPH7:chr9:-:140459344:140459410:140459058:140459536
ENSG00000074266:EED:chr11:+:85977124:85977258:85975305:85988021
ENSG00000108848:LUC7L3:chr17:+:48828657:48828723:48828213:48829560
ENSG00000073008:PVR:chr19:+:45162009:45162168:45161178:45164558

ENSG00000124006:OBSL1:chr2:-:220422064:220422340:220421445:220423946
ENSG00000056586:RC3H2:chr9:-:125620201:125620372:125618157:125620947
ENSG00000089159:PXN:chr12:-:120657009:120657894:120653464:120659425
ENSG00000101198:NKAIN4:chr20:-:61873890:61873975:61872858:61875375
ENSG00000257303:RP11-977G19.11:chr12:+:56706190:56706283:56693971:56708312
ENSG00000123562:MORF4L2:chrX:-:102933426:102933579:102931979:102940098
ENSG00000152726:FAM21B:chr10:+:47915877:47915964:47913703:47919941
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ENSG00000243943:ZNF512:chr2:+:27806524:27806583:27806009:27820933
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ENSG00000099998:GGT5:chr22:-:24616459:24616552:24616084:24620963
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ENSG00000250222:CTC-338M12.5:chr5:+:180620341:180620467:180619140:180621233
ENSG00000170791:CHCHD7:chr8:+:57125320:57125468:57124396:57127156
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ENSG00000076685:NT5C2:chr10:-:104860508:104860700:104859776:104860801
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ENSG00000089177:KIF16B:chr20:-:16354901:16355054:16352406:16359449
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ENSG00000204790:CBWD6:chr9:-:69234829:69234952:69229668:69235561
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ENSG00000172831:CES2:chr16:+:66971939:66972144:66969879:66974124
ENSG00000144283:PKP4:chr2:+:159535092:159535166:159530512:159536940
ENSG00000137216:TMEM63B:chr6:+:44103064:44103103:44102833:44104085
ENSG00000183696:UPP1:chr7:+:48139266:48139384:48134424:48141420
ENSG00000128591:FLNC:chr7:+:128490029:128490128:128489632:128490437
ENSG00000110888:CAPRIN2:chr12:-:30872012:30872159:30869610:30873744
ENSG00000164924:YWHAZ:chr8:-:101964156:101964353:101961128:101965077
ENSG00000124608:AARS2:chr6:-:44274985:44275131:44274768:44278730
ENSG00000143702:CEP170:chr1:-:243349080:243349374:243336148:243349560
ENSG00000100241:SBF1:chr22:-:50895462:50895540:50895102:50897683
ENSG00000151276:MAGI1:chr3:-:65361419:65361620:65350621:65364935
ENSG00000166169:POLL:chr10:-:103342519:103342648:103340173:103347002
ENSG00000138326:RPS24:chr10:+:79799958:79799983:79797062:79800372
ENSG00000120963:ZNF706:chr8:-:102214560:102214675:102213971:102216877
ENSG00000159147:DONSON:chr21:-:34955793:34955972:34954552:34956895
ENSG00000186575:NF2:chr22:+:30079008:30079053:30074312:30090740
ENSG00000151466:SCLT1:chr4:-:129864150:129864343:129812292:129867161

Table 3a. IRIS prediction of AS-derived TCR and CAR-T targets for 22 GBM samples. a. Prioritized TCR targets listed by unique splice junction.

AS_event	AS_event	AS_event
ENSG00000117625:RCOR3:chr1:+:211486061:211486303:211477482:211486765	ENSG00000144283:PKP4:chr2:+:159389949:159390046:159389828:159433782	ENSG00000145725:PPIP5K2:chr5:+:102523014:102523077:102522140:102526542
ENSG00000163531:NFASC:chr1:+:204971723:204971876:204957934:204978684	ENSG00000122490:PQLC1:chr18:-:77679183:77679400:77664183:77693968	ENSG00000173210:ABLIM3:chr5:+:148622053:148622101:148619451:148624443
ENSG00000100296:THOC5:chr22:-:29927066:29927099:29925228:29927819	ENSG00000143847:PPFIA4:chr1:+:203025911:203026078:203025636:203028305	ENSG00000139323:POC1B:chr12:-:89818937:89819156:89815034:89853414
ENSG00000107263:RAPGEF1:chr9:-:134479347:134479440:134477536:134497182	ENSG00000140416:TPM1:chr15:+:63356262:63356341:63354844:63358094	ENSG00000076685:NT5C2:chr10:-:104860508:104860700:104859776:104860801
ENSG00000076864:RAP1GAP:chr1:-:21930327:21930405:21929406:21932558	ENSG00000089159:PXN:chr12:-:120654075:120654919:120653464:120659425	ENSG00000004534:RBM6:chr3:+:50036872:50036946:50000118:50085677
ENSG00000198208:RPS6KL1:chr14:-:75375803:75375864:75375635:75376245	ENSG00000033627:ATP6V0A1:chr17:+:40666306:40666478:40665996:40673044	ENSG00000162961:DPY30:chr2:-:32117060:32117203:32108531:32142994
ENSG00000070476:ZXDC:chr3:-:126160607:126160789:126158570:126180377	ENSG00000122490:PQLC1:chr18:-:77693968:77694022:77664183:77703328	ENSG00000158806:NPM2:chr8:+:21883157:21883283:21883032:21890640
ENSG00000166348:USP54:chr10:-:75294357:75294528:75290593:75296026	ENSG00000099957:P2RX6:chr22:+:21380299:21380365:21380187:21380708	ENSG00000168894:RNF181:chr2:+:85823979:85824054:85823772:85824226
ENSG00000022567:SLC45A4:chr8:-:142231675:142231864:142229928:142264087	ENSG00000095637:SORBS1:chr10:-:97110972:97111133:97106209:97114638	ENSG00000078328:RBFOX1:chr16:+:7680604:7680685:7657340:7703816
ENSG00000124831:LRRFIP1:chr2:+:238666098:238666191:238664830:238667371	ENSG00000102125:TAZ:chrX:+:153642437:153642527:153641904:153647881	ENSG00000058668:ATP2B4:chr1:+:203702350:203702528:203696699:203708673
ENSG00000138614:INTS14:chr15:-:65899496:65899763:65897574:65903435	ENSG00000137497:NUMA1:chr11:-:71723446:71723488:71721900:71723940	ENSG00000181929:PRKAG1:chr12:-:49399525:49399635:49399326:49406844
ENSG00000140678:ITGAX:chr16:+:31381301:31381419:31374695:31382404	ENSG00000110851:PRDM4:chr12:-:108145423:108145986:108140201:108147700	ENSG00000137965:IFI44:chr1:+:79126238:79126376:79125168:79128388
ENSG00000012660:ELOVL5:chr6:-:53138017:53138142:53135525:53139887	ENSG00000241343:RPL36A:chrX:+:100647037:100647083:100646810:100650322	ENSG00000160201:U2AF1:chr21:-:44521475:44521542:44520629:44524424
ENSG00000089157:RPLP0:chr12:-:120636356:120636434:120635265:120636656	ENSG00000117308:GALE:chr1:-:24122998:24123076:24122755:24123186	ENSG00000133816:MICAL2:chr11:+:12270730:12270793:12261132:12277189
ENSG00000131149:GSE1:chr16:+:85698620:85698734:85697220:85699581	ENSG00000162004:CCDC78:chr16:-:775445:775580:775293:775793	ENSG00000066651:TRMT11:chr6:+:126327987:126328100:126320759:126329537
ENSG00000196652:ZKSCAN5:chr7:+:99117449:99117532:99110214:99123435	ENSG00000125733:TRIP10:chr19:+:6746039:6746207:6745005:6746462	ENSG00000165495:PKNOX2:chr11:+:125281641:125281761:125267958:125298907
ENSG00000119950:MXI1:chr10:+:112004585:112004631:111988079:112038937	ENSG00000140859:KIFC3:chr16:-:57793036:57793065:57792821:57793639	ENSG00000076984:MAP2K7:chr19:+:7970692:7970740:7968953:7974639
ENSG00000132676:DAP3:chr1:+:155706796:155706854:155701824:155707947	ENSG00000140521:POLG:chr15:-:89861169:89861268:89860767:89861771	ENSG00000136153:LMO7:chr13:+:76412275:76412332:76410593:76414220

ENSG00000163531:NFASC:chr1:+:204919684:204919702:204913534:204921138
ENSG00000206562:METTL6:chr3:-:15457004:15457090:15456421:15457278
ENSG00000124440:HIF3A:chr19:+:46832337:46832735:46828896:46834412
ENSG00000112425:EPM2A:chr6:-:145956380:145956622:145948829:146007257
ENSG00000108591:DRG2:chr17:+:18001583:18001673:17997287:18007114
ENSG00000136861:CDK5RAP2:chr9:-:123222849:123223025:123220900:123230137
ENSG00000143815:LBR:chr1:-:225597992:225598118:225594534:225599038
ENSG00000198176:TFDP1:chr13:+:114291934:114292211:114291015:114294434
ENSG00000172366:MCRIP2:chr16:+:696471:696608:692249:697416
ENSG00000004864:SLC25A13:chr7:-:95864113:95864229:95838289:95906507
ENSG00000178026:LRRC75B:chr22:-:24984646:24985233:24984297:24985788
ENSG00000206503:HLA-A:chr6:+:29912276:29912411:29912174:29912835
ENSG00000143164:DCAF6:chr1:+:167992225:167992285:167974031:168012262
ENSG00000213199:ASIC3:chr7:+:150748258:150748406:150748182:150748896
ENSG00000168894:RNF181:chr2:+:85823979:85824054:85823772:85824567
ENSG00000134278:SPIRE1:chr18:-:12459753:12459927:12454482:12463349
ENSG00000102317:RBM3:chrX:+:48434202:48434471:48433671:48434701
ENSG00000134775:FHOD3:chr18:+:33952642:33952707:33935608:34081894
ENSG00000102710:SUPT20H:chr13:-:37622700:37622736:37622073:37625624
ENSG00000026950:BTN3A1:chr6:+:26410121:26410142:26409961:26410233
ENSG00000118900:UBN1:chr16:+:4921155:4921302:4920973:4922884
ENSG00000204536:CCHCR1:chr6:-:31122272:31122576:31118899:31124507
ENSG00000112357:PEX7:chr6:+:137193335:137193391:137167319:137219279
ENSG00000117305:HMGCL:chr1:-:24140679:24140828:24134813:24143164
ENSG00000108312:UBTF:chr17:-:42289711:42289822:42289374:42290186
ENSG00000141504:SAT2:chr17:-:7530260:7530362:7530111:7530460
ENSG00000167491:GATAD2A:chr19:+:19612777:19612852:19612225:19613139
ENSG00000010361:FUZ:chr19:-:50312634:50312832:50312515:50314619
ENSG00000243156:MICAL3:chr22:-:18310409:18310547:18305826:18314619
ENSG00000112320:SOBP:chr6:+:107908283:107908379:107854814:107979410
ENSG00000074181:NOTCH3:chr19:-:15295105:15295261:15292612:15295716
ENSG00000106077:ABHD11:chr7:-:73151258:73151550:73151021:73151891
ENSG00000068724:TTC7A:chr2:+:47185633:47185691:47184146:47202111
ENSG00000197119:SLC25A29:chr14:-:100761962:100762330:100759714:100765178
ENSG00000115661:STK16:chr2:+:220111834:220111968:220111598:220112136
ENSG00000163939:PBRM1:chr3:-:52588739:52588895:52584833:52595782
ENSG00000105483:CARD8:chr19:-:48726975:48727125:48725112:48733694
ENSG00000139974:SLC38A6:chr14:+:61510167:61510221:61509930:61512063
ENSG00000136518:ACTL6A:chr3:+:179293190:179293292:179292255:179294004
ENSG00000169598:DFFB:chr1:+:3782847:3782962:3782564:3784537
ENSG00000147099:HDAC8:chrX:-:71715005:71715118:71684581:71787738
ENSG00000170791:CHCHD7:chr8:+:57125320:57125448:57124396:57127156
ENSG00000170946:DNAJC24:chr11:+:31436357:31436496:31392406:31447833
ENSG00000119844:AFTPH:chr2:+:64806619:64808407:64800202:64812555
ENSG00000166833:NAV2:chr11:+:20104137:20104146:20101755:20104552
ENSG00000136044:APPL2:chr12:-:105570653:105570794:105569860:105571003
ENSG00000147100:SLC16A2:chrX:+:73749047:73749276:73745728:73751167
ENSG00000008382:MPND:chr19:+:4354945:4355018:4354417:4357249
ENSG00000076685:NT5C2:chr10:-:104860419:104860700:104859776:104860801
ENSG00000196586:MYO6:chr6:+:76604947:76604977:76602407:76608089
ENSG00000038002:AGA:chr4:-:178357429:178357505:178355643:178358558
ENSG00000102878:HSF4:chr16:+:67203181:67203251:67203004:67203533
ENSG00000072210:ALDH3A2:chr17:+:19576463:19576588:19575269:19578808
ENSG00000106351:AGFG2:chr7:+:100154216:100154249:100153358:100159881
ENSG00000116731:PRDM2:chr1:+:14095533:14095668:14075982:14099572
ENSG00000151914:DST:chr6:-:56329482:56329554:56328562:56330875
ENSG00000079277:MKNK1:chr1:-:47051545:47051646:47049001:47059784
ENSG00000187609:EXD3:chr9:-:140249018:140249225:140248829:140250719
ENSG00000117868:ESYT2:chr7:-:158545471:158545534:158542414:158552176
ENSG00000146147:MLIP:chr6:+:54095511:54095715:54067031:54122105
ENSG00000124222:STX16:chr20:+:57234678:57234690:57227143:57242545
ENSG00000128591:FLNC:chr7:+:128490029:128490128:128489632:128490437
ENSG00000135127:BICDL1:chr12:+:120510314:120510533:120509605:120518690

ENSG00000096746:HNRNPH3:chr10:+:70097614:70097753:70097090:70098259
ENSG00000114520:SNX4:chr3:-:125208251:125208307:125199163:125216184
ENSG00000072195:SPEG:chr2:+:220353219:220353387:220353032:220353499
ENSG00000120860:WASHC3:chr12:-:102455025:102455124:102444069:102455689
ENSG00000170004:CHD3:chr17:+:7810918:7811020:7810806:7811211
ENSG00000132600:PRMT7:chr16:+:68381113:68381197:68380183:68386150
ENSG00000162591:MEGF6:chr1:-:3413551:3413683:3413347:3413796
ENSG00000168904:LRRC28:chr15:+:99903310:99903470:99901716:99926234
ENSG00000143951:WDPCP:chr2:-:63380629:63380709:63380080:63401804
ENSG00000127419:TMEM175:chr4:+:941496:941680:926328:944208
ENSG00000166927:MS4A7:chr11:+:60160157:60160259:60157069:60161259
ENSG00000173210:ABLIM3:chr5:+:148618810:148618840:148617166:148619321
ENSG00000106077:ABHD11:chr7:-:73152205:73152402:73152065:73152657
ENSG00000103197:TSC2:chr16:+:2127598:2127727:2126586:2129032
ENSG00000076351:SLC46A1:chr17:-:26729255:26729339:26727783:26731633
ENSG00000139631:CSAD:chr12:-:53565109:53565225:53564286:53565665
ENSG00000135063:FAM189A2:chr9:+:72001474:72002599:72000863:72003073
ENSG00000143207:RFWD2:chr1:-:176145045:176145143:176118210:176153768
ENSG00000076685:NT5C2:chr10:-:104899162:104899236:104866463:104934614
ENSG00000095637:SORBS1:chr10:-:97135729:97135813:97127456:97141441
ENSG00000077380:DYNC1I2:chr2:+:172569276:172569336:172563887:172571837
ENSG00000172889:EGFL7:chr9:+:139564057:139564173:139563125:139564365
ENSG00000159147:DONSON:chr21:-:34955793:34955972:34954552:34956895
ENSG00000243943:ZNF512:chr2:+:27806524:27806583:27806009:27820933
ENSG00000198176:TFDP1:chr13:+:114292132:114292211:114291015:114294434
ENSG00000100813:ACIN1:chr14:-:23536522:23537880:23535217:23538684
ENSG00000151276:MAGI1:chr3:-:65361419:65361620:65350621:65364935
ENSG00000100722:ZC3H14:chr14:+:89063077:89063152:89044484:89073586
ENSG00000141644:MBD1:chr18:-:47800555:47800720:47800233:47801498
ENSG00000177943:MAMDC4:chr9:+:139752637:139752728:139752549:139752851
ENSG00000135486:HNRNPA1:chr12:+:54675578:54675725:54675286:54675873
ENSG00000145390:USP53:chr4:+:120189422:120189575:120188637:120190845
ENSG00000095637:SORBS1:chr10:-:97131740:97131806:97127456:97135729
ENSG00000073921:PICALM:chr11:-:85701292:85701442:85693046:85707868
ENSG00000204842:ATXN2:chr12:-:111891480:111891649:111890644:111893832
ENSG00000126214:KLC1:chr14:+:104159889:104159971:104158762:104166991
ENSG00000132405:TBC1D14:chr4:+:7011603:7011675:7002978:7012379
ENSG00000005243:COPZ2:chr17:-:46105041:46105155:46103841:46106490
ENSG00000143537:ADAM15:chr1:+:155033589:155033669:155033308:155033890
ENSG00000156170:NDUFAF6:chr8:+:96046235:96046350:96044326:96047681
ENSG00000176155:CCDC57:chr17:-:80136349:80136481:80130736:80141649
ENSG00000104613:INTS10:chr8:+:19677889:19678029:19677187:19679949
ENSG00000136153:LMO7:chr13:+:76381615:76382335:76378677:76383289
ENSG00000122481:RWDD3:chr1:+:95709766:95710254:95699871:95712311
ENSG00000067113:PLPP1:chr5:-:54786787:54786942:54763977:54830399
ENSG00000139631:CSAD:chr12:-:53565056:53565225:53564286:53565665
ENSG00000196476:C20orf96:chr20:-:270199:270317:264722:270899
ENSG00000196781:TLE1:chr9:-:84267098:84267203:84249216:84300590
ENSG00000136717:BIN1:chr2:-:127815048:127815177:127808488:127816586
ENSG00000162735:PEX19:chr1:-:160253319:160253429:160252899:160254844
ENSG00000189046:ALKBH2:chr12:-:109527813:109528012:109526317:109530311
ENSG00000141258:SGSM2:chr17:+:2270564:2270699:2268635:2274555
ENSG00000172661:WASHC2C:chr10:+:46272720:46272873:46268806:46274400
ENSG00000166377:ATP9B:chr18:+:76973961:76974038:76967012:77013380
ENSG00000185164:NOMO2:chr16:-:18539334:18539393:18538946:18540822
ENSG00000197343:ZNF655:chr7:+:99160816:99160889:99158318:99161485
ENSG00000196312:MFSD14C:chr9:-:99735134:99735230:99721179:99775540
ENSG00000126457:PRMT1:chr19:+:50183128:50183182:50180573:50183743
ENSG00000103202:NME4:chr16:+:448207:448394:447313:448989
ENSG00000004455:AK2:chr1:-:33497144:33497262:33490168:33502336
ENSG00000130803:ZNF317:chr19:+:9268509:9268751:9268070:9269501
ENSG00000171103:TRMT61B:chr2:-:29083983:29084174:29075365:29092444
ENSG00000197343:ZNF655:chr7:+:99160810:99160889:99158318:99161485

ENSG00000110066:KMT5B:chr11:-:67946936:67947004:67942650:67947598
ENSG00000138162:TACC2:chr10:+:123988860:123989001:123987523:123996909

ENSG00000133226:SRRM1:chr1:+:24973569:24973699:24973280:24975349

ENSG00000111144:LTA4H:chr12:-:96397615:96397698:96396842:96400091
ENSG00000172785:CBWD1:chr9:-:151304:151427:146158:152033
ENSG00000183474:GTF2H2C:chr5:+:68863979:68864034:68862880:68868271
ENSG00000122490:PQLC1:chr18:-:77679183:77679400:77664183:77703328
ENSG00000103174:NAGPA:chr16:-:5078297:5078399:5078186:5078880
ENSG00000172869:DMXL1:chr5:+:118542528:118542591:118539131:118552595
ENSG00000110888:CAPRIN2:chr12:-:30873744:30873849:30869610:30876192
ENSG00000110888:CAPRIN2:chr12:-:30872012:30873849:30869610:30876192
ENSG00000181163:NPM1:chr5:+:170818308:170818428:170815010:170832305
ENSG00000136699:SMPD4:chr2:-:130918758:130918845:130914969:130921946
ENSG00000158863:FAM160B2:chr8:+:21947271:21947365:21946809:21951950
ENSG00000184182:UBE2F:chr2:+:238903385:238903451:238881867:238933982
ENSG00000104852:SNRNP70:chr19:+:49605370:49605430:49604728:49607890
ENSG00000007376:RPUSD1:chr16:-:837076:837179:836928:837353
ENSG00000123349:PFDN5:chr12:+:53690027:53690059:53689423:53691633
ENSG00000170525:PFKFB3:chr10:+:6270158:6270181:6268328:6274857
ENSG00000102181:CD99L2:chrX:-:149983334:149983409:149963959:149999703
ENSG00000085978:ATG16L1:chr2:+:234182366:234182423:234181698:234183321
ENSG00000004455:AK2:chr1:-:33497144:33497262:33487304:33502336
ENSG00000160818:GPATCH4:chr1:-:156567826:156567913:156566270:156568018

ENSG00000073921:PICALM:chr11:-:85687665:85687725:85670103:85692171

ENSG00000141556:TBCD:chr17:+:80867160:80867183:80863929:80869633
ENSG00000213625:LEPROT:chr1:+:65895613:65895731:65891061:65897485
ENSG00000126777:KTN1:chr14:+:56139889:56139973:56139730:56142552
ENSG00000204642:HLA-F:chr6:+:29692807:29693083:29692225:29693223
ENSG00000196712:NF1:chr17:+:29579955:29580018:29576137:29585361
ENSG00000144674:GOLGA4:chr3:+:37402733:37402796:37396678:37407570
ENSG00000163531:NFASC:chr1:+:204931235:204931286:204926954:204937376
ENSG00000125388:GRK4:chr4:+:3021367:3021558:3015555:3024140
ENSG00000163964:PIGX:chr3:+:196455572:196455626:196455007:196457862
ENSG00000139116:KIF21A:chr12:-:39709733:39709772:39705355:39713707
ENSG00000134905:CARS2:chr13:-:111350264:111350314:111340365:111353784
ENSG00000111554:MDM1:chr12:-:68710360:68710390:68710033:68715126
ENSG00000188157:AGRN:chr1:+:987372:987396:987195:988840
ENSG00000090565:RAB11FIP3:chr16:+:541133:541268:539000:546823
ENSG00000104852:SNRNP70:chr19:+:49605370:49605442:49604728:49607890
ENSG00000112983:BRD8:chr5:-:137502206:137502416:137501797:137503622
ENSG00000129116:PALLD:chr4:+:169846383:169846429:169846229:169847363
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ENSG00000133872:SARAF:chr8:-:29931392:29931567:29927575:29940362
ENSG00000146574:CCZ1B:chr7:-:6852606:6852668:6851694:6854394
ENSG00000100209:HSCB:chr22:+:29141851:29141996:29140692:29147228
ENSG00000072110:ACTN1:chr14:-:69345705:69345786:69345240:69346678
ENSG00000165795:NDRG2:chr14:-:21491022:21491064:21490656:21491399
ENSG00000100320:RBFOX2:chr22:-:36152151:36152191:36142608:36155934
ENSG00000136717:BIN1:chr2:-:127809830:127809938:127808819:127810997

ENSG00000071564:TCF3:chr19:-:1632330:1632404:1627425:1646353
ENSG00000139567:ACVRL1:chr12:+:52307342:52307554:52307134:52308222
ENSG00000062194:GPBP1:chr5:+:56532939:56532999:56531859:56542126
ENSG00000141446:ESCO1:chr18:-:19120537:19120661:19119970:19140819
ENSG00000122490:PQLC1:chr18:-:77693968:77694022:77679400:77703328
ENSG00000100105:PATZ1:chr22:-:31724772:31724910:31723295:31731677
ENSG00000073921:PICALM:chr11:-:85701292:85701442:85695016:85707868
ENSG00000161956:SENP3:chr17:+:7469011:7469081:7468904:7470243
ENSG00000067066:SP100:chr2:+:231314886:231314970:231313865:231325976
ENSG00000143434:SEMA6C:chr1:-:151108923:151109055:151108639:151109332
ENSG00000068724:TTC7A:chr2:+:47178740:47178872:47177665:47183977
ENSG00000168256:NKIRAS2:chr17:+:40174582:40174658:40174490:40175671
ENSG00000132199:ENOSF1:chr18:-:691203:691276:691106:693881
ENSG00000183077:AFMID:chr17:+:76201683:76201834:76198832:76202026
ENSG00000064655:EYA2:chr20:+:45700823:45700891:45644936:45717877
ENSG00000169062:UPF3A:chr13:+:115056963:115057019:115052104:115057108
ENSG00000196712:NF1:chr17:+:29694242:29694296:29687721:29701030
ENSG00000100813:ACIN1:chr14:-:23559190:23559310:23551045:23559730
ENSG00000166073:GPR176:chr15:-:40099206:40099398:40094455:40212055
ENSG00000137814:HAUS2:chr15:+:42852979:42853068:42851606:42853467
ENSG00000169598:DFFB:chr1:+:3789577:3789701:3789136:3800070
ENSG00000175575:PAAF1:chr11:+:73598075:73598144:73589864:73598398
ENSG00000166169:POLL:chr10:-:103345618:103345913:103340173:103347002
ENSG00000099864:PALM:chr19:+:740351:740483:736078:746284
ENSG00000165630:PRPF18:chr10:+:13639644:13639671:13639535:13642243
ENSG00000132153:DHX30:chr3:+:47857452:47857618:47852201:47859511
ENSG00000133816:MICAL2:chr11:+:12277189:12277297:12261132:12278331
ENSG00000157734:SNX22:chr15:+:64444441:64444525:64444049:64444836
ENSG00000160613:PCSK7:chr11:-:117078369:117078451:117077876:117078685
ENSG00000139620:KANSL2:chr12:-:49072818:49072933:49065745:49073437
ENSG00000095485:CWF19L1:chr10:-:102016018:102016233:102013296:102027327
ENSG00000133794:ARNTL:chr11:+:13376779:13376843:13375995:13378285
ENSG00000124193:SRSF6:chr20:+:42087792:42088060:42087149:42088410
ENSG00000197343:ZNF655:chr7:+:99160810:99160889:99160117:99161485
ENSG00000137601:NEK1:chr4:-:170359233:170359410:170354816:170384393
ENSG00000006194:ZNF263:chr16:+:3335680:3335754:3334205:3336022
ENSG00000110921:MVK:chr12:+:110019199:110019355:110017751:110023826
ENSG00000005801:ZNF195:chr11:-:3382972:3383119:3381795:3392204
ENSG00000134874:DZIP1:chr13:-:96251618:96251678:96246340:96258256
ENSG00000111731:C2CD5:chr12:-:22611417:22611519:22610095:22622642
ENSG00000103449:SALL1:chr16:-:51172598:51176056:51171463:51185076
ENSG00000153250:RBMS1:chr2:-:161138767:161138815:161137875:161141285
ENSG00000159761:C16orf86:chr16:+:67701198:67701428:67700975:67701780
ENSG00000163125:RPRD2:chr1:+:150414356:150414434:150413499:150415706
ENSG00000089157:RPLP0:chr12:-:120636356:120636573:120635265:120636656
ENSG00000113163:COL4A3BP:chr5:-:74695134:74695212:74685512:74696029
ENSG00000197694:SPTAN1:chr9:+:131355261:131355321:131353904:131356453
ENSG00000183077:AFMID:chr17:+:76198783:76198832:76187141:76202026
ENSG00000110514:MADD:chr11:+:47310518:47310578:47308085:47310941
ENSG00000091009:RBM27:chr5:+:145631273:145631438:145616995:145634505
ENSG00000127952:STYXL1:chr7:-:75643059:75643205:75634722:75651168
ENSG00000168802:CHTF8:chr16:-:69154955:69155073:69154552:69155338
ENSG00000095203:EPB41L4B:chr9:-:111947768:111947885:111945077:111954557
ENSG00000156414:TDRD9:chr14:+:104516201:104516274:104516017:104518317
ENSG00000169241:SLC50A1:chr1:+:155109303:155109427:155108852:155110036
ENSG00000275052:PPP4R3B:chr2:-:55805382:55805478:55804492:55806814
ENSG00000029363:BCLAF1:chr6:-:136590278:136590441:136589477:136590574
ENSG00000122678:POLM:chr7:-:44113729:44114129:44113624:44118338
ENSG00000136280:CCM2:chr7:+:45111348:45111471:45109560:45112324
ENSG00000107317:PTGDS:chr9:+:139873674:139873853:139872144:139874397
ENSG00000077232:DNAJC10:chr2:+:183604271:183604436:183601113:183605025
ENSG00000058063:ATP11B:chr3:+:182620268:182620354:182616560:182631648
ENSG00000163138:PACRGL:chr4:+:20709425:20709493:20706437:20728907

ENSG00000093167:LRRFIP2:chr3:-:37107714:37107816:37107433:37114280
ENSG00000112234:FBXL4:chr6:-:99365249:99365595:99353546:99374352
ENSG00000147364:FBXO25:chr8:+:382885:382935:381444:385614
ENSG00000119650:IFT43:chr14:+:76524984:76525017:76488737:76548637
ENSG00000102710:SUPT20H:chr13:-:37584688:37584792:37583946:37586328
ENSG00000117625:RCOR3:chr1:+:211486061:211486177:211477482:211486765
ENSG00000106009:BRAT1:chr7:-:2580463:2580528:2579522:2580612
ENSG00000124608:AARS2:chr6:-:44274985:44275131:44274768:44278730
ENSG00000076685:NT5C2:chr10:-:104871501:104871562:104866463:104934614
ENSG00000175224:ATG13:chr11:+:46685546:46685645:46681035:46686398
ENSG00000141644:MBD1:chr18:-:47797838:47797910:47796188:47799046
ENSG00000126777:KTN1:chr14:+:56130672:56130759:56128330:56137446
ENSG00000100105:PATZ1:chr22:-:31724772:31724845:31723295:31731677
ENSG00000144935:TRPC1:chr3:+:142455220:142455375:142443573:142467099
ENSG00000007541:PIGQ:chr16:+:631198:631341:630972:632247
ENSG00000100503:NIN:chr14:-:51233024:51233114:51230682:51233497
ENSG00000196792:STRN3:chr14:-:31398406:31398517:31382863:31404368
ENSG00000144744:UBA3:chr3:-:69124580:69124661:69120768:69126948
ENSG00000050820:BCAR1:chr16:-:75271080:75271242:75269884:75276367
ENSG00000165650:PDZD8:chr10:-:119100490:119100613:119078485:119133866
ENSG00000169045:HNRNPH1:chr5:-:179046269:179046408:179045324:179047892
ENSG00000154114:TBCEL:chr11:+:120924338:120924441:120918376:120925760
ENSG00000143776:CDC42BPA:chr1:-:227247073:227247112:227235711:227257477
ENSG00000133816:MICAL2:chr11:+:12277189:12277297:12270793:12278331
ENSG00000144283:PKP4:chr2:+:159535092:159535166:159530512:159536940
ENSG00000139624:CERS5:chr12:-:50526744:50526847:50524477:50528328
ENSG00000136861:CDK5RAP2:chr9:-:123222849:123222945:123220900:123230137
ENSG00000134138:MEIS2:chr15:-:37388489:37388608:37387815:37390167
ENSG00000139631:CSAD:chr12:-:53564960:53565225:53564286:53565665
ENSG00000198208:RPS6KL1:chr14:-:75375803:75375893:75375635:75377950
ENSG00000115084:SLC35F5:chr2:-:114475329:114475427:114472772:114476730
ENSG00000138614:INTS14:chr15:-:65899496:65899780:65897574:65903435
ENSG00000257218:GATC:chr12:+:120892735:120892833:120884632:120894878
ENSG00000148187:MRRF:chr9:+:125048317:125048445:125047566:125054027
ENSG00000105953:OGDH:chr7:+:44695916:44695961:44687358:44706334
ENSG00000139116:KIF21A:chr12:-:39724043:39724064:39720126:39724547
ENSG00000180098:TRNAU1AP:chr1:+:28887624:28887772:28887244:28887857
ENSG00000116754:SRSF11:chr1:+:70696777:70696886:70694238:70697541
ENSG00000122367:LDB3:chr10:+:88446825:88447029:88441560:88451652
ENSG00000076864:RAP1GAP:chr1:-:21930150:21930405:21929406:21932558
ENSG00000127603:MACF1:chr1:+:39930766:39930784:39929358:39934286
ENSG00000140307:GTF2A2:chr15:-:59944404:59944525:59934461:59949604
ENSG00000099917:MED15:chr22:+:20929399:20929519:20922918:20936897
ENSG00000157637:SLC38A10:chr17:-:79223869:79223893:79220861:79225292
ENSG00000015153:YAF2:chr12:-:42592937:42593037:42555567:42631400
ENSG00000105698:USF2:chr19:+:35760705:35760906:35760602:35761349
ENSG00000061656:SPAG4:chr20:+:34206844:34206920:34206642:34207116
ENSG00000173226:IQCB1:chr3:-:121491403:121491560:121489421:121500589
ENSG00000168961:LGALS9:chr17:+:25970550:25970646:25969374:25972339
ENSG00000127419:TMEM175:chr4:+:942198:942403:941942:944208
ENSG00000159063:ALG8:chr11:-:77849755:77849813:77838482:77850539
ENSG00000124787:RPP40:chr6:-:4998949:4999075:4996654:5000042
ENSG00000005884:ITGA3:chr17:+:48165588:48165730:48165233:48166473
ENSG00000113240:CLK4:chr5:-:178046838:178047674:178045779:178050256
ENSG00000115760:BIRC6:chr2:+:32815872:32816045:32800433:32818981
ENSG00000166377:ATP9B:chr18:+:77135393:77135426:77134101:77137246
ENSG00000137411:VARS2:chr6:+:30887871:30887993:30887625:30888109
ENSG00000128159:TUBGCP6:chr22:-:50658620:50660303:50658444:50660456
ENSG00000183077:AFMID:chr17:+:76201683:76201819:76198832:76202026
ENSG00000162241:SLC25A45:chr11:-:65146846:65147032:65144547:65147337
ENSG00000048028:USP28:chr11:-:113677210:113677306:113675768:113679019
ENSG00000134444:KIAA1468:chr18:+:59947592:59947675:59947089:59947878
ENSG00000124275:MTRR:chr5:+:7873485:7873625:7871036:7875370

ENSG00000135093:USP30:chr12:+:109495730:109495913:109494596:109505328
ENSG00000103034:NDRG4:chr16:+:58534891:58534981:58528912:58537701
ENSG00000153113:CAST:chr5:+:96062497:96062563:96058402:96063192
ENSG00000151466:SCLT1:chr4:-:129864150:129864343:129812292:129867161
ENSG00000085978:ATG16L1:chr2:+:234182636:234182687:234182423:234183321
ENSG00000173947:PIFO:chr1:+:111890295:111890394:111889671:111892727
ENSG00000166979:EVA1C:chr21:+:33829904:33830028:33785321:33840003
ENSG00000169241:SLC50A1:chr1:+:155110036:155110198:155108852:155110454
ENSG00000047346:FAM214A:chr15:-:52892323:52892432:52885933:52893282
ENSG00000130731:METTL26:chr16:-:685517:685774:684797:686093
ENSG00000132589:FLOT2:chr17:-:27212874:27212965:27211333:27215962
ENSG00000090554:FLT3LG:chr19:+:49982219:49982304:49979823:49983554
ENSG00000118007:STAG1:chr3:-:136287606:136287703:136261037:136323150
ENSG00000175287:PHYHD1:chr9:+:131700035:131700223:131698924:131703723
ENSG00000166444:ST5:chr11:-:8751496:8752756:8747756:8772167
ENSG00000135596:MICAL1:chr6:-:109772802:109772903:109767975:109773448
ENSG00000159596:TMEM69:chr1:+:46156645:46156782:46153947:46158875
ENSG00000142949:PTPRF:chr1:+:44067741:44067768:44064584:44069086
ENSG00000185024:BRF1:chr14:-:105707601:105707751:105695250:105718843
ENSG00000127527:EPS15L1:chr19:-:16487932:16488065:16472795:16495939
ENSG00000130731:METTL26:chr16:-:685611:685774:684797:686093
ENSG00000116731:PRDM2:chr1:+:14099572:14099683:14075982:14142921
ENSG00000137842:TMEM62:chr15:+:43470804:43470909:43461875:43473378
ENSG00000060339:CCAR1:chr10:+:70516029:70516240:70515293:70517050
ENSG00000070808:CAMK2A:chr5:-:149607754:149607849:149602780:149618262
ENSG00000116604:MEF2D:chr1:-:156446285:156446306:156445029:156446803
ENSG00000124831:LRRFIP1:chr2:+:238647874:238647952:238636578:238657006
ENSG00000197535:MYO5A:chr15:-:52641014:52641023:52638658:52643450
ENSG00000068400:GRIPAP1:chrX:-:48838206:48838284:48838103:48839390
ENSG00000127603:MACF1:chr1:+:39844132:39844195:39838268:39844874
ENSG00000197530:MIB2:chr1:+:1560937:1561033:1560808:1562029
ENSG00000089177:KIF16B:chr20:-:16362742:16362775:16362392:16385457
ENSG00000205571:SMN2:chr5:+:69372347:69372401:69366578:69372845
ENSG00000075413:MARK3:chr14:+:103966492:103966537:103958371:103969218
ENSG00000165795:NDRG2:chr14:-:21486615:21486630:21486398:21486921
ENSG00000105662:CRTC1:chr19:+:18854917:18854965:18853836:18856632
ENSG00000175224:ATG13:chr11:+:46686398:46686509:46681035:46686932
ENSG00000105426:PTPRS:chr19:-:5216730:5216778:5215606:5218430
ENSG00000160055:TMEM234:chr1:-:32681984:32682118:32681396:32682489
ENSG00000170791:CHCHD7:chr8:+:57125320:57125468:57124396:57127156
ENSG00000102882:MAPK3:chr16:-:30128457:30128606:30128324:30128990
ENSG00000008441:NFIX:chr19:+:13189426:13189549:13186485:13192493
ENSG00000276087:RP11-507M3:chr2:+:24362239:24362320:24358057:24369617
ENSG00000116750:UCHL5:chr1:-:192989436:192989511:192985525:192990226
ENSG00000130731:METTL26:chr16:-:685280:685340:684797:686093
ENSG00000114268:PFKFB4:chr3:-:48562997:48563102:48561263:48572944
ENSG00000139613:SMARCC2:chr12:-:56558086:56558152:56557549:56558431
ENSG00000168802:CHTF8:chr16:-:69154955:69155073:69152428:69155338
ENSG00000109339:MAPK10:chr4:-:87010358:87010430:86989108:87022204
ENSG00000213995:NAXD:chr13:+:111279785:111279894:111267994:111286891
ENSG00000056586:RC3H2:chr9:-:125620201:125620372:125618157:125620947
ENSG00000136527:TRA2B:chr3:-:185649364:185649640:185644522:185655612
ENSG00000143537:ADAM15:chr1:+:155034379:155034593:155033965:155034720
ENSG00000136717:BIN1:chr2:-:127815048:127815177:127808819:127816586
ENSG00000137177:KIF13A:chr6:-:17771344:17771449:17765177:17772138
ENSG00000081692:JMJD4:chr1:-:227920629:227920728:227920377:227921114
ENSG00000153391:INO80C:chr18:-:33059263:33059375:33058313:33077682
ENSG00000118454:ANKRD13C:chr1:-:70736538:70736639:70728530:70740402
ENSG00000061938:TNK2:chr3:-:195596367:195596412:195595580:195596984
ENSG00000175575:PAAF1:chr11:+:73597973:73598144:73589864:73598398
ENSG00000149179:C11orf49:chr11:+:47008758:47008861:46958402:47073938
ENSG00000168958:MFF:chr2:+:228211941:228212100:228205096:228217229
ENSG00000130731:METTL26:chr16:-:684888:685063:684797:686093

ENSG00000111790:FGFR1OP2:chr12:+:27113447:27113561:27110676:27116274	ENSG00000124831:LRRFIP1:chr2:+:238659842:238659914:238657967:238661951

Table 3b. IRIS prediction of AS-derived TCR and CAR-T targets for 22 GBM samples. b. Prioritized CAR-T targets listed by unique splice junction.

AS_event	AS_event	AS_event
ENSG00000005884:ITGA3:chr17:+:48165588:48165730:48165233:48166473	ENSG00000121753:ADGRB2:chr1:-:32205710:32205779:32205635:32205947	ENSG00000166377:ATP9B:chr18:+:76973961:76974038:76967012:77013380
ENSG00000026950:BTN3A1:chr6:+:26410121:26410142:26409961:26410233	ENSG00000130958:SLC35D2:chr9:-:99098998:99099066:99086451:99122435	ENSG00000166377:ATP9B:chr18:+:77132824:77132882:77119462:77133897
ENSG00000050767:COL23A1:chr5:-:177683866:177683929:177683398:177684523	ENSG00000131748:STARD3:chr17:+:37814961:37815073:37814775:37815303	ENSG00000166377:ATP9B:chr18:+:77135393:77135426:77134101:77137246
ENSG00000054277:OPN3:chr1:-:241767561:241767881:241761299:241803183	ENSG00000135424:ITGA7:chr12:-:56094045:56094177:56092701:56094682	ENSG00000166927:MS4A7:chr11:+:60160157:60160259:60157069:60161259
ENSG00000058063:ATP11B:chr3:+:182620268:182620354:182616560:182631648	ENSG00000135424:ITGA7:chr12:-:56094045:56094177:56093773:56094682	ENSG00000166979:EVA1C:chr21:+:33829904:33830028:33785321:33840003
ENSG00000066056:TIE1:chr1:+:43773466:43773595:43773243:43774656	ENSG00000136856:SLC2A8:chr9:+:130159654:130159817:130159565:130162185	ENSG00000168785:TSPAN5:chr4:-:99407888:99408035:99403326:99428828
ENSG00000067113:PLPP1:chr5:-:54786787:54786942:54763977:54830399	ENSG00000139567:ACVRL1:chr12:+:52307342:52307554:52307134:52308222	ENSG00000168890:TMEM150A:chr2:-:85828428:85828605:85828230:85829006
ENSG00000067113:PLPP1:chr5:-:54786787:54786942:54771278:54830399	ENSG00000140678:ITGAX:chr16:+:31381301:31381419:31374695:31382404	ENSG00000169992:NLGN2:chr17:+:7311330:7312031:7308929:7315475
ENSG00000069849:ATP1B3:chr3:+:141620977:141621069:141595752:141622461	ENSG00000142949:PTPRF:chr1:+:44067741:44067768:44064584:44069086	ENSG00000172663:TMEM134:chr11:-:67232526:67232571:67232327:67234782
ENSG00000069849:ATP1B3:chr3:+:141620977:141621069:141596404:141622461	ENSG00000143434:SEMA6C:chr1:-:151108923:151109055:151108639:151109332	ENSG00000173264:GPR137:chr11:+:64055536:64055686:64055418:64055811
ENSG00000073008:PVR:chr19:+:45162009:45162168:45157286:45164558	ENSG00000143434:SEMA6C:chr1:-:151110791:151110882:151110581:151111105	ENSG00000177663:IL17RA:chr22:+:17586742:17586844:17586492:17588616
ENSG00000073008:PVR:chr19:+:45162009:45162168:45161178:45164558	ENSG00000144857:BOC:chr3:+:112991915:112992188:112991550:112993221	ENSG00000177943:MAMDC4:chr9:+:139752637:139752728:139752549:139752851
ENSG00000074181:NOTCH3:chr19:-:15295105:15295261:15292612:15295716	ENSG00000144935:TRPC1:chr3:+:142496473:142496605:142467302:142521010	ENSG00000184916:JAG2:chr14:-:105617327:105617441:105617248:105617619
ENSG00000076351:SLC46A1:chr17:-:26729255:26729339:26727783:26731633	ENSG00000147100:SLC16A2:chrX:+:73749047:73749276:73745728:73751167	ENSG00000186470:BTN3A2:chr6:+:26373124:26373325:26370831:26373503

ENSG00000084234:APLP2:chr11:+:129993506:129993674:129992408:129996594
ENSG00000089472:HEPH:chrX:+:65474876:65474983:65428088:65478681
ENSG00000090554:FLT3LG:chr19:+:49982219:49982304:49979823:49983554
ENSG00000091129:NRCAM:chr7:-:107880402:107880614:107875132:107953108
ENSG00000092421:SEMA6A:chr5:-:115808580:115808631:115803443:115808768
ENSG00000099957:P2RX6:chr22:+:21380299:21380365:21380187:21380708
ENSG00000099957:P2RX6:chr22:+:21380299:21380618:21380187:21380708
ENSG00000099998:GGT5:chr22:-:24616459:24616552:24616084:24620963
ENSG00000101187:SLCO4A1:chr20:+:61299363:61299536:61299262:61299828
ENSG00000102181:CD99L2:chrX:-:149983334:149983409:149963959:149999703
ENSG00000102312:PORCN:chrX:+:48372514:48372529:48371240:48372627
ENSG00000105426:PTPRS:chr19:-:5218797:5218809:5218543:5219320
ENSG00000114948:ADAM23:chr2:+:207474633:207474724:207460886:207482302
ENSG00000117114:ADGRL2:chr1:+:82418670:82418709:82417826:82421560

ENSG00000117335:CD46:chr1:+:207941123:207941168:207940540:207943665

ENSG00000147852:VLDLR:chr9:+:2651414:2651498:2650516:2651873
ENSG00000152154:TMEM178A:chr2:+:39931220:39931334:39893514:39944149
ENSG00000152154:TMEM178A:chr2:+:39934188:39934326:39893514:39944149
ENSG00000152154:TMEM178A:chr2:+:39934188:39934326:39931334:39944149
ENSG00000160613:PCSK7:chr11:-:117078369:117078451:117077876:117078685
ENSG00000160781:PAQR6:chr1:-:156214932:156215029:156214702:156215325
ENSG00000162341:TPCN2:chr11:+:68848867:68848967:68846488:68902902
ENSG00000162341:TPCN2:chr11:+:68852677:68852754:68851484:68902902
ENSG00000163531:NFASC:chr1:+:204919684:204919702:204913534:204921138
ENSG00000163531:NFASC:chr1:+:204931235:204931286:204926954:204937376
ENSG00000163531:NFASC:chr1:+:204971723:204971876:204957934:204978684
ENSG00000163681:SLMAP:chr3:+:57911571:57911661:57908750:57913022
ENSG00000164199:ADGRV1:chr5:+:90445846:90446038:90398157:90459598
ENSG00000166073:GPR176:chr15:-:40099206:40099398:40094455:40212055

ENSG00000166073:GPR176:chr15:-:40099206:40099459:40094455:40212055

ENSG00000196312:MFSD14C:chr9:-:99735134:99735230:99721179:99775540
ENSG00000196557:CACNA1H:chr16:+:1262510:1262528:1262138:1263779
ENSG00000196776:CD47:chr3:-:107768465:107768498:107766139:107776323
ENSG00000198053:SIRPA:chr20:+:1902040:1902358:1896101:1905409
ENSG00000204580:DDR1:chr6:+:30852314:30852487:30848902:30856464
ENSG00000204580:DDR1:chr6:+:30853401:30853457:30848902:30856464
ENSG00000204580:DDR1:chr6:+:30853401:30853457:30850760:30856464
ENSG00000204580:DDR1:chr6:+:30853401:30853457:30852487:30856464
ENSG00000204642:HLA-F:chr6:+:29692807:29693083:29692225:29693223
ENSG00000206503:HLA-A:chr6:+:29912276:29912393:29912174:29912835
ENSG00000206503:HLA-A:chr6:+:29912276:29912411:29912174:29912835
ENSG00000213199:ASIC3:chr7:+:150748258:150748406:150748182:150748896
ENSG00000234745:HLA-B:chr6:-:31322883:31323000:31322442:31323093
ENSG00000250644:RP11-295K3.1:chr11:-:1768896:1769349:1756659:1775032
ENSG00000258555:SPECC1L-ADORA2A:chr22:+:24829098:24829704:24765288:24836550

Table 4. Summary results for seven selected AS-derived tumor-associated epitopes for dextramer-based T-cell recognition testing

#	Epitope	SEQ ID NO:	AS_event	Junction_ peptide form	Num HLA	Num samples
1	VLGPWFFGEI	22	ENSG00000137842:TMEM62:chr15:+:43470804:43470909:43461875:43473378	inc	1	5
2	SLSDFKFMA	556	ENSG00000089159:PXN:chr12:-:120657009:120657894:120653076:120659425	skp	1	5
3	FLASKIGRLV	27	ENSG00000184381:PLA2G6:chr22:-:38524275:38524437:38522456:38525460	inc	1	5
4	KIGRLVTRK	29	ENSG00000184381:PLA2G6:chr22:-:38524275:38524437:38522456:38525460	inc	2	6
5	SQDHFHVFV	227	ENSG00000116001:TIA1:chr2:-:70456190:70456223:70454954:70456395	inc	1	5
6	VVHPFFLAQK	557	ENSG00000137814:HAUS2:chr15:+:42852979:42853068:42851606:42853467	skp	2	6
7	YAIVWVNGV	62	ENSG00000169919:GUSB:chr7:-:65444713:65444898:65444528:65445210	inc	6	13

	Sample/patient IDs							
#	1142	2964	2907	1325	2924	2870	LB3336	2867
1	HLA-A*02:01|208.731342595	-	-	-	-	-	-	-
2	HLA-A*02:01|38.1895012937	-	-	-	-	-	-	-
3	HLA-A*02:01|46.2224678849	-	-	-	-	-	-	-
4	-	HLA-A*03:01|68.3395469768	HLA-A*03:01|68.3395469768	-	-	-	HLA-A*11:01|118.90547129	HLA-A*03:01|68.3395469768
5	HLA-A*02:01|141.321119161	-	-	-	-	-	-	-
6	-	HLA-A*03:01|64.1047060319	HLA-A*03:01|64.1047060319	-	-	-	HLA-A*11:01|61.044235149	HLA-A*03:01|64.1047060319
7	HLA-A*02:01|82.9570933347	-	-	HLA-C*03:03|365.682008381	-	-	HLA-C*08:01|388.2	HLA-C*08:01|388.2
	Sample/patient IDs							

#	1989	2158	LB3367	LB3054	LB3001	3404	LB3120	
1	-	-	-	-	HLA-A*02:01|208.731342595	-	HLA-A*02:01|208.731342595	
2	-	-	-	-	HLA-A*02:01|38.1895012937	-	HLA-A*02:01|38.1895012937	
3	-	-	-	-	HLA-A*02:01|46.2224678849	-	HLA-A*02:01|46.2224678849	
4	-	-	-	-	-	-	-	
5	-	-	-	-	HLA-A*02:01|141.321119161	-	HLA-A*02:01|141.321119161	
6	-	-	-	-	-	-	-	
7	HLA-C*03:04|70.1	HLA-C*12:03|12.928544714	-	-	HLA-A*02:01|82.9570933347	HLA-A*68:02|39.0637358822	HLA-C*12:03|12.928544714;HLA-C*03:04|70.1;HLA-A*02:01|82.9570933347	
	Sample/patient IDs							
#	2899	3374	3372	3244	2675	LB2938	803	
1	HLA-A*02:01|208.731342595	HLA-A*02:01|208.731342595	-	-	-	-	-	
2	HLA-A*02:01|38.1895012937	HLA-A*02:01|38.1895012937	-	-	-	-	-	
3	HLA-A*02:01|46.2224678849	HLA-A*02:01|46.2224678849	-	-	-	-	-	
4	-	-	-	-	-	HLA-A*03:01|68.3395469768	HLA-A*03:01|68.3395469768	
5	HLA-A*02:01|141.321119161	HLA-A*02:01|141.321119161	-	-	-	-	-	
6	-	-	-	-	-	HLA-A*03:01|64.1047060319	HLA-A*03:01|64.1047060319	

7	HLA-C*03:04|70.1;HLA-A*02:01|82.9570933347	HLA-A*02:01|82.9570933347	-	HLA-C*12:03|12.928544714	-	-	HLA-C*03:04|70.1	

Table 5. Summary results of dextramer-based T-cell recognition testing of seven AS-derived tumor epitopes using PBMCs and TILs from six patients and six healthy donors with two different HLA types. Data are shown normalized to results with nonhuman epitope NI3233 as a negative control. A common virus found in 50-80% of the population, cytomegalovirus (CMV) was included as a control (Macguire et al., Methods, 2017). n/a, results not available. Green, 'positive' reactivity; yellow, 'marginal' reactivity; red, 'negative' reactivity.

a. Results for HLA-A03:01

		HLA-A03:01 Epitope with PE/APC Double Staining					HLA-A03:01 Epitope with PE Single Staining					HLA-A03:01 Epitope with APC Single Staining			
Sample/ Patient ID (Cell subset)		**VVHPFFLAQK (SEQ ID NO:557)**	**KIGRLVTRK (SEQ ID NO:29)**	**NI3233 (control)**	**CMV (control)**	**Sample/Patient ID (Cell subset)**	**VVHPFFLAQK (SEQ ID NO:557)**	**KIGRLVTRK (SEQ ID NO:29)**	**NI3233 (con-trol)**	**CMV (con-trol)**	**Sample/Patient ID (Cell subset)**	**VVHPFFLAQK (SEQ ID NO:557)**	**KIGRLVTRK (SEQ ID NO:29)**	**NI3233 (con-trol)**	**CMV (con-trol)**
LB803 (PBMCs)		0.062%	0.201%	0.019%	1.771%	**LB803 (PBMCs)**	0.007%	0.168%	0.002%	1.778%	**LB803 (PBMCs)**	0.091%	0.048%	0.019%	0.641%
LB2867 (PBMCs)		n/a	0.034%	0.009%	1.521%	**LB2867 (PBMCs)**	n/a	0.062%	0.004%	1.876%	**LB2867 (PBMCs)**	n/a	0.001%	0.013%	0.397%
LB2867 (TILs)		0.010%	1.692%	0.008%	1.080%	**LB2867 (TILs)**	0.002%	2.309%	0.001%	1.539%	**LB2867 (TILs)**	0.014%	0.142%	0.008%	0.030%
LB2907 (PBMCs)		0.370%	0.280%	0.000%	0.110%	**LB2907 (PBMCs)**	0.029%	0.070%	0.000%	0.034%	**LB2907 (PBMCs)**	0.330%	0.150%	0.000%	0.030%
LB2964 (TILs)		0.002%	0.010%	0.003%	0.011%	**LB2964 (TILs)**	0.001%	0.005%	0.000%	0.007%	**LB2964 (TILs)**	0.002%	0.007%	0.003%	0.005%
Healthy Donor 1 (PBMCs)		0.051%	0.169%	0.011%	0.119%	**Healthy Donor 1 (PBMCs)**	0.031%	0.140%	0.000%	0.110%	**Healthy Donor 1 (PBMCs)**	0.020%	0.008%	0.011%	0.009%
Healthy Donor 2 (PBMCs)		0.000%	0.000%	0.016%	0.004%	**Healthy Donor 2 (PBMCs)**	0.006%	0.003%	0.001%	0.003%	**Healthy Donor 2 (PBMCs)**	0.000%	0.000%	0.018%	0.005%

Healthy Donor 3 (PBMCs)	0.000%	0.000%	0.009%	2.231%	**Healthy Donor 3 (PBMCs)**	0.003%	0.000%	0.000%	2.210%	**Healthy Donor 3 (PBMCs)**	0.000%	0.000%	0.012%	2.208%

b. Results for HLA-A02:01

	HLA-A02:01 Epitopes with PE/APC Double Staining						
Sample/Patient ID (Cell subset)	**VLGPWFFGEI (SEQ ID NO:22)**	**SQDHFHVFV (SEQ ID NO:227)**	**YAIVWVNGV (SEQ ID NO:62)**	**FLASKIGRLV (SEQ ID NO:27)**	**SLSDFKFMA (SEQ ID NO:556)**	**NI3233 (control)**	**CMV (control)**
LB1142 (PBMCs)	0.012%	0.020%	0.000%	0.072%	0.072%	0.068%	0.000%
LB2899 (TILs)	0.034%	0.022%	0.000%	0.075%	0.017%	0.012%	6.428%
Healthy Donor 1 (PBMCs)	0.362%	0.053%	0.013%	0.302%	0.021%	0.008%	2.062%
Healthy Donor 2 (PBMCs)	0.094%	0.034%	0.005%	0.134%	0.084%	0.026%	0.124%
Healthy Donor 3 (PBMCs)	0.094%	0.020%	0.003%	0.136%	0.023%	0.004%	10.096%

	HLA-A02:01 Epitopes with PE Single Staining						
Sample/Patient ID (Cell subset)	**VLGPWFFGEI (SEQ ID NO:22)**	**SQDHFHVFV (SEQ ID NO:227)**	**YAIVWVNGV (SEQ ID NO:62)**	**FLASKIGRLV (SEQ ID NO:27)**	**SLSDFKFMA (SEQ ID NO:556)**	**NI3233 (control)**	**CMV (control)**
LB1142 (PBMCs)	0.022%	0.000%	0.000%	0.028%	0.008%	0.025%	0.009%
LB2899 (TILs)	0.000%	0.000%	0.000%	0.001%	0.001%	0.001%	6.419%
Healthy Donor 1 (PBMCs)	0.003%	0.000%	0.002%	0.016%	0.004%	0.000%	2.010%
Healthy Donor 2 (PBMCs)	0.023%	0.009%	0.003%	0.019%	0.048%	0.001%	0.069%
Healthy Donor 3 (PBMCs)	0.000%	0.001%	0.000%	0.005%	0.005%	0.000%	5.020%

	HLA-A02:01 Epitopes with APC Single Staining						
Sample/Patient ID (Cell subset)	VLGPWFFGEI (SEQ ID NO:22)	SQDHFHVFV (SEQ ID NO:227)	YAIVWVNGV (SEQ ID NO:62)	FLASKIGRLV (SEQ ID NO:27)	SLSDFKFMA (SEQ ID NO:556)	NI3233 (control)	CMV (control)
LB1142 (PBMCs)	0.029%	0.039%	0.000%	0.129%	0.099%	0.071%	0.000%
LB2899 (TILs)	0.037%	0.024%	0.001%	0.081%	0.018%	0.011%	6.440%
Healthy Donor 1 (PBMCs)	0.389%	0.065%	0.018%	0.309%	0.024%	0.001%	2.069%
Healthy Donor 2 (PBMCs)	0.132%	0.048%	0.006%	0.172%	0.092%	0.028%	0.112%
Healthy Donor 3 (PBMCs)	0.195%	0.036%	0.006%	0.265%	0.051%	0.005%	10.095%

Table 6. Summary results for TCR clonotypes of KIGRLVTRK (SEQ ID NO:29)-positive T cells from one patient, profiled by single-cell and bulk RNA-seq based approaches. Amino acid sequences of TCR CDR3 and frequencies of clonotypes by scRNA-seq, pairSEQ, and immunoSEQ are shown.

	scRNAseq			Adaptive pairSEQ			Adaptive immunoSEQ		
Clono-type	TCR-alpha CDR3 aa	TRC-beta CDR3 aa	Frequency	TCR-alpha CDR3 aa	TRC-beta CDR3 aa	Frequency	TCR-alpha CDR3 aa	TRC-beta CDR3 aa	Frequency
1	CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	38.8876%	CAMRPLGGYNKLIF (SEQ ID NO:32)	CASSQAANEQFF (SEQ ID NO:33)	15.83%	N/A	CASSQAANEQFF (SEQ ID NO:33)	69.61%
2	CAMRPLGGYNKLIF (SEQ ID NO:32)	CASSQAANEQFF (SEQ ID NO:33)	22.5520%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	15.54%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	7.87%
3	CAEEGDRDYKLSF (SEQ ID NO:34)	CASTGRSGRSEQYF (SEQ ID NO:35)	13.7415%	CAVIVTGNQFYF (SEQ ID NO:559)	CASSEDTYGYTF (SEQ ID NO:560)	8.09%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	4.87%

4	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	4.6272%	CALTGPQANAGGTSYGKLTF (SEQ ID NO:561)	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	3.69%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	4.56%
5	*GRDDKIIF (SEQ ID NO:613)	CATTLPGDTEAFF (SEQ ID NO:37)	1.73%	CALSGREYGNKLVF (SEQ ID NO:563)	CASSYLAGGFRDEQFF (SEQ ID NO:564)	3.33%	N/A	CASSFQTVGSGSYEQYF (SEQ ID NO:562)	2.64%
6	CATANNAGNMLTF (SEQ ID NO:745)	CASSLDRHQPQHF (SEQ ID NO:39)	1.29%	CAASTGATNKLIF (SEQ ID NO:566)	CASSQTRTGNQPQHF (SEQ ID NO:565)	3.28%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	1.62%
7	CALWEGQGGSEKLVF (SEQ ID NO:678)	CASSLEARAPSGNTIYF (SEQ ID NO:41)	1.24%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	2.54%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	1.19%
8	CAVGAGTGTASKLTF (SEQ ID NO:719)	CASSLELAGGRDTQYF (SEQ ID NO:43)	1.19%	CVVNPHGSSNTGKLIF (SEQ ID NO:568)	CASSLVGQGAYNEQFF (SEQ ID NO:567)	2.09%	N/A	CASTGRSGRSEQYF (SEQ ID NO:35)	0.83%
9		CASSQAANEQFF (SEQ ID NO:33)	0.56%	CVVRRDSGGSNYKLTF (SEQ ID NO:569)	CASSLDPGRALGYTF (SEQ ID NO:570)	1.91%	N/A	CASSQTRTGNQPQHF (SEQ ID NO:565)	0.82%
10	CAVFQGGSEKLVF (SEQ ID NO:714);CAAADFSGTYKYIF (SEQ ID NO:715)	CASSPEPQGANFYEQYF (SEQ ID NO:572)	0.49%	CAEEGDRDYKLSF (SEQ ID NO:34)	CASTGRSGRSEQYF (SEQ ID NO:35)	1.88%	N/A	CASSFTRVAYEQYF (SEQ ID NO:571)	0.68%
11	CAMRPLGGYNKLIF (SEQ ID NO:32)	CASSQAANEQFF (SEQ ID NO:33); CASSFGVSYEQYF (SEQ ID NO:31)	0.40%	N/A	CASSFQTVGSGSYEQYF (SEQ ID NO:562)	1.72%	N/A	CASSLAAGGLSEQFF (SEQ ID NO:573)	0.57%
12	CALSEGSNFGNEKLTF (SEQ ID NO:581)	CASSEGTVLDEQYF (SEQ ID NO:574)	0.35%	CAESKLDAGNMLTF (SEQ ID NO:575)	CASSLVGQGLGEQYF (SEQ ID NO:576)	1.67%	N/A	CARRRVLVAWDCSGANVLTF (SEQ ID NO:577)	0.41%
13	CAMRPLGGYNKLIF (SEQ ID NO:32)	CASSFGVSYEQYF (SEQ ID NO:31)	0.33%	CAARDSSYKLIF (SEQ ID NO:579)	CASSASDSYNEQFF (SEQ ID NO:578)	1.35%	N/A	CASSLVGQGAYNEQFF (SEQ ID NO:567)	0.31%
14	CAAGGNYGGSQGNLIF (SEQ ID NO:746)	CASSLGSSTQYF (SEQ ID NO:580)	0.33%	CALSEGSNFGNEKLTF (SEQ ID NO:581)	CASSEGTVLDEQYF (SEQ ID NO:574)	1.11%	N/A	CSATGTYNEQFF (SEQ ID NO:582)	0.30%

15	CAEEGDRDYKLSF (SEQ ID NO:34); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.28%	N/A	CASSLAAGGLSEQFF (SEQ ID NO:573)	1.07%	N/A	CASSFNPQGREKLFF (SEQ ID NO:583)	0.25%
16	CALTGPQANAGGTSYGKLTF (SEQ ID NO:561)	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.28%	CVVSNNAGNMLTF (SEQ ID NO:584)	CASSPGPVQETQYF (SEQ ID NO:585)	0.80%	N/A	CASSASDSYNEQFF (SEQ ID NO:578)	0.23%
17	CAMRPLGGYNKLIF (SEQ ID NO:32); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSQAANEQFF (SEQ ID NO:33); CASSFGVSYEQYF (SEQ ID NO:31)	0.26%	CAVTSRGGSNYKLTF (SEQ ID NO:586)	CARRRVLVAWDCSGANVLTF (SEQ ID NO:577)	0.79%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.17%
18	CAVIVTGNQFYF (SEQ ID NO:559)	CASSEDTYGYTF (SEQ ID NO:560)	0.26%	CAVQGYQKVTF (SEQ ID NO:588)	CASSFTRVAYEQYF (SEQ ID NO:571)	0.66%	N/A	CASSSVSSGNTIYF (SEQ ID NO:587)	0.16%
19	CAEEGDRDYKLSF (SEQ ID NO:34)	CASSFGVSYEQYF (SEQ ID NO:31)	0.23%	CAVNIRGANNLFF (SEQ ID NO:589)	CASSSRLAGGQVSYEQYF (SEQ ID NO:590)	0.60%	N/A	CASSWTSGGMNEQFF (SEQ ID NO:591)	0.12%
20	CATDYRTGGSYIPTF (SEQ ID NO:747)	CASSFLTDSYNEQFF (SEQ ID NO:592)	0.19%	CAVRPSGRLMF (SEQ ID NO:593)	CSATGTYNEQFF (SEQ ID NO:582)	0.58%	N/A	CASSQGALGNTEAFF (SEQ ID NO:594)	0.11%
21	CALSEGSNFGNEKLTF (SEQ ID NO:581)	CQQHTDRDQETQYF (SEQ ID NO:595);CASSEGTVLDEQYF (SEQ ID NO:574)	0.19%	CAIPYGYNKLIF (SEQ ID NO:596)	CAWSGTGGSEAFF (SEQ ID NO:597)	0.58%	N/A	CATTLPGDTEAFF (SEQ ID NO:37)	0.10%
22	CALSGREYGNKLVF (SEQ ID NO:563)	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.19%	CLVGGLYGGSQGNLIF (SEQ ID NO:601)	CASSLADPTDTQYF (SEQ ID NO:600)	0.57%	N/A	CASSSRTVVNYGYTF (SEQ ID NO:598)	0.10%
23	CAVRDPSREPGRKAYLRT (SEQ ID NO:748);CAMRPLGGYNKLIF (SEQ ID NO:32)	CASSQAANEQFF (SEQ ID NO:33)	0.16%	CAADAGNNRKLIW (SEQ ID NO:602)	CASSYTPGPRGEQYF (SEQ ID NO:603)	0.57%	N/A	CSARRGGAPNTEAFF (SEQ ID NO:599)	0.10%
24	CAMRPLGGYNKLIF (SEQ ID NO:32);CAEEGDR	CASSQAANEQFF (SEQ ID NO:33);CASTGRS	0.16%	CAAKGGTSYGKLTF (SEQ ID NO:605)	CASSFNPQGREKLFF (SEQ ID NO:583)	0.53%	N/A	CASKPTSGSVNEQFF (SEQ ID NO:604)	0.09%

	DYKLSF (SEQ ID NO:34)	GRSEQYF (SEQ ID NO:35)							
25	CAAVNYGQNFVF (SEQ ID NO:619); CAASSGGTASKL TF (SEQ ID NO:620)	CASSASGNQPQH F (SEQ ID NO:606)	0.16%	CAESTGGYNKLIF (SEQ ID NO:607)	CSVETGVNQP QHF (SEQ ID NO:608)	0.52%	N/A	CAWSGTGGSE AFF (SEQ ID NO:597)	0.09%
26	CALSEVSSGAGS YQLTF (SEQ ID NO:621)	CASSFQTVGSGS YEQYF (SEQ ID NO:562)	0.14%	CAASAALNRDDK IIF (SEQ ID NO:611)	CASCLPGGNEK LFF (SEQ ID NO:610)	0.49%	N/A	CASSATSGKHE QYF (SEQ ID NO:609)	0.08%
27	CAESPSGGSYIPT F (SEQ ID NO:622)	CASSPVGWREQF F (SEQ ID NO:612)	0.14%	N/A	CASSWTSGGM NEQFF (SEQ ID NO:591)	0.46%	N/A	CASSPGPVQET QYF (SEQ ID NO:585)	0.08%
28		CASTGRSGRSEQ YF (SEQ ID NO:35)	0.14%	*GRDDKIIF (SEQ ID NO:613)	CATTLPGDTEA FF (SEQ ID NO:37)	0.45%	N/A	CASSQAANEQF F (SEQ ID NO:33)	0.07%
29	CAVRGATKLSF (SEQ ID NO:623)	CASSPPDSYGYTF (SEQ ID NO:614)	0.12%	CAVGNTGGFKTI F (SEQ ID NO:615)	CASKPTSGSVN EQFF (SEQ ID NO:604)	0.44%	N/A	CASSLADPTDT QYF (SEQ ID NO:600)	0.07%
30	CAASTGATNKLIF (SEQ ID NO:566)	CASSQTRTGNQP QHF (SEQ ID NO:565)	0.12%	CALVTGANNLFF (SEQ ID NO:617)	CASSQGALGN TEAFF (SEQ ID NO:594)	0.43%	N/A	CASRVWGADT QYF (SEQ ID NO:616)	0.07%
31	CAASTPGEKLTF (SEQ ID NO:624)	CASSYDGENTEA FF (SEQ ID NO:618)	0.12%	CALRGRSGGSYIP TF (SEQ ID NO:625)	CASSQSLTGGL QPQHF (SEQ ID NO:626)	0.43%	N/A	CASGLVPRPSG GESNTGELFF (SEQ ID NO:627)	0.07%
32	CAVGNTGGFKTI F (SEQ ID NO:615)	CASKPTSGSVNE QFF (SEQ ID NO:604)	0.12%	CALTLFSGGYNK LIF (SEQ ID NO:629)	CASSSVSSGNTI YF (SEQ ID NO:587)	0.41%	N/A	CASSPGNNQPQ HF (SEQ ID NO:628)	0.06%
33	CAFDSGYSTLTF (SEQ ID NO:749)	CSARPPGGGYTF (SEQ ID NO:630)	0.12%	CALSAASGGSYIP TF (SEQ ID NO:631)	CSARRGGAPN TEAFF (SEQ ID NO:599)	0.40%	N/A	CSARMTTQET QYF (SEQ ID NO:632)	0.05%
34	CVVNPHGSSNTG KLIF (SEQ ID NO:568)	CASSLVGQGAYN EQFF (SEQ ID NO:567)	0.09%	CAVSEVASGSRL TF (SEQ ID NO:634)	CASSATSGKHE QYF (SEQ ID NO:609)	0.33%	N/A	CASIGRSMGTD TQYF (SEQ ID NO:633)	0.05%
35	CAMRPLGGYNK LIF (SEQ ID NO:32);	CASSFGVSYEQY F (SEQ ID NO:31)	0.09%	N/A	CASSPLRVGDY GYTF (SEQ ID NO:635)	0.32%	N/A	CASRSRLRPSG RSTDTQYF	0.04%

	CAVHEIQGAQKLVF (SEQ ID NO:30)							(SEQ ID NO:636)	
36	CALWEGQGGSEKLVF (SEQ ID NO:678)	CASSLEARAPSGNTIYF (SEQ ID NO:41); CASSFGVSYEQYF (SEQ ID NO:31)	0.09%	PLKGNDMRF (SEQ ID NO:637)	CASRSRLRPSGRSTDTQYF (SEQ ID NO:636)	0.30%	N/A	CASSPLRVGDYGYTF (SEQ ID NO:635)	0.04%
37	CAVFQGGSEKLVF (SEQ ID NO:714)	CASSPEPQGANFYEQYF (SEQ ID NO:572)	0.09%	CAPAGGTSYGKLTF (SEQ ID NO:638)	CASSGSDGLEQFF (SEQ ID NO:639)	0.29%	N/A	CASTGTRSYNEQFF (SEQ ID NO:640)	0.04%
38	CAVDRDQGNLIF (SEQ ID NO:750)	CSVEGSLAQYF (SEQ ID NO:642)	0.09%	CVVSNNAGNMLTF (SEQ ID NO:584)	CASSPGNNQPQHF (SEQ ID NO:628)	0.29%	N/A	CASSTGTSGRAREQYF (SEQ ID NO:641)	0.04%
39	CAVRGTGNQFYF (SEQ ID NO:751)	CSVVGTASSGNTIYF (SEQ ID NO:643)	0.09%	CAVREYNFNKFYF (SEQ ID NO:644)	CASSTQGGTDTQYF (SEQ ID NO:645)	0.29%	N/A	CASCLPGGNEKLFF (SEQ ID NO:610)	0.03%
40	CAVRDGASNYQLIW (SEQ ID NO:752)	CASSDGGEQFF (SEQ ID NO:648)	0.07%	CAERGSNTGKLIF (SEQ ID NO:647)	CASSPTVGAYGYTF (SEQ ID NO:646)	0.27%	N/A	CASSLVGQGLGEQYF (SEQ ID NO:576)	0.03%
41	CAIPYGYNKLIF (SEQ ID NO:596)	CAWSGTGGSEAFF (SEQ ID NO:597)	0.07%	N/A	CASSLYSGQFF (SEQ ID NO:649)	0.27%	N/A	CASSTQGGTDTQYF (SEQ ID NO:645)	0.03%
42	CALSENILTGANNLFF (SEQ ID NO:753)	CASSRPEGVNQPQHF (SEQ ID NO:652)	0.07%	CIVIVYSGGGADGLTF (SEQ ID NO:651)	CASSSRTSGGDEQYF (SEQ ID NO:650)	0.25%	N/A	CASSEGTVLDEQYF (SEQ ID NO:574)	0.03%
43	CALSERAGNQFYF (SEQ ID NO:754)	CASSDGTGDYEQYF (SEQ ID NO:653)	0.07%	CAVSFTGGGNKLTF (SEQ ID NO:654)	CASIGRSMGTDTQYF (SEQ ID NO:633)	0.24%	N/A	CASSAEAYSGYGYTF (SEQ ID NO:655)	0.03%
44	CALWEGQGGSEKLVF (SEQ ID NO:678)	CASSQAANEQFF (SEQ ID NO:33);CASSLEARAPSGNTIYF (SEQ ID NO:41)	0.07%	CAVGGYSTLTF (SEQ ID NO:656)	CASGLVPRPSGGESNTGELFF (SEQ ID NO:627)	0.23%	N/A	CASSQSLTGGLQPQHF (SEQ ID NO:626)	0.03%
45	CAVQGYQKVTF (SEQ ID NO:588)	CASSFTRVAYEQYF (SEQ ID NO:571)	0.07%	CGTEGGGSNYKLTF (SEQ ID NO:657)	CASLFERGSTEAFF (SEQ ID NO:658)	0.22%	N/A	CASSPTVGAYGYTF (SEQ ID NO:646)	0.02%

46	CAGAYYGQNFVF (SEQ ID NO:755)	CASSLGGGSSYEQYF (SEQ ID NO:661)	0.07%	CAVGRSSASKIIF (SEQ ID NO:660)	CASSQQTETSYGYTF (SEQ ID NO:659)	0.22%	N/A	CASSSRLAGGQVSYEQYF (SEQ ID NO:590)	0.02%
47	CAVTNRDDKIIF (SEQ ID NO:756)	CASSPATTMGIDTQYF (SEQ ID NO:662)	0.07%	CAASGYSSASKIIF (SEQ ID NO:663)	CASTGTRSYNEQFF (SEQ ID NO:640)	0.21%	N/A	CASSSTATPADEQFF (SEQ ID NO:664)	0.02%
48	CAVRYGQNFVF (SEQ ID NO:757)	CASSREGDQPQHF (SEQ ID NO:666)	0.07%	N/A	CASSPGNSQPQHF (SEQ ID NO:665)	0.21%	N/A	CASSGSDGLEQFF (SEQ ID NO:639)	0.02%
49	CLVGWDQGGKLIF (SEQ ID NO:758)	CASSLVSDNEQFF (SEQ ID NO:667)	0.07%	CAASRSRGSTLGRLYF (SEQ ID NO:668)	CASSTGTSGRAREQYF (SEQ ID NO:641)	0.21%	N/A	CASSLEARAPSGNTIYF (SEQ ID NO:41)	0.02%
50	CVVSNNAGNMLTF (SEQ ID NO:584)	CASSPGPVQETQYF (SEQ ID NO:585)	0.07%	CAVIIQGAQKLVF (SEQ ID NO:669)	CASSSRTVVNYGYTF (SEQ ID NO:598)	0.20%	N/A	CASSSRTSGGDEQYF (SEQ ID NO:650)	0.02%
51		CASSLEARAPSGNTIYF (SEQ ID NO:41)	0.07%	CAVEATGTYKYIF (SEQ ID NO:670)	CASSDGQIAFF (SEQ ID NO:671)	0.18%	N/A	CASTTGSGRINNEQFF (SEQ ID NO:672)	0.02%
52	CAVRDPSREPGRKAYLRT (SEQ ID NO:748)	CASSQAANEQFF (SEQ ID NO:33)	0.05%	N/A	CASTTGSGRINNEQFF (SEQ ID NO:672)	0.16%	N/A	CASSRPGTSGTDTQYF (SEQ ID NO:673)	0.02%
53	CAVRDQRRDDKIIF (SEQ ID NO:759)	CASSLPGTSGRYSYEQYF (SEQ ID NO:674)	0.05%	CIVRPYGGSQGNLIF (SEQ ID NO:675)	CASISSNGEAFF (SEQ ID NO:676)	0.16%	N/A	CASSLLAVTDTQYF (SEQ ID NO:677)	0.02%
54	CAVNAGNNRKLIW (SEQ ID NO:760)	CASSSGTSGGRHTYEQYF (SEQ ID NO:679)	0.05%	CALWEGQGGSEKLVF (SEQ ID NO:678)	CASSLEARAPSGNTIYF (SEQ ID NO:41)	0.16%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.02%
55	CVVDSGAGSYQLTF (SEQ ID NO:761)	CATELRYTGELFF (SEQ ID NO:680)	0.05%	CAVNTGNQFYF (SEQ ID NO:683)	CASSELIPTF (SEQ ID NO:686)	0.16%	N/A	CASSYTPGPRGEQYF (SEQ ID NO:603)	0.02%
56	CAVDLREGRRALTF (SEQ ID NO:762);CVVSKGSARQLTF (SEQ ID NO:763)	CASSSGTGNQPQHF (SEQ ID NO:681)	0.05%	CAASIAGGGADGLTF (SEQ ID NO:684)	CASSPGGGQPQHF (SEQ ID NO:687)	0.14%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%

57	CAMGGSTLGRLYF (SEQ ID NO:764);CAVSSVDIPTF (SEQ ID NO:946)	CASSETPRDSFF (SEQ ID NO:682)	0.05%	CAECNNAGNMLTF (SEQ ID NO:685)	CASSPQGGAGNTIYF (SEQ ID NO:688)	0.14%	N/A	CASSLYSGQFF (SEQ ID NO:649)	0.01%
58	CAARDSSYKLIF (SEQ ID NO:579)	CASSASDSYNEQFF (SEQ ID NO:578)	0.05%	CAVGADTGRRALTF (SEQ ID NO:690)	CASSARDRAVQYF (SEQ ID NO:689)	0.13%	N/A	CASSPGNSQPQHF (SEQ ID NO:665)	0.01%
59	CAASMAGTALIF (SEQ ID NO:765)	CSVYIATNEKLFF (SEQ ID NO:691)	0.05%	CALNDNAGNMLTF (SEQ ID NO:692)	CASSAEAYSGYGYTF (SEQ ID NO:655)	0.13%	N/A	CASSQQTETSYGYTF (SEQ ID NO:659)	0.01%
60	CAPNAGNNRKLIW (SEQ ID NO:766)	CATSDFRGGGLAKNIQYF (SEQ ID NO:694);CASSPLAGVYEQFF (SEQ ID NO:695)	0.05%	N/A	CASSQRGGGLRGTDTQYF (SEQ ID NO:693)	0.12%	N/A	CASSDGQIAFF (SEQ ID NO:671)	0.01%
61	CAMRPLGGYNKLIF (SEQ ID NO:32); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSQAANEQFF (SEQ ID NO:33)	0.05%	CAMRLNYGGSQGNLIF (SEQ ID NO:697)	CASSSTATPADEQFF (SEQ ID NO:664)	0.11%	N/A	CASSQVFGTGASPLHF (SEQ ID NO:696)	0.01%
62	CALKVGSQGNLIF (SEQ ID NO:767)	CASSINSNQPQHF (SEQ ID NO:698)	0.05%	CAMSSNQGGKLIF (SEQ ID NO:699)	CSARMTTQETQYF (SEQ ID NO:632)	0.11%	N/A	CASLFERGSTEAFF (SEQ ID NO:658)	0.01%
63	CATNDYKLSF (SEQ ID NO:768)	CASSLADRGDEKLFF (SEQ ID NO:702)	0.05%	CAVISNFGNEKLTF (SEQ ID NO:701)	CASSQGARTSYTF (SEQ ID NO:700)	0.09%	N/A	CASSPQGGAGNTIYF (SEQ ID NO:688)	0.01%
64	CATDNDYKLSF (SEQ ID NO:769)	CASSWTSGGMNEQFF (SEQ ID NO:591)	0.05%	CAAPRGGAQKLVF (SEQ ID NO:703)	CASSLMRLAGGRDEQFF (SEQ ID NO:704)	0.09%	N/A	CASSELIPTF (SEQ ID NO:686)	0.01%
65	CATANNAGNMLTF (SEQ ID NO:745)		0.05%	CVVSAFNNDMRF (SEQ ID NO:707)	CATSRQGSNQPQHF (SEQ ID NO:706)	0.09%	N/A	CASRDSSGSSYNEQFF (SEQ ID NO:705)	0.01%
66	CALSEAGSSNTGKLIF (SEQ ID NO:770)	CASSPGQRYSNQPQHF (SEQ ID NO:708)	0.05%	CAGLMKTSYDKVIF (SEQ ID NO:709)	CASSPGHTIYF (SEQ ID NO:710)	0.09%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%

67	CALSAYFNAGGTSYGKLTF (SEQ ID NO:771)	CASSPGQGRQPQHF (SEQ ID NO:713)	0.05%	CAVNSGGGADGLTF (SEQ ID NO:712)	CASSQVFGTGASPLHF (SEQ ID NO:696)	0.08%	N/A	CASSTARAQYF (SEQ ID NO:711)	0.01%
68	CALWEGQGGSEKLVF (SEQ ID NO:678); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.05%	CAVFQGGSEKLVF (SEQ ID NO:714);CAAADFSGTYKYIF (SEQ ID NO:715)	CASSPEPQGANFYEQYF (SEQ ID NO:572)	0.08%	N/A	CSARAYNEQFF (SEQ ID NO:716)	0.01%
69	CALWEGQGGSEKLVF (SEQ ID NO:678)	CASSFGVSYEQYF (SEQ ID NO:31)	0.05%	CAVNGQGNNRLAF (SEQ ID NO:717)	CATSGGASNQPQHF (SEQ ID NO:718)	0.08%	N/A	CATSRQGSNQPQHF (SEQ ID NO:706)	0.01%
70	CALGVNTGFQKLVF (SEQ ID NO:772);CALSDTSGGYNKLIF (SEQ ID NO:773)	CASNTRESYEQYF (SEQ ID NO:720)	0.05%	CAVGAGTGTASKLTF (SEQ ID NO:719)	CASSLELAGGRDTQYF (SEQ ID NO:43)	0.08%	N/A	CSVETGVNQPQHF (SEQ ID NO:608)	0.01%
71	CAVIIQGAQKLVF (SEQ ID NO:669)	CASSSRTVVNYGYTF (SEQ ID NO:598)	0.05%	N/A	CASSQDNRVHEQYF (SEQ ID NO:721)	0.07%	N/A	CASSFRGYEKLFF (SEQ ID NO:722)	0.01%
72	CAVHGTDKLIF (SEQ ID NO:774)	CASSLVRTADTQYF (SEQ ID NO:725)	0.05%	CAPVSSGSARQLTF (SEQ ID NO:724)	CASTPTGLPYGYTF (SEQ ID NO:723)	0.07%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.01%
73	CAVTIQGAQKLVF (SEQ ID NO:775)	CASTNLAGGYNEQFF (SEQ ID NO:726)	0.05%	CAVEGGSQGNLIF (SEQ ID NO:727)	CASSLSASGVTDTQYF (SEQ ID NO:728)	0.06%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%
74	CAVFQGGSEKLVF (SEQ ID NO:714)	CASSFGVSYEQYF (SEQ ID NO:31)	0.05%	CAVSDLGDGQKLLF (SEQ ID NO:731)	CASSFRGYEKLFF (SEQ ID NO:722)	0.06%	N/A	CASSIAGSGGDEQFF (SEQ ID NO:729)	0.01%
75	CAGEFYYF (SEQ ID NO:776)	CATSSNGYNEQFF (SEQ ID NO:733)	0.05%	CAAGGYQKVTF (SEQ ID NO:732)	CASTFGRGNIQYF (SEQ ID NO:730)	0.06%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%
76	CAGDRGTDNYGQNFVF (SEQ ID NO:777)	CASSLVSFSPETQYF (SEQ ID NO:734)	0.05%	CALDMRNYGQNFVF (SEQ ID NO:739)	CASSQDLQKNIQYF (SEQ ID NO:958)	0.06%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.01%

77	*GRDDKIIF (SEQ ID NO:613)	CASSSARGVPEAFF (SEQ ID NO:735)	0.05%	CAENTRAGGTSYGKLTF (SEQ ID NO:740)	CASSTARAQYF (SEQ ID NO:711)	0.06%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
78	CAYRSAETSGSRLTF (SEQ ID NO:778)	CASSSREALYEQYF (SEQ ID NO:736)	0.05%	CAYRSARSGAGSYQLTF (SEQ ID NO:741)	CASRVWGADTQYF (SEQ ID NO:616)	0.06%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
79	CAVDIGTSYGKLTF (SEQ ID NO:779)	CASSWTPSEQFF (SEQ ID NO:737)	0.05%	CAVGTSGTYKYIF (SEQ ID NO:742)	CASSRPGTSGTDTQYF (SEQ ID NO:673)	0.05%	N/A	CASSLGSSTQYF (SEQ ID NO:580)	0.01%
80	CLVGGGDYKLSF (SEQ ID NO:780)	CASSLGPPPLHF (SEQ ID NO:738)	0.05%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.05%	N/A	CASTGQSSYNEQFF (SEQ ID NO:743)	0.01%
81	CLVGGLYGGSQGNLIF (SEQ ID NO:601)	CASSLADPTDTQYF (SEQ ID NO:600)	0.05%	CALGVNTGFQKLVF (SEQ ID NO:772);CALSDTSGGYNKLIF (SEQ ID NO:773)	CASNTRESYEQYF (SEQ ID NO:720)	0.05%	N/A	CASSLAAGGLSEQFF (SEQ ID NO:573)	0.01%
82	CAVGGYSTLTF (SEQ ID NO:656)	CASGLVPRPSGGESNTGELFF (SEQ ID NO:627)	0.05%	CAVEGPGGSQGNLIF (SEQ ID NO:782)	CSVVSPDYGYTF (SEQ ID NO:796)	0.04%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
83	CAEEGDRDYKLSF (SEQ ID NO:34); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31); CASTGRSGRSEQYF (SEQ ID NO:35)	0.05%	CAAIQGAQKLVF (SEQ ID NO:783)	CASSDQGIDIQYF (SEQ ID NO:797)	0.04%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
84	CAEEGDRDYKLSF (SEQ ID NO:34)	CASSQAANEQFF (SEQ ID NO:33); CASSFGVSYEQYF (SEQ ID NO:31)	0.05%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.04%	N/A	CASSFIQPQHF (SEQ ID NO:802)	0.01%
85	CAEEGDRDYKLSF (SEQ ID NO:34)	CASSFGVSYEQYF (SEQ ID NO:31); CASTGRSGRSEQYF (SEQ ID NO:35)	0.05%	CAEESNDYKLSF (SEQ ID NO:784);CAESDYQLIW (SEQ ID NO:785)	CASSQRGHGYTF (SEQ ID NO:787)	0.04%	N/A	CASTGRSGRSEQYF (SEQ ID NO:35)	0.01%
86	CAEEGDRDYKLSF (SEQ ID NO:34)	CASSQAANEQFF (SEQ ID NO:33);CASTGRSGRSEQYF (SEQ ID NO:35)	0.05%	*GRDDKIIF (SEQ ID NO:613)	CASSPEAGGANGYTF (SEQ ID NO:798)	0.04%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%

87	CAVTSRGGSNYKLTF (SEQ ID NO:586)	CARRRVLVAWDCSGANVLTF (SEQ ID NO:577);CASSLAAGGLSEQFF (SEQ ID NO:573)	0.05%	CAVTSYGNNRLAF (SEQ ID NO:789)	CASRDSSGSSYNEQFF (SEQ ID NO:705)	0.04%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
88	CVVSLFYGGATNKLIF (SEQ ID NO:781)	CASSASHSSYNEQFF (SEQ ID NO:744)	0.05%	CAVGDDYKLSF (SEQ ID NO:790)	CASSLPGAPTDTQYF (SEQ ID NO:799)	0.04%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
89	CVVRRDSGGSNYKLTF (SEQ ID NO:569)	CASSLDPGRALGYTF (SEQ ID NO:570)	0.05%	CAVDSGGSNYKLTF (SEQ ID NO:791)	CASTIWRGYTEAFF (SEQ ID NO:800)	0.04%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
90	CAVGAGTGTASKLTF (SEQ ID NO:719)		0.05%	CLQGGSARQLTF (SEQ ID NO:792)	CASSIAGSGGDEQFF (SEQ ID NO:729)	0.04%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.01%
91	CAVSEGGGYQKVTF (SEQ ID NO:803)	CASSETVPSLDEQYF (SEQ ID NO:810)	0.05%	N/A	CASSFERGGETQYF (SEQ ID NO:801)	0.03%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%
92	CAVSDGAGGTSYGKLTF (SEQ ID NO:804)	CASSLTPLAWNEQFF (SEQ ID NO:811)	0.05%	CAYRSLTGNQFYF (SEQ ID NO:793)	CASSPGQVWNGYTF (SEQ ID NO:822)	0.03%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.01%
93	CAVHEIQGAQKLVF (SEQ ID NO:30)		0.05%	CAVTLNNAGNMLTF (SEQ ID NO:794)	CASSRTGGYSGPQHF (SEQ ID NO:823)	0.03%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.01%
94	CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31); CASTGRSGRSEQYF (SEQ ID NO:35)	0.05%	CAVNTGFQKLVF (SEQ ID NO:795)	CASSLGADTQYF (SEQ ID NO:824)	0.03%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
95	CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSQAANEQFF (SEQ ID NO:33); CASSFGVSYEQYF (SEQ ID NO:31)	0.05%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.03%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
96	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.05%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.03%	N/A	CASSGGITDSQFF (SEQ ID NO:828)	0.01%

97	N/A	CASSPGQGSYGYTF (SEQ ID NO:812)	0.05%	CAVGGYSSASKIIF (SEQ ID NO:819)	CASSFIQPQHF (SEQ ID NO:802)	0.03%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%
98	N/A	CASSVHPDRGLGEQYF (SEQ ID NO:813)	0.05%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.03%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%
99	CAVRDRGGGKLIF (SEQ ID NO:805)	CASSREGSTDTQYF (SEQ ID NO:814)	0.02%	CAVSDLGDGQKLLF (SEQ ID NO:731)	CASTSGFSGANVLTF (SEQ ID NO:825)	0.03%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%
100	CAVNYQLIW (SEQ ID NO:806)	CASSHRGSSYNEQFF (SEQ ID NO:815);CASSHGGYTF (SEQ ID NO:816)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.03%	N/A	CASSLAAGGLSEQFF (SEQ ID NO:573)	0.01%
101	CAVRDERRGNVLHC (SEQ ID NO:807);CLVGKGFQKLVF (SEQ ID NO:1359)	CASSLVSRGDEQFF (SEQ ID NO:817)	0.02%	N/A	CASSLVGQGAYNEQFF (SEQ ID NO:567)	0.03%	N/A	CASSQGARTSYTF (SEQ ID NO:700)	0.01%
102	CAVIAAGNKLTF (SEQ ID NO:808)	CASSFRSAGGEQFF (SEQ ID NO:818)	0.02%	N/A	CASSLVGQGAYNEQFF (SEQ ID NO:567)	0.03%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%
103	CAVRDPSREPGRKAYLRT (SEQ ID NO:748)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.03%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
104	CAVRDPSREPGRKAYLRT (SEQ ID NO:748)	CASSQAANEQFF (SEQ ID NO:33); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVSFTHPDYKLSF (SEQ ID NO:820)	CASSQVTGYEQYF (SEQ ID NO:826)	0.03%	N/A	CASSFNPQGREKLFF (SEQ ID NO:583)	0.00%
105	CAVRPSGRLMF (SEQ ID NO:593);CAVKGPQAGTALIF (SEQ ID NO:809)	CSATGTYNEQFF (SEQ ID NO:582)	0.02%	CVVSDIRAGTYKYIF (SEQ ID NO:821)	CASSQNYEQYF (SEQ ID NO:827)	0.03%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.00%
106	CAVRDGASNYQLIW (SEQ ID NO:752); CAVHEIQGAQKL	CASSDGGEQFF (SEQ ID NO:648); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVRSTNAGKSTF (SEQ ID NO:852)	CASSRQRETQYF (SEQ ID NO:861)	0.03%	N/A	CASSAGQGYGYTF (SEQ ID NO:871)	0.00%

	VF (SEQ ID NO:30)								
107	CAVSPPGGFGNVLHC (SEQ ID NO:829)	CATSDSKQGGTHSSYNEQFF (SEQ ID NO:833)	0.02%	N/A	CATSDFDGGGYTF (SEQ ID NO:862)	0.03%	N/A	CTSSQAANEQFF (SEQ ID NO:872)	0.00%
108	CAVSAETGANNLFF (SEQ ID NO:830)	CASSQELGSYEQYF (SEQ ID NO:834)	0.02%	CAYRDYNTDKLIF (SEQ ID NO:853)	CASSDMNTEAFF (SEQ ID NO:863)	0.03%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
109	CAVSSFSGGYNKLIF (SEQ ID NO:831)	CASSPSGGSHSTDTQYF (SEQ ID NO:835)	0.02%	N/A	CASTGQSSYNEQFF (SEQ ID NO:743)	0.03%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
110	CAVIPFSGGYNKLIF (SEQ ID NO:832)	CASSPSGGPHSTDTQYF (SEQ ID NO:836)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSLELAGGRDTQYF (SEQ ID NO:43)	0.00%
111	CAVTNFGNEKLTF (SEQ ID NO:838)	CASSQGEGGYTF (SEQ ID NO:837)	0.02%	N/A	CASSLGAAGELFF (SEQ ID NO:864)	0.02%	N/A	CASSPEAGGANGYTF (SEQ ID NO:798)	0.00%
112	CVVSEKGRDNTNAGKSTF (SEQ ID NO:839); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSQERRGNEQFF (SEQ ID NO:845); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVEAGRRALTF (SEQ ID NO:854)	CASRLGTGGTEAFF (SEQ ID NO:865)	0.02%	N/A	CSARDGTYEQYF (SEQ ID NO:873)	0.00%
113	CVVSEKGRDNTNAGKSTF (SEQ ID NO:839)	CASSQERRGNEQFF (SEQ ID NO:845)	0.02%	CAVSETMDSSYKLIF (SEQ ID NO:855)	CASSFRGLSYGYTF (SEQ ID NO:866)	0.02%	N/A	CASSLPGAPTDTQYF (SEQ ID NO:799)	0.00%
114	CVVRASI (SEQ ID NO:840)	CASSFQSTNPSTDTQYF (SEQ ID NO:846)	0.02%	CAVSVSGGSNYKLTF (SEQ ID NO:856)	CSARAYNEQFF (SEQ ID NO:716)	0.02%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.00%
115	CVVSAFNNDMRF (SEQ ID NO:707)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAFISNAGNMLTF (SEQ ID NO:857)	CASSSRTGAYNEQFF (SEQ ID NO:867)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
116	CAVSFTGGGNKLTF (SEQ ID NO:654);CVFPTGANNLFF (SEQ ID NO:841)	CASIGRSMGTDTQYF (SEQ ID NO:633)	0.02%	N/A	CATSDWGGVNTGELFF (SEQ ID NO:868)	0.02%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.00%

117	CAVSSGGYQKVTF (SEQ ID NO:842)	CATAGGQDEKLFF (SEQ ID NO:847)	0.02%	CAVRPGNYGQNFVF (SEQ ID NO:858);CASMLNYGGATNKLIF (SEQ ID NO:859)	CASSPRQERSYNSPLHF (SEQ ID NO:869)	0.02%	N/A	CASTGSFGPQTGYTDTQYF (SEQ ID NO:874)	0.00%
118	CVLDNYGQNFVF (SEQ ID NO:843)	CASSFRDRGSPEQFF (SEQ ID NO:848)	0.02%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
119	CVLDNYGQNFVF (SEQ ID NO:843);CAMRPLGGYNKLIF (SEQ ID NO:32)	CASSQAANEQFF (SEQ ID NO:33);CASSFRDRGSPEQFF (SEQ ID NO:848)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
120	CVVSAFTDKLIF (SEQ ID NO:844)	CASSQAGGRDEQYF (SEQ ID NO:849);CASSLGGRHYNSPLHF (SEQ ID NO:850)	0.02%	PGSRGRRALTF (SEQ ID NO:860)	CASSYAGTSGEQFF (SEQ ID NO:870)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
121	CAVNIRGANNLFF (SEQ ID NO:589);CVVSPSDGGYQKVTF (SEQ ID NO:875)	CASSSRLAGGQVSYEQYF (SEQ ID NO:590)	0.02%	CAAGGNYGGSQGNLIF (SEQ ID NO:746)	CASSLGSSTQYF (SEQ ID NO:580)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
122	CAVRRAGNMLTF (SEQ ID NO:876)	CSARDPSLGRGYEQYF (SEQ ID NO:851)	0.02%	CAMRELDNYGQNFVF (SEQ ID NO:898)	CASSPQALTANTEAFF (SEQ ID NO:913)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
123	CAVGNTGFQKLVF (SEQ ID NO:877)	CASLRTGEGNEQFF (SEQ ID NO:892);CASSPTGEESDTQYF (SEQ ID NO:893)	0.02%	N/A	CASSLVGQGAYNEQFF (SEQ ID NO:567)	0.02%	N/A	CASSGGWDQPQHF (SEQ ID NO:922)	0.00%
124	CAVGNTGFQKLVF (SEQ ID NO:877);CAEEGDRDYKLSF (SEQ ID NO:34)	CASLRTGEGNEQFF (SEQ ID NO:892);CASSPTGEESDTQYF (SEQ ID NO:893);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CAMSPSYNQGGKLIF (SEQ ID NO:899)	CASSRGLTEAFF (SEQ ID NO:914)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%

125	CAVNKEIKAAGNKLTF (SEQ ID NO:878);CARDHSGAGSYQLTF (SEQ ID NO:879)	CASSHMRGGRDEQFF (SEQ ID NO:894)	0.02%	CALSSGAGSYQLTF (SEQ ID NO:900)	CASSYSGTLQETQYF (SEQ ID NO:915)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
126	*GRDDKIIF (SEQ ID NO:613)	CASRRRGLFETQYF (SEQ ID NO:895)	0.02%	N/A	CATTLPGDTEAFF (SEQ ID NO:37)	0.02%	N/A	CASSPGHTIYF (SEQ ID NO:710)	0.00%
127	CAVAASTTDSWGKLQF (SEQ ID NO:880)	CASSPRLGPYEQYF (SEQ ID NO:896)	0.02%	CAASKVQGAQKLVF (SEQ ID NO:901)	CASSIDRGLGEQYF (SEQ ID NO:916)	0.02%	N/A	CASSPTGEESDTQYF (SEQ ID NO:893)	0.00%
128	CAVTDSTDKLIF (SEQ ID NO:881); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVGDSSGTYKYIF (SEQ ID NO:907);CAVDDGQKLLF (SEQ ID NO:908)	CASSQDRGTEAFF (SEQ ID NO:917)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
129	CAVNNAGNMLTF (SEQ ID NO:882);CAASAQQEPF (SEQ ID NO:883)	CASSPVLREQYF (SEQ ID NO:897)	0.02%	CAVGGYSSASKIIF (SEQ ID NO:819)	CASSPQDRVAGDTIYF (SEQ ID NO:918)	0.02%	N/A	CASTGRSGRSEQYF (SEQ ID NO:35)	0.00%
130	CAVNSVDNAGNMLTF (SEQ ID NO:884);CARNNDMRF (SEQ ID NO:885)	CASRDQYGTEKLFF (SEQ ID NO:902)	0.02%	CALSENILTGANNLFF (SEQ ID NO:753)	CASSRPEGVNQPQHF (SEQ ID NO:652)	0.02%	N/A	CATSGGASNQPQHF (SEQ ID NO:718)	0.00%
131	CAVNPEGYNNNDMRF (SEQ ID NO:886)	CASSESRYDEQYF (SEQ ID NO:903)	0.02%	CAMNLGNNNDMRF (SEQ ID NO:891)	CASSYSGGNEQFF (SEQ ID NO:906)	0.02%	N/A	CASTGRSGRSEQYF (SEQ ID NO:35)	0.00%
132	CAVTSGRISNFGNEKLTF (SEQ ID NO:887);CAMGYGQNFVF (SEQ ID NO:888)	CSVEDRGVPYGYTF (SEQ ID NO:904)	0.02%	CAVNFGNEKLTF (SEQ ID NO:909)	CASSPGVAGVKETQYF (SEQ ID NO:919)	0.02%	N/A	CASKPTSGSVNEQFF (SEQ ID NO:604)	0.00%
133	CAAHTGGFKTIF (SEQ ID NO:889)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAVRRAGGGNKLTF (SEQ ID NO:910);CAVRDRTGGFKTIF (SEQ ID NO:911)	CATSRLGQLSTSTDTQYF (SEQ ID NO:920)	0.02%	N/A	CASSLGADTQYF (SEQ ID NO:824)	0.00%

134	CAMGGSTLGRLYF (SEQ ID NO:764)	CASSETPRDSFF (SEQ ID NO:682)	0.02%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.02%	N/A	CASSSTGFETQYF (SEQ ID NO:923)	0.00%
135	CAMSGDSNYQLIW (SEQ ID NO:890)	CATSNHESEQYF (SEQ ID NO:905)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
136	CAMNLGNNNDMRF (SEQ ID NO:891)	CASSYSGGNEQFF (SEQ ID NO:906)	0.02%	CAVGSNTNAGKSTF (SEQ ID NO:912)	CASSQRQGGVEQYF (SEQ ID NO:921)	0.02%	N/A	CASSQRGGGLRGTDTQYF (SEQ ID NO:693)	0.00%
137	CAMSFNNDMRF (SEQ ID NO:960)	CASSLLQGRPNEKLFF (SEQ ID NO:924)	0.02%	CAVRDGGDYKLSF (SEQ ID NO:934)	CASSLAPRLSSSYNEQFF (SEQ ID NO:937)	0.02%	N/A	CASSARDRAVQYF (SEQ ID NO:689)	0.00%
138	CAMSDTGFQKLVF (SEQ ID NO:961)	CSVLTGTGLGGYTF (SEQ ID NO:925)	0.02%	CAAKISGSARQLTF (SEQ ID NO:935)	CASIEGGTPGTQYF (SEQ ID NO:938)	0.02%	N/A	CATSDWGGVNTGELFF (SEQ ID NO:868)	0.00%
139	CAMDTGFQKLVF (SEQ ID NO:962)	CASSLAGGRGDEQYF (SEQ ID NO:926)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASISSNGEAFF (SEQ ID NO:676)	0.00%
140	CAASGYSSASKIIF (SEQ ID NO:663)	CASTGTRSYNEQFF (SEQ ID NO:640)	0.02%	CASRNDYKLSF (SEQ ID NO:936)	CASSPAQGAGYTF (SEQ ID NO:939)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
141	CAASPPGSGGYNKLIF (SEQ ID NO:963)	CASSPRGAGVLNEQFF (SEQ ID NO:927)	0.02%	CALTRVFRGGSNYKLTF (SEQ ID NO:941)	CASSLSVGAGETQYF (SEQ ID NO:940)	0.02%	N/A	CASSQDLQKNIQYF (SEQ ID NO:958)	0.00%
142	CAASTPGEKLTF (SEQ ID NO:624)	CASSYDGENTEAFF (SEQ ID NO:618); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CALRQGDNNDMRF (SEQ ID NO:942)	CASSLSAGANSPLHF (SEQ ID NO:949)	0.02%	N/A	CASSPGGGQPQHF (SEQ ID NO:687)	0.00%
143	CAESDSGAGSYQLTF (SEQ ID NO:964);CLVAPPDARLMF (SEQ ID NO:965)	CASSQAADRPTYEQYF (SEQ ID NO:928)	0.02%	CALSAYFNAGGTSYGKLTF (SEQ ID NO:771)	CASSPGQGRQPQHF (SEQ ID NO:713)	0.02%	N/A	CATTLPGDTEAFF (SEQ ID NO:37)	0.00%
144	CAESDYQLIW (SEQ ID NO:785)	CASSQRGHGYTF (SEQ ID NO:787)	0.02%	N/A	CASSTQTGTSDIQYF (SEQ ID NO:950)	0.02%	N/A	CASSLSVGAGETQYF (SEQ ID NO:940)	0.00%

145	CAERDPGNMLTF (SEQ ID NO:966)	CASSWTNRGGN QPQHF (SEQ ID NO:929)	0.02%	N/A	CASSMTSGDT GELFF (SEQ ID NO:951)	0.01%	N/A	CASKPTSGSVN EQFF (SEQ ID NO:604)	0.00%
146	CAEYSGYALNF (SEQ ID NO:967)	CASSLEYSGGKS GEQFF (SEQ ID NO:930)	0.02%	CAVRLISGGSNY KLTF (SEQ ID NO:1111)	CASSSPTRELY SNQPQHF (SEQ ID NO:952)	0.01%	N/A	CATSDFDGGG YTF (SEQ ID NO:862)	0.00%
147	CAMRGGYAGNK LTF (SEQ ID NO:968)	CASSLGGNQPQH F (SEQ ID NO:931)	0.02%	N/A	CASSYLAGGFR DEQFF (SEQ ID NO:564)	0.01%	N/A	CASSWGRENT EAFF (SEQ ID NO:959)	0.00%
148	CAMSPSYNQGGK LIF (SEQ ID NO:899)	CASSRGLTEAFF (SEQ ID NO:914)	0.02%	CAARKTSGTYKY IF (SEQ ID NO:944)	CASSQVSSGSY EQYF (SEQ ID NO:953)	0.01%	N/A	CASSQDNRVH EQYF (SEQ ID NO:721)	0.00%
149	CAMRGVYNQGG KLIF (SEQ ID NO:969)	CASSRGLTEAFF (SEQ ID NO:914)	0.02%	CAARKTSGTYKY IF (SEQ ID NO:945)	CASSPTQDINQ PQHF (SEQ ID NO:954)	0.01%	N/A	CASTGRSGRSE QYF (SEQ ID NO:35)	0.00%
150	CAMRVHNSGAG SYQLTF (SEQ ID NO:970);CAEEGD RDYKLSF (SEQ ID NO:34)	CASSSTGTNQPQ HF (SEQ ID NO:932);CASTGR SGRSEQYF (SEQ ID NO:35)	0.02%	CAVSSVDIPTF (SEQ ID NO:946);CAMGGS TLGRLYF (SEQ ID NO:764)	CASSETPRDSF F (SEQ ID NO:682)	0.01%	N/A	CASSFRGLSYG YTF (SEQ ID NO:866)	0.00%
151	CAMRGMNRDDK IIF (SEQ ID NO:971)	CATMSGAGNTIY F (SEQ ID NO:933);CASSQA ANEQFF (SEQ ID NO:33)	0.02%	CAPDPAGGTSYG KLTF (SEQ ID NO:947);CILRGSD SNYQLIW (SEQ ID NO:948)	CASSQDLRILN TQYF (SEQ ID NO:955)	0.01%	N/A	CASSFGVSYEQ YF (SEQ ID NO:31)	0.00%
152	CAMRPLGGYNK LIF (SEQ ID NO:32)	CASSQAANEQFF (SEQ ID NO:33);CASTGRS GRSEQYF (SEQ ID NO:35)	0.02%	CAPNQGGKLIF (SEQ ID NO:956)	CASSLLAGSNH EQYF (SEQ ID NO:957)	0.01%	N/A	CASSLSASGVT DTQYF (SEQ ID NO:728)	0.00%
153	CAMRPLGGYNK LIF (SEQ ID NO:32)	CASSQDLQKNIQ YF (SEQ ID NO:958)	0.02%	CAASKGSSNTGK LIF (SEQ ID NO:975)	CASSPNNPGNT IYF (SEQ ID NO:988)	0.01%	N/A	CASSVATGQG LEDTQYF (SEQ ID NO:996)	0.00%
154	CAMRPLGGYNK LIF (SEQ ID NO:32);CALSEGS NFGNEKLTF (SEQ ID NO:581)	CASSQAANEQFF (SEQ ID NO:33);CASSEGT VLDEQYF (SEQ ID NO:574)	0.02%	CAFMKPGGSNYK LTF (SEQ ID NO:976);CAAAVK GNQFYF (SEQ ID NO:977)	CASTPMALGTE AFF (SEQ ID NO:989)	0.01%	N/A	CASSQAANEQF F (SEQ ID NO:33)	0.00%

155	CAMRPLGGYNKLIF (SEQ ID NO:32);CALKIGSQGNLIF (SEQ ID NO:972)	CASSINSNQPQHF (SEQ ID NO:698)	0.02%	CAVGLNTGFQKLVF (SEQ ID NO:978)	CASSGQPQHF (SEQ ID NO:990)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
156	CAMRPLGGYNKLIF (SEQ ID NO:32);CLVGDLYGGATNKLIF (SEQ ID NO:973)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAITGSNYQLIW (SEQ ID NO:979)	CASSLAGTSGSGADTQYF (SEQ ID NO:991)	0.01%	N/A	CASSLADPTDTQYF (SEQ ID NO:600)	0.00%
157	CAMRPLGGYNKLIF (SEQ ID NO:32);CAVFQGGSEKLVF (SEQ ID NO:714);CAAADFSGTYKYIF (SEQ ID NO:715)	CASSPEPQGANFYEQYF (SEQ ID NO:572)	0.02%	CALRLTQGGSEKLVF (SEQ ID NO:980)	CASSSTGFETQYF (SEQ ID NO:923)	0.01%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
158	CAMRPLGGYNKLIF (SEQ ID NO:32);CALWEGQGGSEKLVF (SEQ ID NO:678); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSQAANEQFF (SEQ ID NO:33); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.00%
159	CAMRPLGGYNKLIF (SEQ ID NO:32)	CASGPQF (SEQ ID NO:1242);CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAAVNYGQNFVF (SEQ ID NO:619);CAASSGGTASKLTF (SEQ ID NO:620)	CASSASGNQPQHF (SEQ ID NO:606)	0.01%	N/A	CASTFGRGNIQYF (SEQ ID NO:730)	0.00%
160	CAMRPLGGYNKLIF (SEQ ID NO:32);CAASSGGTASKLTF (SEQ ID NO:620)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	CALSEYSGAGSYQLTF (SEQ ID NO:981)	CASSNNRGRSNQPQHF (SEQ ID NO:992)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
161	CAMRPLGGYNKLIF (SEQ ID NO:32)	CASSQAANEQFF (SEQ ID NO:33);CASSFRDRGSPEQFF (SEQ ID NO:848)	0.02%	CAASRVTGGGNKLTF (SEQ ID NO:982)	CASSSHVSRGRETQYF (SEQ ID NO:993)	0.01%	N/A	CASSFQTVGSGSYEQYF (SEQ ID NO:562)	0.00%

162	CAMRPLGGYNKLIF (SEQ ID NO:32);CAVFQGGSEKLVF (SEQ ID NO:714);CAAADFSGTYKYIF (SEQ ID NO:715)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	N/A	CATSDWGGVNTGELFF (SEQ ID NO:868)	0.01%	N/A	CASSLYAGNYGYTF (SEQ ID NO:997)	0.00%
163	CAMRPLGGYNKLIF (SEQ ID NO:32);CAVGAGTGTASKLTF (SEQ ID NO:719)	CASSQAANEQFF (SEQ ID NO:33);CASSLELAGGRDTQYF (SEQ ID NO:43)	0.02%	CIARSDMKTSYDKVIF (SEQ ID NO:983)	CASKEKNRAYGYTF (SEQ ID NO:994)	0.01%	N/A	CAISGKLGGSYEQYF (SEQ ID NO:998)	0.00%
164	CAMRPLGGYNKLIF (SEQ ID NO:32);CALSEVSSGAGSYQLTF (SEQ ID NO:621)	CASSFQTVGSGSYEQYF (SEQ ID NO:562);CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAVDLREGRRALTF (SEQ ID NO:762);CVVSKGSARQLTF (SEQ ID NO:763)	CASSSGTGNQPQHF (SEQ ID NO:681)	0.01%	N/A	CASSGGQIQETQYF (SEQ ID NO:999)	0.00%
165	CAMRPLGGYNKLIF (SEQ ID NO:32)	CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CIPLDDNDYKLSF (SEQ ID NO:984);CIAQRAQGGSEKLVF (SEQ ID NO:985)	CASSAGQGYGYTF (SEQ ID NO:871)	0.01%	N/A	CASNTRESYEQYF (SEQ ID NO:720)	0.00%
166	CAMRPLGGYNKLIF (SEQ ID NO:32);CAFIPTYDKVIF (SEQ ID NO:1001)	CASSQEPGSVHNEQFF (SEQ ID NO:974)	0.02%	CAVWGSGGYQKVTF (SEQ ID NO:986);CAVRENNARLMF (SEQ ID NO:987)	CASSLGSGGEAFF (SEQ ID NO:995)	0.01%	N/A	CAWDGTGNTEAFF (SEQ ID NO:1000)	0.00%
167	CAMRLNYGGSQGNLIF (SEQ ID NO:697)	CASSSTATPADEQFF (SEQ ID NO:664)	0.02%	N/A	CASSFLTDSYNEQFF (SEQ ID NO:592)	0.01%	N/A	CASREDRASYEQYF (SEQ ID NO:1031)	0.00%
168	CAMDQRNEKLTF (SEQ ID NO:1002)	CASSWTSGNRDEQFF (SEQ ID NO:1011)	0.02%	CAFMKPGGSNYKLTF (SEQ ID NO:976);CAAAVKGNQFYF (SEQ ID NO:977)	CASHFNKEKLFF (SEQ ID NO:1029)	0.01%	N/A	CASSLAPRLSSSYNEQFF (SEQ ID NO:937)	0.00%
169	CALKVGSQGNLIF (SEQ ID NO:767)	CASSINSNTEAFF (SEQ ID NO:1012)	0.02%	CAAGGTSYGKLTF (SEQ ID NO:1018)	CASSFTGEGQPQHF (SEQ ID NO:1030)	0.01%	N/A	CASTPTGLPYGYTF (SEQ ID NO:723)	0.00%

170	CATDDNYGQNFVF (SEQ ID NO:1003)	CASSLYDPGNTEAFF (SEQ ID NO:1013)	0.02%	CAVRDRDDKIIF (SEQ ID NO:1019)	CASSPTIRASHYGYTF (SEQ ID NO:1033)	0.01%	N/A	CASSVGLADYNEQFF (SEQ ID NO:1032)	0.00%
171	CATDAPDSGAGSYQLTF (SEQ ID NO:1004);CATDYRTGGSYIPTF (SEQ ID NO:747);CASNGPAKTGANNLFF (SEQ ID NO:1005)	CASRTINEQFF (SEQ ID NO:1014)	0.02%	CAVEDRNDMRF (SEQ ID NO:1020);CAVTNFGNEKLTF (SEQ ID NO:838)	CASSQGEGGYTF (SEQ ID NO:837)	0.01%	N/A	CASRTQGWDDGYTF (SEQ ID NO:1056)	0.00%
172	CATDAPDSGAGSYQLTF (SEQ ID NO:1004)	CASRTINEQFF (SEQ ID NO:1014)	0.02%	CAHDSYGGATNKLIF (SEQ ID NO:1021);LFF (SEQ ID NO:1022)	CATSRGGGSYEQYF (SEQ ID NO:1049)	0.01%	N/A	CASSDGGEQFF (SEQ ID NO:648)	0.00%
173	CATANNAGNMLTF (SEQ ID NO:745)	CASSLDRHQPQHF (SEQ ID NO:39);CASSPEPQGANFYEQYF (SEQ ID NO:572)	0.02%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.01%	N/A	CATSRLGQLSTSTDTQYF (SEQ ID NO:920)	0.00%
174	CATANNAGNMLTF (SEQ ID NO:745); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVYLGSNQFYF (SEQ ID NO:1023)	CASSLATGGMGELFF (SEQ ID NO:1050)	0.01%	N/A	CASSFNPQGREKLFF (SEQ ID NO:583)	0.00%
175	CATANNAGNMLTF (SEQ ID NO:745)	CASSLDRHQPQHF (SEQ ID NO:39); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSLADPTDTQYF (SEQ ID NO:600)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
176	CATDAAGTYKYIF (SEQ ID NO:1006)	CASSSGTSGRAASFNEQFF (SEQ ID NO:1015)	0.02%	CGTDLPFNDYKLSF (SEQ ID NO:1024)	CASSLLAVTDTQYF (SEQ ID NO:677)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
177	CATVQISNFGNEKLTF (SEQ ID NO:1007);CALISGGTSYGKLTF (SEQ ID NO:1008)	CASSAGQLQETQYF (SEQ ID NO:1016)	0.02%	CIVRIPFSSGSARQLTF (SEQ ID NO:1025)	CASSQARNYNEQFF (SEQ ID NO:1051)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%

178	CAPAGGTSYGKLTF (SEQ ID NO:638)	CASSGSDGLEQFF (SEQ ID NO:639)	0.02%	CAATPSGNTPLVF (SEQ ID NO:1026);CAPYGGATNKLIF (SEQ ID NO:1027)	CASSQGGRGHIQYF (SEQ ID NO:1052)	0.01%	N/A	CASSLGAAGELFF (SEQ ID NO:864)	0.00%
179	CATDYRTGGSYIPTF (SEQ ID NO:747);CLVGGGDYKLSF (SEQ ID NO:780)	CASSLGPPPLHF (SEQ ID NO:738)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.00%
180	CALSGFSSARQLTF (SEQ ID NO:1009);CALEEDSNYQLIW (SEQ ID NO:1010)	CASSPGLAGLNEQFF (SEQ ID NO:1017)	0.02%	CASLSGAGSYQLTF (SEQ ID NO:1028)	CASSLIQGNEQFF (SEQ ID NO:1053)	0.01%	N/A	CASSYGYEQYF (SEQ ID NO:1057)	0.00%
181	CALSEAGSARQLTF (SEQ ID NO:1034)	CASSNSGRGADTQYF (SEQ ID NO:1040)	0.02%	CAASILGRNQFYF (SEQ ID NO:1045)	CASSWGRENTEAFF (SEQ ID NO:959)	0.01%	N/A	CASSYSGTLQETQYF (SEQ ID NO:915)	0.00%
182	CALSGSYSGAGSYQLTF (SEQ ID NO:1035)	CASSLIAGDTDTQYF (SEQ ID NO:1041)	0.02%	CIVRVPIYNQGGKLIF (SEQ ID NO:1046);CAVKAPNTGNQFYF (SEQ ID NO:1047)	CASNQGQGLPNQPQHF (SEQ ID NO:1054)	0.01%	N/A	CASSQVETQYF (SEQ ID NO:1058)	0.00%
183	CALSVSGNTPLVF (SEQ ID NO:1036)	CASLPSADSNQPQHF (SEQ ID NO:1042)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
184	CALSEATSGTYKYIF (SEQ ID NO:1037);CAGQAGFGNVLHC (SEQ ID NO:1038)	CASSFVGAKNIQYF (SEQ ID NO:1043)	0.02%	CAGQRDSGGYQKVTF (SEQ ID NO:1048)	CASSVHPDRGLGEQYF (SEQ ID NO:813)	0.01%	N/A	GYTF (SEQ ID NO:1059)	0.00%
185	CALSEAEGGGADGLTF (SEQ ID NO:1039)	CASSLGISDEKLFF (SEQ ID NO:1044)	0.02%	CAVTDSTDKLIF (SEQ ID NO:881)	CASSLDTLYTEAFF (SEQ ID NO:1055)	0.01%	N/A	CSARPPGGGYTF (SEQ ID NO:630)	0.00%
186	CALSEGSNFGNEKLTF (SEQ ID NO:581)	CASSFGVSYEQYF (SEQ ID NO:31);CASSEGTVLDEQYF (SEQ ID NO:574)	0.02%	N/A	CASKPTSGSVNEQFF (SEQ ID NO:604)	0.01%	N/A	CASSYETNYRGYNVNTEAFF (SEQ ID NO:1060)	0.00%

187	CALSEGSNFGNEKLTF (SEQ ID NO:581)	CQQHTDRDQETQYF (SEQ ID NO:595)	0.02%	CAVSDPSGTYKYIF (SEQ ID NO:1066)	CASSFTGDSQAPLHF (SEQ ID NO:1085)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
188	CALSETSNSGGSNYKLTF (SEQ ID NO:1061)	CASSLSNPGLRGYTF (SEQ ID NO:1076)	0.02%	CAMRVHNSGAGSYQLTF (SEQ ID NO:970)	CASSSTGTNQPQHF (SEQ ID NO:932)	0.01%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.00%
189	CALWEGQGGSEKLVF (SEQ ID NO:678)		0.02%	CAVSVSSGNTPLVF (SEQ ID NO:1067)	CASSLAAGLTYEQYF (SEQ ID NO:1086)	0.01%	N/A	CASSLNAGTEAFF (SEQ ID NO:1088)	0.00%
190	CALWEGQGGSEKLVF (SEQ ID NO:678);CAEEGDRDYKLSF (SEQ ID NO:34)	CASSLEARAPSGNTIYF (SEQ ID NO:41)	0.02%	CAATSRGSRLTF (SEQ ID NO:1068)	CASSARWADTEAFF (SEQ ID NO:1087)	0.01%	N/A	CASSFNPQGREKLFF (SEQ ID NO:583)	0.00%
191	CALGVNTGFQKLVF (SEQ ID NO:772); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVSLGSGGSNYKLTF (SEQ ID NO:1069)	CASSPPGTGANYGYTF (SEQ ID NO:1090)	0.01%	N/A	CASSSTGTNQPQHF (SEQ ID NO:932)	0.00%
192	CAVEEGGYQKVTF (SEQ ID NO:1062);CAVRGGGYQKVTF (SEQ ID NO:1063)	CASRGLTTYEQYF (SEQ ID NO:1077)	0.02%	N/A	CASSDGQIAFF (SEQ ID NO:671)	0.01%	N/A	CSAKTGVRRYF (SEQ ID NO:1089)	0.00%
193	CAVVSSGSARQLTF (SEQ ID NO:1064)	CASSLGAGGLETQYF (SEQ ID NO:1078)	0.02%	CAVSDLGSGYALNF (SEQ ID NO:1070)	CASSVGLADYNEQFF (SEQ ID NO:1032)	0.01%	N/A	CASSSGTGNQPQHF (SEQ ID NO:681)	0.00%
194	CAVRSTNAGKSTF (SEQ ID NO:852)	CASSRQRETQYF (SEQ ID NO:861)	0.02%	CAGLYGGSQGNLIF (SEQ ID NO:1071)	CASSRTRGADTQYF (SEQ ID NO:1091)	0.01%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.00%
195	CAVRRDGGATNKLIF (SEQ ID NO:1065)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAYRSARGAQKLVF (SEQ ID NO:1072)	CASSRTSIEKLFF (SEQ ID NO:1092)	0.01%	N/A	CSVEGSLAQYF (SEQ ID NO:642)	0.00%
196	CAVRRDGGATNKLIF (SEQ ID NO:1065);CALDE	CATSDWGGVNTGELFF (SEQ ID NO:868)	0.02%	N/A	CATTLPGDTEAFF (SEQ ID NO:37)	0.01%	N/A	CASSPEPQGANFYEQYF (SEQ ID NO:572)	0.00%

	GNNARLMF (SEQ ID NO:1073)								
197	CAVRRDGGATNKLIF (SEQ ID NO:1065)	CATSDWGGVNTGELFF (SEQ ID NO:868)	0.02%	CAYTGNAGKSTF (SEQ ID NO:1081)	CASSEPTSNSPLHF (SEQ ID NO:1093)	0.01%	N/A	CASSQVSRYSGNTIYF (SEQ ID NO:1098)	0.00%
198	CAAKGDQTGANNLFF (SEQ ID NO:1074)	CASSYPASGTFEQYF (SEQ ID NO:1079)	0.02%	CAASELGSNYKLTF (SEQ ID NO:1082)	CASSNGRYRFTF (SEQ ID NO:1094)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
199	CAVLTNSGYALNF (SEQ ID NO:1075)	CASTSSGTQYF (SEQ ID NO:1080)	0.02%	CIDTNSNSGYALNF (SEQ ID NO:1083)	CASSLAGRETQYF (SEQ ID NO:1095)	0.01%	N/A	CASHFNKEKLFF (SEQ ID NO:1029)	0.00%
200	CAAGGNYGGSQGNLIF (SEQ ID NO:746)	CASSFGVSYEQYF (SEQ ID NO:31);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CACAAGNKLTF (SEQ ID NO:1084)	CASSIPQGSKTEQFF (SEQ ID NO:1096)	0.01%	N/A	CSVEFPSSYNEQFF (SEQ ID NO:1099)	0.00%
201	CAVIVTGNQFYF (SEQ ID NO:559)	CASSEDTYGYTF (SEQ ID NO:560);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	N/A	CASSSNQTPENF (SEQ ID NO:1097)	0.01%	N/A	CASSYAGTSGEQFF (SEQ ID NO:870)	0.00%
202	CAVSVSGGSNYKLTF (SEQ ID NO:856)	CSARAYNEQFF (SEQ ID NO:716)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%	N/A	CASSFQTVGSGSYEQYF (SEQ ID NO:562)	0.00%
203	CAVQGGSEKLVF (SEQ ID NO:1100)	CASQDSNPTETQYF (SEQ ID NO:1106)	0.02%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.01%	N/A	CASSPQALTANTEAFF (SEQ ID NO:913)	0.00%
204	CAVFQGGSEKLVF (SEQ ID NO:714)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAVRLISGGSNYKLTF (SEQ ID NO:1111)	CASSRDGPETQYF (SEQ ID NO:1119)	0.01%	N/A	CASSLGAGLTYEQYF (SEQ ID NO:1128)	0.00%
205	CAVFQGGSEKLVF (SEQ ID NO:714);CAAADFSGTYKYIF (SEQ ID NO:715);CAVGAGTGTASKLTF (SEQ ID NO:719)	CASSPEPQGANFYEQYF (SEQ ID NO:572);CASSLELAGGRDTQYF (SEQ ID NO:43)	0.02%	CAMKVIGGNKLVF (SEQ ID NO:1112)	CASSYETNYRGYNVNTEAFF (SEQ ID NO:1060)	0.01%	N/A	CASSAGVGLAGEAEQFF (SEQ ID NO:1129)	0.00%

206	CAVFQGGSEKLVF (SEQ ID NO:714);CAAADFSGTYKYIF (SEQ ID NO:715)	CASSPEPQGANFYEQYF (SEQ ID NO:572);CASSYSTIGGGRETQYF (SEQ ID NO:1107)	0.02%	N/A	CASTGTRSYNEQFF (SEQ ID NO:640)	0.01%	N/A	CASSQDRGTEAFF (SEQ ID NO:917)	0.00%
207	CAVFQGGSEKLVF (SEQ ID NO:714);CAAADFSGTYKYIF (SEQ ID NO:715)	CASSPEPQGANFYEQYF (SEQ ID NO:572); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAASGRKTSGSRLTF (SEQ ID NO:1113);CAVSDQTGANNLFF (SEQ ID NO:1114)	CASSEALRQPQHF (SEQ ID NO:1120)	0.01%	N/A	CASSDMNTEAFF (SEQ ID NO:863)	0.00%
208	CAVGNTGGFKTIF (SEQ ID NO:615)	CASKPTSGSVNEQFF (SEQ ID NO:604); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CAWSYMTGREQFF (SEQ ID NO:1121)	0.01%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.00%
209	CAVVIPPGKLIF (SEQ ID NO:1101)	CAIRSEGTGFYEQYF (SEQ ID NO:1108)	0.02%	CAASTSSGYALNF (SEQ ID NO:1115)	CASSPRPGGSYEQYF (SEQ ID NO:1122)	0.01%	N/A	CASSLATGGMGELFF (SEQ ID NO:1050)	0.00%
210	CASNGPAKTGANNLFF (SEQ ID NO:1005)	CASRTINEQFF (SEQ ID NO:1014)	0.02%	N/A	CASTGRSGRSEQYF (SEQ ID NO:35)	0.01%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.00%
211	CAASSGGTASKLTF (SEQ ID NO:620)	CASSASGNQPQHF (SEQ ID NO:606)	0.02%	N/A	CASSLQPRSSYEQYF (SEQ ID NO:1123)	0.01%	N/A	CASSLGLTYEQYF (SEQ ID NO:1130)	0.00%
212	CAFDSGYSTLTF (SEQ ID NO:749); CAVHEIQGAQKLVF (SEQ ID NO:30)	CSARPPGGGYTF (SEQ ID NO:630); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAMRGMNRDDKIIF (SEQ ID NO:971)	CATMSGAGNTIYF (SEQ ID NO:933)	0.01%	N/A	CASIEGGTPGTQYF (SEQ ID NO:938)	0.00%
213	CAHDSYGGATNKLIF (SEQ ID NO:1021)	CATSRGGGSYEQYF (SEQ ID NO:1049);CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAARPGQGAQKLVF (SEQ ID NO:1116)	CATSRDPGYEQFF (SEQ ID NO:1124)	0.01%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.00%
214	CIVREPLYNDYKLSF (SEQ ID NO:1102)	CATSDSRLAGGHEQFF (SEQ ID NO:1109)	0.02%	CAVDATDKLIF (SEQ ID NO:1117)	CASRRRTGRGVLYEQYF (SEQ ID NO:1125)	0.01%	N/A	CASTPMALGTEAFF (SEQ ID NO:989)	0.00%

215	CIVSLDI (SEQ ID NO:1103);CGTEFSNTGKLIF (SEQ ID NO:1104)	CASRQGNTEAFF (SEQ ID NO:1110)	0.02%	CAMREGYTGNQFYF (SEQ ID NO:1118)	CASSRIGGSSGNTIYF (SEQ ID NO:1126)	0.01%	N/A	CASSFERGGETQYF (SEQ ID NO:801)	0.00%
216	CIVRVDGDMRF (SEQ ID NO:1105)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSQAKDASNQPQHF (SEQ ID NO:1127)	0.01%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
217	CIVIVYSGGGADGLTF (SEQ ID NO:651)	CASSSRTSGGDEQYF (SEQ ID NO:650)	0.02%	CAFDSGYSTLTF (SEQ ID NO:749)	CSARPPGGGYTF (SEQ ID NO:630)	0.01%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.00%
218	CTILSNFGNEKLTF (SEQ ID NO:1131)	CASNPIGQSNTGELFF (SEQ ID NO:1140)	0.02%	CAASAGDNNDMRF (SEQ ID NO:1148)	CASSGGITDSQFF (SEQ ID NO:828)	0.01%	N/A	CASSVAGTAYGYTF (SEQ ID NO:1160)	0.00%
219	CAASSDDSWGKLQF (SEQ ID NO:1132)	CASSLGQGRQTQYF (SEQ ID NO:1141)	0.02%	CAGYAGGTSYGKLTF (SEQ ID NO:1149)	CASSQVSRYSGNTIYF (SEQ ID NO:1098)	0.01%	N/A	CASSSRTGAYNEQFF (SEQ ID NO:867)	0.00%
220	CAASGNAGNMLTF (SEQ ID NO:1133)	CASSQEFARASSLNEQFF (SEQ ID NO:1142)	0.02%	CAVEDNAGGFKTIF (SEQ ID NO:1150)	CASSPAGRYGYTF (SEQ ID NO:1153)	0.01%	N/A	CSAREKTNSGNTIYF (SEQ ID NO:1167)	0.00%
221	CAASLIENSGYALNF (SEQ ID NO:1134)	CASSPPRGGGYNEQFF (SEQ ID NO:1143)	0.02%	CAVRGATKLSF (SEQ ID NO:623)	CASSPPDSYGYTF (SEQ ID NO:614)	0.01%	N/A	CASSETPRDSFF (SEQ ID NO:682)	0.00%
222	CAASASKTSYDKVIF (SEQ ID NO:1135)	CASRSRLRPSGRSTDTQYF (SEQ ID NO:636)	0.02%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.01%	N/A	CASSRVIQQRKNIQYF (SEQ ID NO:1168)	0.00%
223	CAAKGGTSYGKLTF (SEQ ID NO:605)	CASSFNPQGREKLFF (SEQ ID NO:583)	0.02%	CAMSAAGGYQKVTF (SEQ ID NO:1155)	CASSEVGGGYTF (SEQ ID NO:1158)	0.01%	N/A	CASSPPGTGANYGYTF (SEQ ID NO:1090)	0.00%
224	CAAQQGGSEKLVF (SEQ ID NO:1136)	CASSQQGRVGYTF (SEQ ID NO:1144); CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAGLDSNSGYALNF (SEQ ID NO:1156)	CASSPPDRGQETQYF (SEQ ID NO:1159)	0.01%	N/A	CASSLAAGLTYEQYF (SEQ ID NO:1086)	0.00%
225	*GRDDKIIF (SEQ ID NO:613)	CASSQQGRVGYTF (SEQ ID NO:1144);CATTLP	0.02%	N/A	CASSFNPQGREKLFF (SEQ ID NO:583)	0.01%	N/A	CASTSGFSGANVLTF (SEQ ID NO:825)	0.00%

		GDTEAFF (SEQ ID NO:37)							
226	CAVRDISDDYKLSF (SEQ ID NO:788)	CAISESMGGTYTEAFF (SEQ ID NO:1146)	0.02%	CAVGNTGFQKLVF (SEQ ID NO:877)	CASLRTGEGNEQFF (SEQ ID NO:892)	0.01%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
227	CAVRDNSGAGSYQLTF (SEQ ID NO:1137)	CASSLFGRGANVLTF (SEQ ID NO:1147)	0.02%	CAVGYDKVIF (SEQ ID NO:1157)	CASSQASGIEQFF (SEQ ID NO:1163)	0.01%	N/A	CASSPRQERSYNSPLHF (SEQ ID NO:869)	0.00%
228	CAVEGFGNVLHC (SEQ ID NO:1138)	CASSFSLATSYNEQFF (SEQ ID NO:1151)	0.02%	CAVEDLRGTYKYIF (SEQ ID NO:1161)	CASNRDTGELFF (SEQ ID NO:1164)	0.01%	N/A	CASSLADPTDTQYF (SEQ ID NO:600)	0.00%
229	CAVLSTQTGANNLFF (SEQ ID NO:1139)	CASSSGGIVAFF (SEQ ID NO:1152)	0.02%	N/A	CASSIGTGGLANTGELFF (SEQ ID NO:1165)	0.01%	N/A	CASSYSGGNEQFF (SEQ ID NO:906)	0.00%
230	CAVRDPIRSQGNLIF (SEQ ID NO:1145)	CASSSDLQVGNTIYF (SEQ ID NO:1154)	0.02%	CAVRPSGGGADGLTF (SEQ ID NO:1162)	CASSPTRGMNTEAFF (SEQ ID NO:1166)	0.01%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.00%
231	CAVRDGMEYGNKLVF (SEQ ID NO:1169)	CASRPPGTGEAFF (SEQ ID NO:1172)	0.02%	CARDGGSQGNLIF (SEQ ID NO:1173)	CASRGQGSYTEAFF (SEQ ID NO:1174)	0.01%	N/A	CASSLGSGGEAFF (SEQ ID NO:995)	0.00%
232	CAVVTLGNEKLTF (SEQ ID NO:1170)	CASQPSGSSYNEQFF (SEQ ID NO:1179)	0.02%	CAMRRQGGKLIF (SEQ ID NO:1181)	CASSGEGQGKEKTQYF (SEQ ID NO:1184)	0.01%	N/A	CASSAFPSSGGAGETQYF (SEQ ID NO:1202)	0.00%
233	CAVRIPSGGSYIPTF (SEQ ID NO:1171)	CASSPTSLELFF (SEQ ID NO:1180)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.00%
234	CGTTSNDYKLSF (SEQ ID NO:1175)	CASSLTGDNEQFF (SEQ ID NO:1189)	0.02%	N/A	CASSLVGQGAYNEQFF (SEQ ID NO:567)	0.01%	N/A	CASSPSTGWGSPLHF (SEQ ID NO:1206)	0.00%
235	CGTDLPFNDYKLSF (SEQ ID NO:1024)	CASSLLAVTDTQYF (SEQ ID NO:677)	0.02%	CAVRDSNSGYSTLTF (SEQ ID NO:1182);CAVRDRYQAGTALIF (SEQ ID NO:1183)	CASSTPASGPTGELFF (SEQ ID NO:1185)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
236	CGTEGDDKIIF (SEQ ID NO:1176)	CASSGQANSYEQYF (SEQ ID NO:1190)	0.02%	N/A	CASSELIPTF (SEQ ID NO:686)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%

237	CGTEGDDKIIF (SEQ ID NO:1176)	CASSFGVSYEQYF (SEQ ID NO:31); CASSGQANSYEQYF (SEQ ID NO:1190)	0.02%	N/A	CASSLVGQGAYNEQFF (SEQ ID NO:567)	0.01%	N/A	CTSSVGLADYNEQFF (SEQ ID NO:1207)	0.00%
238	CGASPNTGNQFYF (SEQ ID NO:1177)	CASSQDQGTSGGPTRGTQYF (SEQ ID NO:1191)	0.02%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.01%	N/A	CASSLAGTSGSGADTQYF (SEQ ID NO:991)	0.00%
239	CGASPNTGNQFYF (SEQ ID NO:1177); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVDSWGSTNAGKSTF (SEQ ID NO:1195)	CASSSVGGGYGYTF (SEQ ID NO:1200)	0.01%	N/A	CASSFQTVGSGSYEQYF (SEQ ID NO:562)	0.00%
240	CAGPRSNDYKLSF (SEQ ID NO:1178)	CASGFSGNTEAFF (SEQ ID NO:1192);CASSQAANEQFF (SEQ ID NO:33)	0.02%	N/A	CASSLLGGTYNEKLFF (SEQ ID NO:1201)	0.01%	N/A	CASSLDTLYTEAFF (SEQ ID NO:1055)	0.00%
241	CAGPDSGGGADGLTF (SEQ ID NO:1186)		0.02%	CAVIPDSNYQLIW (SEQ ID NO:1196)	CASSPGQGSYGYTF (SEQ ID NO:812)	0.01%	N/A	CASSLATGGMGELFF (SEQ ID NO:1050)	0.00%
242	CAGTGNQFYF (SEQ ID NO:1187)	CASSDAGYNEQFF (SEQ ID NO:1193)	0.02%	CAVLYGNKLVF (SEQ ID NO:1197);CAGSDSNYQLIW (SEQ ID NO:1198)	CASSHPLREAFF (SEQ ID NO:1205)	0.01%	N/A	CASSLAAGGLSEQFF (SEQ ID NO:573)	0.00%
243	CACAAGNKLTF (SEQ ID NO:1084)	CASSIPQGSKTEQFF (SEQ ID NO:1096)	0.02%	CAGLYGGSQGNLIF (SEQ ID NO:1071)	CASRLAFQREGTDTQYF (SEQ ID NO:1208)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
244	CAFMGVSGSARQLTF (SEQ ID NO:1188)	CASGQGREQAFF (SEQ ID NO:1194)	0.02%	CAGRSYGQNFVF (SEQ ID NO:1199)	CASSLGQNTEAFF (SEQ ID NO:1209)	0.01%	N/A	CASSFTRVAYEQYF (SEQ ID NO:571)	0.00%
245	*GRDDKIIF (SEQ ID NO:613);CAVGAGTGTASKLTF (SEQ ID NO:719)	CATTLPGDTEAFF (SEQ ID NO:37)	0.02%	CAENPFKTSGSRLTF (SEQ ID NO:1203)	CASSLSGNEKLFF (SEQ ID NO:1210)	0.01%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.00%

246	*GRDDKIIF (SEQ ID NO:613)	CATTLPGDTEAFF (SEQ ID NO:37);CASSFGV SYEQYF (SEQ ID NO:31)	0.02%	CAVSVRGNKLVF (SEQ ID NO:1204)	CASSPSGREET QYF (SEQ ID NO:1211)	0.01%	N/A	CASSQAANEQF F (SEQ ID NO:33)	0.00%
247	*GRDDKIIF (SEQ ID NO:613)	CASSFGVSYEQY F (SEQ ID NO:31)	0.02%	CAASHDYKLSF (SEQ ID NO:1223)	CAISGKLGGSY EQYF (SEQ ID NO:998)	0.01%	N/A	CASSRTGYSNQ PQHF (SEQ ID NO:1245)	0.00%
248	*GRDDKIIF (SEQ ID NO:613); CAVHEIQGAQKL VF (SEQ ID NO:30)	CATTLPGDTEAFF (SEQ ID NO:37)	0.02%	N/A	CASSFNPQGRE KLFF (SEQ ID NO:583)	0.01%	N/A	CASSLVGSTGE LFF (SEQ ID NO:1246)	0.00%
249	CALGDDQTGAN NLFF (SEQ ID NO:1212);CAGPD AGGTSYGKLTF (SEQ ID NO:1213)	CASSFDNRGPISY EQYF (SEQ ID NO:1219)	0.02%	N/A	CASSYLAGGFR DEQFF (SEQ ID NO:564)	0.01%	N/A	CASSFQTVGSG SYEQYF (SEQ ID NO:562)	0.00%
250	CACREGNTGKLI F (SEQ ID NO:1214);CAVGT PYNNNDMRF (SEQ ID NO:1215)	CASSNRGSSTDT QYF (SEQ ID NO:1220)	0.02%	CAVGNTGFQKLV F (SEQ ID NO:877)	CASSPTGEESD TQYF (SEQ ID NO:893)	0.01%	N/A	CASSFTRVAYE QYF (SEQ ID NO:571)	0.00%
251	CAYRSADGQKLL F (SEQ ID NO:1216)	CASSFNNDNEQF F (SEQ ID NO:1221)	0.02%	CAVRGTGNQFYF (SEQ ID NO:751)	CSVVGTASSGN TIYF (SEQ ID NO:643)	0.01%	N/A	CASSQAANEQF F (SEQ ID NO:33)	0.00%
252	CAYTGGDGGSQ GNLIF (SEQ ID NO:1217)	CASSLTDFRPEAF F (SEQ ID NO:1222)	0.02%	N/A	CASSPLRVGDY GYTF (SEQ ID NO:635)	0.01%	N/A	CASSEDTYGYT F (SEQ ID NO:560)	0.00%
253	CAYRSPLTGNQF YF (SEQ ID NO:1218)	CASSPLRGPQYF (SEQ ID NO:1237)	0.02%	N/A	CASGDRLNTE AFF (SEQ ID NO:1226)	0.01%	N/A	CASTGRSGRSE QYF (SEQ ID NO:35)	0.00%
254	CAYRSLTGNQFY F (SEQ ID NO:793)	CASSPGQVWNG YTF (SEQ ID NO:822)	0.02%	CAGAYYGQNFVF (SEQ ID NO:755)	CASSLGGGSSY EQYF (SEQ ID NO:661)	0.01%	N/A	CASSLERRGGR RWDNEQFF (SEQ ID NO:1251)	0.00%
255	CAVDSTDSNYQL IW (SEQ ID NO:1228)	CASSQGRGVEQY F (SEQ ID NO:1238)	0.02%	CAMGGATNKLIF (SEQ ID NO:1224);CAADN	CASSVATGQG LEDTQYF (SEQ ID NO:996)	0.01%	N/A	CASSLAAGGLS EQFF (SEQ ID NO:573)	0.00%

				YGQNFVF (SEQ ID NO:1225)					
256	CAVDLDSNYQLIW (SEQ ID NO:1229)	CASSPSAGGNIQYF (SEQ ID NO:1239)	0.02%	CAEILPHNARLMF (SEQ ID NO:1232)	CASSSLGGEAFF (SEQ ID NO:1227)	0.01%	N/A	CASSFTRVAYEQYF (SEQ ID NO:571)	0.00%
257	CAVDPPPMEYGNKLVF (SEQ ID NO:1230)	CASSLEGDQPQHF (SEQ ID NO:1240)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%	N/A	CASSLVGQGAYNEQFF (SEQ ID NO:567)	0.00%
258	CLVGGGDYKLSF (SEQ ID NO:780)	CASSLGPPPLHF (SEQ ID NO:738);CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASGPQF (SEQ ID NO:1242)	0.01%	N/A	CATSAPLDYGYTF (SEQ ID NO:1252)	0.00%
259	CLVGERNFNKFYF (SEQ ID NO:1231)	CASSPGGANTEAFF (SEQ ID NO:1244)	0.02%	CAMREGVDSSASKIIF (SEQ ID NO:1233)	CSAREKTNSGNTIYF (SEQ ID NO:1167)	0.01%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
260	CLVGWDQGGKLIF (SEQ ID NO:758)	CASSLVSDNEQFF (SEQ ID NO:667);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CAASSPGSQGNLIF (SEQ ID NO:1234)	CASSQDGRAGAGYTGELFF (SEQ ID NO:1243)	0.01%	N/A	CAWSGTGGSEAFF (SEQ ID NO:597)	0.00%
261	CLVGDGWNTNAGKSTF (SEQ ID NO:1235)	CASSAKTDAYNEQFF (SEQ ID NO:1247)	0.02%	N/A	CASRPDHYGYTF (SEQ ID NO:1249)	0.01%	N/A	CASSLCLPIIHHNSPLHF (SEQ ID NO:1253)	0.00%
262	CLVGADRYSSASKIIF (SEQ ID NO:1236)	CASSPPGTSGYEQYF (SEQ ID NO:1248)	0.02%	CVVNKGNARLMF (SEQ ID NO:1241)	CSNVKGGNTEAFF (SEQ ID NO:1250)	0.01%	N/A	CSVVGTASSGNTIYF (SEQ ID NO:643)	0.00%
263	CLVGDLYGGATNKLIF (SEQ ID NO:973)	CSVEFPSSYNEQFF (SEQ ID NO:1099)	0.02%	CVVTNTGGFKTIF (SEQ ID NO:1257)	CASTYGQGEYNEQFF (SEQ ID NO:1269)	0.01%	N/A	CASSPTIRASHYGYTF (SEQ ID NO:1033)	0.00%
264	CLVGDPYMNYGGSQGNLIF (SEQ ID NO:1254)	CASSDGTGQNSGTEAFF (SEQ ID NO:1256)	0.02%	CAVRGGGYQKVTF (SEQ ID NO:1063);CAVEEGGYQKVTF (SEQ ID NO:1062)	CASRGLTTYEQYF (SEQ ID NO:1077)	0.01%	N/A	CASSRSEAKNIQYF (SEQ ID NO:1283)	0.00%
265	CLVAPNNDMRF (SEQ ID NO:1255)	CAMPGLLGYGYTF (SEQ ID NO:1263)	0.02%	CAAIQGAQKLVF (SEQ ID NO:783)	CASSPRQERSYNSPLHF (SEQ ID NO:869)	0.01%	N/A	CASSLGPPVLDEQYF (SEQ ID NO:1288)	0.00%

266	CLVGETGNQFYF (SEQ ID NO:1258)	CASTLNPGPGNTIYF (SEQ ID NO:1264)	0.02%	CAAQQGGSEKLVF (SEQ ID NO:1136)	CASSQQGRVGYTF (SEQ ID NO:1144)	0.01%	N/A	CATMSGAGNTIYF (SEQ ID NO:933)	0.00%
267	CLVGETGNQFYF (SEQ ID NO:1258)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	CIVRGFSDGQKLLF (SEQ ID NO:1262)	CASSSEEGSGNTIYF (SEQ ID NO:1270)	0.01%	N/A	CASSPSRGSNQPQHF (SEQ ID NO:1289)	0.00%
268	CAEEGDRDYKLSF (SEQ ID NO:34); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CAVSRGQGAQKLVF (SEQ ID NO:1266)	CASSFTGPGNSPLHF (SEQ ID NO:1281)	0.01%	N/A	CASSPPDSYGYTF (SEQ ID NO:614)	0.00%
269	CAEEGDRDYKLSF (SEQ ID NO:34)	CASSLDRHQPQHF (SEQ ID NO:39);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CTNAGKSTF (SEQ ID NO:1267);CVVSPMDTGRRALTF (SEQ ID NO:1268)	CASSLGRAQNIQYF (SEQ ID NO:1282)	0.01%	N/A	CASSEGIGAEAFF (SEQ ID NO:1295)	0.00%
270	CAEEGDRDYKLSF (SEQ ID NO:34)	CASSLELAGGRDTQYF (SEQ ID NO:43);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CAMREGQTGNQFYF (SEQ ID NO:1277)	CASSVAANNQPQHF (SEQ ID NO:1286)	0.01%	N/A	CASSFGGSGANVLTF (SEQ ID NO:1296)	0.00%
271	CAEEGDRDYKLSF (SEQ ID NO:34)	CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CLVGAGAAGNKLTF (SEQ ID NO:1278)	CASSEWGGVDQPQHF (SEQ ID NO:1287)	0.01%	N/A	CASSLEYSGGKSGEQFF (SEQ ID NO:930)	0.00%
272	CAEKPNRDDKIIF (SEQ ID NO:1259)	CATINGGAPSFYGYTF (SEQ ID NO:1265)	0.02%	CAVEDRNAGNMLTF (SEQ ID NO:1279)	CASSNPGANTEAFF (SEQ ID NO:1291)	0.01%	N/A	CASSYLGGGNEQFF (SEQ ID NO:1297)	0.00%
273	CAETPNRDDKIIF (SEQ ID NO:1260);CAVGAGTGTASKLTF (SEQ ID NO:719)	CSARGDRANSPLHF (SEQ ID NO:1274)	0.02%	CAVTNRDDKIIF (SEQ ID NO:756)	CASSPATTMGIDTQYF (SEQ ID NO:662)	0.01%	N/A	CASSLASLSFSETQYF (SEQ ID NO:1298)	0.00%
274	CAESTGGYNKLIF (SEQ ID NO:607)	CSVETGVNQPQHF (SEQ ID NO:608)	0.02%	CAVGLNTGFQKLVF (SEQ ID NO:978)	CSASSPTGRVSEQYF (SEQ ID NO:1292)	0.01%	N/A	CASSLGQNTEAFF (SEQ ID NO:1209)	0.00%
275	CARTSSNTGKLIF (SEQ ID NO:1261)	CASNDGTYEQYF (SEQ ID NO:1275)	0.02%	TGAGSYQLTF (SEQ ID NO:1284)	CASSESRYDEQYF (SEQ ID NO:903)	0.01%	N/A	CASSLEPSGGRRWDNEQFF (SEQ ID NO:558)	0.00%

276	CALRDSGYALNF (SEQ ID NO:1271)	CAWDGTGNTEAFF (SEQ ID NO:1000)	0.02%	CVVSEKGRDNTNAGKSTF (SEQ ID NO:839);CALGELPGTYKYIF (SEQ ID NO:1285)	CASSQERRGNEQFF (SEQ ID NO:845)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
277	CAVTNDYKLSF (SEQ ID NO:1272)	CASSALAGPEWQFF (SEQ ID NO:1276)	0.02%	CVVSSASGGSYIPTF (SEQ ID NO:1290)	CASSVNSGGARPGELFF (SEQ ID NO:1293)	0.01%	N/A	CASSLESANTGELFF (SEQ ID NO:1299)	0.00%
278	CAVIPREYGNKLVF (SEQ ID NO:1273)	CASSPGTLYEQYF (SEQ ID NO:1280)	0.02%	N/A	CASSGGTGGNTIYF (SEQ ID NO:1294)	0.01%	N/A	CASSQAGGTSGYNEQFF (SEQ ID NO:1300)	0.00%
279	CVVSNNAGNMLTF (SEQ ID NO:584)	CASSPGNNQPQHF (SEQ ID NO:628)	0.02%	CLVGWDQGGKLIF (SEQ ID NO:758)	CASSLVSDNEQFF (SEQ ID NO:667)	0.01%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.00%
280	CAVGARNTPLVF (SEQ ID NO:1301)	CAISDGDRPYNEQFF (SEQ ID NO:1303)	0.02%	N/A	CASSSRLAGGQVSYEQYF (SEQ ID NO:590)	0.01%	N/A	CASSQDGGASGNTIYF (SEQ ID NO:1323)	0.00%
281	CAVGAGTGTASKLTF (SEQ ID NO:719)	CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAVNYQLIW (SEQ ID NO:806);CAVSDNTDKLIF (SEQ ID NO:1304)	CASSHGGYTF (SEQ ID NO:816)	0.01%	N/A	CASLRTGEGNEQFF (SEQ ID NO:892)	0.00%
282	CAVGAGTGTASKLTF (SEQ ID NO:719); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSLELAGGRDTQYF (SEQ ID NO:43);CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	*GRDDKIIF (SEQ ID NO:613)	CASRTPTGKYTEAFF (SEQ ID NO:1307)	0.01%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
283	CAVGAGTGTASKLTF (SEQ ID NO:719); CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CASSGSARQLTF (SEQ ID NO:1305);CAVIVTGNQFYS (SEQ ID NO:1306)	CASSLEQARNNEQFF (SEQ ID NO:1319)	0.01%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.00%
284	CAVGEGNTGTASKLTF (SEQ ID NO:1302)	CASSLYSGQFF (SEQ ID NO:649)	0.02%	CAVGVSSNTGKLIF (SEQ ID NO:1315)	CASSLEGAGTNEQFF (SEQ ID NO:1320)	0.01%	N/A	CASSQEMGVATNEKLFF (SEQ ID NO:1324)	0.00%

285	CAVSDLGDGQKLLF (SEQ ID NO:731)	CASTSGFSGANVLTF (SEQ ID NO:825);CASSFRGYEKLFF (SEQ ID NO:722)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
286	CAVSGGYGKLTF (SEQ ID NO:1308)	CASSAGVGLAGEAEQFF (SEQ ID NO:1129)	0.02%	CALSRAGGTSYGKLTF (SEQ ID NO:1316);CAASKGSNYKLTF (SEQ ID NO:1317)	CASNDLNEPGELFF (SEQ ID NO:1321)	0.01%	N/A	CASSVGLSDLLNEQFF (SEQ ID NO:1325)	0.00%
287	CAVSPGNTPLVF (SEQ ID NO:1309)	CASSLPGEGGRPQHF (SEQ ID NO:1313)	0.02%	CAASASNSGYALNF (SEQ ID NO:1318)	CASSLALDYGYTF (SEQ ID NO:1322)	0.01%	N/A	CASSFQTVGSGSYEQYF (SEQ ID NO:562)	0.00%
288	CAVSEGNTPLVF (SEQ ID NO:1310)	CSAREVGVETQYF (SEQ ID NO:1314)	0.02%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.01%	N/A	CASTGTRSYNEQFF (SEQ ID NO:640)	0.00%
289	CAVSERDDKIIF (SEQ ID NO:1311)	CASSESYGEQFF (SEQ ID NO:1326)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
290	CAVIPDSNYQLIW (SEQ ID NO:1196)	CASSPGQGSYGYTF (SEQ ID NO:812);CANTLSQQTWKHHIF (SEQ ID NO:1327)	0.02%	N/A	CASSPQGGAGNTIYF (SEQ ID NO:688)	0.01%	N/A	CSARGTTVSTDTQYF (SEQ ID NO:1335)	0.00%
291	CAVIPDSNYQLIW (SEQ ID NO:1196)	CASSPGQGSYGYTF (SEQ ID NO:812)	0.02%	*GRDDKIIF (SEQ ID NO:613)	CASSPPTGTYGYTF (SEQ ID NO:1332)	0.01%	N/A	CASSQDQGTSGGPTRGTQYF (SEQ ID NO:1191)	0.00%
292	CAVNTDKLIF (SEQ ID NO:1312)	CASSPWDPQFSGNTIYF (SEQ ID NO:1328)	0.02%	CAVIGLAPVNMLTF (SEQ ID NO:1329);CAVSSYSGSARQLTF (SEQ ID NO:1330)	CASEDQGYTGELFF (SEQ ID NO:1333)	0.01%	N/A	CASKASGGVATQYF (SEQ ID NO:1336)	0.00%
293	CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVEVSYNQGGKLIF (SEQ ID NO:1331)	CASSLDDNSPLHF (SEQ ID NO:1334)	0.01%	N/A	CASSQETGELFF (SEQ ID NO:1337)	0.00%
294	CAVHEIQGAQKLVF (SEQ ID NO:30);	CASSYLAGGFRDEQFF (SEQ ID NO:564);CASSFG	0.02%	CAVRPRGRGKLIF (SEQ ID NO:1360)	CASSPSQGAHYEQYF (SEQ ID NO:1363)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%

	CAVHEIQGAQKLVF (SEQ ID NO:30)	VSYEQYF (SEQ ID NO:31)							
295	CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSYLAGGFRDEQFF (SEQ ID NO:564);CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAGQESTSGTYKYIF (SEQ ID NO:1361)	CSARDGTYEQYF (SEQ ID NO:873)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
296	CAVHEIQGAQKLVF (SEQ ID NO:30)	CASSQAANEQFF (SEQ ID NO:33);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	CAVSVMDTGRRALTF (SEQ ID NO:1362)	CASSSGGLYNEQFF (SEQ ID NO: 1403)	0.01%	N/A	CASSVEPSGGRRWDNEQFF (SEQ ID NO:1353)	0.00%
297	CAVHEIQGAQKLVF (SEQ ID NO:30)	CAISESMAGESYNEQFF (SEQ ID NO:1340);CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	N/A	CASSLIAGANYEQYF (SEQ ID NO:1346)	0.00%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
298	CALSAGSNYQLIW (SEQ ID NO:1338)	CATYGQTGHYGYTF (SEQ ID NO:1341);CASSQAANEQFF (SEQ ID NO:33)	0.02%	CAMSARSGTYKYIF (SEQ ID NO:1344)	CASSGPLGSPLHF (SEQ ID NO:1350)	0.00%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
299	CALFSGGGADGLTF (SEQ ID NO:1339)	CATSPGGESYGYTF (SEQ ID NO:1342)	0.02%	CAASTPGEKLTF (SEQ ID NO:624)	CASSYDGENTEAFF (SEQ ID NO:618)	0.00%	N/A	CASSLEPSGGRRGDNEQFF (SEQ ID NO:1351)	0.00%
300	CALSGREYGNKLVF (SEQ ID NO:563)	CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAVRSDSNYQLIW (SEQ ID NO:1345)	CASSQVETQYF (SEQ ID NO:1058)	0.00%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
301	CALSDRGGSNYKLTF (SEQ ID NO:1347)	CASSQEDRAADTQYF (SEQ ID NO:1343)	0.02%	CAVLYGNKLVF (SEQ ID NO:1197);CAGSDSNYQLIW (SEQ ID NO:1198)	CASSHPLREAFF (SEQ ID NO:1205)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
302	CALSAASGGSYIPTF (SEQ ID NO:631)	CSARRGGAPNTEAFF (SEQ ID NO:599)	0.02%	CAGLYGGSQGNLIF (SEQ ID NO:1071)	CASRLAFQREGTDTQYF (SEQ ID NO:1208)	0.01%	N/A	CAWSGAGPLVHF (SEQ ID NO:1352)	0.00%

303	CALRGRSGGSYIPTF (SEQ ID NO:625)	CASSQSLTGGLQPQHF (SEQ ID NO:626)	0.02%	CAGRSYGQNFVF (SEQ ID NO:1199)	CASSLGQNTEAFF (SEQ ID NO:1209)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
304	N/A	CATSRQGSNQPQHF (SEQ ID NO:706)	0.02%	CAENPFKTSGSRLTF (SEQ ID NO:1203)	CASSLSGNEKLFF (SEQ ID NO:1210)	0.01%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.00%
305	N/A	CASSASHSSYNEQFF (SEQ ID NO:744)	0.02%	CAVSVRGNKLVF (SEQ ID NO:1204)	CASSPSGREETQYF (SEQ ID NO:1211)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
306	N/A	CATRRQGRTDTQYF (SEQ ID NO:1348)	0.02%	CAASHDYKLSF (SEQ ID NO:1223)	CAISGKLGGSYEQYF (SEQ ID NO:998)	0.01%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
307	N/A	CSARGDRANSPLHF (SEQ ID NO:1274)	0.02%	N/A	CASSFNPQGREKLFF (SEQ ID NO:583)	0.01%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.00%
308	N/A	CASRLEGSPLHF (SEQ ID NO:1349);CASSQAANEQFF (SEQ ID NO:33)	0.02%	N/A	CASSYLAGGFRDEQFF (SEQ ID NO:564)	0.01%	N/A	CASSVEPSGGRRWDNEQFF (SEQ ID NO:1353)	0.00%
309	N/A	CASRSRLRPSGRSTDTQYF (SEQ ID NO:636)	0.02%	CAVGNTGFQKLVF (SEQ ID NO:877)	CASSPTGEESDTQYF (SEQ ID NO:893)	0.01%	N/A	CARSWGPRGGRRWDNEQFF (SEQ ID NO:1354)	0.00%
310	N/A	CARRRVLVAWDCSGANVLTF (SEQ ID NO:577);CASSLAAGGLSEQFF (SEQ ID NO:573)	0.02%	CAVRGTGNQFYF (SEQ ID NO:751)	CSVVGTASSGNTIYF (SEQ ID NO:643)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
311	N/A	CASSLTPLAWNEQFF (SEQ ID NO:811)	0.02%	N/A	CASSPLRVGDYGYTF (SEQ ID NO:635)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
312	N/A	CATAGGQDEKLFF (SEQ ID NO:847)	0.02%	N/A	CASGDRLNTEAFF (SEQ ID NO:1226)	0.01%	N/A	CASSEDTYGYTF (SEQ ID NO:560)	0.00%

313	N/A	CASSQAANEQFF (SEQ ID NO:33);CASSFGVSYEQYF (SEQ ID NO:31)	0.02%	CAGAYYGQNFVF (SEQ ID NO:755)	CASSLGGGSSYEQYF (SEQ ID NO:661)	0.01%	N/A	CASSQTRTGNQPQHF (SEQ ID NO:565)	0.00%
314	N/A	CASSQERRGNEQFF (SEQ ID NO:845)	0.02%	CAMGGATNKLIF (SEQ ID NO:1224);CAADNYGQNFVF (SEQ ID NO:1225)	CASSVATGQGLEDTQYF (SEQ ID NO:996)	0.01%	N/A	CASSLDRHQPQHF (SEQ ID NO:39)	0.00%
315	N/A	CASTNLAGGYNEQFF (SEQ ID NO:726)	0.02%	CAEILPHNARLMF (SEQ ID NO:1232)	CASSSLGGEAFF (SEQ ID NO:1227)	0.01%	N/A	CASSHSGGAPYNEQFF (SEQ ID NO:1356)	0.00%
316	N/A	CASSATSGKHEQYF (SEQ ID NO:609)	0.02%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.01%	N/A	CASSFGVSYEQYF (SEQ ID NO:31)	0.00%
317	N/A	CASSLELAGGRDTQYF (SEQ ID NO:43)	0.02%	N/A	CASGPQF (SEQ ID NO:1242)	0.01%	N/A	CASSFTRVAYEQYF (SEQ ID NO:571)	0.00%
318	N/A	CASSFLTDSYNEQFF (SEQ ID NO:592)	0.02%	CAMREGVDSSASKIIF (SEQ ID NO:1233)	CSAREKTNSGNTIYF (SEQ ID NO:1167)	0.01%	N/A	CASSSRLAGGQVSYEQYF (SEQ ID NO:590)	0.00%
319	N/A	CASSDGGEQFF (SEQ ID NO:648)	0.02%	CAASSPGSQGNLIF (SEQ ID NO:1234)	CASSQDGRAGAGYTGELFF (SEQ ID NO:1243)	0.01%	N/A	CASSLDPGRALGYTF (SEQ ID NO:570)	0.00%
320	N/A	CASSFGVSYEQYF (SEQ ID NO:31);CASTGRSGRSEQYF (SEQ ID NO:35)	0.02%	N/A	CASRPDHYGYTF (SEQ ID NO:1249)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
321	N/A	CASSETVPSLDEQYF (SEQ ID NO:810)	0.02%	CVVNKGNARLMF (SEQ ID NO:1241)	CSNVKGGNTEAFF (SEQ ID NO:1250)	0.01%	N/A	CASSQAANEQFF (SEQ ID NO:33)	0.00%
322	N/A	CATTLPGDTEAFF (SEQ ID NO:37)	0.02%	CVVTNTGGFKTIF (SEQ ID NO:1257)	CASTYGQGEYNEQFF (SEQ ID NO:1269)	0.01%	N/A	CAPVPPLDYGYTF (SEQ ID NO:1357)	0.00%

323	N/A	CASSLGEKDGYTF (SEQ ID NO:1355)	0.02%	CAVRGGGYQKVTF (SEQ ID NO:1063);CAVEEGGYQKVTF (SEQ ID NO:1062)	CASRGLTTYEQYF (SEQ ID NO:1077)	0.01%	N/A	CSVYIATNEKLFF (SEQ ID NO:691)	0.00%
324	N/A	CASSPPTGTYGYTF (SEQ ID NO:1332)	0.02%	CAAIQGAQKLVF (SEQ ID NO:783)	CASSPRQERSYNSPLHF (SEQ ID NO:869)	0.01%	N/A	CASSFLGGGQPQHF (SEQ ID NO:1358)	0.00%
325	N/A	CASSEGTVLDEQYF (SEQ ID NO:574)	0.02%	CAAQQGGSEKLVF (SEQ ID NO:1136)	CASSQQGRVGYTF (SEQ ID NO:1144)	0.01%	N/A	CSVYSIQSRANVLTF (SEQ ID NO:943)	0.00%

Table 7A HLA-A*01:01 peptide embodiments

Tumor Diagnosis	TILs remaining	PBMC remaining	epitope	NTEPVKDPY (SEQ ID NO:1364)	YTYQMHGEY (SEQ ID NO:1365)	LLKIDSFELLY (SEQ ID NO:1366)	KIDSFELLYY (SEQ ID NO:1367)	LKIDSFELLYY (SEQ ID NO:1368)	WLEAVYCGCY (SEQ ID NO:1369)	WSVSFTSLY (SEQ ID NO:1370)
			as_event	ENSG00000090924:PLEKHG2:chr19:+:39911588:39911636:39911492:39911714	ENSG00000064655:EYA2:chr20:+:45700823:45700891:45644936:45717877	ENSG00000128973:CLN6:chr15:-:68503893:68504079:68503656:68506627	ENSG00000128973:CLN6:chr15:-:68503893:68504079:68503656:68506627	ENSG00000128973:CLN6:chr15:-:68503893:68504079:68503656:68506627	ENSG00000121964:GTDC1:chr2:-:144714761:144714906:144710410:144764748	ENSG00000159147:DONSON:chr21:-:34955793:34955972:34954552:34956895
			junction_peptide_form	skp	skp	skp	skp	skp	skp	inc
			num_hla	1	11	2	6	1	4	6
			meanPSI	0.8	0.69	0.78	0.78	0.78	0.77	0.83
			Q1PSI	0.75	0.57	0.67	0.67	0.67	0.74	0.78
			Q3PSI	0.88	0.8	1	1	1	0.82	0.92
			deltaPSI	-0.14733856	-0.16464674	-0.11588091	-0.11588091	-0.11588091	-0.07110498	0.2098805

			fc_of_tumor_isoform	3.66	2.17	2.07	2.07	2.07	1.46	1.34
			tissue_matched_normal_panel	1/1	1/1	1/1	1/1	1/1	1/1	1/1
			tumor_panel	1/2	1/2	1/2	1/2	1/2	1/2	2/2
			normal_panel	11/11	7/11	10/11	10/11	10/11	6/11	9/11
			tag	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific
			mappability	0.988888888889;1.0;0.9888888888	0.966666666667;0.962316176471;0.85937987982	1.0;0.8972222553;0.911111111111	1.0;0.8972222553;0.911111111111	1.0;0.8972222553;0.911111111111	0.959259259701;0.809486004038;0.900000000662	0.914814815256;0.925462963184;0.79740740839
			mappability_tag	PASS	PASS	PASS	PASS	PASS	PASS	MID
			meanGeneExp	13.09	12.43	10.98	10.98	10.98	13.29	11.7
			Q1GeneExp	9.42	4.09	7.26	7.26	7.26	11.52	5.84
			Q3GeneExp	15.49	14.25	13.18	13.18	13.18	15.23	11.74
			canonical_match	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical	Q9NYP3
			overlap_with_validation_set	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
			LB3658	-	-	-	HLA-A*03:01|111.666676021;PSI=0.7857;Exp=7.10659	-	-	-
			LB3116	-	-	-	HLA-A*03:01|111.666676	-	-	-

							021;PSI=1.0;Exp=11.5036			
			LB3521	-	-	-	-	-	-	-
GBM-PD1	1.00E+08	1.50E+07	LB3755	HLA-A*01:01|132.715003543;PSI=0.8426;Exp=15.8894	HLA-A*01:01|135.644296657;PSI=1.0;Exp=0.481202	HLA-A*01:01|49.868679783;PSI=0.4444;Exp=11.3429	HLA-A*01:01|152.010342791;PSI=0.4444;Exp=11.3429	HLA-A*01:01|172.4364754;PSI=0.4444;Exp=11.3429	HLA-A*01:01|196.35445269;PSI=0.732;Exp=15.575	HLA-A*01:01|78.3573868745;PSI=0.7179;Exp=6.79611
			LB3560	-	-	-	-	-	-	-
			LB3404	-	-	-	-	-	-	-
			LB4024	-	-	-	-	-	-	-
			LB3598	-	-	-	-	-	-	-
			LB3334	-	-	-	-	-	-	-
			LB3804	-	-	-	HLA-A*03:01|111.666676021;PSI=1.0;Exp=11.1882	-	-	-
GBM-?	3.20E+06	2 vials	LB3330	HLA-A*01:01|132.715003543;PSI=0.4916;Exp=10.7555	HLA-A*01:01|135.644296657;PSI=0.4905;Exp=25.5327	HLA-A*01:01|49.868679783;PSI=1.0;Exp=14.0106	HLA-A*01:01|152.010342791;PSI=1.0;Exp=14.0106	HLA-A*01:01|172.4364754;PSI=1.0;Exp=14.0106	HLA-A*01:01|196.35445269;PSI=0.4136;Exp=11.8293	HLA-A*01:01|78.3573868745;PSI=1.0;Exp=9.03713
			LB3931	-	-	-	-	-	-	-
			LB3933	-	-	-	-	-	-	-
			LB3491	-	-	-	-	-	-	-
GBM-new	None	None	LB3865	HLA-A*01:01|132.715003543;PSI=NaN;Exp=12.7593	HLA-A*01:01|135.644296657;PSI=NaN;Exp=5.24305	HLA-A*01:01|49.868679783;PSI=1.0;Exp=12.7443	HLA-A*03:01|111.666676021,HLA-A*01:01|152.010342791;PSI=1.0;Exp=12.7443	HLA-A*01:01|172.4364754;PSI=1.0;Exp=12.7443	HLA-A*01:01|196.35445269;PSI=NaN;Exp=4.09618	HLA-A*01:01|78.3573868745;PSI=0.6522;Exp=9.59658

			LB3864	-	-	-	HLA-A*03:01|111.666676021;PSI=1.0;Exp=9.44704	-	-	-
			LB3667_P	-	-	-	HLA-A*03:01|111.666676021;PSI=0.5556;Exp=17.5151	-	-	-
			LB3869	-	-	-	-	-	-	-
			LB3883	-	-	-	-	-	-	-
			LB3914	-	-	-	-	-	-	-
			LB3386	-	-	-	-	-	-	-
			LB3284	-	-	-	-	-	-	-
			LB3802	-	-	-	-	-	-	-
			LB3724	-	-	-	-	-	-	-
			LB3607	-	-	-	-	-	-	-
			LB3667	-	-	-	HLA-A*03:01|111.666676021;PSI=1.0;Exp=8.05775	-	-	-
			LB3579	-	-	-	-	-	-	-
			LB3771	-	-	-	-	-	-	-
			LB3388	-	-	-	-	-	-	-
			LB3518	-	-	-	HLA-A*03:01|111.666676021;PSI=0.5652;Exp=60.6273	-	-	-
			LB3571	-	-	-	-	-	-	-
			LB3413	-	-	-	-	-	-	-
			LB2985	-	-	-	-	-	-	-
			LB3382	-	-	-	-	-	-	-

			LB3415	-	-	-	HLA-A*03:01|111.666676021;PSI=0.3514;Exp=5.40968	-	-	-
			LB3166	-	-	-	HLA-A*03:01|111.666676021;PSI=0.9286;Exp=16.1027	-	-	-
			LB3348	-	-	-	-	-	-	-
GBM-new	None	3.20E+07	LB3608	HLA-A*01:01|132.715003543;PSI=0.6939;Exp=23.1889	HLA-A*01:01|135.644296657;PSI=NaN;Exp=15.6381	HLA-A*01:01|49.868679783;PSI=0.9333;Exp=22.8167	HLA-A*03:01|111.666676021,HLA-A*01:01|152.010342791;PSI=0.9333;Exp=22.8167	HLA-A*01:01|172.4364754;PSI=0.9333;Exp=22.8167	HLA-A*01:01|196.35445269;PSI=NaN;Exp=15.254	HLA-A*01:01|78.3573868745;PSI=0.8381;Exp=13.1588
			LB2878	-	-	-	-	-	-	-
			LB3484	-	-	-	-	-	-	-
			LB3872	-	-	-	-	-	-	-
			LB2873	-	-	-	-	-	-	-
GBM-new	None	5.80E+06	LB3606	HLA-A*01:01|132.715003543;PSI=1.0;Exp=13.2476	HLA-A*01:01|135.644296657;PSI=0.4515;Exp=22.4001	HLA-A*01:01|49.868679783;PSI=1.0;Exp=15.6211	HLA-A*01:01|152.010342791;PSI=1.0;Exp=15.6211	HLA-A*01:01|172.4364754;PSI=1.0;Exp=15.6211	HLA-A*01:01|196.35445269;PSI=0.9189;Exp=10.594	HLA-A*01:01|78.3573868745;PSI=1.0;Exp=7.42539
			LB3738	-	-	-	-	-	-	-
			LB3619	-	-	-	-	-	-	-
			LB3810	-	-	-	-	-	-	-
			LB3811	-	-	-	HLA-A*03:01|111.666676021;PSI=1.0;Exp=7.38678	-	-	-

			LB3570	-	-	-	-	-	-	-
			LB2912	-	-	-	HLA-A*03:01|111.666676021;PSI=NaN;Exp=8.20204	-	-	-
GBM-PD1	None	1 vial	LB3944	HLA-A*01:01|132.715003543;PSI=0.5724;Exp=15.8863	HLA-A*01:01|135.644296657;PSI=0.6252;Exp=33.0536	HLA-A*01:01|49.868679783;PSI=NaN;Exp=12.1669	HLA-A*03:01|111.666676021,HLA-A*01:01|152.010342791;PSI=NaN;Exp=12.1669	HLA-A*01:01|172.4364754;PSI=NaN;Exp=12.1669	HLA-A*01:01|196.35445269;PSI=NaN;Exp=11.7426	HLA-A*01:01|78.3573868745;PSI=1.0;Exp=6.01632
			LB2933	-	-	-	-	-	-	-
GBM-new	None	6.70E+06	LB3612	HLA-A*01:01|132.715003543;PSI=0.7088;Exp=8.46541	HLA-A*01:01|135.644296657;PSI=NaN;Exp=14.7366	HLA-A*01:01|49.868679783;PSI=1.0;Exp=9.78051	HLA-A*01:01|152.010342791;PSI=1.0;Exp=9.78051	HLA-A*01:01|172.4364754;PSI=1.0;Exp=9.78051	HLA-A*01:01|196.35445269;PSI=NaN;Exp=11.6363	HLA-A*01:01|78.3573868745;PSI=0.8176;Exp=8.71747
			LB3778	-	-	-	-	-	-	-
			1142	-	-	-	-	-	-	-
			2964	-	HLA-B*35:08|363.7	-	HLA-A*03:01|111.666676021	-	-	-
			2907	-	-	-	HLA-A*03:01|111.666676021	-	-	-
			1325	-	HLA-C*03:03|88.6517984814	-	-	-	-	-
			2924	-	HLA-C*14:02|21.913324327	-	-	-	-	-
GBM-rec	None	None	2870	HLA-A*01:01|132.715003543	HLA-A*01:01|135.644296657	HLA-A*01:01|49.868679783	HLA-A*01:01|1	HLA-A*01:01|172.4364754	HLA-A*01:01|196.35445269	HLA-A*01:01|78.3573868745

							52.010342791			
			LB3336	-	-	-	HLA-A*11:01|111.947487784	-	-	-
			2867	-	-	-	HLA-A*03:01|111.666676021	-	-	-
?	None	None	1989	HLA-A*01:01|132.715003543	HLA-A*01:01|135.644296657;HLA-C*03:04|410.3	HLA-A*01:01|49.868679783	HLA-A*01:01|152.010342791	HLA-A*01:01|172.4364754	HLA-A*01:01|196.35445269	HLA-A*01:01|78.3573868745
?	None	None	2158	HLA-A*01:01|132.715003543	HLA-A*26:01|177.287532427;HLA-A*01:01|135.644296657;HLA-C*12:03|20.6898254443	HLA-A*01:01|49.868679783	HLA-A*01:01|152.010342791	HLA-A*01:01|172.4364754	HLA-A*01:01|196.35445269	HLA-A*26:01|293.171871247;HLA-A*01:01|78.3573868745;HLA-C*12:03|101.243515002
			LB3367	-	-	-	-	-	-	-
?	None	None	LB3054	HLA-A*01:01|132.715003543	HLA-A*01:01|135.644296657	HLA-A*01:01|49.868679783	HLA-A*01:01|152.010342791	HLA-A*01:01|172.4364754	HLA-A*01:01|196.35445269	HLA-A*01:01|78.3573868745
			LB3001	-	HLA-A*30:02|86.3125131795	-	HLA-A*30:02|291.046552821	-	HLA-A*30:02|247.961903329	HLA-A*30:02|105.263393456
			3404	-	-	-	-	-	-	-
			LB3120	-	HLA-B*15:01|92.979088308;HLA-C*12:03|20.6898254443;HLA-	-	HLA-B*15:01|485.297368226	-	HLA-B*15:01|168.509526524	HLA-B*15:01|243.4263507;HLA-C*12:03|101.243515002

					C*03:04|410.3					
?	9.00E+07	None	2899	HLA-A*01:01|132.715003543	HLA-A*01:01|135.644296657;HLA-B*15:01|92.979088308;HLA-C*03:04|410.3	HLA-A*01:01|49.868679783	HLA-A*01:01|152.010342791;HLA-B*15:01|485.297368226	HLA-A*01:01|172.4364754	HLA-A*01:01|196.35445269;HLA-B*15:01|168.509526524	HLA-A*01:01|78.3573868745;HLA-B*15:01|243.4263507
?	1 vial	2 vials	3374	HLA-A*01:01|132.715003543	HLA-A*01:01|135.644296657	HLA-A*01:01|49.868679783	HLA-A*01:01|152.010342791	HLA-A*01:01|172.4364754	HLA-A*01:01|196.35445269	HLA-A*01:01|78.3573868745
			3372	-	HLA-C*14:02|21.913324327	-	-	-	-	-
			3244	-	HLA-C*12:03|20.6898254443;HLA-C*15:02|471.580570951	-	-	-	-	HLA-C*12:03|101.243515002
			2675	-	HLA-A*29:01|5.4;HLA-A*30:02|86.3125131795	HLA-A*29:01|167.9	HLA-A*29:01|92.4;HLA-A*30:02|291.046552821	-	HLA-A*29:01|223.3;HLA-A*30:02|247.961903329	HLA-A*29:01|9.2;HLA-A*30:02|105.263393456
			LB2938	-	-	-	HLA-A*03:01|111.666676021	-	-	-
			803	-	HLA-A*30:02|86.3125131795;HLA-C*03:04|410.3	-	HLA-A*03:01|111.666676021;HLA-A*30:02|291.046552821	-	HLA-A*30:02|247.961903329	HLA-A*30:02|105.263393456

Table 7B HLA-A*02:01 peptide embodiments

Tumor Diagnosis	Cells Remaining	epitope	NLLTTCSTV (SEQ ID NO:1371)	FTVTVTEPL (SEQ ID NO:1372)	YLEAKADLV (SEQ ID NO:1373)	YLDQLNHILA (SEQ ID NO:1374)	FLQEPLQVFNV (SEQ ID NO:1375)	FLPRGTPAL (SEQ ID NO:1376)	RMAEHHSFWV (SEQ ID NO:1377)	HLLRIFCTI (SEQ ID NO:1378)	SLSEVDIPSI (SEQ ID NO:1379)	LILKGIFCTI (SEQ ID NO:1380)	STWGGFDEL (SEQ ID NO:1381)
		as_event	ENSG00000115307:AUP1:chr2:-:74755543:74755616:74755133:74756258	ENSG00000124006:OBSL1:chr2:-:220422064:220422340:220421445:220423946	ENSG00000173264:GPR137:chr11:+:64055536:64055686:64055418:64055811	ENSG00000102882:MAPK3:chr16:-:30128457:30128606:30128324:30128990	ENSG00000110888:CAPRIN2:chr12:-:30872012:30873849:30869610:30876192	ENSG00000130734:ATG4D:chr19:+:10657864:10657929:10655806:10662572	ENSG00000197530:MIB2:chr1:+:156093715610330:15608081562029	ENSG00000152154:TMEM178A:chr2:+:39934188:39934326:39931334:39944149	ENSG00000135317:SNX14:chr6:-:86277251:86277295:86267778:86281854	ENSG00000152154:TMEM178A:chr2:+:39934188:39934326:39893514:39944149	ENSG00000073921:PICALM:chr11:-:85701292:85701442:85693046:85707868
		junction_peptide_form	skp	skp	skp	skp	skp	skp	skp	skp	skp	skp	skp
		num_hla	1	9	1	1	1	7	1	4	1	1	4
		meanPSI	0.68	0.86	0.91	0.86	0.61	0.7	0.8	0.69	0.6	0.86	0.68
		Q1PSI	0.49	0.76	0.88	0.82	0.52	0.51	0.76	0.61	0.58	0.78	0.47
		Q3PSI	0.95	1	0.95	0.92	0.69	1	0.87	0.75	0.64	0.95	0.87
		deltaPSI	-0.2907525	-0.11745417	-0.0684096	-0.1016771	-0.2913299	-0.2027176	-0.1040743	-0.2609795	-0.2114651	-0.1254085	-0.2106166
		fc_of_tumor_isoform	11.24	5.53	4.36	3.87	3.86	3.03	2.12	5.93	2.11	9.39	2.90
		tissue_matched_normal_panel	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
		tumor_panel	1/2	1/2	1/2	1/2	2/2	1/2	1/2	2/2	1/2	2/2	2/2

		normal_panel	10/11	9/11	9/11	11/11	10/11	7/11	11/11	7/11	9/11	10/11	9/11
		tag	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific
		mappability	1.0;0.878924101999;0.988888888889	0.870370370812;0.933333333333;0.988888888889	0.92962962985;0.96111111111;1.0	1.0;0.950000000331;0.892592593034	0.916666666667;0.95;0.92222222222	0.885185185406;0.965985578757;0.955555555556	0.948148148859;0.983333333333;1.0	0.925925926367;0.964814814925;1.0	0.940740740961;0.886363637041;0.883333333333	1.0;0.964814814925;1.0	0.977777777778;0.9;0.922222222222
		mappability_tag	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
		meanGeneExp	60.52	45.84	26.84	42.78	13.43	18	33.53	11.08	23.69	11.08	56.28
		Q1GeneExp	49.5	25.32	22.19	31.94	9.3	11.21	18.54	6.07	17.03	6.07	46.57
		Q3GeneExp	66.57	62.99	31.66	48.69	15.31	23.56	32.27	15.97	30.09	15.97	69.82
		canonical_match	Q9Y679	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical	NonCanonical
		overlap_with_validation_set	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
GBM-new	9.00E+06	LB3348	HLA-A*02:01|76.6614096321;PSI=0.7102;Exp=74.4904	HLA-A*02:01|215.548562721;PSI=0.9286;Exp=74.6547	HLA-A*02:01|92.43128442;PSI=0.9489;Exp=36.7204	HLA-A*02:01|144.888167987;PSI=0.7697;Exp=38.7829	HLA-A*02:01|134.636291305;PSI=0.8727;Exp=8.41674	HLA-A*02:01|27.2539188711;PSI=0.7762;Exp=20.8916	HLA-A*02:01|37.6355477373;PSI=0.8021;Exp=100.231	HLA-A*02:01|140.932214008;PSI=0.8348;Exp=17.7503	HLA-A*02:01|80.882433811;PSI=0.7763;Exp=21.9197	HLA-A*02:01|388.730694216;PSI=0.922;Exp=17.7503	HLA-A*02:01|312.538580131;PSI=0.6842;Exp=17.8044
GBM-rec	Yes (low counts)	LB3484	HLA-A*02:01|76.6614	HLA-A*02:01|215.548	HLA-A*02:01|92.4312	HLA-A*02:01|144.888	HLA-A*02:01|134.636	HLA-A*02:01|27.2539	HLA-A*02:01|37.6355	HLA-A*02:01|140.932	HLA-A*02:01|80.8824	HLA-A*02:01|388.730	HLA-A*02:01|312.538

			096321;PSI=0.7012;Exp=79.8166	562721;PSI=NaN;Exp=69.4729	8442;PSI=0.9298;Exp=57.0311	167987;PSI=0.7862;Exp=59.7864	291305;PSI=0.8738;Exp=5.82644	188711;PSI=0.4012;Exp=32.232	477373;PSI=0.8188;Exp=23.6385	214008;PSI=0.5331;Exp=12.3154	33811;PSI=0.6595;Exp=23.5465	694216;PSI=0.7297;Exp=12.3154	580131;PSI=NaN;Exp=62.5282
GBM-PD1	1.40E+06	LB3667_P	HLA-A*02:01|76.6614096321;PSI=1.0;Exp=81.4137	HLA-A*02:01|215.548562721;PSI=0.6821;Exp=37.5304	HLA-A*02:01|92.43128442;PSI=0.9199;Exp=79.1791	HLA-A*02:01|144.888167987;PSI=0.7778;Exp=39.0693	HLA-A*02:01|134.636291305;PSI=0.6247;Exp=4.47357	HLA-A*02:01|27.2539188711;PSI=0.5915;Exp=20.6671	HLA-A*02:01|37.6355477373;PSI=0.9078;Exp=9.06001	HLA-A*02:01|140.932214008;PSI=0.5333;Exp=24.7515	HLA-A*02:01|80.882433811;PSI=0.4937;Exp=16.8417	HLA-A*02:01|388.730694216;PSI=0.704;Exp=24.7515	HLA-A*02:01|312.538580131;PSI=0.441;Exp=51.353
GBM-PD1	5e6 + CD45's	LB3872	HLA-A*02:01|76.6614096321;PSI=0.6783;Exp=98.3987	HLA-A*02:01|215.548562721;PSI=0.8693;Exp=85.5524	HLA-A*02:01|92.43128442;PSI=0.8913;Exp=68.9229	HLA-A*02:01|144.888167987;PSI=0.8564;Exp=43.5918	HLA-A*02:01|134.636291305;PSI=0.4909;Exp=17.3207	HLA-A*02:01|27.2539188711;PSI=0.1955;Exp=25.913	HLA-A*02:01|37.6355477373;PSI=0.7971;Exp=30.262	HLA-A*02:01|140.932214008;PSI=0.596;Exp=32.6984	HLA-A*02:01|80.882433811;PSI=0.4978;Exp=26.9345	HLA-A*02:01|388.730694216;PSI=0.9148;Exp=32.6984	HLA-A*02:01|312.538580131;PSI=0.1831;Exp=50.4812
		PSI Values Only												
GBM-?		LB3348	PSI=0.7102	PSI=0.9286	PSI=0.9489	PSI=0.7697	PSI=0.8727	PSI=0.7762;Exp=20.8916	HLA-A*02:01|37.6355477373;PSI=0.8021;Exp=100.231	PSI=0.8348	HLA-A*02:01|80.882433811;PSI=0.7763;Exp=21.9197	PSI=0.922	PSI=0.6842;Exp=17.8044
GBM-rec		LB3484	PSI=0.7012	PSI=NaN	PSI=0.9298	PSI=0.7862	PSI=0.8738	PSI=0.4012	HLA-A*02:01|37.6355477373;PSI=0.8188;Exp=23.6385	PSI=0.5331	HLA-A*02:01|80.882433811;PSI=0.6595;Exp=23.5465	PSI=0.7297	PSI=NaN

GBM-PD1		LB3667_P	PSI=1.0	PSI=0.6821	PSI=0.9199	PSI=0.7778	PSI=0.6247	PSI=0.5915	HLA-A*02:01|37.6355477373;PSI=0.9078;Exp=9.06001	PSI=0.5333	HLA-A*02:01|80.882433811;PSI=0.4937;Exp=16.8417	PSI=0.704	PSI=0.441
GBM-PD1		LB3872	PSI=0.6783	PSI=0.8693	PSI=0.8913	PSI=0.8564	PSI=0.4909	PSI=0.1955	HLA-A*02:01|37.6355477373;PSI=0.7971;Exp=30.262	PSI=0.596	HLA-A*02:01|80.882433811;PSI=0.4978;Exp=26.9345	PSI=0.9148	PSI=0.1831
		Expr Values Only											
GBM-?		LB3348	Exp=74.4904	Exp=74.6547	Exp=36.7204	Exp=38.7829	Exp=8.41674	Exp=20.8916	Exp=17.7503	Exp=17.7503	Exp=17.8044		
GBM-rec		LB3484	Exp=79.8166	Exp=69.4729	Exp=57.0311	Exp=59.7864	Exp=5.82644	Exp=32.232		Exp=12.3154	Exp=12.3154	Exp=62.5282	
GBM-PD1		LB3667_P	Exp=81.4137	Exp=37.5304	Exp=79.1791	Exp=39.0693	Exp=4.47357	Exp=20.6671	Exp=24.7515	Exp=24.7515	Exp=51.353		
GBM-PD1		LB3872	Exp=98.3987	Exp=85.5524	Exp=68.9229	Exp=43.5918	Exp=17.3207	Exp=25.913		Exp=32.6984	Exp=32.6984	Exp=50.4812	
GBM-new	a lot	LB2878	HLA-A*02:01|76.6614096321;PSI=NaN;Exp=45.8943	HLA-A*02:01|215.548562721;PSI=NaN;Exp=41.7994	HLA-A*02:01|92.43128442;PSI=0.9551;Exp=19.3054	HLA-A*02:01|144.888167987;PSI=0.918;Exp=20.7238	HLA-A*02:01|134.636291305;PSI=0.7778;Exp=12.0616	HLA-A*02:01|27.2539188711;PSI=1.0;Exp=16.952	HLA-A*02:01|37.6355477373;PSI=0.7928;Exp=29.4093	HLA-A*02:01|140.932214008;PSI=0.5042;Exp=23.7336	HLA-A*02:01|80.882433811;PSI=NaN;Exp=23.7729	HLA-A*02:01|388.730694216;PSI=NaN;Exp=23.7336	HLA-A*02:01|312.538580131;PSI=NaN;Exp=47.9216
GBM-new	1.50E+07	LB3348	HLA-A*02:01|76.6614096321;	HLA-A*02:01|215.548562721;	HLA-A*02:01|92.43128442;PS	HLA-A*02:01|144.888167987;	HLA-A*02:01|134.636291305;	HLA-A*02:01|27.2539188711;	HLA-A*02:01|37.6355477373;	HLA-A*02:01|140.932214008;	HLA-A*02:01|80.882433811;P	HLA-A*02:01|388.730694216;	HLA-A*02:01|312.538580131;

			PSI=0.7102;Exp=74.4904	PSI=0.9286;Exp=74.6547	I=0.9489;Exp=36.7204	PSI=0.7697;Exp=38.7829	PSI=0.8727;Exp=8.41674	PSI=0.7762;Exp=20.8916	PSI=0.8021;Exp=100.231	PSI=0.8348;Exp=17.7503	SI=0.7763;Exp=21.9197	PSI=0.922;Exp=17.7503	PSI=0.6842;Exp=17.8044
GBM-rec	5.00E+06	LB3386	HLA-A*02:01|76.6614096321;PSI=0.198;Exp=75.0333	HLA-A*02:01|215.548562721;PSI=0.811;Exp=102.968	HLA-A*02:01|92.43128442;PSI=0.9403;Exp=34.6509	HLA-A*02:01|144.888167987;PSI=0.8103;Exp=39.6758	HLA-A*02:01|134.636291305;PSI=0.7281;Exp=13.7332	HLA-A*02:01|27.2539188711;PSI=0.5616;Exp=36.1776	HLA-A*02:01|37.6355477373;PSI=0.8058;Exp=47.1911	HLA-A*02:01|140.932214008;PSI=0.5526;Exp=14.7699	HLA-A*02:01|80.882433811;PSI=0.4354;Exp=21.5787	HLA-A*02:01|388.730694216;PSI=0.7742;Exp=14.7699	HLA-A*02:01|312.538580131;PSI=0.2821;Exp=39.9268
GBM-rec	7.80E+06	LB3484	HLA-A*02:01|76.6614096321;PSI=0.7012;Exp=79.8166	HLA-A*02:01|215.548562721;PSI=NaN;Exp=69.4729	HLA-A*02:01|92.43128442;PSI=0.9298;Exp=57.0311	HLA-A*02:01|144.888167987;PSI=0.7862;Exp=59.7864	HLA-A*02:01|134.636291305;PSI=0.8738;Exp=5.82644	HLA-A*02:01|27.2539188711;PSI=0.4012;Exp=32.232	HLA-A*02:01|37.6355477373;PSI=0.8188;Exp=23.6385	HLA-A*02:01|140.932214008;PSI=0.5331;Exp=12.3154	HLA-A*02:01|80.882433811;PSI=0.6595;Exp=23.5465	HLA-A*02:01|388.730694216;PSI=0.7297;Exp=12.3154	HLA-A*02:01|312.538580131;PSI=NaN;Exp=62.5282
GBM-new	Yes	LB3570	HLA-A*02:01|76.6614096321;PSI=0.4216;Exp=92.6196	HLA-A*02:01|215.548562721;PSI=NaN;Exp=46.0624	HLA-A*02:01|92.43128442;PSI=0.9215;Exp=35.0582	HLA-A*02:01|144.888167987;PSI=0.8676;Exp=44.4346	HLA-A*02:01|134.636291305;PSI=0.5877;Exp=16.1834	HLA-A*02:01|27.2539188711;PSI=0.6433;Exp=23.2262	HLA-A*02:01|37.6355477373;PSI=0.9525;Exp=59.5663	HLA-A*02:01|140.932214008;PSI=0.7324;Exp=16.36	HLA-A*02:01|80.882433811;PSI=NaN;Exp=17.2887	HLA-A*02:01|388.730694216;PSI=NaN;Exp=16.36	HLA-A*02:01|312.538580131;PSI=0.7661;Exp=45.2489
GBM-new	3.20E+07	LB3598	HLA-A*02:01|76.6614096321;PSI=NaN;Exp=70.5063	HLA-A*02:01|215.548562721;PSI=NaN;Exp=65.577	HLA-A*02:01|92.43128442;PSI=1.0;Exp=97.0072	HLA-A*02:01|144.888167987;PSI=0.8689;Exp=52.6473	HLA-A*02:01|134.636291305;PSI=0.5536;Exp=3.25387	HLA-A*02:01|27.2539188711;PSI=NaN;Exp=37.3093	HLA-A*02:01|37.6355477373;PSI=1.0;Exp=44.8408	HLA-A*02:01|140.932214008;PSI=0.6032;Exp=72.646	HLA-A*02:01|80.882433811;PSI=NaN;Exp=19.5963	HLA-A*02:01|388.730694216;PSI=NaN;Exp=72.646	HLA-A*02:01|312.538580131;PSI=NaN;Exp=11.1257
GBM-new	5.80E+06	LB3606	HLA-A*02:01|76.6614096321;PSI=1.0;Exp=89.9652	HLA-A*02:01|215.548562721;PSI=NaN;Exp=53.2923	HLA-A*02:01|92.43128442;PSI=0.9032;Exp=54.3114	HLA-A*02:01|144.888167987;PSI=0.8201;Exp	HLA-A*02:01|134.636291305;PSI=0.6156;Exp	HLA-A*02:01|27.2539188711;PSI=NaN;Exp=35.109	HLA-A*02:01|37.6355477373;PSI=0.7963;Exp	HLA-A*02:01|140.932214008;PSI=0.4522;Exp	HLA-A*02:01|80.882433811;PSI=0.5205;Exp=15.0806	HLA-A*02:01|388.730694216;PSI=0.7816;Exp	HLA-A*02:01|312.538580131;PSI=NaN;Exp=38.8818

						=62.0419	=6.63403		=30.7953	=57.6896		=57.6896	
GBM-PD1	3.50E+07	LB3667_P	HLA-A*02:01|76.6614096321;PSI=1.0;Exp=81.4137	HLA-A*02:01|215.548562721;PSI=0.6821;Exp=37.5304	HLA-A*02:01|92.43128442;PSI=0.9199;Exp=79.1791	HLA-A*02:01|144.888167987;PSI=0.7778;Exp=39.0693	HLA-A*02:01|134.636291305;PSI=0.6247;Exp=4.47357	HLA-A*02:01|27.2539188711;PSI=0.5915;Exp=20.6671	HLA-A*02:01|37.6355477373;PSI=0.9078;Exp=9.06001	HLA-A*02:01|140.932214008;PSI=0.5333;Exp=24.7515	HLA-A*02:01|80.882433811;PSI=0.4937;Exp=16.8417	HLA-A*02:01|388.730694216;PSI=0.704;Exp=24.7515	HLA-A*02:01|312.538580131;PSI=0.441;Exp=51.353
GBM-PD1	4.50E+06	LB3738	HLA-A*02:01|76.6614096321;PSI=1.0;Exp=31.4966	HLA-A*02:01|215.548562721;PSI=1.0;Exp=47.0162	HLA-A*02:01|92.43128442;PSI=0.9301;Exp=21.0541	HLA-A*02:01|144.888167987;PSI=0.8554;Exp=19.2176	HLA-A*02:01|134.636291305;PSI=0.621;Exp=11.0891	HLA-A*02:01|27.2539188711;PSI=0.0;Exp=7.37745	HLA-A*02:01|37.6355477373;PSI=0.93;Exp=22.4331	HLA-A*02:01|140.932214008;PSI=0.6729;Exp=0.495587	HLA-A*02:01|80.882433811;PSI=0.5673;Exp=7.9695	HLA-A*02:01|388.730694216;PSI=1.0;Exp=0.495587	HLA-A*02:01|312.538580131;PSI=0.9111;Exp=27.8479
GBM-PD1	3.00E+07	LB3778	HLA-A*02:01|76.6614096321;PSI=NaN;Exp=17.687	HLA-A*02:01|215.548562721;PSI=1.0;Exp=24.967	HLA-A*02:01|92.43128442;PSI=0.8189;Exp=15.0835	HLA-A*02:01|144.888167987;PSI=0.9697;Exp=18.4314	HLA-A*02:01|134.636291305;PSI=0.6986;Exp=4.55368	HLA-A*02:01|27.2539188711;PSI=0.675;Exp=5.64169	HLA-A*02:01|37.6355477373;PSI=0.8245;Exp=20.0435	HLA-A*02:01|140.932214008;PSI=1.0;Exp=3.89409	HLA-A*02:01|80.882433811;PSI=0.3392;Exp=11.939	HLA-A*02:01|388.730694216;PSI=1.0;Exp=3.89409	HLA-A*02:01|312.538580131;PSI=0.7544;Exp=25.4987
GBM-PD1	7.00E+06	LB3802	HLA-A*02:01|76.6614096321;PSI=1.0;Exp=24.6527	HLA-A*02:01|215.548562721;PSI=1.0;Exp=37.4108	HLA-A*02:01|92.43128442;PSI=0.9325;Exp=14.8715	HLA-A*02:01|144.888167987;PSI=0.8579;Exp=17.4732	HLA-A*02:01|134.636291305;PSI=0.7841;Exp=10.5371	HLA-A*02:01|27.2539188711;PSI=1.0;Exp=6.49666	HLA-A*02:01|37.6355477373;PSI=0.8339;Exp=16.0274	HLA-A*02:01|140.932214008;PSI=0.9022;Exp=1.75055	HLA-A*02:01|80.882433811;PSI=0.6679;Exp=10.8914	HLA-A*02:01|388.730694216;PSI=0.5299;Exp=1.75055	HLA-A*02:01|312.538580131;PSI=0.7838;Exp=36.5375
GBM-new	6.50E+06	LB3883	HLA-A*02:01|76.6614096321;PSI=0.7886;Exp=73.7825	HLA-A*02:01|215.548562721;PSI=0.8505;Exp=93.2394	HLA-A*02:01|92.43128442;PSI=0.8956;Exp=57.2512	HLA-A*02:01|144.888167987;PSI=0.7439;Exp=54.8545	HLA-A*02:01|134.636291305;PSI=0.722;Exp=5.60051	HLA-A*02:01|27.2539188711;PSI=1.0;Exp=31.5877	HLA-A*02:01|37.6355477373;PSI=0.8179;Exp=21.1917	HLA-A*02:01|140.932214008;PSI=0.4157;Exp=23.2842	HLA-A*02:01|80.882433811;PSI=0.25;Exp=16.384	HLA-A*02:01|388.730694216;PSI=0.8778;Exp=23.2842	HLA-A*02:01|312.538580131;PSI=1.0;Exp=43.6037

GBM-PD1	< 10e6	LB3914	HLA-A*02:01|76.6614096321;PSI=NaN;Exp=22.9798	HLA-A*02:01|215.548562721;PSI=0.8;Exp=20.6998	HLA-A*02:01|92.43128442;PSI=0.9372;Exp=14.8156	HLA-A*02:01|144.888167987;PSI=0.9633;Exp=18.9921	HLA-A*02:01|134.636291305;PSI=0.4;Exp=7.02732	HLA-A*02:01|27.2539188711;PSI=1.0;Exp=4.75366	HLA-A*02:01|37.6355477373;PSI=0.6739;Exp=18.5007	HLA-A*02:01|140.932214008;PSI=0.8026;Exp=10.5907	HLA-A*02:01|80.882433811;PSI=0.6432;Exp=14.1801	HLA-A*02:01|388.730694216;PSI=0.8934;Exp=10.5907	HLA-A*02:01|312.538580131;PSI=NaN;Exp=55.0117
GBM-PD1	< 10e6	LB4024	HLA-A*02:01|76.6614096321;PSI=0.5908;Exp=40.9701	HLA-A*02:01|215.548562721;PSI=0.8526;Exp=60.1696	HLA-A*02:01|92.43128442;PSI=0.8859;Exp=33.9611	HLA-A*02:01|144.888167987;PSI=0.8948;Exp=94.8624	HLA-A*02:01|134.636291305;PSI=0.5619;Exp=7.51328	HLA-A*02:01|27.2539188711;PSI=0.6093;Exp=15.98	HLA-A*02:01|37.6355477373;PSI=0.8173;Exp=16.719	HLA-A*02:01|140.932214008;PSI=0.5916;Exp=19.4243	HLA-A*02:01|80.882433811;PSI=0.5188;Exp=13.5334	HLA-A*02:01|388.730694216;PSI=0.8912;Exp=19.4243	HLA-A*02:01|312.538580131;PSI=0.931;Exp=42.146
		PSI Values Only												
		LB2878	PSI=NaN	PSI=NaN	PSI=0.9551	PSI=0.918;	PSI=0.7778	PSI=1.0	HLA-A*02:01|37.6355477373;PSI=0.7928;Exp=29.4093	PSI=0.5042	HLA-A*02:01|80.882433811;PSI=NaN;Exp=23.7729	PSI=NaN	PSI=NaN
		LB3348	PSI=0.7102	PSI=0.9286	PSI=0.9489	PSI=0.7697	PSI=0.8727	PSI=0.7762;Exp=20.8916	HLA-A*02:01|37.6355477373;PSI=0.8021;Exp=100.231	PSI=0.8348	HLA-A*02:01|80.882433811;PSI=0.7763;Exp=21.9197	PSI=0.922	PSI=0.6842;Exp=17.8044
		LB3386	PSI=0.198	PSI=0.811	PSI=0.9403	PSI=0.8103	PSI=0.7281	PSI=0.5616	HLA-A*02:01|37.6355477373;PSI=0.8058;Exp	PSI=0.5526	HLA-A*02:01|80.882433811;PSI=0.43	PSI=0.7742	PSI=0.2821

									=47.1911		54;Exp=21.5787		
		LB3484	PSI=0.7012	PSI=NaN	PSI=0.9298	PSI=0.7862	PSI=0.8738	PSI=0.4012	HLA-A*02:01|37.6355477373;PSI=0.8188;Exp=23.6385	PSI=0.5331	HLA-A*02:01|80.882433811;PSI=0.6595;Exp=23.5465	PSI=0.7297	PSI=NaN
		LB3570	PSI=0.4216	PSI=NaN	PSI=0.9215	PSI=0.8676	PSI=0.5877	PSI=0.6433	HLA-A*02:01|37.6355477373;PSI=0.9525;Exp=59.5663	PSI=0.7324	HLA-A*02:01|80.882433811;PSI=NaN;Exp=17.2887	PSI=NaN	PSI=0.7661
		LB3598	PSI=NaN	PSI=NaN	PSI=1.0	PSI=0.8689	PSI=0.5536	PSI=NaN	HLA-A*02:01|37.6355477373;PSI=1.0;Exp=44.8408	PSI=0.6032	HLA-A*02:01|80.882433811;PSI=NaN;Exp=19.5963	PSI=NaN	PSI=NaN
		LB3606	PSI=1.0	PSI=NaN	PSI=0.9032	PSI=0.8201	PSI=0.6156	PSI=NaN	HLA-A*02:01|37.6355477373;PSI=0.7963;Exp=30.7953	PSI=0.4522	HLA-A*02:01|80.882433811;PSI=0.5205;Exp=15.0806	PSI=0.781	PSI=NaN
		LB3667_P	PSI=1.0	PSI=0.6821	PSI=0.9199	PSI=0.7778	PSI=0.6247	PSI=0.5915	HLA-A*02:01|37.6355477373;PSI=0.9078;Exp=9.06001	PSI=0.5333	HLA-A*02:01|80.882433811;PSI=0.4937;Exp=16.8417	PSI=0.704	PSI=0.441
		LB3738	PSI=1.0	PSI=1.0	PSI=0.9301	PSI=0.8554	PSI=0.621	PSI=0.0	HLA-A*02:01	PSI=0.6729	HLA-A*02:01	PSI=1.0	PSI=0.9111

									|37.6355477373;PSI=0.93;Exp=22.4331		|80.882433811;PSI=0.5673;Exp=7.9695		
		LB3778	PSI=NaN	PSI=1.0	PSI=0.8189	PSI=0.9697	PSI=0.6986	PSI=0.675	HLA-A*02:01|37.6355477373;PSI=0.8245;Exp=20.0435	PSI=1.0;	HLA-A*02:01|80.882433811;PSI=0.3392;Exp=11.939	PSI=1.0	PSI=0.7544
		LB3802	PSI=1.0	PSI=1.0	PSI=0.9325	PSI=0.8579	PSI=0.7841	PSI=1.0	HLA-A*02:01|37.6355477373;PSI=0.8339;Exp=16.0274	PSI=0.9022	HLA-A*02:01|80.882433811;PSI=0.6679;Exp=10.8914	PSI=0.5299	PSI=0.7838
		LB3883	PSI=0.7886	PSI=0.8505	PSI=0.8956	PSI=0.7439	PSI=0.722	PSI=1.0	HLA-A*02:01|37.6355477373;PSI=0.8179;Exp=21.1917	PSI=0.4157	HLA-A*02:01|80.882433811;PSI=0.25;Exp=16.384	PSI=0.8778	PSI=1.0
		LB3914	PSI=NaN	PSI=0.8	PSI=0.9372	PSI=0.9633	PSI=0.4	PSI=1.0	HLA-A*02:01|37.6355477373;PSI=0.6739;Exp=18.5007	PSI=0.8026	HLA-A*02:01|80.882433811;PSI=0.6432;Exp=14.1801	PSI=0.8934	PSI=NaN
		LB4024	PSI=0.5908	PSI=0.8526	PSI=0.8859	PSI=0.8948	PSI=0.5619	PSI=0.6093	HLA-A*02:01|37.6355477373;PSI=0.8	PSI=0.5916	HLA-A*02:01|80.882433811;PSI=0.51	PSI=0.8912	PSI=0.931

									173;Exp=16.719		88;Exp=13.5334		
		Expr Values Only											
		LB2878	Exp=45.8943	Exp=41.7994	Exp=19.3054	Exp=20.7238	Exp=12.0616	Exp=16.952	HLA-A*02:01|37.6355477373;PSI=0.7928;Exp=29.4093	Exp=23.7336	HLA-A*02:01|80.882433811;PSI=NaN;Exp=23.7729	Exp=23.7336	Exp=47.9216
		LB3348	Exp=74.4904	Exp=74.6547	Exp=36.7204	Exp=38.7829	Exp=8.41674	Exp=20.8916		Exp=17.7503		Exp=17.7503	Exp=17.8044
		LB3386	Exp=75.0333	Exp=102.968	Exp=34.6509	Exp=39.6758	Exp=13.7332	Exp=36.1776	HLA-A*02:01|37.6355477373;PSI=0.8058;Exp=47.1911	Exp=14.7699	HLA-A*02:01|80.882433811;PSI=0.4354;Exp=21.5787	Exp=14.7699	Exp=39.9268
		LB3484	Exp=79.8166	Exp=69.4729	Exp=57.0311	Exp=59.7864	Exp=5.82644	Exp=32.232		Exp=12.3154		Exp=12.3154	Exp=62.5282
		LB3570	Exp=92.6196	Exp=46.0624	Exp=35.0582	Exp=44.4346	Exp=16.1834	Exp=23.2262	HLA-A*02:01|37.6355477373;PSI=0.9525;Exp=59.5663	Exp=16.36	HLA-A*02:01|80.882433811;PSI=NaN;Exp=17.2887	Exp=16.36	Exp=45.2489
		LB3598	Exp=70.5063	Exp=65.577	Exp=97.0072	Exp=52.6473	Exp=3.25387	Exp=37.3093	HLA-A*02:01|37.6355477373;PSI=1.0;Exp=44.8408	Exp=72.646	HLA-A*02:01|80.882433811;PSI=NaN;Exp=19.5963	Exp=72.646	Exp=11.1257

		LB3606	Exp=89.9652	Exp=53.2923	Exp=54.3114	Exp=62.0419	Exp=6.63403	Exp=35.109	HLA-A*02:01|37.6355477373;PSI=0.7963;Exp=30.7953	Exp=57.6896	HLA-A*02:01|80.882433811;PSI=0.5205;Exp=15.0806	Exp=57.6896	Exp=38.8818
		LB3667_P	Exp=81.4137	Exp=37.5304	Exp=79.1791	Exp=39.0693	Exp=4.47357	Exp=20.6671	Exp=24.7515	Exp=24.7515	Exp=51.353		
		LB3738	Exp=31.4966	Exp=47.0162	Exp=21.0541	Exp=19.2176	Exp=11.0891	Exp=7.37745	HLA-A*02:01|37.6355477373;PSI=0.93;Exp=22.4331	Exp=0.495587	HLA-A*02:01|80.882433811;PSI=0.5673;Exp=7.9695	Exp=0.495587	Exp=27.8479
		LB3778	Exp=17.687	Exp=24.967	Exp=15.0835	Exp=18.4314	Exp=4.55368	Exp=5.64169	HLA-A*02:01|37.6355477373;PSI=0.8245;Exp=20.0435	Exp=3.89409	HLA-A*02:01|80.882433811;PSI=0.3392;Exp=11.939	Exp=3.89409	Exp=25.4987
		LB3802	Exp=24.6527	Exp=37.4108	Exp=14.8715	Exp=17.4732	Exp=10.5371	Exp=6.49666	HLA-A*02:01|37.6355477373;PSI=0.8339;Exp=16.0274	Exp=1.75055	HLA-A*02:01|80.882433811;PSI=0.6679;Exp=10.8914	Exp=1.75055	Exp=36.5375
		LB3883	Exp=73.7825	Exp=93.2394	Exp=57.2512	Exp=54.8545	Exp=5.60051	Exp=31.5877	HLA-A*02:01|37.6355477373;PSI=0.8179;Exp=21.1917	Exp=23.2842	HLA-A*02:01|80.882433811;PSI=0.25;Exp=16.384	Exp=23.2842	Exp=43.6037
		LB3914	Exp=22.9798	Exp=20.6998	Exp=14.8156	Exp=18.9921	Exp=7.02732	Exp=4.75366	HLA-A*02:01	Exp=10.5907	HLA-A*02:01	Exp=10.5907	Exp=55.0117

									|37.6355477373;PSI=0.6739;Exp=18.5007		|80.882433811;PSI=0.6432;Exp=14.1801		
		LB4024	Exp=40.9701	Exp=60.1696	Exp=33.9611	Exp=94.8624	Exp=7.51328	Exp=15.98	HLA-A*02:01|37.6355477373;PSI=0.8173;Exp=16.719	Exp=19.4243	HLA-A*02:01|80.882433811;PSI=0.5188;Exp=13.5334	Exp=19.4243	Exp=42.146

Table 7C-1 HLA-A*03:01 peptide embodiments

Tumor Diagnosis	TILs remaining	PBMC Remaining	epitope	TVTEPLLVK (SEQ ID NO:1382)	GLQTRAFWK (SEQ ID NO:1383)	ILLYKNKRK (SEQ ID NO:1384)	KGFSIDSGK (SEQ ID NO:1395)	LLQGDTPVRK (SEQ ID NO:1385)	SLDWETPSK (SEQ ID NO:1386)	KEMRPARAK (SEQ ID NO:1387)	RAVTNHSVYY (SEQ ID NO:1388)
			as_event	ENSG00000124006:OBSL1:chr2:-:220422064:220422340:220421445:220423946	ENSG00000213995:NAXD:chr13:+:111279785:111279894:111267994:111286891	ENSG00000105426:PTPRS:chr19:-:5218797:5218809:5218543:5219320	ENSG00000135424:ITGA7:chr12:-:56094045:56094177:56092701:56094682	ENSG00000133110:POSTN:chr13:-:38143436:38143520:38138697:38143880	ENSG00000130803:ZNF317:chr19:+:92685099:9268751:9268070:9269501	ENSG00000095637:SORBS1:chr10:-:97110965:97111133:97106209:97114638	ENSG00000169738:DCXR:chr17:-:79994289:79994324:79994184:79994419
			junction_peptide_form	skp	skp	skp	skp	inc	skp	skp	skp
			num_hla	2	2	1	1	2	2	2	5
			meanPSI	0.86	0.86	0.81	0.76	0.78	0.67	0.74	0.68
			Q1PSI	0.76	0.79	0.79	0.65	0.7	0.54	0.65	0.63
			Q3PSI	1	0.92	0.88	0.95	1	0.85	0.92	0.74
			deltaPSI	-0.11745417	-0.1148569	-0.14997525	-0.17939215	0.56988939	-0.23746398	-0.17985192	-0.21937263

			fc_of_tumor_isoform	5.53	5.12	4.37	3.81	3.71	3.52	3.30	3.18
			tissue_matched_normal_panel	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
			tumor_panel	1/2	1/2	2/2	2/2	2/2	1/2	2/2	1/2
			normal_panel	9/11	11/11	9/11	10/11	11/11	8/11	9/11	11/11
			tag	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific
			mappability	0.870370370812;0.933333333333;0.988888888889	1.0;0.964814814925;0.988888888889	0.833333333333;1.0;1.0	0.922222222222;0.899074074295;0.929629629845	0.798888889617;0.830332341648;0.977777777778	0.602379255369;0.988888888889;0.851111111707	0.988888888889;0.943518518739;0.922222222222	0.944444444444;0.957142857143;0.988888888889
			mappability_tag	PASS	PASS	PASS	PASS	MID	MID	PASS	PASS
			meanGeneExp	45.84	17.78	39.22	70.12	26.79	13.66	31.35	51.98
			Q1GeneExp	25.32	12.52	20.47	23.4	0.36	11.02	15.24	31.42
			Q3GeneExp	62.99	22.12	52.05	104.24	25.93	16.87	44.12	59.71
			canonical_match	NonCanonical	NonCanonical	NonCanonical	NonCanonical	Q15063	NonCanonical	NonCanonical	NonCanonical
			overlap_with_validation_set	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
GBM-PD1	None	1.50E+07	LB3658	HLA-A*03:01|208.48573055;PSI=0.8857;Exp=20.4866	HLA-A*03:01|80.017830959;PSI=0.838;Exp=7.38987	HLA-A*03:01|164.472840555;PSI=0.8664;Exp=81.7972	HLA-A*03:01|479.024452697;PSI=0.9092;Exp=16.6967	HLA-A*03:01|275.936831392;PSI=0.9879;Exp=26.7422	HLA-A*03:01|325.933767655;PSI=0.8154;Exp=7.02972	HLA-A*03:01|261.731672867;PSI=0.8602;Exp=29.0428	HLA-A*03:01|359.444725975;PSI=0.8109;Exp=25.4283

GBM-ndw	None	None	LB3116	HLA-A*03:01|208.48573055; PSI=0.8349; Exp=71.2357	HLA-A*03:01|80.017830959; PSI=0.8807; Exp=10.3855	HLA-A*03:01|164.472840555;PSI=0.8807;Exp=70.0058	HLA-A*03:01|479.02445269; PSI=0.3754; Exp=175.51	HLA-A*03:01|275.936831392;PSI=0.9882;Exp=41.2789	HLA-A*03:01|325.933767655;PSI=0.6746;Exp=17.4652	HLA-A*03:01|261.73167286; PSI=0.4565; Exp=10.0721	HLA-A*03:01|359.444725975;PSI=0.6531;Exp=48.4138
			LB3521	-	-	-	-	-	-	-	-
			LB3755	-	-	-	-	-	-	-	-
			LB3560	-	-	-	-	-	-	-	-
			LB3404	-	-	-	-	-	-	-	-
			LB4024	-	-	-	-	-	-	-	-
			LB3598	-	-	-	-	-	-	-	-
			LB3334	-	-	-	-	-	-	-	-
GBM-PD1	None	None	LB3804	HLA-A*03:01|208.48573055; PSI=0.8462; Exp=30.709	HLA-A*03:01|80.017830959; PSI=0.8191; Exp=7.55512	HLA-A*03:01|164.472840555;PSI=0.8548;Exp=56.8095	HLA-A*03:01|479.02445269; PSI=0.486; Exp=10.0564	HLA-A*03:01|275.936831392;PSI=0.9033;Exp=18.5945	HLA-A*03:01|325.933767655;PSI=0.7083;Exp=13.4752	HLA-A*03:01|261.73167286; PSI=0.9301; Exp=13.4276	HLA-A*03:01|359.444725975;PSI=0.3779;Exp=16.4146
			LB3330	-	-	-	-	-	-	-	-
			LB3931	-	-	-	-	-	-	-	-
			LB3933	-	-	-	-	-	-	-	-
			LB3491	-	-	-	-	-	-	-	-
GBM-new	None	None	LB3865	HLA-A*03:01|208.48573055; PSI=NaN;Exp=76.8135	HLA-A*03:01|80.017830959; PSI=NaN;Exp=4.63342	HLA-A*03:01|164.472840555;PSI=1.0;Exp=4.89151	HLA-A*03:01|479.02445269; PSI=1.0;Exp=332.147	HLA-A*03:01|275.936831392;PSI=0.7951;Exp=51.3237	HLA-A*03:01|325.933767655;PSI=1.0;Exp=15.7981	HLA-A*03:01|261.73167286; PSI=1.0;Exp=10.5235	HLA-A*03:01|359.444725975;PSI=0.5126;Exp=48.0229
GBM-PD1	None	4.00E+06	LB3864	HLA-A*03:01|208.48573055; PSI=NaN;Exp=83.5069	HLA-A*03:01|80.017830959; PSI=0.7927; Exp=16.9033	HLA-A*03:01|164.472840555;PSI=0.9197;Exp=60.0456	HLA-A*03:01|479.02445269; PSI=1.0;Exp=31.8391	HLA-A*03:01|275.936831392;PSI=0.9874;Exp=1.92735	HLA-A*03:01|325.933767655;PSI=0.7647;Exp=8.66598	HLA-A*03:01|261.73167286; PSI=0.9627; Exp=60.5956	HLA-A*03:01|359.444725975;PSI=0.4498;Exp=56.6732
GBM-PD1	None	9.50E+06	LB3667_P	HLA-A*03:01|208.48573055; PSI=0.6821;	HLA-A*03:01|80.017830959; PSI=0.8857;	HLA-A*03:01|164.472840555;PSI=0.81;	HLA-A*03:01|479.02445269; PSI=1.0;Exp=36.7748	HLA-A*03:01|275.936831392;PSI=0.998	HLA-A*03:01|325.933767655;PSI=0.783	HLA-A*03:01|261.73167286; PSI=0.7293;	HLA-A*03:01|359.444725975;PSI=0.452

				Exp=37.5304	Exp=9.89526	Exp=70.6468		1;Exp=108.09	8;Exp=14.3983	Exp=19.2624	;Exp=65.891
			LB3869	-	-	-	-	-	-	-	-
			LB3883	-	-	-	-	-	-	-	-
			LB3914	-	-	-	-	-	-	-	-
			LB3386	-	-	-	-	-	-	-	-
			LB3284	-	-	-	-	-	-	-	-
			LB3802	-	-	-	-	-	-	-	-
			LB3724	-	-	-	-	-	-	-	-
			LB3607	-	-	-	-	-	-	-	-
GBM-PD1	None	9.50E+06	LB3667	HLA-A*03:01|208.48573055;PSI=0.8095;Exp=28.1043	HLA-A*03:01|80.017830959;PSI=0.8514;Exp=5.0966	HLA-A*03:01|164.472840555;PSI=0.871;Exp=98.8834	HLA-A*03:01|479.02445269;PSI=0.5953;Exp=36.4681	HLA-A*03:01|275.936831392;PSI=0.9986;Exp=53.1781	HLA-A*03:01|325.933767655;PSI=0.9636;Exp=8.26845	HLA-A*03:01|261.73167286;PSI=0.8507;Exp=40.9214	HLA-A*03:01|359.444725975;PSI=0.8587;Exp=14.8368
			LB3579	-	-	-	-	-	-	-	-
			LB3771	-	-	-	-	-	-	-	-
			LB3388	-	-	-	-	-	-	-	-
GBM-new	None	1 vial	LB3518	HLA-A*03:01|208.48573055;PSI=0.6111;Exp=37.546	HLA-A*03:01|80.017830959;PSI=0.6395;Exp=7.18774	HLA-A*03:01|164.472840555;PSI=0.8747;Exp=48.3845	HLA-A*03:01|479.02445269;PSI=0.5677;Exp=6.01588	HLA-A*03:01|275.936831392;PSI=0.8883;Exp=0.79955	HLA-A*03:01|325.933767655;PSI=0.4545;Exp=18.4034	HLA-A*03:01|261.73167286;PSI=0.8209;Exp=3.07287	HLA-A*03:01|359.444725975;PSI=0.6426;Exp=61.9658
			LB3571	-	-	-	-	-	-	-	-
			LB3413	-	-	-	-	-	-	-	-
			LB2985	-	-	-	-	-	-	-	-
			LB3382	-	-	-	-	-	-	-	-
GBM-rec	5.00E+06	None	LB3415	HLA-A*03:01|208.48573055;PSI=0.86;Exp=41.0176	HLA-A*03:01|80.017830959;PSI=0.7339;Exp=11.1101	HLA-A*03:01|164.472840555;PSI=0.7253;Exp=224.032	HLA-A*03:01|479.02445269;PSI=0.5124;Exp=4.67915	HLA-A*03:01|275.936831392;PSI=NaN;Exp=0	HLA-A*03:01|325.933767655;PSI=0.5;Exp=18.3159	HLA-A*03:01|261.73167286;PSI=0.3966;Exp=16.7822	HLA-A*03:01|359.444725975;PSI=0.6965;Exp=54.1611
GBM-new	1.00E+07	2 vials	LB3166	HLA-A*03:01|208.48573055;	HLA-A*03:01|80.017830959;PSI=0.8074;	HLA-A*03:01|164.472840555;PSI=0.938	HLA-A*03:01|479.02445269;PSI=0.8458;	HLA-A*03:01|275.936831392;PSI=0.908	HLA-A*03:01|325.933767655;PSI=0.659	HLA-A*03:01|261.73167286;	HLA-A*03:01|359.444725975;PSI=0.603

				PSI=NaN;Exp=41.8087	Exp=23.5324	9;Exp=64.7527	Exp=82.1131	6;Exp=2.08244	3;Exp=10.8844	PSI=0.7414;Exp=16.451	2;Exp=45.2665
			LB3348	-	-	-	-	-	-	-	-
GBM-new	None	3.20E+07	LB3608	HLA-A*03:01|208.48573055;PSI=NaN;Exp=57.8724	HLA-A*03:01|80.017830959;PSI=0.8553;Exp=24.7715	HLA-A*03:01|164.472840555;PSI=0.8245;Exp=67.0993	HLA-A*03:01|479.02445269;PSI=0.4375;Exp=48.867	HLA-A*03:01|275.936831392;PSI=1.0;Exp=6.54335	HLA-A*03:01|325.933767655;PSI=0.3;Exp=9.48093	HLA-A*03:01|261.73167286;PSI=0.6014;Exp=17.5825	HLA-A*03:01|359.444725975;PSI=0.4383;Exp=123.592
			LB2878	-	-	-	-	-	-	-	-
			LB3484	-	-	-	-	-	-	-	-
			LB3872	-	-	-	-	-	-	-	-
			LB2873	-	-	-	-	-	-	-	-
			LB3606	-	-	-	-	-	-	-	-
			LB3738	-	-	-	-	-	-	-	-
			LB3619	-	-	-	-	-	-	-	-
			LB3810	-	-	-	-	-	-	-	-
GBM-PD1	None	2.50E+07	LB3811	HLA-A*03:01|208.48573055;PSI=0.931;Exp=55.4443	HLA-A*03:01|80.017830959;PSI=0.9703;Exp=5.44264	HLA-A*03:01|164.472840555;PSI=0.8702;Exp=84.5466	HLA-A*03:01|479.02445269;PSI=0.8631;Exp=30.1859	HLA-A*03:01|275.936831392;PSI=0.4882;Exp=1.83486	HLA-A*03:01|325.933767655;PSI=0.907;Exp=8.10345	HLA-A*03:01|261.73167286;PSI=0.8412;Exp=23.8327	HLA-A*03:01|359.444725975;PSI=0.4859;Exp=18.0988
			LB3570	-	-	-	-	-	-	-	-
GBM-new	None	None	LB2912	HLA-A*03:01|208.48573055;PSI=1.0;Exp=40.3581	HLA-A*03:01|80.017830959;PSI=0.4096;Exp=10.4298	HLA-A*03:01|164.472840555;PSI=0.9606;Exp=88.5827	HLA-A*03:01|479.02445269;PSI=1.0;Exp=20.2117	HLA-A*03:01|275.936831392;PSI=1.0;Exp=1.48672	HLA-A*03:01|325.933767655;PSI=0.7444;Exp=14.9645	HLA-A*03:01|261.73167286;PSI=0.1286;Exp=16.1158	HLA-A*03:01|359.444725975;PSI=0.0;Exp=31.2348
GBM-PD1	None	1 vial	LB3944	HLA-A*03:01|208.48573055;PSI=1.0;Exp=54.6886	HLA-A*03:01|80.017830959;PSI=0.7892;Exp=19.3552	HLA-A*03:01|164.472840555;PSI=0.7291;Exp=55.0341	HLA-A*03:01|479.02445269;PSI=0.4706;Exp=60.1386	HLA-A*03:01|275.936831392;PSI=0.9683;Exp=46.7932	HLA-A*03:01|325.933767655;PSI=0.6186;Exp=16.4447	HLA-A*03:01|261.73167286;PSI=0.7417;Exp=16.9717	HLA-A*03:01|359.444725975;PSI=0.2896;Exp=59.3865
			LB2933	-	-	-	-	-	-	-	-
			LB3612	-	-	-	-	-	-	-	-
			LB3778	-	-	-	-	-	-	-	-

			1142	-	-	-	-	-	-	HLA-B*44:02|296.872227974	-
GBM-rec (DC Vax)	None	3.00E+07	2964	HLA-A*03:01|208.48573055	HLA-A*03:01|80.017830959	HLA-A*03:01|164.472840555	HLA-A*03:01|479.02445269	HLA-A*03:01|275.936831392	HLA-A*03:01|325.933767655	HLA-A*03:01|261.73167286	HLA-A*03:01|359.444725975
GBM-rec	None	~10 vials	2907	HLA-A*03:01|208.48573055	HLA-A*03:01|80.017830959	HLA-A*03:01|164.472840555	HLA-A*03:01|479.02445269	HLA-A*03:01|275.936831392	HLA-A*03:01|325.933767655	HLA-A*03:01|261.73167286	HLA-A*03:01|359.444725975
			1325	-	-	-	-	-	-	-	-
			2924	-	-	-	-	-	-	-	-
			2870	-	-	-	-	-	-	-	-
			LB3336	HLA-A*11:01|36.0957182154	HLA-A*11:01|81.980453317	-	-	HLA-A*11:01|460.174873754	HLA-A*11:01|126.861030627	-	HLA-A*11:01|482.406975764
GBM-PD1	2.00E+07	None	2867	HLA-A*03:01|208.48573055	HLA-A*03:01|80.017830959	HLA-A*03:01|164.472840555	HLA-A*03:01|479.02445269	HLA-A*03:01|275.936831392	HLA-A*03:01|325.933767655	HLA-A*03:01|261.73167286	HLA-A*03:01|359.444725975
			1989	-	-	-	-	-	-	-	-
			2158	-	-	-	-	-	-	-	-
			LB3367	-	-	-	-	-	-	-	-
			LB3054	-	-	-	-	-	-	-	-
			LB3001	-	-	-	-	-	-	-	HLA-A*30:02|65.7953777362
			3404	-	-	-	-	-	-	-	-
			LB3120	-	-	-	-	-	-	-	HLA-B*15:01|48.2919586049
			2899	-	-	-	-	-	-	-	HLA-B*15:01|48.2919586049
			3374	-	-	-	-	-	-	-	-
			3372	-	-	-	-	-	-	HLA-B*44:02|29	-

										6.872227974	
			3244	-	-	-	-	-	-	-	-
			2675	-	-	-	-	-	-	-	HLA-A*29:01|184.1;HLA-A*30:02|65.7953777362
GBM-rec	5.00E+07		LB2938	HLA-A*03:01|208.48573055	HLA-A*03:01|80.017830959	HLA-A*03:01|164.472840555	HLA-A*03:01|479.02445269	HLA-A*03:01|275.936831392	HLA-A*03:01|325.933767655	HLA-A*03:01|261.73167286	HLA-A*03:01|359.444725975
GBM-rec	None	None	803	HLA-A*03:01|208.48573055	HLA-A*03:01|80.017830959	HLA-A*03:01|164.472840555	HLA-A*03:01|479.02445269	HLA-A*03:01|275.936831392	HLA-A*03:01|325.933767655	HLA-A*03:01|261.73167286	HLA-A*03:01|359.444725975;HLA-A*30:02|65.7953777362

Table 7C-2 HLA-A*03:01 peptide embodiments

Tumor Diagnosis	TILs remaining	PBMC Remaining	epitope	SVYYPSECSK (SEQ ID NO:1389)	QLFEGMKAFK (SEQ ID NO:1390)	ALGQLFEGMK (SEQ ID NO:1391)	KMFRKLHNSY (SEQ ID NO:1392)	RLQARPRLGR (SEQ ID NO:1393)	RIPYKVVARR (SEQ ID NO:1394)	ISWMKGVPGK (SEQ ID NO:786)	KIDSFELLYY (SEQ ID NO:1367)
			as_event	ENSG00000169738:DCXR:chr17:-:79994289:79994324:79994184:79994419	ENSG00000105552:BCAT2:chr19:-:49310256:49310331:49303554:49314240	ENSG00000105552:BCAT2:chr19:-:49310256:49310331:49303554:49314240	ENSG00000167515:TRAPPC2L:chr16:+:88925752:88925851:88925197:88926300	ENSG00000084693:AGBL5:chr2:+:27291915:27291962:27291612:27292440	ENSG00000117308:GALE:chr1:-:24122998:24123076:24122755:24123186	ENSG00000072195:SPEG:chr2:+:220353219:220353387:220353032:220353499	ENSG00000128973:CLN6:chr15:-:68503893:68504079:68503656:68506627
			junction_peptide_form	skp	skp	skp	skp	skp	skp	skp	skp
			num_hla	2	2	1	5	2	2	2	6

			meanPSI	0.68	0.37	0.37	0.51	0.53	0.78	0.91	0.78
			Q1PSI	0.63	0.25	0.25	0.36	0.4	0.7	0.86	0.67
			Q3PSI	0.74	0.46	0.46	0.66	0.64	0.85	0.97	1
			deltaPSI	-0.21937263	-0.42872933	-0.42872933	-0.33070982	-0.27504723	-0.12762619	-0.05290968	-0.11588091
			fc_of_tumor_isoform	3.18	3.16	3.16	3.05	2.44	2.43	2.38	2.07
			tissue_matched_normal_panel	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
			tumor_panel	1/2	1/2	1/2	2/2	2/2	1/2	1/2	1/2
			normal_panel	11/11	11/11	11/11	11/11	11/11	11/11	7/11	10/11
			tag	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific	associated;recurrent;specific
			mappability	0.9444444444444;0.957142857143;0.988888888889	1.0;0.878486106396;0.988888888889	1.0;0.878486106396;0.988888888889	0.751851852073;0.885185185406;0.677777777778	0.966666666667;0.957446808511;0.95	0.874074074295;0.952457275146;1.0	0.933333333333;0.966666666832;0.955555555556	1.0;0.897222222553;0.911111111111
			mappability_tag	PASS	PASS	PASS	MID	PASS	PASS	PASS	PASS
			meanGeneExp	51.98	14.04	14.04	33.04	10.42	10.71	23.85	10.98
			Q1GeneExp	31.42	8.24	8.24	23.59	7.53	7.57	13.3	7.26
			Q3GeneExp	59.71	18.16	18.16	38.86	12.38	12.9	29.09	13.18
			canonical_match	NonCanonical	O15382	NonCanonical	NonCanonical	Q8NDL9	NonCanonical	NonCanonical	NonCanonical
			overlap_with_validation_set	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE

GBM-PD1	None	1.50E+07	LB3658	HLA-A*03:01|30.3740655035;PSI=0.8109;Exp=25.4283	HLA-A*03:01|49.7990754857;PSI=0.0766;Exp=4.16081	HLA-A*03:01|256.90390855;PSI=0.0766;Exp=4.16081	HLA-A*03:01|168.535140059;PSI=0.6869;Exp=13.6283	HLA-A*03:01|76.687185499;PSI=0.3188;Exp=6.96294	HLA-A*03:01|303.700280923;PSI=0.8292;Exp=4.80752	HLA-A*03:01|148.162551399;PSI=0.975;Exp=12.7955	HLA-A*03:01|111.666676021;PSI=0.7857;Exp=7.10659
GBM-ndw	None	None	LB3116	HLA-A*03:01|30.3740655035;PSI=0.6531;Exp=48.4138	HLA-A*03:01|49.7990754857;PSI=NaN;Exp=22.7113	HLA-A*03:01|256.90390855;PSI=NaN;Exp=22.7113	HLA-A*03:01|168.535140059;PSI=NaN;Exp=26.6608	HLA-A*03:01|76.687185499;PSI=0.3842;Exp=9.7237	HLA-A*03:01|303.700280923;PSI=0.8213;Exp=12.0779	HLA-A*03:01|148.162551399;PSI=0.9314;Exp=36.3056	HLA-A*03:01|111.666676021;PSI=1.0;Exp=11.5036
			LB3521	-	-	-	-	-	-	-	-
			LB3755	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791;PSI=0.4444;Exp=11.3429
			LB3560	-	-	-	-	-	-	-	-
			LB3404	-	-	-	-	-	-	-	-
			LB4024	-	-	-	-	-	-	-	-
			LB3598	-	-	-	-	-	-	-	-
			LB3334	-	-	-	-	-	-	-	-
GBM-PD1	None	None	LB3804	HLA-A*03:01|30.3740655035;PSI=0.3779;Exp=16.4146	HLA-A*03:01|49.7990754857;PSI=0.1989;Exp=11.4874	HLA-A*03:01|256.90390855;PSI=0.1989;Exp=11.4874	HLA-A*03:01|168.535140059;PSI=NaN;Exp=9.06054	HLA-A*03:01|76.687185499;PSI=0.503;Exp=6.39262	HLA-A*03:01|303.700280923;PSI=0.8421;Exp=3.922	HLA-A*03:01|148.162551399;PSI=0.7273;Exp=4.98352	HLA-A*03:01|111.666676021;PSI=1.0;Exp=11.1882
			LB3330	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791;PSI=1.0;Exp=14.0106
			LB3931	-	-	-	-	-	-	-	-
			LB3933	-	-	-	-	-	-	-	-
			LB3491	-	-	-	-	-	-	-	-
GBM-new	None	None	LB3865	HLA-A*03:01|30.	HLA-A*03:01|49.	HLA-A*03:01|25	HLA-A*03:01|16	HLA-A*03:01|76.	HLA-A*03:01|30	HLA-A*03:01|14	HLA-A*03:01|11

				3740655035;PSI=0.5126;Exp=48.0229	7990754857;PSI=NaN;Exp=48.2656	6.90390855;PSI=NaN;Exp=48.2656	8.535140059;PSI=NaN;Exp=63.1351	687185499;PSI=0.2913;Exp=8.58791	3.700280923;PSI=0.8146;Exp=19.3929	8.162551399;PSI=NaN;Exp=58.4136	1.666676021,HLA-A*01:01|152.010342791;PSI=1.0;Exp=12.7443
GBM-PD1	None	4.00E+06	LB3864	HLA-A*03:01|30.3740655035;PSI=0.4498;Exp=56.6732	HLA-A*03:01|49.7990754857;PSI=0.3892;Exp=7.31418	HLA-A*03:01|256.90390855;PSI=0.3892;Exp=7.31418	HLA-A*03:01|168.535140059;PSI=0.7786;Exp=71.9334	HLA-A*03:01|76.687185499;PSI=0.4402;Exp=13.7975	HLA-A*03:01|303.700280923;PSI=0.6166;Exp=8.44985	HLA-A*03:01|148.162551399;PSI=1.0;Exp=22.6416	HLA-A*03:01|111.666676021;PSI=1.0;Exp=9.44704
GBM-PD1	None	9.50E+06	LB3667_P	HLA-A*03:01|30.3740655035;PSI=0.452;Exp=65.891	HLA-A*03:01|49.7990754857;PSI=0.583;Exp=7.79778	HLA-A*03:01|256.90390855;PSI=0.583;Exp=7.79778	HLA-A*03:01|168.535140059;PSI=NaN;Exp=38.5944	HLA-A*03:01|76.687185499;PSI=0.3855;Exp=24.228	HLA-A*03:01|303.700280923;PSI=0.6029;Exp=9.31553	HLA-A*03:01|148.162551399;PSI=1.0;Exp=5.89619	HLA-A*03:01|111.666676021;PSI=0.5556;Exp=17.5151
			LB3869	-	-	-	-	-	-	-	-
			LB3883	-	-	-	-	-	-	-	-
			LB3914	-	-	-	-	-	-	-	-
			LB3386	-	-	-	-	-	-	-	-
			LB3284	-	-	-	-	-	-	-	-
			LB3802	-	-	-	-	-	-	-	-
			LB3724	-	-	-	-	-	-	-	-
			LB3607	-	-	-	-	-	-	-	-
GBM-PD1	None	9.50E+06	LB3667	HLA-A*03:01|30.3740655035;PSI=0.8587;Exp=14.8368	HLA-A*03:01|49.7990754857;PSI=0.3062;Exp=4.81125	HLA-A*03:01|256.90390855;PSI=0.3062;Exp=4.81125	HLA-A*03:01|168.535140059;PSI=NaN;Exp=6.02762	HLA-A*03:01|76.687185499;PSI=0.5125;Exp=9.41816	HLA-A*03:01|303.700280923;PSI=0.7116;Exp=3.50746	HLA-A*03:01|148.162551399;PSI=0.7209;Exp=7.22624	HLA-A*03:01|111.666676021;PSI=1.0;Exp=8.05775
			LB3579	-	-	-	-	-	-	-	-
			LB3771	-	-	-	-	-	-	-	-
			LB3388	-	-	-	-	-	-	-	-
GBM-new	None	1 vial	LB3518	HLA-A*03:01|30.3740655035;PSI=0.6426	HLA-A*03:01|49.7990754857;PSI=0.2296	HLA-A*03:01|256.90390855;PSI=0.2296;	HLA-A*03:01|168.535140059;PSI=NaN;	HLA-A*03:01|76.687185499;PSI=0.2504;	HLA-A*03:01|303.700280923;PSI=0.852	HLA-A*03:01|148.162551399;PSI=0.888	HLA-A*03:01|111.666676021;PSI=0.565

				;Exp=61.9658	;Exp=23.1389	Exp=23.1389	Exp=38.7218	Exp=15.6254	5;Exp=12.9371	9;Exp=5.20371	2;Exp=60.6273
			LB3571	-	-	-	-	-	-	-	-
			LB3413	-	-	-	-	-	-	-	-
			LB2985	-	-	-	-	-	-	-	-
			LB3382	-	-	-	-	-	-	-	-
GBM-rec	5.00E+06	None	LB3415	HLA-A*03:01|30.3740655035;PSI=0.6965;Exp=54.1611	HLA-A*03:01|49.7990754857;PSI=0.8225;Exp=11.2931	HLA-A*03:01|256.90390855;PSI=0.8225;Exp=11.2931	HLA-A*03:01|168.535140059;PSI=NaN;Exp=15.881	HLA-A*03:01|76.687185499;PSI=0.5643;Exp=11.1364	HLA-A*03:01|303.700280923;PSI=0.4677;Exp=6.96308	HLA-A*03:01|148.162551399;PSI=0.9149;Exp=7.84823	HLA-A*03:01|111.666676021;PSI=0.3514;Exp=5.40968
GBM-new	1.00E+07	2 vials	LB3166	HLA-A*03:01|30.3740655035;PSI=0.6032;Exp=45.2665	HLA-A*03:01|49.7990754857;PSI=0.111;Exp=9.70689	HLA-A*03:01|256.90390855;PSI=0.111;Exp=9.70689	HLA-A*03:01|168.535140059;PSI=NaN;Exp=38.9127	HLA-A*03:01|76.687185499;PSI=0.6097;Exp=9.87983	HLA-A*03:01|303.700280923;PSI=0.7797;Exp=9.43708	HLA-A*03:01|148.162551399;PSI=0.8889;Exp=12.8782	HLA-A*03:01|111.666676021;PSI=0.9286;Exp=16.1027
			LB3348	-	-	-	-	-	-	-	-
GBM-new	None	3.20E+07	LB3608	HLA-A*03:01|30.3740655035;PSI=0.4383;Exp=123.592	HLA-A*03:01|49.7990754857;PSI=0.2823;Exp=10.8311	HLA-A*03:01|256.90390855;PSI=0.2823;Exp=10.8311	HLA-A*03:01|168.535140059;PSI=0.7832;Exp=116.07	HLA-A*03:01|76.687185499;PSI=0.2345;Exp=21.9999	HLA-A*03:01|303.700280923;PSI=0.7124;Exp=12.6139	HLA-A*03:01|148.162551399;PSI=1.0;Exp=19.6733	HLA-A*03:01|111.666676021,HLA-A*01:01|152.010342791;PSI=0.9333;Exp=22.8167
			LB2878	-	-	-	-	-	-	-	-
			LB3484	-	-	-	-	-	-	-	-
			LB3872	-	-	-	-	-	-	-	-
			LB2873	-	-	-	-	-	-	-	-
			LB3606	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791;PSI=1.0;Exp=15.6211
			LB3738	-	-	-	-	-	-	-	-
			LB3619	-	-	-	-	-	-	-	-

			LB3810	-	-	-	-	-	-	-	-
GBM-PD1	None	2.50E+07	LB3811	HLA-A*03:01|30.3740655035;PSI=0.4859;Exp=18.0988	HLA-A*03:01|49.7990754857;PSI=0.0864;Exp=7.9484	HLA-A*03:01|256.90390855;PSI=0.0864;Exp=7.9484	HLA-A*03:01|168.535140059;PSI=NaN;Exp=10.6078	HLA-A*03:01|76.687185499;PSI=0.2482;Exp=6.98463	HLA-A*03:01|303.700280923;PSI=0.7554;Exp=5.8747	HLA-A*03:01|148.162551399;PSI=0.7949;Exp=14.8362	HLA-A*03:01|111.666676021;PSI=1.0;Exp=7.38678
			LB3570	-	-	-	-	-	-	-	-
GBM-new	None	None	LB2912	HLA-A*03:01|30.3740655035;PSI=0.0;Exp=31.2348	HLA-A*03:01|49.7990754857;PSI=NaN;Exp=8.68636	HLA-A*03:01|256.90390855;PSI=NaN;Exp=8.68636	HLA-A*03:01|168.535140059;PSI=NaN;Exp=35.4783	HLA-A*03:01|76.687185499;PSI=0.6545;Exp=18.9776	HLA-A*03:01|303.700280923;PSI=0.2164;Exp=17.7743	HLA-A*03:01|148.162551399;PSI=0.6042;Exp=35.9184	HLA-A*03:01|111.666676021;PSI=NaN;Exp=8.20204
GBM-PD1	None	1 vial	LB3944	HLA-A*03:01|30.3740655035;PSI=0.2896;Exp=59.3865	HLA-A*03:01|49.7990754857;PSI=0.1877;Exp=9.30797	HLA-A*03:01|256.90390855;PSI=0.1877;Exp=9.30797	HLA-A*03:01|168.535140059;PSI=NaN;Exp=53.8258	HLA-A*03:01|76.687185499;PSI=0.7514;Exp=15.7755	HLA-A*03:01|303.700280923;PSI=0.5861;Exp=15.7044	HLA-A*03:01|148.162551399;PSI=0.1667;Exp=10.6916	HLA-A*03:01|111.666676021,HLA-A*01:01|152.010342791;PSI=NaN;Exp=12.1669
			LB2933	-	-	-	-	-	-	-	-
			LB3612	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791;PSI=1.0;Exp=9.78051
			LB3778	-	-	-	-	-	-	-	-
			1142	-	-	-	-	-	-	-	-
GBM-rec (DC Vax)	None	3.00E+07	2964	HLA-A*03:01|30.3740655035	HLA-A*03:01|49.7990754857	HLA-A*03:01|256.90390855	HLA-A*03:01|168.535140059	HLA-A*03:01|76.687185499;HLA-A*74:03|353.8	HLA-A*74:03|334.5;HLA-A*03:01|303.700280923	HLA-A*03:01|148.162551399	HLA-A*03:01|111.666676021
GBM-rec	None	~10 vials	2907	HLA-A*03:01|30.3740655035	HLA-A*03:01|49.7990754857	HLA-A*03:01|256.90390855	HLA-A*03:01|168.535140059	HLA-A*03:01|76.687185499	HLA-A*03:01|303.700280923	HLA-A*03:01|148.162551399	HLA-A*03:01|111.666676021

			1325	-	-	-	-	-	-	-	-
			2924	-	-	-	-	-	-	-	-
			2870	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791
			LB3336	HLA-A*11:01|15.3704339619	HLA-A*11:01|175.649175952	-	-	-	-	HLA-A*11:01|156.142776175	HLA-A*11:01|111.947487784
GBM-PD1	2.00E+07	None	2867	HLA-A*03:01|30.3740655035	HLA-A*03:01|49.7990754857	HLA-A*03:01|256.90390855	HLA-A*03:01|168.535140059	HLA-A*03:01|76.687185499	HLA-A*03:01|303.700280923	HLA-A*03:01|148.162551399	HLA-A*03:01|111.666676021
			1989	-	-	-	HLA-A*32:03|175.7	-	-	-	HLA-A*01:01|152.010342791
			2158	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791
			LB3367	-	-	-	-	-	-	-	-
			LB3054	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791
			LB3001	-	-	-	HLA-A*30:02|35.6867799277	-	-	-	HLA-A*30:02|291.046552821
			3404	-	-	-	-	-	-	-	-
			LB3120	-	-	-	HLA-B*15:01|9.68619030398	-	-	-	HLA-B*15:01|485.297368226
			2899	-	-	-	HLA-B*15:01|9.68619030398	-	-	-	HLA-A*01:01|152.010342791;HLA-B*15:01|48

											5.297368226
			3374	-	-	-	-	-	-	-	HLA-A*01:01|152.010342791
			3372	-	-	-	-	-	-	-	-
			3244	-	-	-	-	-	-	-	-
			2675	-	-	-	HLA-A*29:01|230.9;HLA-A*30:02|35.6867799277	-	-	-	HLA-A*29:01|92.4;HLA-A*30:02|291.046552821
GBM-rec	5.00E+07		LB2938	HLA-A*03:01|30.3740655035	HLA-A*03:01|49.7990754857	HLA-A*03:01|256.90390855	HLA-A*03:01|168.535140059	HLA-A*03:01|76.687185499	HLA-A*03:01|303.700280923	HLA-A*03:01|148.162551399	HLA-A*03:01|111.666676021
GBM-rec	None	None	803	HLA-A*03:01|30.3740655035	HLA-A*03:01|49.7990754857	HLA-A*03:01|256.90390855	HLA-A*03:01|168.535140059;HLA-A*30:02|35.6867799277	HLA-A*03:01|76.687185499	HLA-A*03:01|303.700280923	HLA-A*03:01|148.162551399	HLA-A*03:01|111.666676021;HLA-A*30:02|291.046552821

* * *

[0319] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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[0320] The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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WHAT IS CLAIMED IS:

1. A method to synthesize an antigenic peptide, comprising:

identifying alternative splice events in RNA-seq data derived from neoplastic tissue;

obtaining a reference panel of alternative splicing events that includes splice junction data, wherein the reference panel of alternative splicing events is derived from healthy matched tissue, other tissues of the body, or a second neoplastic tissue that is similar;

detecting a neoplastic alternative splicing event in the neoplastic tissue by comparing the alternative splice events derived from neoplastic tissue with the reference panel of alternative splicing events;

selecting an alternative isoform from the neoplastic tissue RNA-seq data that is detected to have the neoplastic alternative splicing event;

generating a peptide derived based on a nucleotide sequence that spans across a splice junction of the detected neoplastic alternative splicing event of the selected alternative isoform.

2. The method as in claim 1, wherein the selected isoform is selected based on the neoplastic splicing event being present at a greater level in the neoplastic tissue as compared to the healthy matched tissue or the other tissues of the body of the reference panel.

3. The method as in claims 1 or 2, wherein the selected isoform is selected based on the neoplastic splicing event being present at a greater level in the second neoplastic tissue as compared to the healthy matched tissue or the other tissues of the body of the reference panel.

4. The method as in claims 1, 2 or 3, wherein the alternative splice event is a skipped exon, an included exon, an alternative 3' splice site, and alternative 5' splice site, or a retained intron.

5. The method as in any of claims 1 to 4, wherein the alternative splice events in the RNA-seq data are identified using the rMATS package.

6. The method as in any of claims 1 to 5, wherein the neoplastic alternative splicing event is determined by the relative abundance or prevalence of alternative isoforms in the neoplastic tissue as compared to the reference tissue panel.

7. The method as in any of claims 1 to 6, wherein the reference tissue panel includes alternative splicing events from healthy tissue having the same tissue origin as the neoplastic tissue.

8. The method as in claims 6 or 7, wherein the relative abundance of alternative isoform is determined by the relative expression of the alternative isoform in the neoplastic tissue, as compared to the relative expression of the alternative isoform in the reference tissue panel.

9. The method as in claim 6 or 7, wherein the prevalence of the alternative isoform is determined by the number of samples expressing the alternative isoform within a neoplastic

tissue panel, as compared to the number of samples expressing the alternative isoform within the reference tissue panel.

10. The method as in any of claims 1 to 9, wherein the selection of at least one alternative isoform is based upon a statistical inference of the significance of the neoplastic alternative splicing event.

11. The method as in any of claims 1 to 10, wherein the generated peptide is determined to be a T Cell Receptor (TCR) target.

12. The method as in claim 11, wherein the generated peptide has computed median HLA binding affinity (IC_{50}) less than 500 nM.

13. The method as in any of claims 1 to 2, wherein the generated peptide is a part of an extracellular domain.

14. The method as in any of claims 1 to 13, wherein the generated peptide was identified in mass spectrometry data.

15. The method as in any of claims 1 to 14, wherein the generated peptide is synthesized via solid-phase peptide synthesis.

16. The method as in any of claims 1 to 14, wherein the generated peptide is synthesized via molecular expression in a host cell.

17. The method as in any of claims 1 to 16, wherein the generated peptide is utilized in an assay to determine peptide immunogenicity.

18. The method as in any of claims 1 to 16, wherein the generated peptide is utilized in an assay to determine recognition by T cells.

19. The method as in any of claims 1 to 16, wherein the generated peptide is utilized in a peptide vaccine for treatment of the neoplasm.

20. The method as in any of claims 1 to 16, wherein the generated peptide is utilized to develop modified T cell receptors of T cells.

21. The method as in any of claims 1 to 16, wherein the generated peptide is utilized to develop antibodies.

22. The method as in any of claims 1 to 16, wherein the generated peptide is utilized develop chimeric antigen receptors of T cells.

23. The method as in any of claims 1 to 23, wherein the neoplasm is one of: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), anal cancer, astrocytomas, basal cell carcinoma, bile duct cancer, bladder cancer, breast cancer, Burkitt's lymphoma, cervical cancer, chronic lymphocytic leukemia (CLL) chronic myelogenous leukemia (CML), chronic myeloproliferative neoplasms, colorectal cancer, diffuse large B-cell lymphoma,

endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, Ewing sarcoma, fallopian tube cancer, follicular lymphoma, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, hairy cell leukemia, hepatocellular cancer, Hodgkin lymphoma, hypopharyngeal cancer, Kaposi sarcoma, Kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, Merkel cell cancer, mesothelioma, mouth cancer, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic neuroendocrine tumors, pharyngeal cancer, pituitary tumor, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, skin cancer, small cell lung cancer, small intestine cancer, squamous neck cancer, T cell lymphoma, testicular cancer, thymoma, thyroid cancer, uterine cancer, vaginal cancer, or vascular tumors.

24. The method as in any of claims 1 to 23, wherein the neoplastic tissue is sourced from a tumor biopsy, a nodal biopsy, a surgical resection, or a liquid/soft biopsy.

25. An engineered T-cell Receptor (TCR) comprising:

a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:30 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:31;

a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:32 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:33;

a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:34 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:35;

a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:36 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:37;

a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:38 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:39;

a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:40 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:41;

a TCR alpha (TCR-a) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:42 and a TCR beta (TCR-b) CDR3 comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO:43; or

a TCR-a and TCR-b CDR3 comprising an amino acid sequence with at least 90% sequence identity to a TCR-a and TCR-b CDR3 pair from a clonotype listed in Table 6.

26. The TCR of claim 25, wherein the TCR comprises:

a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:44 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:45;

a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:46 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:47; or

a TCR alpha (TCR-a) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:48 and a TCR beta (TCR-b) variable region comprising an amino acid sequence with at least 80% sequence identity to SEQ ID NO:49.

27. The TCR of claim 25 or 26, wherein the TCR comprises or consists of a bispecific TCR.

28. The TCR of claim 27, wherein the bispecific TCR comprises an scFv that targets or selectively binds CD3.

29. The TCR of any one of claims 25-28, wherein the soluble TCR is further defined as a single-chain TCR (scTCR), wherein the α chain and the β chain are covalently attached via a flexible linker.

30. The TCR of 2526any one of claims 25-29, wherein the TCR comprises a modification or is chimeric.

31. One or more nucleic acids encoding the TCR of claim 25 or 30.

32. The nucleic acid(s) of claim 31, wherein the nucleic acid comprises a cDNA encoding the TCR.

33. A nucleic acid vector comprising the nucleic acid(s) of claim 31 or 32.

34. The vector of claim 33, wherein the vector comprises the TCR alpha and TCR beta genes.

35. A cell comprising the TCR of claim 25 or 30, the nucleic acid(s) of claim 31 or 32, or the vector of claim 33 or 34.

36. The cell of claim 35, wherein the cell is an immune cell.

37. The cell of claim 35 or 36, wherein the cell comprises a stem cell, progenitor cell, T cell, NK cell, invariant NK cell, NKT cell, mesenchymal stem cell (MSC), induced pluripotent stem (iPS) cell, regulatory T cell, CD8+ T cell, CD4+ T cell, or γδ T cell.

38. The cell of claim 37, wherein the cell comprises a hematopoietic stem or progenitor cell, a T cell, or an induced pluripotent stem cell (iPSC).

39. The cell of any one of claims 35-38, wherein the cell is autologous.

40. The host of any one of claims 35-38, wherein the cell is allogeneic.

41. The cell of any one of claims 35-40, wherein the cell is isolated from a cancer patient.

42. The cell of any one of claims 35-41, wherein the cell is a HLA-A type.

43. The cell of claim 42, wherein the cell is a HLA-A*03:01 type, HLA-A*01:01, or HLA-A*02:01.

44. A composition comprising the cell of any one of claims 35-43.

45. The composition of claim 44, wherein the composition has been determined to be serum-free, mycoplasma-free, endotoxin-free, and/or sterile.

46. A method comprising transferring the nucleic acid of any one of claims 32 or 33 or the vector of claim 34 into a cell.

47. The method of claim 46, wherein the method further comprises culturing the cell in media, incubating the cell at conditions that allow for the division of the cell, screening the cell, and/or freezing the cell.

48. A method for treating brain cancer in a subject comprising administering the composition of claim 44 or 45 to a subject.

49. The method of claim 48, wherein the brain cancer comprises glioblastoma or glioma.

50. The method of any one of claims 48-49, wherein the subject has previously been treated for the cancer.

51. The method of claim 50, wherein the subject has been determined to be resistant to the previous treatment.

52. The method of any one of claims 48-51, wherein the method further comprises the administration of an additional therapy.

53. The method of any one of claims 48-52, wherein the cancer comprises stage I, II, III, or IV cancer.

54. The method of any one of claims 48-53, wherein the cancer comprises metastatic and/or recurrent cancer.

55. A peptide from the TRIM11 protein comprising at least 6 contiguous amino acids from the TRIM11 and comprising the amino acids QD, which correspond to the amino acids at positions 168-169 of SEQ ID NO:1.

56. A peptide from the RCOR3 protein comprising at least 6 contiguous amino acids from the RCOR3 and comprising the amino acids QG, which correspond to the amino acids at positions 358-359 of SEQ ID NO:2.

57. A peptide from the FAM76B protein comprising at least 6 contiguous amino acids from the FAM76B and comprising the amino acids DS, which correspond to the amino acids at positions 230-231 of SEQ ID NO:3.

58. A peptide from the SLMAP protein comprising at least 6 contiguous amino acids from the SLMAP and comprising the amino acids NP, which correspond to the amino acids at positions 332-333 of SEQ ID NO:4.

59. A peptide from the TMEM62 protein comprising at least 6 contiguous amino acids from the TMEM62 and comprising the amino acids LG, which correspond to the amino acids at positions 495-496 of SEQ ID NO:5.

60. A peptide from the PLA2G6 protein comprising at least 6 contiguous amino acids from the PLA2G6 and comprising the amino acids RL, which correspond to the amino acids at positions 395-396 of SEQ ID NO:6.

61. A peptide comprising at least 6 contiguous amino acids from one of SEQ ID NOS:786 or 1364-1395.

62. A peptide having at least 70% sequence identity to a peptide of SEQ ID NO:786 or 1364-1395.

63. A peptide comprising at least 6 contiguous amino acids from a peptide of Table 1a, Table 1b, Table 1c, or 4, wherein the peptide comprises an alternative splice site junction.

64. A peptide comprising at least 6 contiguous amino acids encoded by an alternatively spliced nucleic acid, wherein the at least 6 contiguous amino acids are encoded on a nucleic acid that comprises an alternative splice site junction, and wherein the alternative splice site junction is an AS event selected from an AS event in Table 3a or 3b.

65. The peptide of claim 64, wherein the AS event is selected from an AS event in Table 3a.

66. The peptide of claim 65, wherein the AS event is selected from an AS event in Table 3b.

67. The peptide of claim 55, wherein the peptide comprises an amino acid sequence selected from SEQ ID NO:7-9.

68. The peptide of claim 56, wherein the peptide comprises an amino acid sequence of SEQ ID NO:10.

69. The peptide of claim 57, wherein the peptide comprises an amino acid sequence of SEQ ID NO:11 or 12.

70. The peptide of claim 58, wherein the peptide comprises an amino acid sequence selected from SEQ ID NO:13-15.

71. The peptide of claim 59, wherein the peptide comprises an amino acid sequence selected from SEQ ID NO:16-22.

72. The peptide of claim 60, wherein the peptide comprises an amino acid sequence selected from SEQ ID NO:23-29.

73. The peptide of any one of claims 55-72, wherein the peptide comprises at least 10 amino acids.

74. The peptide of any one of claims 55-72, wherein the peptide consists of 10 amino acids.

75. The peptide of any one of claims 55-74, wherein the peptide is less than 20 amino acids in length.

76. The peptide of any one of claims 55-75, wherein the peptide is modified.

77. The peptide of claim 76, wherein the modification comprises conjugation to a molecule.

78. The peptide of claim 76 or 77, wherein the molecule comprises an antibody, a lipid, an adjuvant, or a detection moiety.

79. A composition comprising the peptide of any one of claims 55-78.

80. The composition of claim 79, wherein the composition is formulated as a vaccine.

81. The composition of claim 79 or 80, wherein the composition further comprises an adjuvant.

82. A nucleic acid encoding for the peptide of any one of claims 55-78.

83. An expression vector comprising the nucleic acid of claim 82.

84. A host cell comprising the nucleic acid of claim 82 or the expression vector of claim 83.

85. An in vitro isolated dendritic cell comprising the peptide of any one of claims 55-78, the nucleic acid of claim 82, or the expression vector of claim 83.

86. The dendritic cell of claim 85, wherein the dendritic cell comprises a mature dendritic cell.

87. The dendritic cell of claim 85 or 86, wherein the cell is a cell with an HLA type selected from HLA-A, HLA-B, or HLA-C.

88. The dendritic cell of claim 85 or 86, wherein the cell is a cell with an HLA type selected from HLA-A*02:01, HLA-A*03:01, HLA-A*23:01, HLA-A*68:02, HLA-B*07:05, HLA-B*18:01, HLA-B*40:01, HLA-C*03:03, HLA-C*14:02, or HLA-C*15:02.

89. A method of making a cell comprising transferring the nucleic acid of claim 82 or the expression vector of claim 83 into the cell.

90. The method of claim 89, wherein the method further comprises isolating the expressed peptide or polypeptide.

91. An *in vitro* method for making a dendritic cell vaccine comprising contacting a mature dendritic cell *in vitro* with a peptide of any one of claims 55-78.

92. The method of claim 91, wherein the method further comprises screening the dendritic cell for one or more cellular properties.

93. The method of claim 91 or 92, wherein the method further comprises contacting the cell with one or more cytokines or growth factors.

94. The method of claim 93, wherein the one or more cytokines or growth factors comprises GM-CSF.

95. The method of claim 92, wherein the cellular property comprises cell surface expression of one or more of CD86, HLA, and CD14.

96. The method of any one of claims 91-95, wherein the dendritic cell is derived from a CD34+ hematopoietic stem or progenitor cell.

97. The method of any one of claims 91-95, wherein the dendritic cell is derived from a peripheral blood monocyte (PBMC).

98. The method of any one of claims 91-95, wherein the dendritic cells are cells in which the DCs are derived are isolated by leukaphereses.

99. An *in vitro* composition comprising a dendritic cell and the peptide of any one of claims 55-78.

100. The composition of claim 99, wherein the composition further comprises one or more cytokines, growth factors, or adjuvants.

101. The composition of claim 100, wherein the composition comprises GM-CSF.

102. The composition of claim 101, wherein the peptide and GM-CSF are linked.

103. The composition of claim any one of claims 99-103, wherein the composition is determined to be serum-free, mycoplasma-free, endotoxin-free, and sterile.

104. The composition of any one of claims 99-103, wherein the peptide is on the surface of the dendritic cell.

105. The composition of claim 104, wherein the peptide is bound to a MHC molecule on the surface of the dendritic cell.

106. The composition of any one of claims 99-105, wherein the composition is enriched for dendritic cells expressing CD86 on the surface of the cell.

107. The composition of any one of claims 99-106, wherein the dendritic cell comprises a monocyte-derived dendritic cell.

108. The composition of any one of claims 99-106, wherein the dendritic cell is derived from a CD34+ hematopoietic stem or progenitor cell.

109. The composition of any one of claims 99-106, wherein the dendritic cell is derived from a peripheral blood monocyte (PBMC).

110. The composition of any one of claims 99-109, wherein the dendritic cells or the cells in which the DCs are derived from are isolated by leukaphereses.

111. An engineered T-cell Receptor (TCR) or chimeric antigen receptor (CAR) that specifically recognizes the peptide of any one of claims 55-78.

112. A cell comprising the TCR or CAR of claim 111.

113. The cell of claim 112, wherein the cell comprises at least one TCR and at least one CAR and wherein the TCR and CAR each recognize a different peptide.

114. The cell of claim 112 or 113, wherein the cell comprises a stem cell, a progenitor cell, or a T cell.

115. The cell of claim 114, wherein the cell comprises a hematopoietic stem or progenitor cell, a T cell, or an induced pluripotent stem cell (iPSC).

116. An antibody or antigen binding fragment thereof that specifically recognizes the peptide of any one of claims 55-78.

117. A method of treating a subject for brain cancer comprising administering the peptide of any one of claims 55-78, the composition of any one of claims 79-81 or 99-110, the dendritic cell of any one of claims 85-88, or the cell of any one of claims 112-115 or the antibody or antigen binding fragment of claim 116.

118. The method of claim 117, wherein the method comprises administering a cell or a composition comprising a cell and wherein the cell comprises an autologous cell.

119. The method of claim 117 or 118, wherein the cancer comprises glioblastoma or glioma.

120. The method of any one of claims 117-119, wherein the subject has previously been treated for the cancer.

121. The method of claim 120, wherein the subject has been determined to be resistant to the previous treatment.

122. The method of any one of claims 117-121, wherein the method further comprises the administration of an additional therapy.

123. The method of any one of claims 117-122, wherein the cancer comprises stage I, II, III, or IV cancer.

124. The method of any one of claims 117-123, wherein the cancer comprises metastatic and/or recurrent cancer.

125. A method of activating or expanding peptide-specific T cells comprising contacting a starting population of T cells from a mammalian subject and preferably from a blood sample from the mammalian subject cells ex vivo with the peptide of any one of claims 55-78 thereby activating, stimulating proliferation, and/or expanding peptide-specific T cells in the starting population.

126. The method of claim 125, wherein contacting is further defined as co-culturing the starting population of T cells with antigen presenting cells (APCs), wherein the APCs can present the peptide of any one of claims 55-78 on their surface.

127. The method of claim 126, wherein the APCs are dendritic cells.

128. The method of claim 127, wherein the dendritic cells are autologous dendritic cells obtained from the mammalian subject.

129. The method of claim 125, wherein contacting is further defined as co-culturing the starting population of T cells with artificial antigen presenting cells (aAPCs).

130. The method of claim 129, wherein the artificial antigen presenting cells (aAPCs) comprise or consist of poly(lactide-co-glycolide) (PLGA), K562 cells, paramagnetic beads coated with CD3 and CD28 agonist antibodies, beads or microparticles coupled with an HLA-dimer and anti-CD28, or nanosize-aAPCs (nano-aAPC) that are preferably less than 100 nm in diameter.

131. The method of any one of claims 125-130, wherein the T cells are CD8+ T cells or CD4+ T cells.

132. The method of any one of claims 125-131, wherein the T cells are cytotoxic T lymphocytes (CTLs).

133. The method of any one of claims 125-132, wherein the starting population of cells comprises or consists of peripheral blood mononuclear cells (PBMCs).

134. The method of claim 133, wherein the method further comprises isolating or purifying the T cells from the peripheral blood mononuclear cells (PBMCs).

135. The method of any one of claims 125-134, wherein the mammalian subject is a human.

136. The method of any one of claims 125-135, wherein the method further comprises reinfusing or administering the activated or expanded peptide-specific T cells to the subject.

137. A peptide-specific T cell activated or expanded according to any one of claims 125-136.

138. A pharmaceutical composition comprising the peptide-specific T cells activated or expanded according to any one of claims 125-136.

a

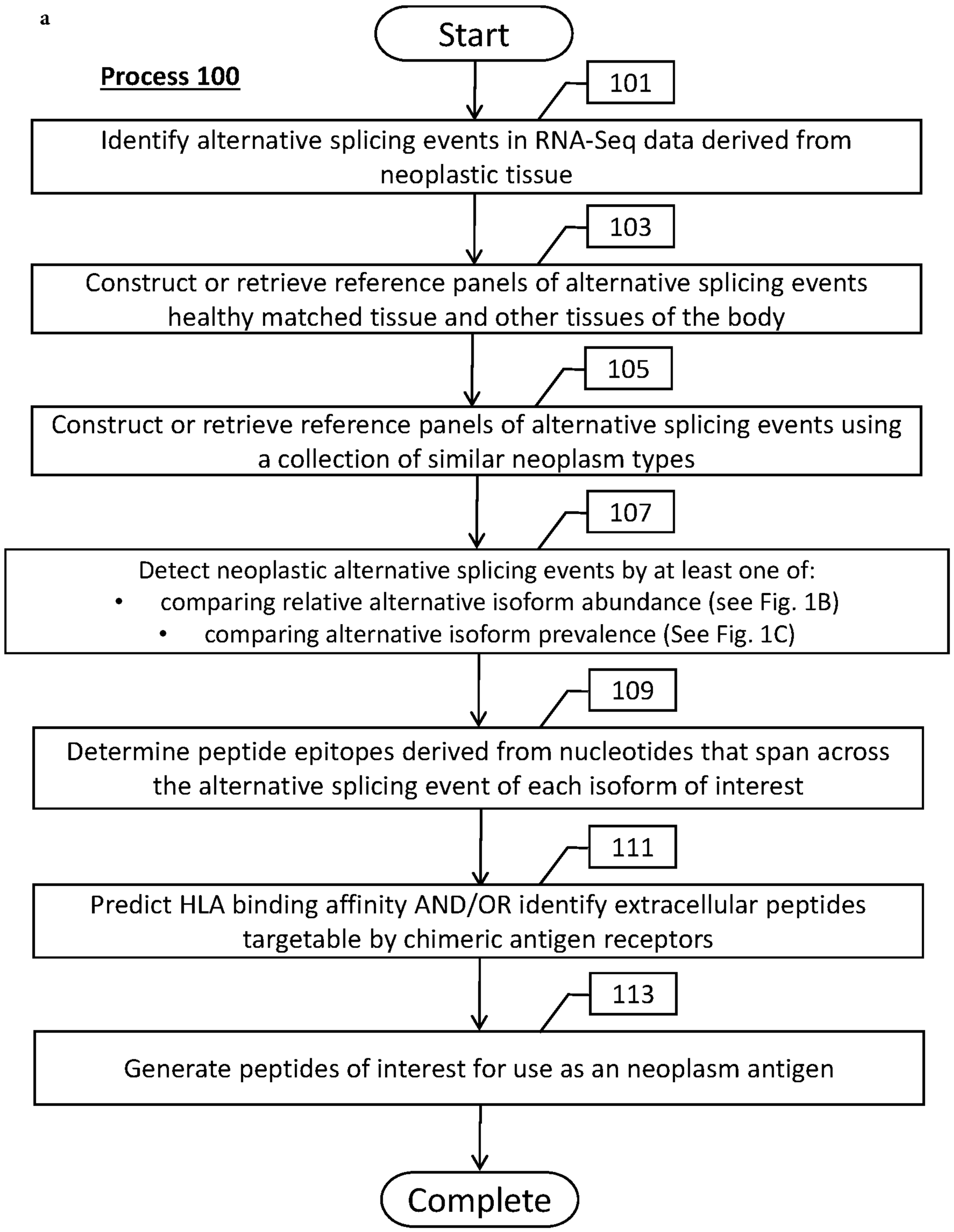

Start
Process 100
101
Identify alternative splicing events in RNA-Seq data derived from neoplastic tissue
103
Construct or retrieve reference panels of alternative splicing events healthy matched tissue and other tissues of the body
105
Construct or retrieve reference panels of alternative splicing events using a collection of similar neoplasm types
107
Detect neoplastic alternative splicing events by at least one of:
• comparing relative alternative isoform abundance (see Fig. 1B)
• comparing alternative isoform prevalence (See Fig. 1C)
109
Determine peptide epitopes derived from nucleotides that span across the alternative splicing event of each isoform of interest
111
Predict HLA binding affinity AND/OR identify extracellular peptides targetable by chimeric antigen receptors
113
Generate peptides of interest for use as an neoplasm antigen
Complete

Fig. 1A

b

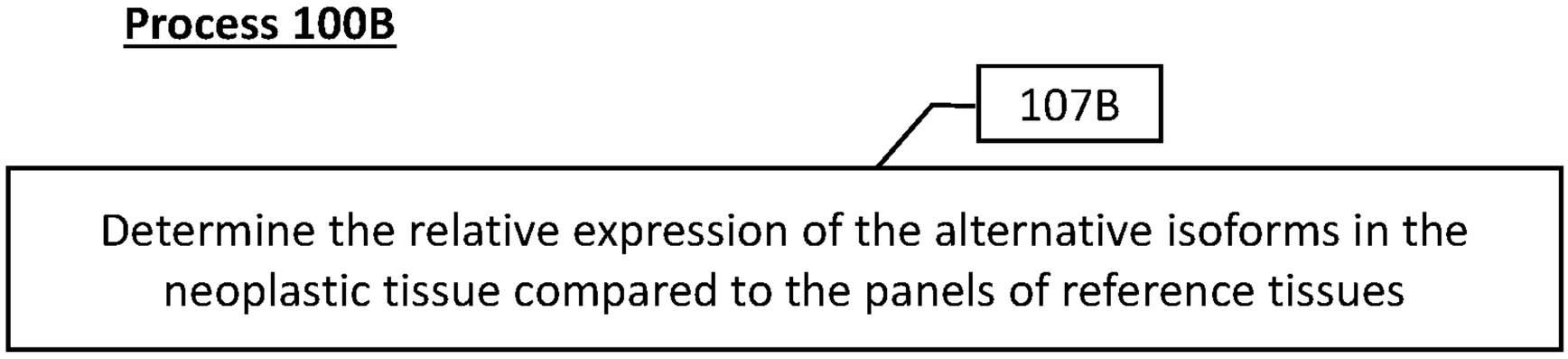


Fig. 1B

c

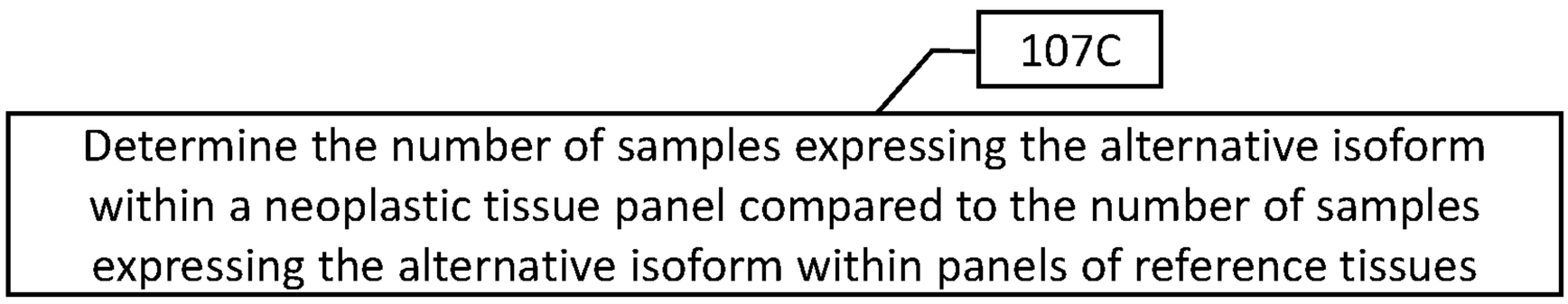


Fig. 1C

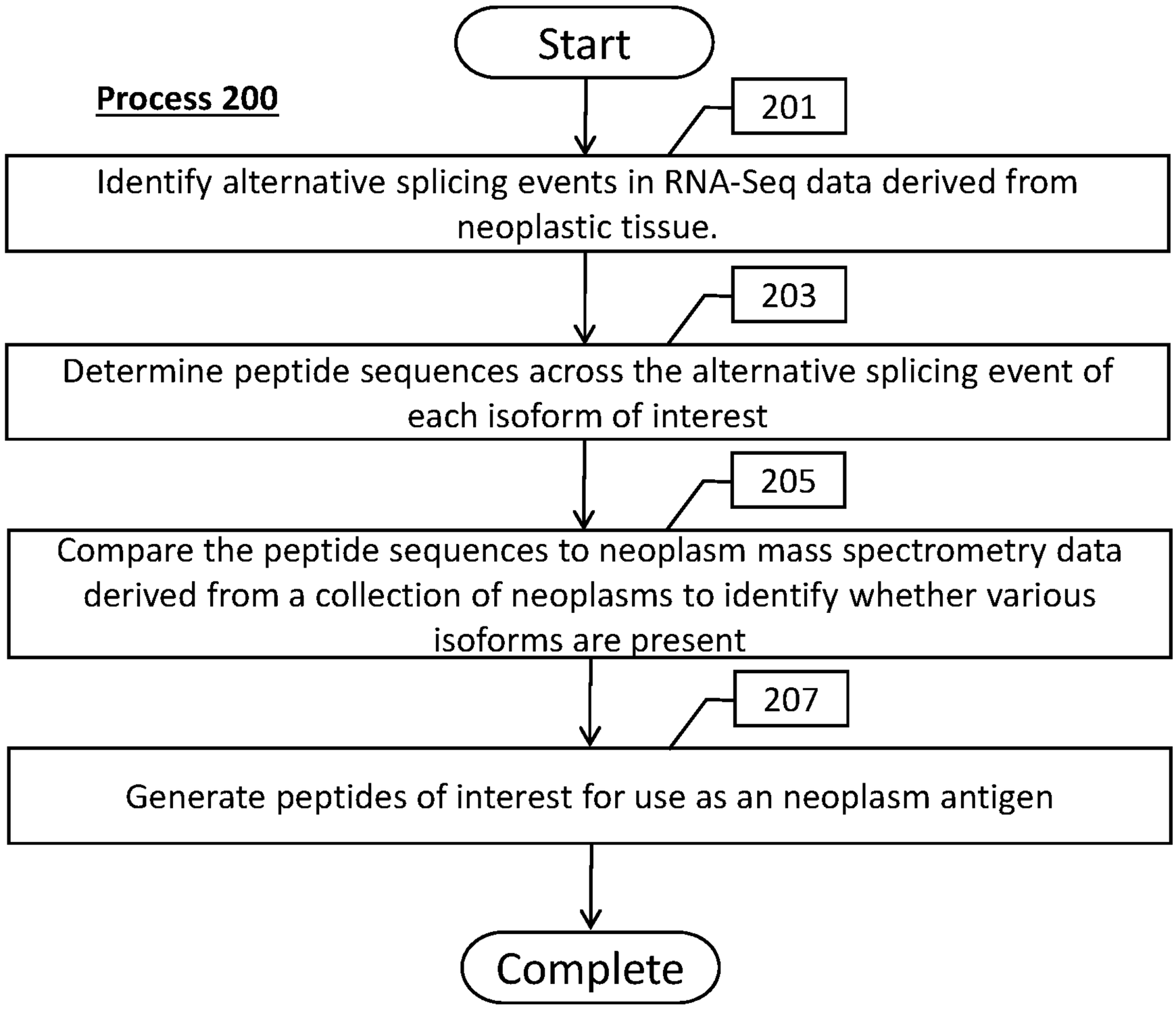

Start
Process 200
201
Identify alternative splicing events in RNA-Seq data derived from neoplastic tissue.
203
Determine peptide sequences across the alternative splicing event of each isoform of interest
205
Compare the peptide sequences to neoplasm mass spectrometry data derived from a collection of neoplasms to identify whether various isoforms are present
207
Generate peptides of interest for use as an neoplasm antigen
Complete

Fig. 2

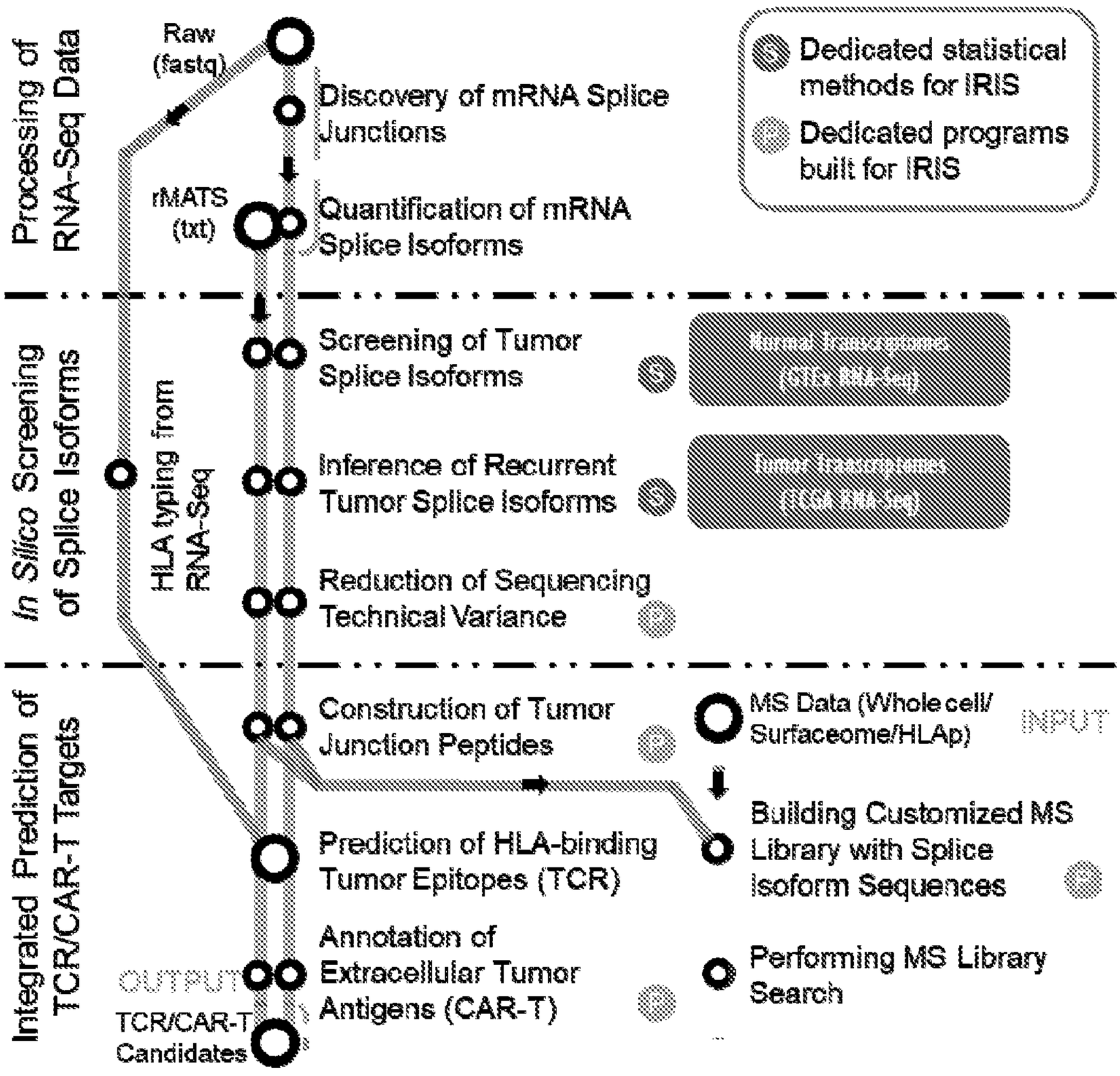

Processing of RNA-Seq Data
Raw (fastq)
Discovery of mRNA Splice Junctions
rMATS (txt)
Quantification of mRNA Splice Isoforms
In Silico Screening of Splice Isoforms
HLA typing from RNA-Seq
Screening of Tumor Splice Isoforms
Inference of Recurrent Tumor Splice Isoforms
Reduction of Sequencing Technical Variance
Integrated Prediction of TCR/CAR-T Targets
Construction of Tumor Junction Peptides
Prediction of HLA-binding Tumor Epitopes (TCR)
Annotation of Extracellular Tumor Antigens (CAR-T)
OUTPUT
TCR/CAR-T Candidates
MS Data (Whole cell/ Surfaceome/HLAp)
INPUT
Building Customized MS Library with Splice Isoform Sequences
Performing MS Library Search
Dedicated statistical methods for IRIS
Dedicated programs built for IRIS

Fig. 3

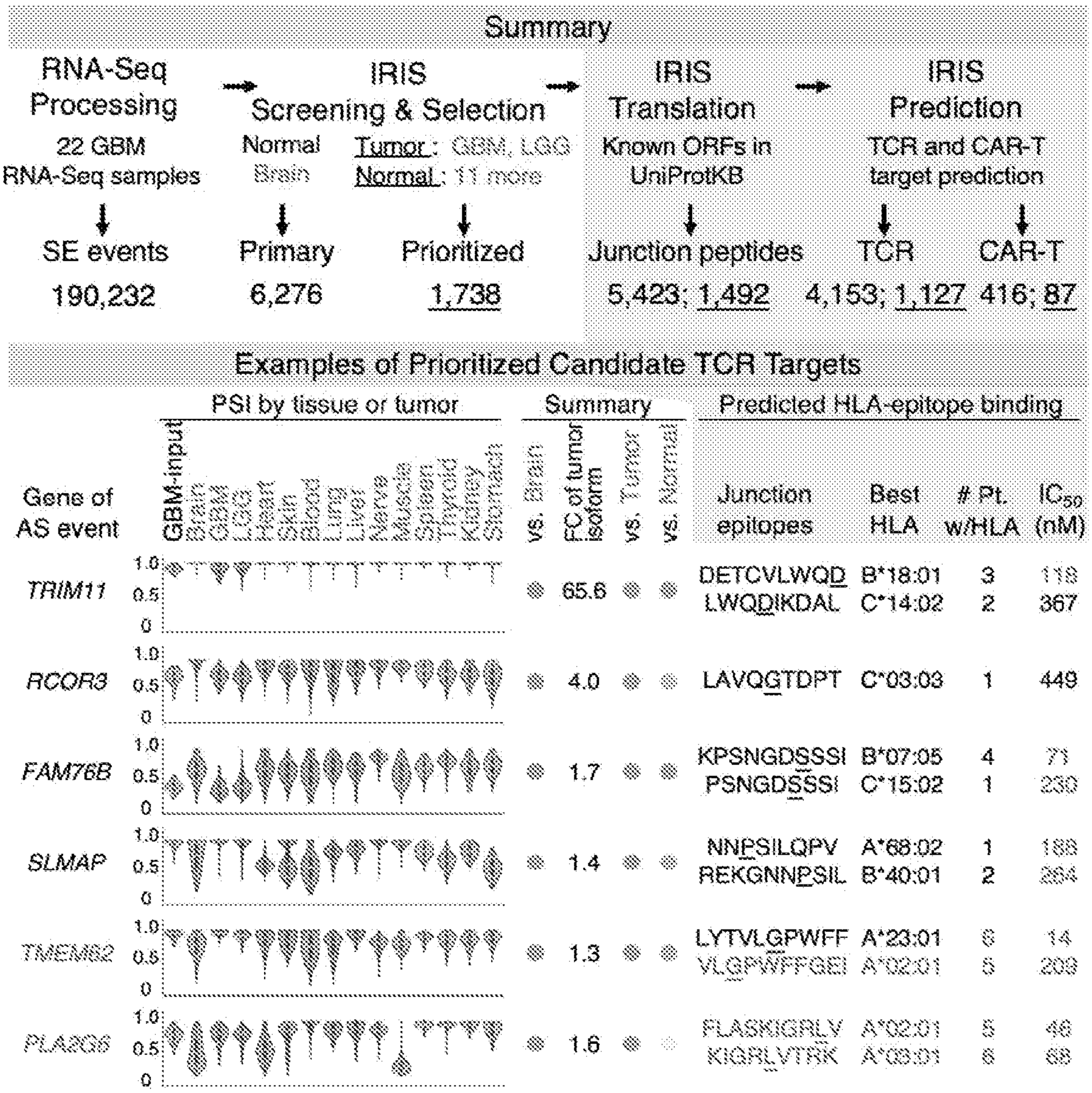

Summary
RNA-Seq Processing
22 GBM RNA-Seq samples
SE events
190,232
IRIS Screening & Selection
Normal Brain
Primary
6,276
Tumor: GBM, LGG
Normal: 11 more
Prioritized
1,738
IRIS Translation
Known ORFs in UniProtKB
Junction peptides
5,423; 1,492
IRIS Prediction
TCR and CAR-T target prediction
TCR
4,153; 1,127
CAR-T
416; 87
Examples of Prioritized Candidate TCR Targets
PSI by tissue or tumor
GBM-input
Brain
GBM
Lung
Breast
Skin
Blood
Lung
Liver
Uterus
Muscle
Spleen
Thyroid
Kidney
Stomach
Summary
vs. Brain
FC of tumor isoform
vs. Tumor
vs. Normal
Predicted HLA-epitope binding
Gene of AS event
Junction epitopes
Best HLA
Pt. w/HLA
IC50 (nM)
TRIM11
65.6
DETCVLWQD B*18:01 3 118
LWQDIKDAL C*14:02 2 367
RCOR3
4.0
LAVQGTDPT C*03:03 1 449
FAM76B
1.7
KPSNGDSSSI B*07:05 4 71
PSNGDSSSI C*15:02 1 230
SLMAP
1.4
NNPSILQPV A*68:02 1 188
REKGNNPSIL B*40:01 2 264
TMEM82
1.3
LYTVLGPWFF A*23:01 6 14
VLGPWFFGEI A*02:01 5 209
PLA2G6
1.6
FLASKIGRLV A*02:01 5 46
KIGRLVTRK A*03:01 6 68

FIG. 4

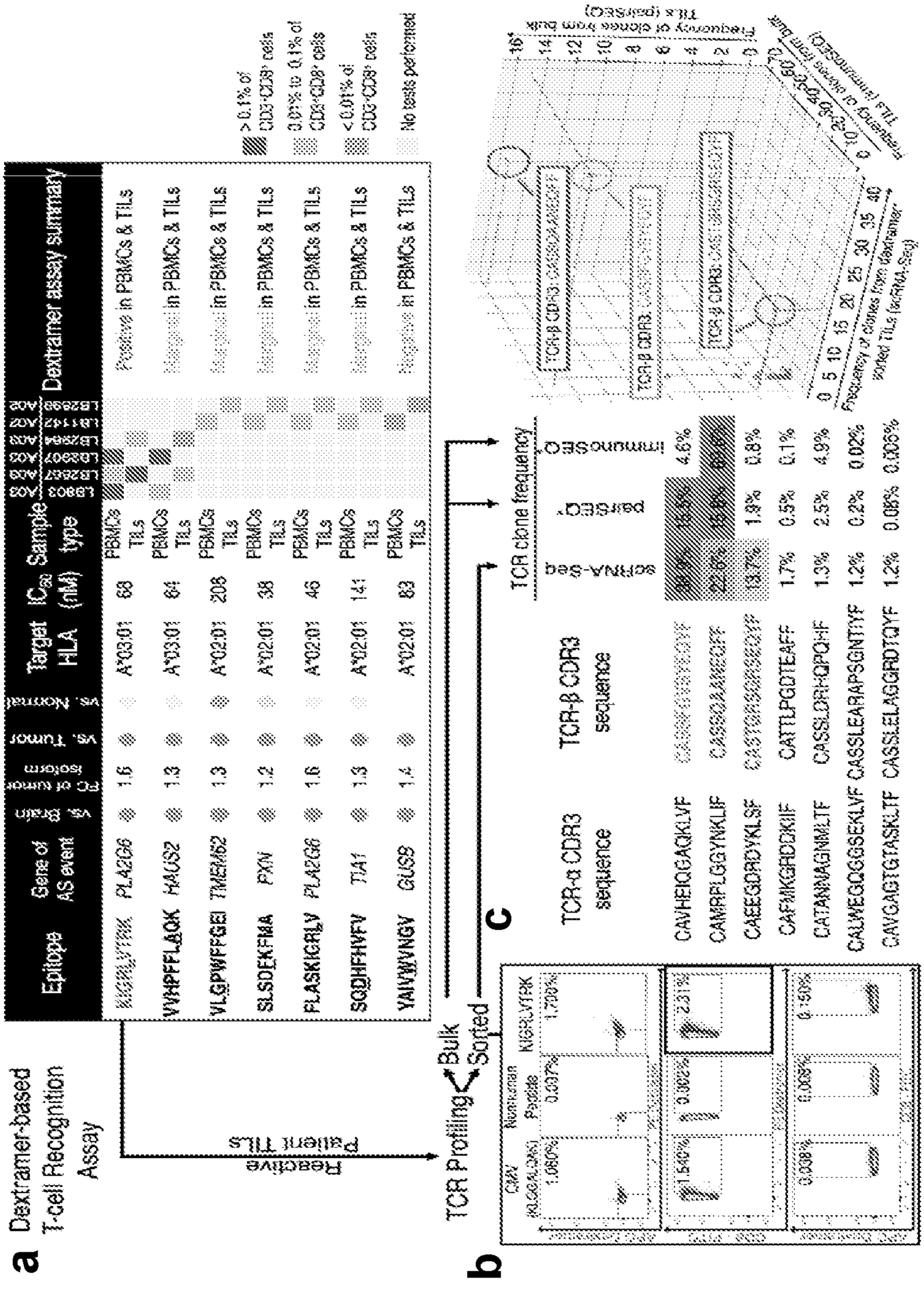
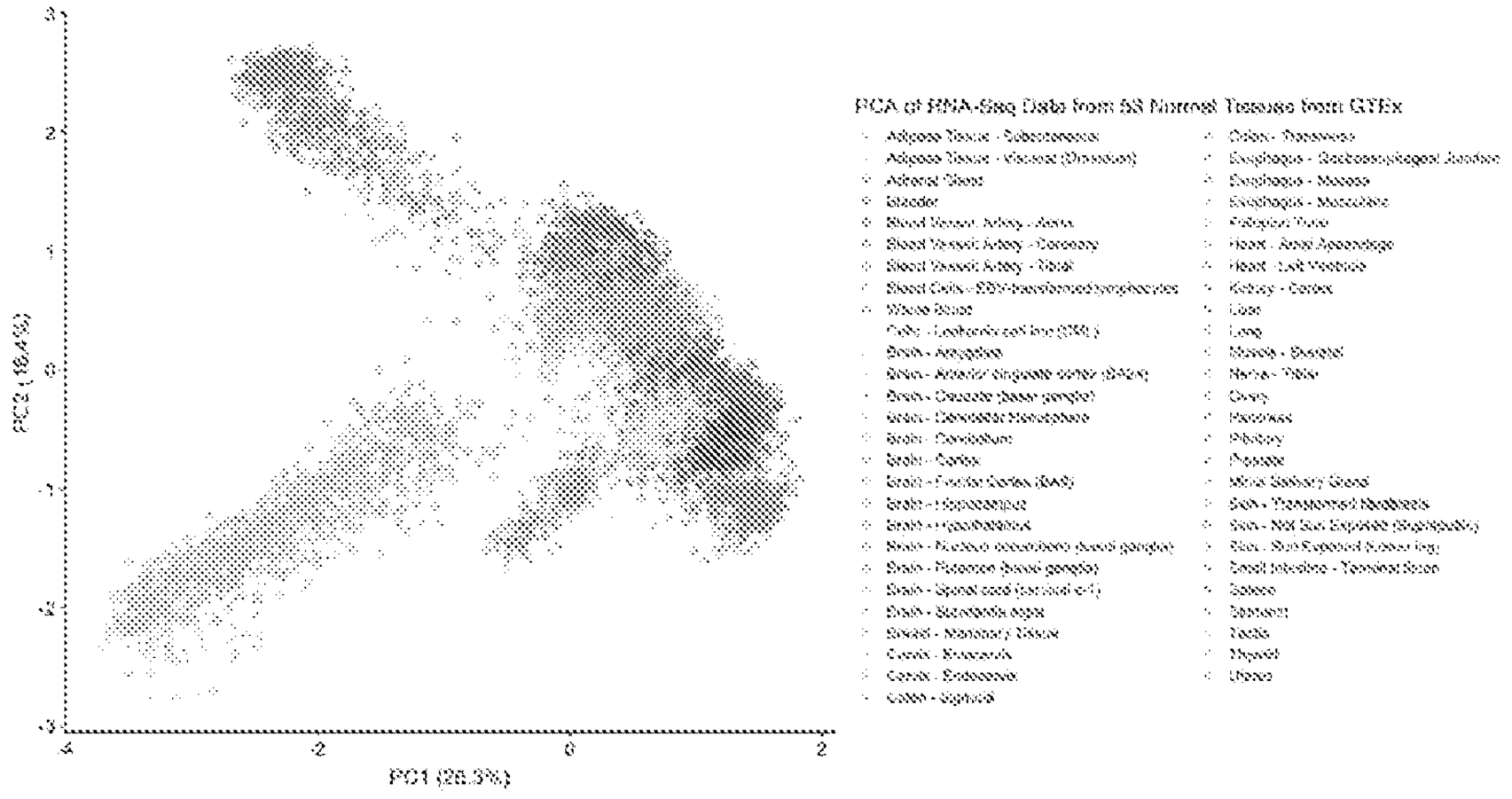

Dextramer-based T-cell Recognition Assay
Reactive Patient TILs
TCR Profiling
Bulk
Sorted
Epitope
Gene of AS event
Target HLA
IC50 (nM)
Sample type
Dextramer assay summary
KIGRNLYTRK
PLA2G6
A*03:01
68
PBMCs
TILs
Positive in PBMCs & TILs
VVHPFFLAQK
HAUS2
A*03:01
64
PBMCs
TILs
Negative in PBMCs & TILs
VLGPWFFGEI
TMEM62
A*02:01
208
PBMCs
TILs
Negative in PBMCs & TILs
SLSDEKFMA
PXN
A*02:01
38
PBMCs
TILs
Negative in PBMCs & TILs
FLASKKGRLV
PLA2G6
A*02:01
46
PBMCs
TILs
Negative in PBMCs & TILs
SQDHFHVFV
TIA1
A*02:01
141
PBMCs
TILs
Negative in PBMCs & TILs
YAIVWVNGV
GUSB
A*02:01
83
PBMCs
TILs
Negative in PBMCs & TILs
> 0.1% of CD3+CD8+ cells
0.01% to 0.1% of CD3+CD8+ cells
< 0.01% of CD3+CD8+ cells
No tests performed
TCR clone frequency
TCR-β CDR3 sequence
scRNA-seq
pairSEQ
Immunoseq
CASSIRSSYEQYF
CASSQAMEQFF
CASSRDRGGEQYF
CATTLPGDTEAFF
CASSLDRHQPQHF
CASSLEARAPSGNTIYF
CASSELAGGRDTQYF
TCR-β CDR3 sequence
Frequency of clones from bulk TILs (scRNA-Seq)
Frequency of clones from dextramer sorted TILs (scRNA-Seq)
TCR-α CDR3 sequence
CAVHEIQGAQKLVF
CAMRFLGGYNKLIF
CAEESGDRDYKLSF
CAFMKGRDDKIIF
CATANNAGNMLTF
CALMEGQGGSEKLVF
CAVGAGTGTASKLTF
Gene
Normal Peptide
NIGRLVTRK
a
b
c

FIG. 5A-C

a



b

Summary of 53 Normal Tissues from GTEx

Tissue	Sample Size	Events Detected	Events Selected	Tissue	Sample Size	Events Detected	Events Selected
Adipose	620	293,489	177,621	**Prostate**	119	145,285	77,386
Adrenal	159	154,489	103,394	**Salivary**	70	129,283	68,459
Blood	595	305,861	185,067	**Skin**	974	342,777	212,661
Blood Vessel	750	303,856	190,594	**Small Intestine**	104	152,146	84,231
Bone Marrow	102	177,163	126,033	**Spleen**	118	141,698	93,070
Brain	1,409	399,295	221,221	**Stomach**	204	176,310	114,884
Breast	218	197,683	133,548	**Testis**	203	263,987	179,735
Cervix	11	76,175	46,535	**Thyroid**	361	224,850	149,243
Colon	376	224,758	147,544	**Uterus**	90	145,394	101,249
Esophagus	790	281,759	177,670	**Vagina**	97	148,627	101,789
Fallopian	7	69,716	41,180	**Muscle**	475	221,632	132,075
Heart	489	223,803	133,012	**Nerve**	335	222,251	146,857
Kidney	36	102,385	68,138	**Ovary**	108	151,202	103,115
Liver	136	135,295	79,865	**Pancreas**	197	140,163	83,941
Lung	374	273,703	180,005	**Pituitary**	124	158,497	105,267

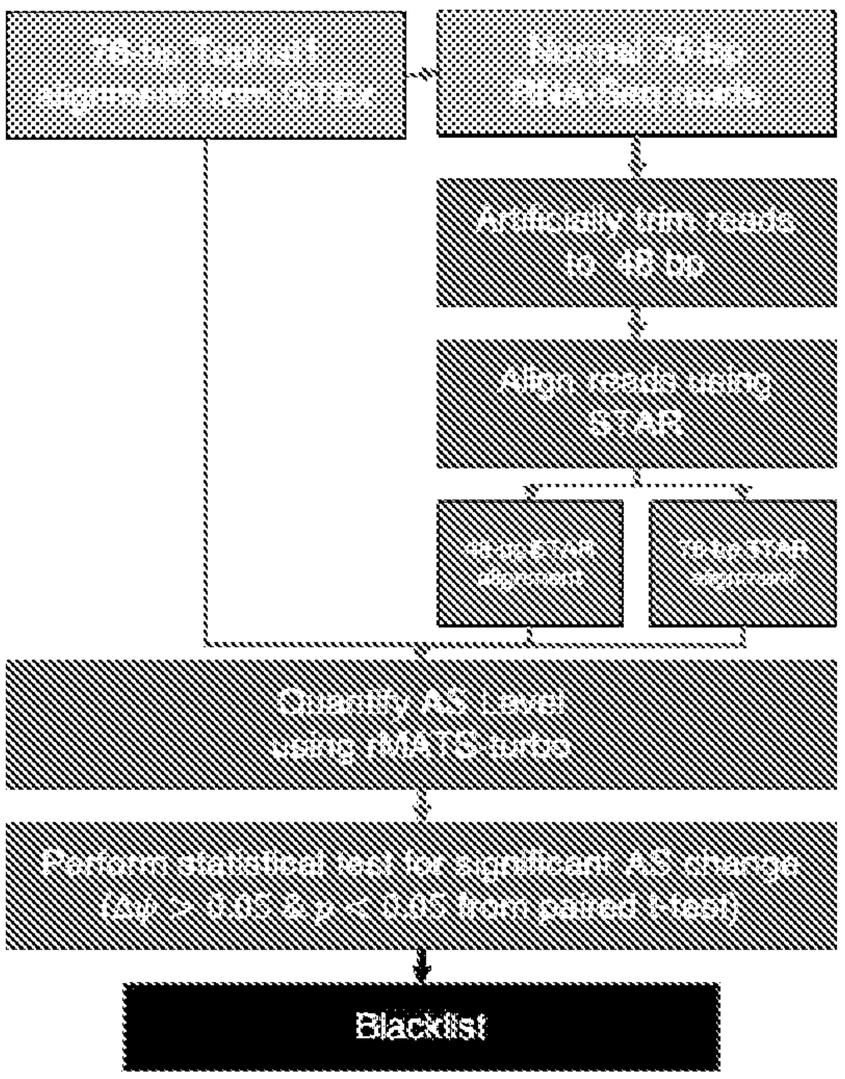
c

Summary of Tumor Reference Panel

Tumor	Sample Size	Events Detected	Events Selected
GBM	162	288,380	190,854
LGG	516	321,777	206,734

FIG. 6A-C

a



* 48-bp Tophat1 alignment is excluded as it does not reflect technical conditions in the study.

b

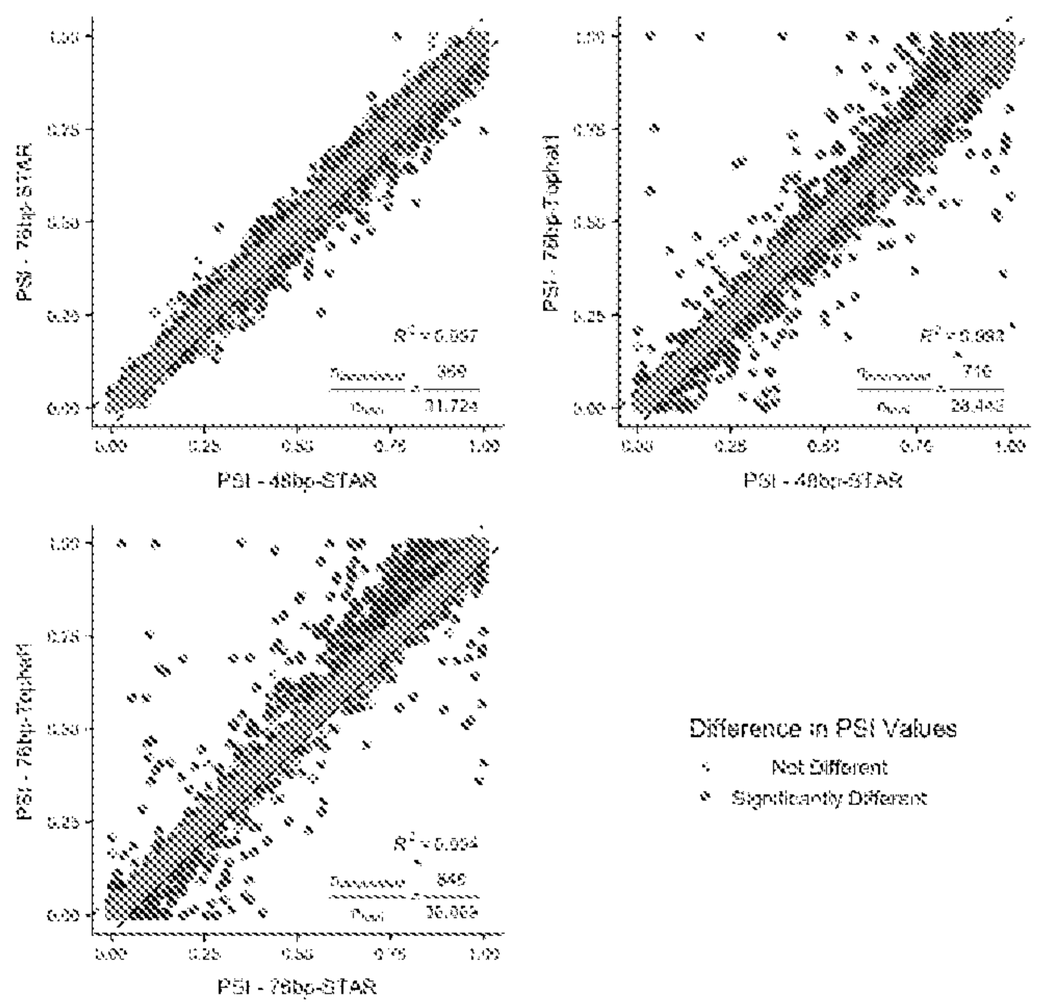
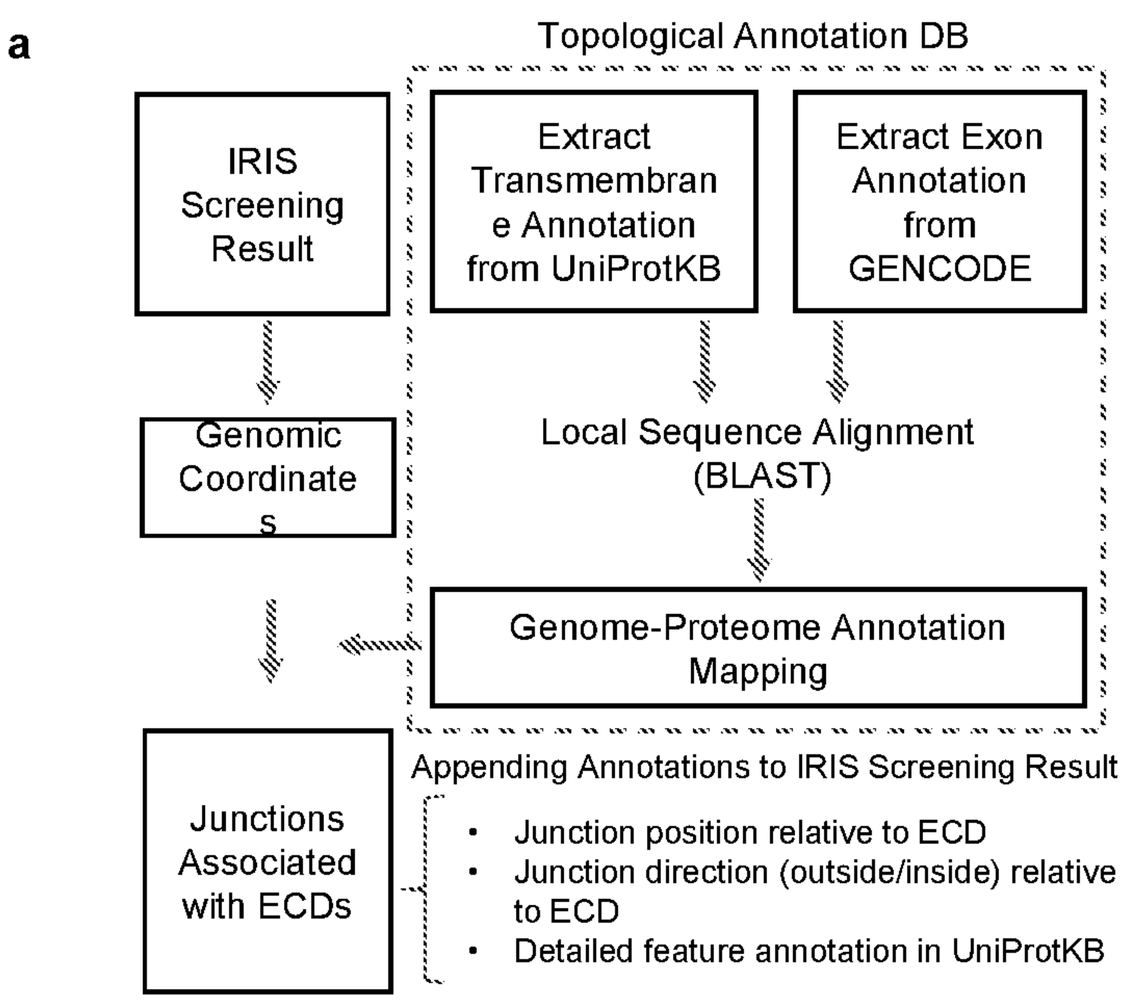


FIG. 7A-B


a
Topological Annotation DB
IRIS Screening Result
Extract Transmembrane Annotation from UniProtKB
Extract Exon Annotation from GENCODE
Genomic Coordinates
Local Sequence Alignment (BLAST)
Genome-Proteome Annotation Mapping
Junctions Associated with ECDs
Appending Annotations to IRIS Screening Result
• Junction position relative to ECD
• Junction direction (outside/inside) relative to ECD
• Detailed feature annotation in UniProtKB

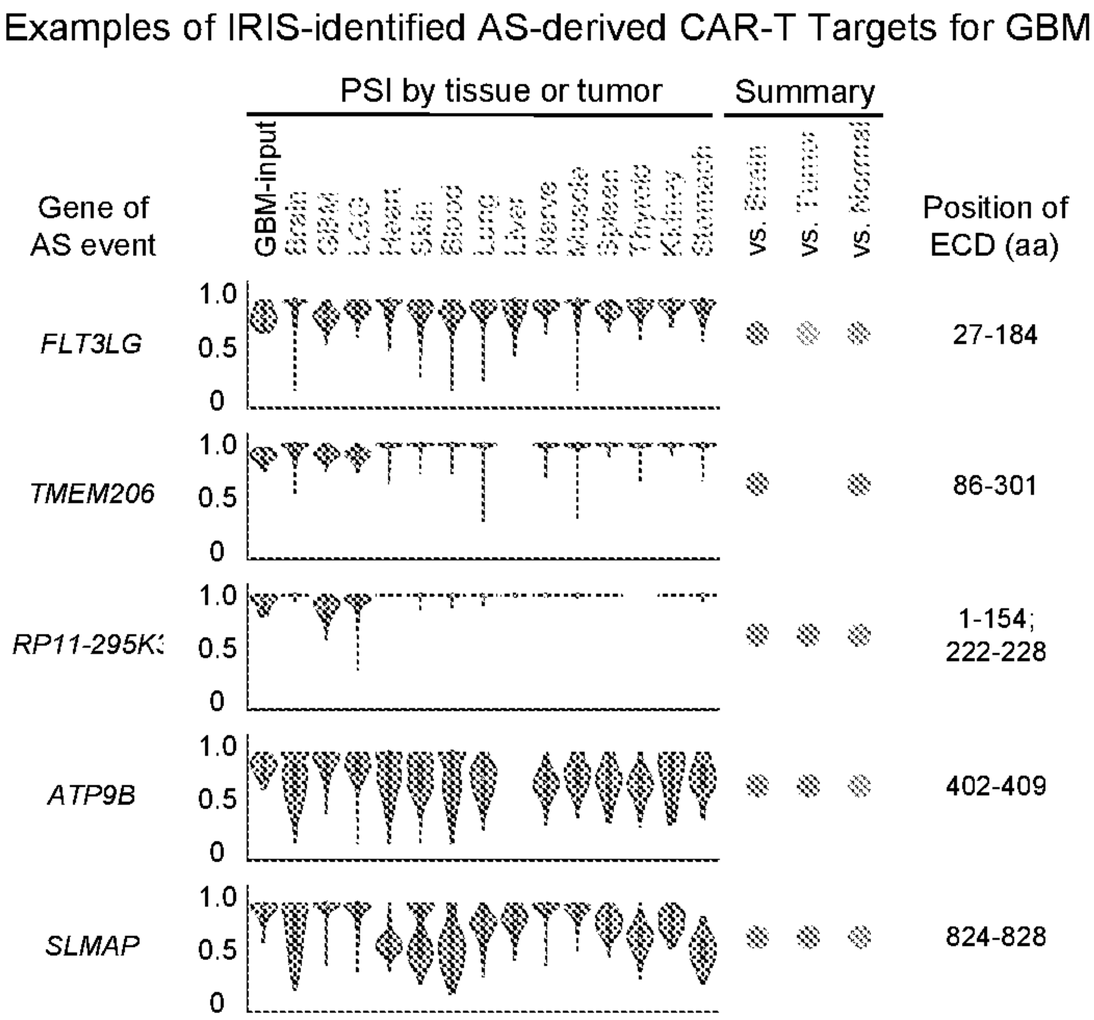
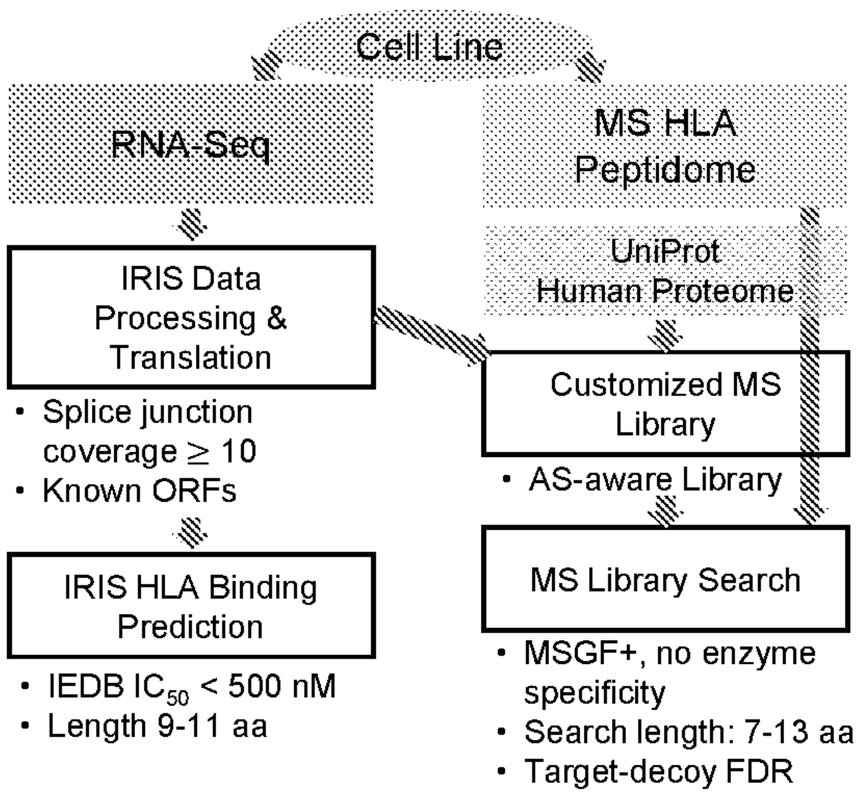

b Examples of IRIS-identified AS-derived CAR-T Targets for GBM
PSI by tissue or tumor
Summary
Gene of AS event
GBM-input
Brain
GBM
LGG
Heart
Skin
Blood
Lung
Liver
Nerve
Muscle
Spleen
Thyroid
Kidney
Stomach
vs. Brain
vs. Tumor
vs. Normal
Position of ECD (aa)
FLT3LG
27-184
TMEM206
86-301
RP11-295K3
1-154; 222-228
ATP9B
402-409
SLMAP
824-828
1.0
0.5
0

FIG. 8A-B

a



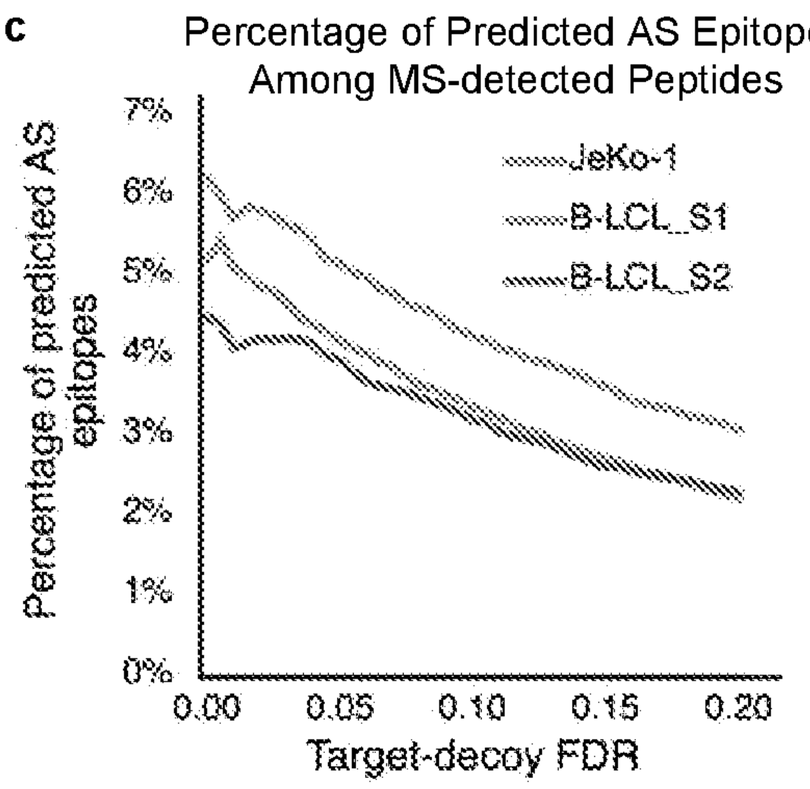
b

Summary of AS Epitope Presentation via IRIS MS Module (FDR 5%)

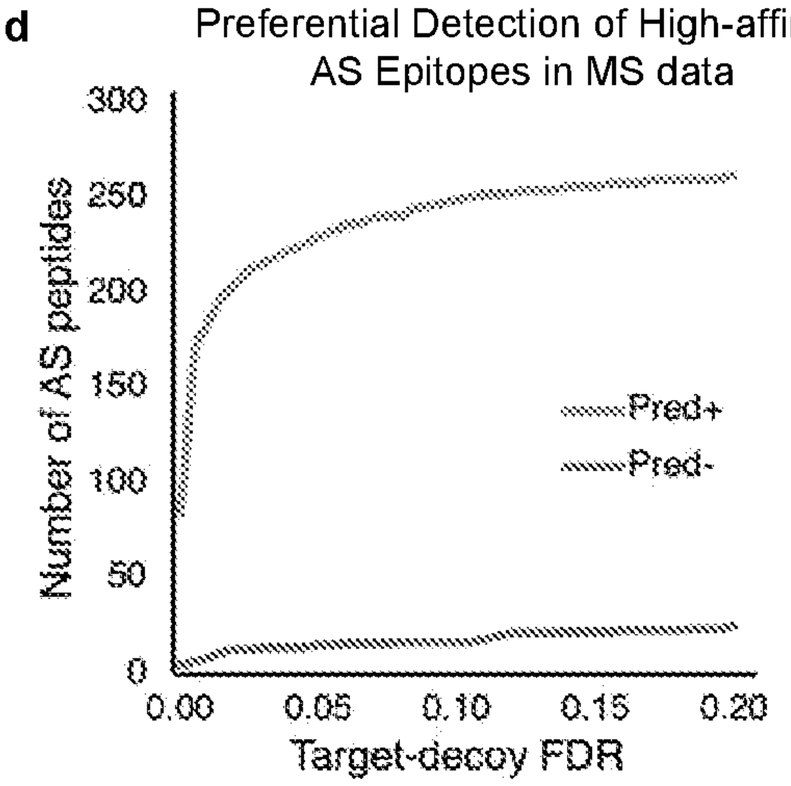
Feature	JeKo-1	B-LCL-S1	B-LCL-S2
PSMs	31,427	15,049	10,197
Unique peptides	5,514	3,472	2,169
Predicted AS epitopes*	78,332	103,032	79,742
MS-validated AS epitopes	230	178	85

*IC_{50} < 500 nM

c



d



e

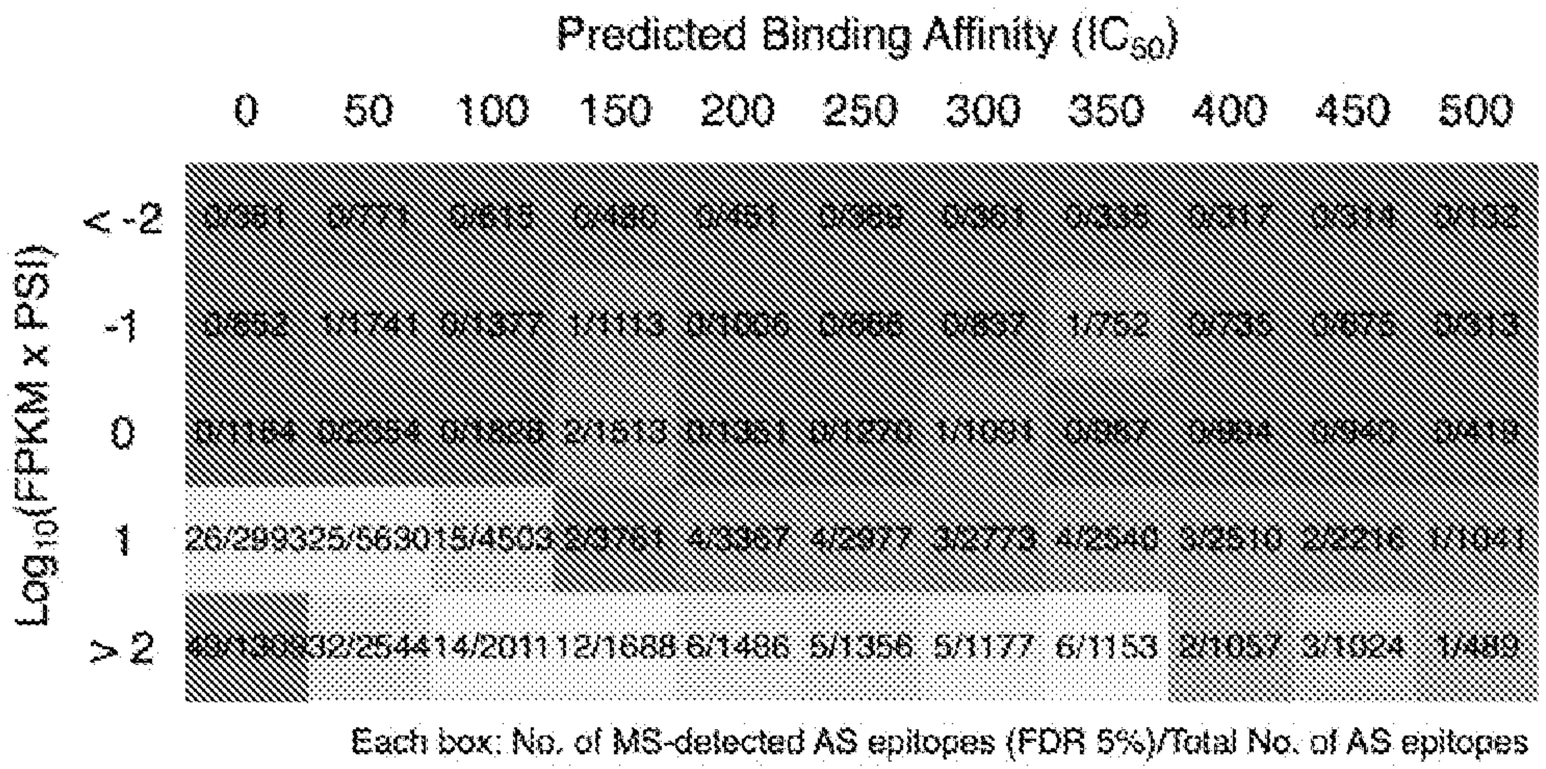
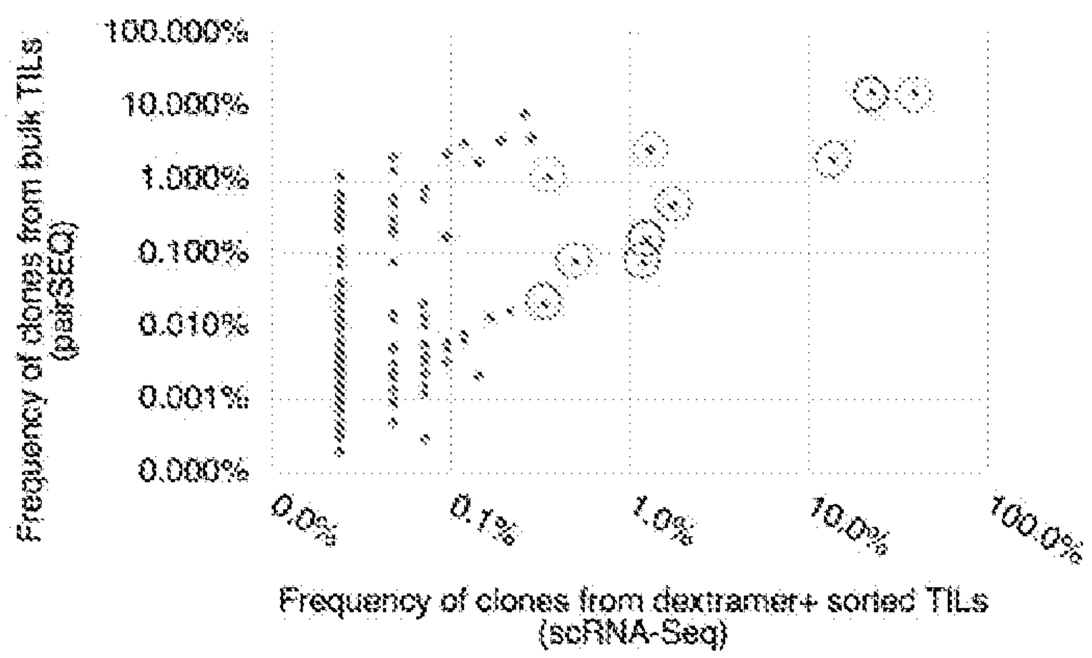


FIG. 9A-E

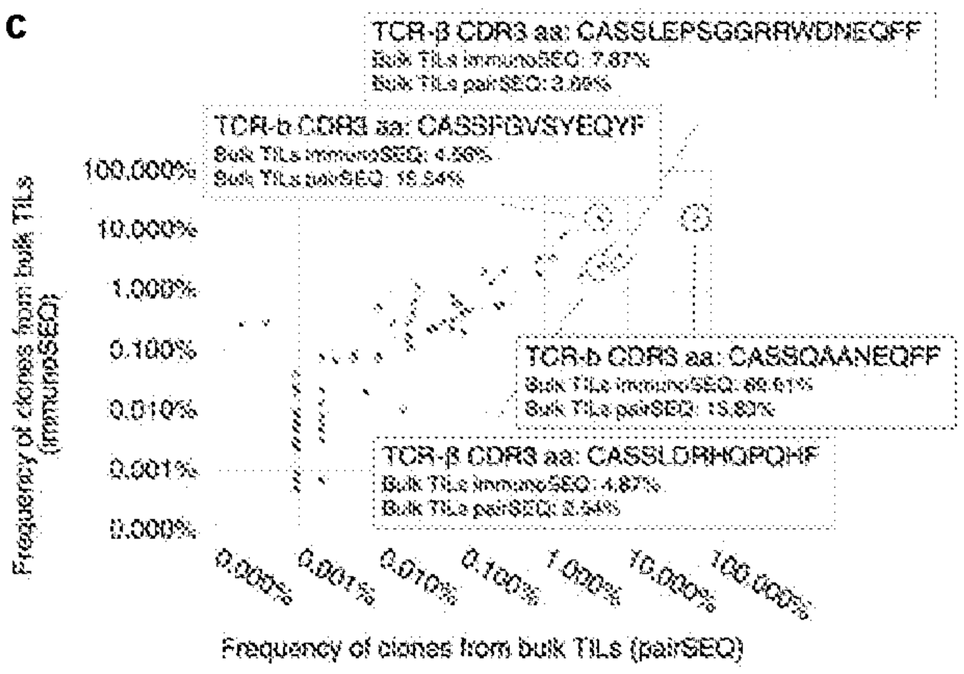
a



b

Amino acid sequence		TCR clone frequency	
TCR-α CDR3	TCR-β CDR3	Dextramer+ sorted TILs (scRNA-Seq)	Bulk TILs (pairSEQ)
CAVHEIQGAQKLVF	CASSFGVSYEQYF	38.8876%	15.5410%
CAMRPLGGYNKLIF	CASSQAANEQFF	22.5520%	15.8253%
CAEEGDRDYKLSF	CASTGRSGRSEQYF	13.7415%	1.8803%
CAFMKGRDDKIIF	CATTLPGDTEAFF	1.7294%	0.4544%
CATANNAGNMLTF	CASSLDRHQPQHF	1.2853%	2.5376%
CALWEGQGGSEKLVF	CASSLEARAPSGNTIYF	1.2386%	0.1565%
CAVGAGTGTASKLTF	CASSLELAGGRDTQYF	1.1919%	0.0778%
CAVFQGGSEKLVF; CAAADFSGTYKYIF	CASSPEPQGANFYEQYF	0.4908%	0.0802%
CALSEGSNFGNEKLTF	CASSEGTVLDEQYF	0.3505%	1.1144%
CAAGGNYGGSQGNLIF	CASSLGSSTQYF	0.3272%	0.0214%

c



d

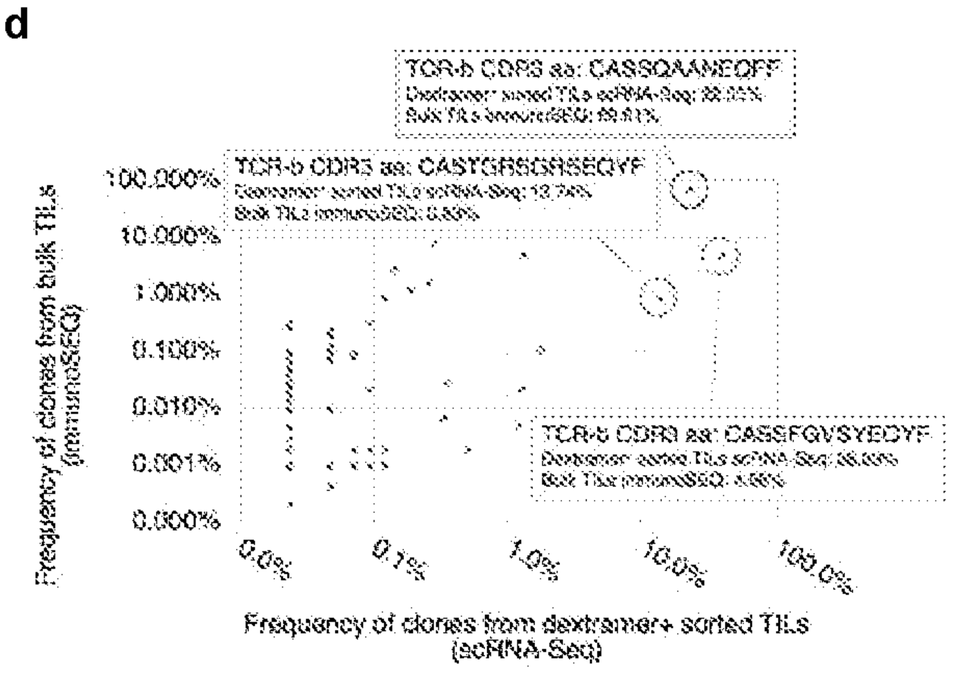


FIG. 10A-D

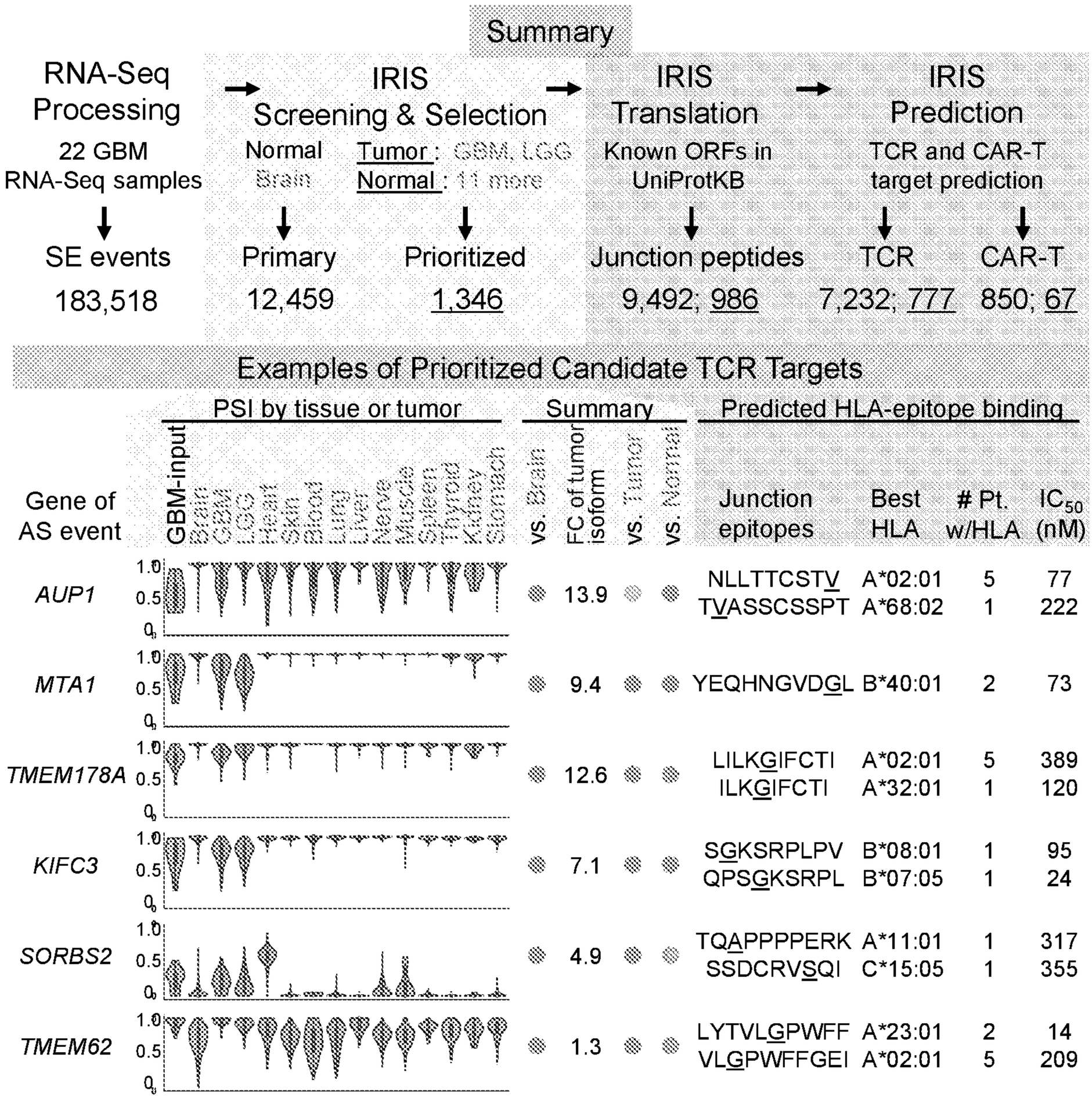

Summary
RNA-Seq Processing
22 GBM RNA-Seq samples
SE events
183,518
IRIS Screening & Selection
Normal Brain
Tumor: GBM, LGG
Normal: 11 more
Primary
12,459
Prioritized
1,346
IRIS Translation
Known ORFs in UniProtKB
Junction peptides
9,492; 986
IRIS Prediction
TCR and CAR-T target prediction
TCR
7,232; 777
CAR-T
850; 67
Examples of Prioritized Candidate TCR Targets
PSI by tissue or tumor
GBM-input
Brain
GBM
LGG
Heart
Skin
Blood
Lung
Liver
Nerve
Muscle
Spleen
Thyroid
Kidney
Stomach
Summary
vs. Brain
FC of tumor isoform
vs. Tumor
vs. Normal
Predicted HLA-epitope binding
Gene of AS event
Junction epitopes
Best HLA
Pt. w/HLA
IC50 (nM)
AUP1
13.9
NLLTTCSTV A*02:01 5 77
TVASSCSSPT A*68:02 1 222
MTA1
9.4
YEQHNGVDGL B*40:01 2 73
TMEM178A
12.6
LILKGIFCTI A*02:01 5 389
ILKGIFCTI A*32:01 1 120
KIFC3
7.1
SGKSRPLPV B*08:01 1 95
QPSGKSRPL B*07:05 1 24
SORBS2
4.9
TQAPPPPERK A*11:01 1 317
SSDCRVSQI C*15:05 1 355
TMEM62
1.3
LYTVLGPWFF A*23:01 2 14
VLGPWFFGEI A*02:01 5 209

Fig. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/59476

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/68, G16B 20/00, A61K 38/05 (2021.01)

CPC - C12Q 1/6886, C07K 14/47, C12Q 2600/156, G16B 20/00

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)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/0101170 A1 (THE BROAD INSTITUTE INC. et al.) 14 April 2016 (14.04.2016) para [0009],]0050], [0093], [0109]-[0100], [0111], [0162], [0446], [0471]; Figure 11C	1-3
A	US 2019/0279742 A1 (GRITSTONE ONCOLOGY, INC.) 12 September 2019 (12.09.2019) Entire document	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
09 March 2021	MAR 25 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/59476

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/59476

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims 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. ☒ Claims Nos.: 4-24, 29-54, 75-138
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:

---See 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 claims 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 claims Nos.:
1-3

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/US 20/59476

Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-3: drawn to a method to synthesize an antigenic peptide.

Groups II+: Claims 25-28, drawn to an engineered T-cell Receptor. Group II+ will be searched upon payment of additional fees. The composition may be searched, for example, to the extent that TCR-a CDR3, TCR-b CDR3, TCR-a variable region and TCR-b variable region encompasses SEQ ID NOs: 30, 31, 44 and 45, respectively, for an additional fee and election as such. It is believed that claims 25-28 read on this exemplary invention. Additional TCR-a CDR3, TCR-b CDR3, TCR-a variable region and TCR-b variable region will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected TCR-a CDR3, TCR-b CDR3, TCR-a variable region and TCR-b variable region. Failure to clearly identify how any paid additional invention fees are to be applied to the"+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be an engineered T-cell Receptor. comprising TCR-a CDR3, TCR-b CDR3, TCR-a variable region and TCR-b variable region of SEQ ID NOs: 32, 33, 46 and 47, respectively (Claims 25-28).

Groups III+: Claims 55-74, drawn to a peptide comprising at least 6 contiguous amino acids encoded by an alternatively spliced nucleic acid. Group III+ will be searched upon payment of additional fees. The composition may be searched, for example, to the extent that the alternatively splice peptide encompasses TRIM11 SEQ ID NO: 7 for an additional fee and election as such. It is believed that claims 55, (73-74)(in part) read on this exemplary invention. Additional alternatively spliced peptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected alternatively spliced peptide(s). Failure to clearly identify how any paid additional invention fees are to be applied to the"+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be an alternatively spliced peptide encompasses RCOR3 of SEQ ID NO: 10 (Claims 56, (73 74)(in part)).

The inventions listed as Groups I, II+ and III+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I includes the special technical feature of a method which differs from the special technical feature of a composition, as disclosed by Groups II+ and III+.

Groups II+ include the special technical feature of an engineered TCR, not required by Groups III+.

Group III+ includes the special technical feature of an alternatively spliced peptide, not required by Groups II+.

The inventions of Groups II+ each include the special technical feature of a specific set of TCR-a CDR3, TCR-b CDR3, TCR-a variable region and TCR-b variable region, not required by any of the other inventions of Groups II+.

The inventions of Groups III+ each include the special technical feature of a specific alternatively spliced peptide, not required by any of the other inventions of Groups III+

Common Technical Features

The inventions of Groups I and III+ share the technical feature of an alternatively spliced peptide.

However, these shared technical features do not represent a contribution over prior art in view of US 2016/0101170 A1 to The Broad Institute Inc. et al. (hereinafter "Broad Inst").

---See Supplemental Box ---

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/59476

Box No. III Observations where unity of invention is lacking

Broad Inst discloses (instant claim 1) a method to synthesize an antigenic peptide (para [0009] - "a method of making a personalized neoplasia vaccine for a subject diagnosed as having a neoplasia, which includes identifying a plurality of mutations in the neoplasia, analyzing the plurality of mutations to identify a subset of at least five neo-antigenic mutations predicted to encode neo-antigenic peptides"; para [0162] - "The isolated tumor specific neo-antigenic peptides described herein can be produced in vitro, e.g., in the laboratory, by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable trans formed host"), comprising: identifying alternative splice events in RNA-seq data derived from neoplastic tissue (para [0050] - "Mutation may also refer to patterns in the sequence of RNA from a patient that are not attributable to expected variations based on known information for an individual gene and are reasonably considered to be novel variations in, for example, the splicing pattern of one or more genes that has been specifically altered in the tumor cells of the patient"; para [0093] - "candidate neoepitopes may be predicted using well-validated algorithms, e.g., Net-MHCpan, and their identification may be refined by experimental validation for peptide-HLA binding and by confirmation of gene expression at the RNA level"); obtaining a reference panel of alternative splicing events that includes splice junction data (para [0471] - "According to the techniques herein, bioinformatic analysis may be used to estimate clonality of mutations. For example, the ABSOLUTE algorithm (Carter et al., 2012, Lan dau et al., 2013) may be used to estimate tumor purity, ploidy, absolute copy numbers and clonality of mutations"; para [0446] - "The other two classes of mutations, frameshifts and splice-site mutations, mutations at exon-intron junctions, have the potential to generate longer stretches of novel amino acid sequences entirely specific to the tumor (neo-open reading frames, or neoORFs), with a higher number of neoantigen peptides per given alteration") (Note: ABSOLUTE incorporates splice junction data), wherein the reference panel of alternative splicing events is derived from healthy matched tissue, other tissues of the body, or a second neoplastic tissue that is similar (para [0109] - "identifying all, or nearly all, mutations in the neoplasia/tumor at the DNA level using whole genome, whole exome, e.g., only captured exons, or RNA sequencing of tumor versus matched germline samples from each patient"); detecting a neoplastic alternative splicing event in the neoplastic tissue by comparing the alternative splice events derived from neoplastic tissue with the reference panel of alternative splicing events (para [0471] - "According to the techniques herein, bioinformatic analysis may be used to estimate clonality of mutations. For example, the ABSOLUTE algorithm (Carter et al., 2012, Lan dau et al., 2013) may be used to estimate tumor purity, ploidy, absolute copy numbers and clonality of mutations"; para [0446] - "The other two classes of mutations, frameshifts and splice-site mutations (mutations at exon-intron junctions) have the potential to generate longer stretches of novel amino acid sequences entirely specific to the tumor (neo-open reading frames, or neoORFs), with a higher number of neoantigen peptides per given alteration"); selecting an alternative isoform from the neoplastic tissue RNA-seq data that is detected to have the neoplastic alternative splicing event (para [0111] - "synthesizing the plurality of candidate neo-antigen peptides selected from the sets of all neoORF peptides and predicted binding peptides for use in a cancer vaccine"); generating a peptide derived based on a nucleotide sequence that spans across a splice junction of the detected neoplastic alternative splicing event of the selected alternative isoform (para [0111] - "synthesizing the plurality of candidate neo-antigen peptides selected from the sets of all neoORF peptides and predicted binding peptides for use in a cancer vaccine"; para [0469] - "Table 13 shows the distribution of mutation classes, summed neoORF sizes and number of predicted binding peptides across 13 cancers"; Figure 11C illustrates a selected Neo-ORF).

Broad Inst discloses (instant claim 64) a peptide (para [0162] - "The isolated tumor specific neo-antigenic peptides described herein can be produced in vitro, e.g., in the laboratory, by any suitable method known in the art. Such methods range from direct protein synthetic methods to constructing a DNA sequence encoding isolated polypeptide sequences and expressing those sequences in a suitable trans formed host") comprising at least 6 contiguous amino acids (para [0016] - "In another embodiment, the at least about 20 neoantigenic peptides range from about 5 to about 50 amino acids in length") encoded by an alternatively spliced nucleic acid (para [0149] - "tumor specific neo-antigens may be produced in vivo by introducing molecules, e.g., DNA, RNA, viral expression systems, and the like, that encode tumor specific neo-antigens into a subject, whereupon the encoded tumor specific neo-antigens are expressed"; para [0050] - "Mutation may also refer to patterns in the sequence of RNA from a patient that are not attributable to expected variations based on known information for an individual gene and are reasonably considered to be novel variations in, for example, the splicing pattern of one or more genes that has been specifically altered in the tumor cells of the patient"), wherein the at least 6 contiguous amino acids are encoded on a nucleic acid that comprises an alternative splice site junction (para [0446] - "The other two classes of mutations, frameshifts and splice-site mutations, mutations at exon-intron junctions, have the potential to generate longer stretches of novel amino acid sequences entirely specific to the tumor (neo-open reading frames, or neoORFs), with a higher number of neoantigen peptides per given alteration") (Note: ABSOLUTE incorporates splice junction data"; para [0149] - "tumor specific neo-antigens may be produced in vivo by introducing molecules, e.g., DNA, RNA, viral expression systems, and the like, that encode tumor specific neo-antigens into a subject, whereupon the encoded tumor specific neo-antigens are expressed), and wherein the alternative splice site junction is an AS event selected from an AS event (para [0050] - "Mutation may also refer to patterns in the sequence of RNA from a patient that are not attributable to expected variations based on known information for an individual gene and are reasonably considered to be novel variations in, for example, the splicing pattern of one or more genes that has been specifically altered in the tumor cells of the patient").

The inventions of Groups II+ share the technical feature of an engineered TCR. However, these shared technical features do not represent a contribution over prior art in view of US 2019/0279742 A1 to Gritstone Oncology, Inc. (hereinafter "Gritstone").

Gritstone discloses (instant claim 25) an engineered T-cell Receptor (TCR) (para [0604] - "a polynucleotide encoding a TCR disclosed herein, wherein the polynucleotide is introduced via genetic engineering, and thereby express recombinant or genetically engineered TCRs"), comprising: a TCR alpha (TCR-a) CDR3, a TCR beta (TCR-b) CDR3 (pg 81, Supplementary Table 6 - alpha CDR3, beta-CDR3).

Gritstone discloses (instant claim 26) a TCR alpha (TCR-a) and a TCR beta (TCR-b) variable region (pg 81-82, Supplementary Table 6 - full length alpha VJ; full length beta V(D)J).

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I, II+ and III+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

---See Supplemental Box ---

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/59476

Box No. III Observations where unity of invention is lacking

Item 4 (continued):
Claims 4-24, 29-54, 75-138 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).