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(54) **Titre : PROCÉDES ET MATÉRIAUX POUR LE CIBLAGE D'ANTIGÈNES TUMORAUX**
(54) **Title: METHODS AND MATERIALS FOR TARGETING TUMOR ANTIGENS**

(57) **Abrégé/Abstract:**

This document relates to methods and materials for treating a mammal having cancer. For example, this document provides T cell receptors (TCRs) that can bind to a modified peptide (e.g., a tumor antigen). In some cases, methods of using T cells expressing one or more TCRs that can bind to a modified peptide (e.g., a tumor antigen) to treat a mammal having cancer are provided.



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(54) Title: METHODS AND MATERIALS FOR TARGETING TUMOR ANTIGENS

(57) Abstract: This document relates to methods and materials for treating a mammal having cancer. For example, this document provides T cell receptors (TCRs) that can bind to a modified peptide (e.g., a tumor antigen). In some cases, methods of using T cells expressing one or more TCRs that can bind to a modified peptide (e.g., a tumor antigen) to treat a mammal having cancer are provided.



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METHODS AND MATERIALS FOR TARGETING TUMOR ANTIGENS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Patent Application Serial No. 63/168,878, filed on March 31, 2021. The disclosure of the prior application is considered part of (and is
5 incorporated by reference in) the disclosure of this application.

BACKGROUND

1. Technical Field

This document relates to methods and materials for treating a mammal having cancer. For example, this document provides T cell receptors (TCRs) that can bind to a modified
10 peptide (*e.g.*, a tumor antigen). In some cases, T cells expressing one or more TCRs provided herein can be administered to a mammal having cancer to treat the mammal.

2. Background Information

Immune checkpoint blockade (ICB) has revolutionized cancer treatment; however, the efficacy of ICB agents, such as programmed cell death protein 1 (PD-1) signaling
15 inhibitors (*e.g.*, anti-PD-1 antibodies and anti-PD-L1 antibodies), is predicated upon CD8 T cell-mediated anti-tumor immunity (Tumeh et al., *Nature* 515:568-571 (2014)), and most patients do not respond ICB agents. PD-1 blockade “unleashes” CD8 T cells, including those specific for **m**utation-associated **n**eoantigens (MANAs), but factors in the tumor microenvironment can inhibit responses by dampening MANA-specific T cell function.
20 Recent advances in single cell transcriptomics are revealing global T cell dysfunction programs in tumor-infiltrating lymphocytes (TIL). However, the vast majority of TIL do not recognize tumor antigens.

SUMMARY

There is a continuing need in the art to develop new methods to diagnose, monitor,
25 and effectively treat cancers. For example, the identification of therapeutic targets highly

specific to cancer cells is one of the greatest challenges for developing an effective cancer therapy.

This document provides methods and materials for treating a mammal having cancer. For example, this document provides TCRs that can bind to a modified p53 peptide (*e.g.*, a modified p53 peptide present in a peptide- human leukocyte antigen (HLA) complex) such as a p53 polypeptide having a R to L substitution at amino acid residue 248 (*e.g.*, p53 R248L peptide). In some cases, T cells expressing one or more TCRs that can bind to a modified p53 peptide (*e.g.*, a modified p53 peptide present in a peptide-HLA complex) can be administered to a mammal having a cancer (*e.g.*, a cancer containing one or more cancer cells expressing the modified p53 peptide) to treat the mammal.

As demonstrated herein, T cell receptors (TCRs) were identified that target (*e.g.*, target and bind to) the p53 R248L MANA. MANAs can be used as highly specific cancer targets because they are not present in normal tissue(s). The ability to specifically target MANAs provides a tumor-specific method to diagnose and/or treat cancer. For example, TCRs specifically targeting MANAs can be used in T cells (*e.g.*, T cells expressing a chimeric antigen receptor (CARTs)) to treat a mammal having cancer. Further, TCRs that can bind to a MANA can be used to provide a widely applicable and genetically predictable off-the-shelf targeted cancer immunotherapy.

In general, one aspect of this document features TCRs that can bind to a modified p53 polypeptide comprising a R to L substitution at amino acid residue 248 (R248L). The modified p53 polypeptide can include a p53 R248L peptide comprising, consisting essentially of, or consisting of the amino acid sequence set forth in any one of SEQ ID NOs:1-40. The TCR can include an alpha (α) chain including a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44. The TCR can include a beta (β) chain including a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48. The TCR can include an α chain that includes a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44, and can include a β chain that includes a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48.

In another aspect, this document features T cells comprising a TRC that can bind to a modified p53 polypeptide comprising a R248L substitution. The modified p53 polypeptide can include a p53 R248L peptide comprising, consisting essentially of, or consisting of the

amino acid sequence set forth in any one of SEQ ID NOs:1-40. The TCR can include an alpha (α) chain including a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44. The TCR can include a beta (β) chain including a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48. The TCR can include an α chain that includes a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44, and can include a β chain that includes a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48. The T cell can be a human T cell. The T cell can be a non-human T cell.

In another aspect, this document features nucleic acids encoding a TRC that can bind to a modified p53 polypeptide comprising a R248L substitution. The modified p53 polypeptide can include a p53 R248L peptide comprising, consisting essentially of, or consisting of the amino acid sequence set forth in any one of SEQ ID NOs:1-40. The TCR can include an alpha (α) chain including a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44. The TCR can include a beta (β) chain including a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48. The TCR can include an α chain that includes a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44, and can include a β chain that includes a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48. The nucleic acid can be in the form of a vector. The vector can be an expression vector. The vector can be a viral vector.

In another aspect, this document features T cells including a nucleic acid encoding a TRC that can bind to a modified p53 polypeptide comprising a R248L substitution, where the nucleic acid encodes the TCR. The T cell can be a human T cell. The T cell can be a non-human T cell.

In another aspect, this document features methods for treating a mammal having cancer. The methods can include, or consist essentially of, administering to a mammal having cancer a T cell including a TRC that can bind to a modified p53 polypeptide comprising a R248L substitution or a T cell including nucleic acid encoding a TRC that can bind to a modified p53 polypeptide comprising a R248L substitution, where the cancer includes a cancer cell expressing the modified p53 polypeptide. The cancer cell expressing the modified p53 polypeptide can presents a p53 R248L peptide in a peptide-HLA complex. The p53 R248L peptide comprising, consisting essentially of, or consisting of the amino acid sequence set forth in any one of SEQ ID NOs:1-40. The mammal can be a human. The

cancer can be a non-small cell lung cancer (NSCLC), a colon adenocarcinoma, a rectal adenocarcinoma, a head and neck squamous cell carcinoma, a pancreatic adenocarcinoma, melanomas, a urothelial carcinoma, a uterine corpus endometrial carcinoma, or a uterine carcinoma. The method also can include administering a checkpoint inhibitor to the

5 mammal. The checkpoint inhibitor can be an anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) antibody, an anti-PD-1 (programmed death 1) antibody, an anti-PD-L1 (programmed death 1 ligand) antibody, an anti-LAG3 (lymphocyte activation gene 3) antibody, an anti-Tim3 (T cell immunoglobulin and mucin domain-containing protein 3) antibody, an anti-TIGIT (T cell immunoreceptor with Ig and ITIM domains) antibody, an

10 anti-VISTA (V-domain Ig suppressor of T cell activation) antibody, an anti-CD47 (cluster of differentiation 47) antibody, an anti-SIRPalpha (signal regulatory protein alpha) antibody, an anti-B7-H3 (B7 homolog 3) antibody, an anti-B7-H4 (B7 homolog 4) antibody, an anti-neuritin antibody, an anti-neuropilin antibody, an anti-IL-35 (interleukin 35), an IDO (indoleamine-pyrrole 2,3-dioxygenase) inhibitor, an A2AR (adenosine A2A receptor)

15 inhibitor, an arginase inhibitor, or a glutaminase inhibitor. The method also can include administering a co-stimulatory molecule to the mammal. The co-stimulator molecule can be an agonist of a co-stimulatory receptor. The agonist of a co-stimulatory receptor can be an anti-GITR (glucocorticoid-induced TNFR-related) antibody, an anti-CD27 (cluster of

20 differentiation 27) antibodies antibody, an anti-4-1BB (CD137; cluster of differentiation 137) antibody, an anti-OX40 (CD134; cluster of differentiation 134) antibody, an anti-ICOS (inducible T-cell costimulator) antibody, or an anti-CD40 (cluster of differentiation 40) antibody.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention

25 pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are

30 illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

5 Figures 1A-1E. Profiling single immune cells in anti-PD-1-treated lung cancer with combined scRNA-Seq/TCRSeq. Figure 1A: Graphical overview of the experimental design. Single cell RNAseq/TCRseq was performed on T cells derived from resected tumor, adjacent NL, and tumor-draining lymph nodes (TDLN) of lung cancer patients treated with neoadjuvant PD-1 blockade. The MANAFEST and ViraFEST assays were performed to
10 identify mutation associated neoantigen (MANA)/EBV/Influenza A- specific T cells. Antigen specific T cell clones were linked with transcriptomic profiles by using the TCRb chain as a biologic barcode. Figure 1B: 2D projection of expression profiles of 560,916 T cells from post-treatment tumor, adjacent normal lung, and tumor-draining lymph node using UMAP. Broad immune cell subsets were annotated and marked by color code. Figure 1C:
15 Heatmap of the top 5 differential genes for respective immune cell subsets. Figure 1D: 2D UMAP red-scale projection of canonical T cell subset marker genes (CD8A, CD4, and FOXP3), cell subset selective genes (GZMK, TCF7, ZNF683, CXCL13, SLC4A10, and MKI67), and well defined immune checkpoints (PDCD1, CTLA4, HAVCR2, TIGIT, ENTPD1, LAG3). Figure 1E: Principal component analysis (PCA) of pseudobulk gene
20 expression for post treatment tumor (yellow) vs adjacent NL (dark blue) and MPR (light blue) vs non-MPR (red).

 Figures 2A-2I. Transcriptional characterization of antigen specific T cells in NSCLC treated with neoadjuvant PD-1 blockade. Figure 2A: MANAFEST assays were performed on the peripheral blood of 4 MPR and 5 non-MPR. An example MANAFEST assay is
25 shown for patient MD01-004. Data are shown as the frequency of MANAFEST+clonotypes after in vitro culture for clonotypes found only in the MANAFEST assay (blue) and clonotypes found in the MANAFEST assay and also detected in single cell analysis of TIL (green). Red bars represent 4 individual clonotypes tested in the Jurkat reporter system shown in Figure 2B. Figure 2B: Specificity of four clones positive by MANAFEST for the

p53 R248L-derived NSSCMGGMNLR (SEQ ID NO:1) neoantigen (MANA 12, red box and red bars) were confirmed in a dose-dependent manner by cloning and transfection into a Jurkat NFAT luciferase reporter system (Figure 2B, top). A known HLA A*11-restricted EBV-specific TCR was also cloned as a control (green). To enable ligand-dependent TCR signaling capacity comparisons between TCRs with variable transfection efficiencies, data are shown as the log fold change in luminescence relative to TCR-transfected Jurkats cultured without peptide. Pre- and post-treatment tissue (Figure 2B, middle) and peripheral blood (Figure 2B, bottom) representation were also visualized for these four clonotypes and these data are shown as the frequency among all TCRs detected by bulk TCRseq. Figure C: 2D projection of expression profiles of 235,851 CD8 T cells from tumor, adjacent normal lung, and draining lymph node using UMAP. CD8 T cell subsets are annotated and marked by color code. Figure 2D: 2D projection of MANA/EBV/Influenza A specific T cells on total merged CD8 UMAP. TRB aa sequence was used as a biological barcode to match MANA/EBV/Influenza A specific T cell clonotypes identified from the FEST assay with single cell VDJ profile. Figure 2E: 2D projection of MANA/EBV/Influenza A specific T cells on CD8 UMAP of post treatment tumor and adjacent NL. TIL and NL T cells were down-sampled to equal number of cells before visualization. Bar plot shows proportion of antigen specific T cells among total CD8 T cells by tissue compartment (blue bar, adjacent NL; yellow bar, post treatment tumor). Dot plot shows proportion of antigen specific T cells, stratified by CD8 T cell subsets, with size of the dot representing proportion among total CD8 T cells (blue dot, adjacent NL; yellow dot, post treatment tumor). Figure 2F: MANA/EBV/Influenza A specific gene programs in the TIL expressed as a heatmap. Figure 2G: Expression levels of key transcriptional regulators, memory makers, tissue resident markers, T cell immune checkpoints and CD8 effector/activation genes among MANA-specific T cells (red), Influenza A-specific T cells (blue) and EBV-specific T cells (purple). Figure 2H: Waterfall plot showing the top 30 differential genes comparing flu-specific T cells and MANA-specific T cells. Figure 2I: IL7 functional experiment for MANA- and influenza A-specific T cells. Ridge plot shows the composite IL7-upregulated gene set score for MANA-specific T cells vs Influenza A specific T cells within TIL cultured with MANA/Influenza A peptide at titrating concentrations of IL7 (0 μ g, 0.1 μ g, 1 μ g, and 10 μ g,

left panel). A dose response curve of the mean (with standard error) IL7-upregulated gene set score against different titrations of IL7 is shown (right panel).

Figures 3A-3F. Differential MANA-specific gene programs in MPR vs. non-MPR tumors. Figure 3A: Heatmap of differential genes of tumor infiltrating MANA-specific T from MPR and non-MPR. Figure 3B: IL7R expression of MANA specific CD8 clones in MRPs and non-MPR at clonal level (each dot representing a unique clone). Wilcoxon rank sum test, **: $0.001 < P < 0.01$. Figure 3C: T cell immune checkpoint score (derived from expression of CTLA4, PDCD1, LAG3, HAVCR2, TIGIT, ENTPD1) of single cell RNA-Seq/TCR-Seq profiled MANA specific CD8 cells and influenza A specific CD8 cells in MRPs and non-MPR. Each dot represents a single cell. Wilcoxon rank sum test, ****: $P < 0.0001$, ns: $P > 0.05$. Figure 3D: Top 30 ranked genes that are expression correlated with T cell immune checkpoints comprising the checkpoint score in MPR and non-MPR. Figure 3E: Gene program differences between MPR and non-MPR in top genes that are expression correlated with T cell immune checkpoints (derived from Figure 3D). Figure 3F: MANA specific T cell tracking across tumor, adjacent NL, tumor draining LN, and longitudinal blood from a patient achieving a pathologic complete response after 4 weeks of neoadjuvant nivolumab. MANA-specific T cells were labeled as red triangle and corresponding cell types were annotated with dashed lines.

Figure 4. Neoantigen-specific TCRs identified by the MANAFEST assay. MANAFEST assay for 3 MPR and 3 non-MPR patients. MANAFEST+ expansions observed in each patient are shown for clonotypes only found in the MANAFEST assays (blue), clonotypes found in the MANAFEST assay and detected in single cell TIL (green) and clonotypes detected in single cell TIL and validated by cloning and transfection into a Jurkat NFAT luciferase reporter system (red). MANAFEST data are shown as the frequency of MANAFEST+ clonotypes among CD8+ T cells after 10 day culture. Significant MANAFEST+ expansions were not observed in nonMPR patient, MD01-019 (data not shown). MANAFEST+ expansions for MPR patient, MD01-005, have been previously shown.

Figures 5A – 5D. Validation of MANA-specific TCRs identified by the MANAFEST assay. Seven MANA-specific clonotypes identified by MANAFEST in three

patients were confirmed by cloning and transfection into a Jurkat NFAT luciferase reporter system in a dose-dependent manner. Figure 5A: In MD01-005, two clonotypes recognize ARVCF-derived EVIVPLSGW (SEQ ID NO:49) MANA. In vitro binding and stability assays demonstrate the affinity kinetics of each relevant MANA, the corresponding wild-type peptide for their restricting HLA class I allele. Blank, or no peptide was used as a negative control for each assay; known HLA-matched epitopes were used as positive controls. Data are shown as counts per second with increasing peptide concentration for binding assays (top) or absorbance in presence of urea for stability assays (bottom). Data points indicate the mean of two independent experiments $\pm$ SD. Figure 5B: In MD01-004, four clonotypes recognize p53 R248L-derived NSSCMGGMNLR (SEQ ID NO:1) MANA. *In vitro* binding and stability assays demonstrate the affinity kinetics of each relevant MANA, the corresponding wild-type peptide for their restricting HLA class I allele. Blank, or no peptide was used as a negative control for each assay; known HLA-matched epitopes were used as positive controls. Data are shown as counts per second with increasing peptide concentration for binding assays (top) or absorbance in presence of urea for stability (bottom). Data points indicate the mean of two independent experiments $\pm$ SD. Figure 5C: In MD043-011, one clonotype recognizes CARM1-derived neoantigens. Low level expansion of the CASSLDPYEQYF (SEQ ID NO:50) clone, which did not meet our standard cutoff for antigen specificity, was observed in response to three neoantigens, MD043-011-MANA 24 – MANA31 and -MANA 36, which all encompassed the same core epitope resulting from a CARM1 R208W mutation, AQAGAWKIYAV (SEQ ID NO:51), AQAGAWKIY (SEQ ID NO:52), FAAQAGAWKIY (SEQ ID NO:53), respectively. Figure 5D: The COS-7 cell line was transfected with HLA-A*68:01 plasmid and p53 R248L mutant plasmid or p53 wild type plasmid. HLA- and p53-transfected COS-7 cells, autologous APC loaded with MD01-004-MANA12, and HLA-A*68:01-transfected COS-7 were co-cultured with CD8⁺ Jurkat reporter cells expression the MD01-004-MANA12-reactive TCR, CATTGGQNTEAFF (SEQ ID NO:45).

Figure 6. Peripheral dynamics and cross-compartment representation of MANA-specific T cells. Bulk TCRseq was performed on pre- and post-treatment tissue and peripheral blood. MANA-specific TCR β clone representation is shown as the frequency

among all TCRs detected by TCRseq. TDLN, tumor draining lymph node; DLN, draining lymph node.

Figure 7. Virus-specific TCR identified by the ViraFEST assay. The ViraFEST assay was performed on 1 MPR and 2 non-MPR to identify influenza-specific TCR β clones.

5 Influenza A pools consisting of overlapping peptides from the matrix protein of H1N1 and H3N2 and the nucleocapsid protein of H1N1 and H3N2 were used to stimulate peripheral blood T cells in vitro for 10 days. viraFEST+ expansions are shown for each patient. Clonotypes only found in the MANAFEST assays (blue) and clonotypes found in the MANAFEST assay and detected in single cell TIL (green) are shown as the frequency among
10 all CD8+ T cells detected by TCRseq.

Figure 8. Peripheral dynamics and cross-compartment representation of CEF-specific T cells. CEF+ TCR β clonotypes were identified in three MPR and one non-MPR (data previously shown as positive controls in MANAFEST assays in Fig. 2 and Fig. 5). Pre- and post-treatment tissue and peripheral blood representation were visualized for each clonotype
15 and these data are shown as the frequency among all TCRs detected by TCRseq. TDLN, tumor draining lymph node; DLN, draining lymph node.

Figure 9. Peripheral dynamics and cross-compartment representation of flu-specific T cells. Peripheral blood T cells were tested for reactivity against peptide pools representing the matrix protein and nucleoprotein of H1N1 and H3N2 using the ViraFEST assay. Flu-specific cells were identified in one MPR and two no-MPR. Pre- and post-treatment tissue and peripheral blood representation were visualized for flu-specific clonotypes. Data are shown as the frequency among all TCRs detected by TCRseq. TDLN, tumor draining lymph node; DLN, draining lymph node.
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Figure 10. 2D UMAP red-scale projection of canonical T cell subset marker genes, cell subset selective genes and immune checkpoints on CD8 T cell subsets.
25

Figures 11A – 11B. Clonal tracking of MANA-specific T cells across tissue compartments. Figure 11A: MANA specific T cells were found in tumor, adjacent NL, and tumor draining LN at tumor resection and in a distant brain metastasis from a patient with 75% residual tumor at resection and early relapse. The scatterplot shows the average
30 expression of genes comparing the post-treatment tumor at resection vs. the distant brain

metastasis. The top differential genes are labeled in red. Figure 11B: MANA specific T cells were detected in the post-treatment tumor and tumor draining LN from a patient with partial response (40% percent residual tumor).

Figures 12A-12D. Identification and characterization of T cell receptors specific for a p53 R248L-derived neoantigen in NSCLC treated with neoadjuvant PD-1 blockade. Figure 12A: MANAFEST assay was performed in non-MPR patient, MD01-004, in which 41 neoantigen-specific and 2 CMV/EBV/flu (CEF)-specific TCRb CDR3 clonotypes were identified. Figure 12B: Four of these clones were specific for the hotspot p53 R248L-derived MANA (MD01-004-MANA12), whose specificities were validated by TCR cloning into the Jurkat/NFAT-luciferase system. Additionally, clones specific for p53 R248L-derived MANA were found at appreciable frequency in the pre- and post-treatment tumor, despite the tumor not attaining MPR. Notably, these MANA-specific clones were detected at very low frequency (median: 0.001%, range: 0-0.038%) in the peripheral blood across all available timepoints, thereby highlighting the sensitivity of the MANAFEST assay. Peptide dose-response curves were comparable to the positive control EBV-specific TCR, suggesting these TCRs were capable of strong ligand-dependent signaling (sometimes referred to as functional avidity). Figure 12C: Endogenous processing and HLA A*68:01-restricted presentation of MD01-004-MANA12 was confirmed by transfection of HLA A*6801 and R248L-mutated p53 into a COS-7 cell line and co-culture with a MD01-004- MANA12-reactive TCR. Figure 12D: *in vitro* binding and stability assays demonstrate the affinity kinetics of each relevant MANA, the corresponding wild-type peptide for their restricting HLA class I allele.

Figure 13. Table 6. MANAs tested by MANAFEST.

Figure 14. Table 8. Antigen-specific TCR clonotypes identified by the MANAFEST and viraFEST assays.

DETAILED DESCRIPTION

This document provides methods and materials for treating a mammal having cancer. For example, this document provides TCRs that can bind to a modified p53 peptide (*e.g.*, a modified p53 peptide present in a peptide-HLA complex) such as a p53 R248L peptide. In some cases, T cells expressing TCRs that can bind to a modified p53 peptide (*e.g.*, a modified

p53 peptide present in a peptide-HLA complex) can be administered to a mammal having a cancer (*e.g.*, a cancer containing one or more cancer cells expressing the modified p53 peptide) to treat the mammal.

As used herein, a modified peptide is a peptide derived from a modified polypeptide.

5 A modified polypeptide can be any appropriate modified polypeptide (*e.g.*, a polypeptide having a disease-causing mutation such as a mutation in an oncogenic or a mutation in a tumor suppressor gene). A modified peptide can have one or more amino acid modifications (*e.g.*, substitutions) relative to a WT peptide (*e.g.*, a peptide derived from a WT polypeptide from which the modified polypeptide is derived). A modified peptide also can be referred to
10 as a mutant peptide. In some cases, a modified peptide can be a tumor antigen. Examples of tumor antigens include, without limitation, MANAs, tumor-associated antigens, and tumor-specific antigens. A modified peptide can be any appropriate length. In some cases, a modified peptide can be from about 8 amino acids to about 11 amino acids in length. For example, a modified peptide can be about 11 amino acids in length. A modified peptide can
15 be derived from any modified polypeptide. In some cases, a modified peptide described herein can be derived from a p53 polypeptide. A modified peptide can include any appropriate modification. In some cases, modified peptides described herein can include one or more modifications (*e.g.*, mutations) shown in Table 1.

20 Table 1. Modified peptides.

Protein of origin	Mutation	Mutant Peptide	SEQ ID NO:
p53	R248L	NSSCMGGMNLR	1
p53	R248L	CNSSCMGGMNL	2
p53	R248L	NSSCMGGMNLRP	3
p53	R248L	SSCMGGMNLRP	4
p53	R248L	SCMGGMNLRPIS	5
p53	R248L	CMGGMNLRPIL	6
p53	R248L	MGGMNLRPILT	7
p53	R248L	GGMNLRPILTI	8

p53	R248L	GMNLRPILTII	9
p53	R248L	MNLRPILTIIT	10
p53	R248L	NLRPILTIITL	11
p53	R248L	LRPILTIITLE	12
p53	R248L	CNSSCMGGMN	13
p53	R248L	NSSCMGGMNL	14
p53	R248L	SSCMGGMNLR	15
p53	R248L	SCMGGMNLRP	16
p53	R248L	CMGGMNLRPI	17
p53	R248L	MGGMNLRPIL	18
p53	R248L	GGMNLRPILT	19
p53	R248L	GMNLRPILTI	20
p53	R248L	MNLRPILTII	21
p53	R248L	NLRPILTIIT	22
p53	R248L	LRPILTIITL	23
p53	R248L	SSCMGGMNL	24
p53	R248L	SCMGGMNLR	25
p53	R248L	CMGGMNLRP	26
p53	R248L	MGGMNLRPI	27
p53	R248L	GGMNLRPIL	28
p53	R248L	GMNLRPILT	29
p53	R248L	MNLRPILTI	30
p53	R248L	NLRPILTII	31
p53	R248L	LRPILTIIT	32
p53	R248L	SCMGGMNL	33
p53	R248L	CMGGMNLR	34
p53	R248L	MGGMNLRP	35
p53	R248L	GGMNLRPI	36
p53	R248L	GMNLRPIL	37
p53	R248L	MNLRPILT	38
p53	R248L	NLRPILTI	39

p53	R248L	LRPILTII	40
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In some cases, a modified p53 peptide described herein (e.g., a p53 R248L peptide) can be a peptide that is not 100% identical to the mutant peptides set forth in Table 1, but retains the R to L substitution at amino acid residue 248. For example, a modified p53 peptide can include one or more (e.g., one, two, three, four, five, or more) amino acid substitutions relative to a peptide set forth in Table 1.

A modified peptide described herein (e.g., a p53 R248L peptide) can be in a complex with an HLA. An HLA can be any appropriate HLA allele. In some cases, an HLA can be a class I HLA (e.g., HLA-A, HLA-B, and HLA-C) allele. In some cases, an HLA can be a class II HLA (e.g., HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR) allele. An example of a HLA allele that a modified peptide described herein can complex with includes, without limitation, A*68 (e.g., A*68:01).

This document provides TCRs that can bind to a modified peptide described herein (e.g., a p53 R248L peptide). In some cases, a TCR that can bind to a modified peptide described herein does not target (e.g., does not bind to) an uncomplexed modified peptide described herein (e.g., a modified peptide described herein that is not present in a complex (e.g., a peptide-HLA complex)). In some cases, a TCR that can bind to a modified peptide described herein does not target (e.g., does not bind to) a WT peptide (e.g., a peptide derived from a WT polypeptide from which the modified polypeptide is derived).

A TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can be any appropriate type of TCR. Examples of TCRs that can bind to a modified peptide described herein (e.g., can be designed to bind to a modified peptide described herein) such as a p53 R248L peptide include, without limitation, chimeric antigen receptors (CARs), TCRs, and TCR mimics.

A TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can include any appropriate alpha (α) chain and any appropriate beta (β) chain. For example, a TCR that can bind to a modified p53 peptide described herein can include an α chain having three complementarity determining regions (TCR α CDRs) and a β chain having three CDRs (TCR β CDRs).

An α chain of a TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can include any appropriate CDRs. For example, an α chain of a TCR that can bind to a modified p53 peptide described herein can include one of the CDR3s set forth below:

5

Table 2. TCR α -CDR sequences

	Sequence	SEQ ID NO
TCR α CDR3	CILSGANNLFF	41
TCR α CDR3	CILYGGATNKLIF	42
TCR α CDR3	CILNNNDMRF	43
TCR α CDR3	CILKTNSGNTPLVF	44

A β chain of a TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can include any appropriate CDRs. For example, a β chain of a TCR that can bind to a modified p53 peptide described herein can include one of the CDR3s set forth below:

10

Table 3. TCR β -CDR sequences

	Sequence	SEQ ID NO
TCR β CDR3	CATTGGQNTEAFF	45
TCR β CDR3	CASQSGILPWEQFF	46
TCR β CDR3	CAISEWRAGSTDTQYF	47
TCR β CDR3	CASSEVQGASNEKLFF	48

15

In some cases, a TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can have one or more CDRs that are not 100% identical to the CDRs set forth in Table 2 and Table 3, but retain the ability to bind to the modified p53 peptide. For

example, a CDR that includes one or more (e.g., one, two, three, four, five, or more) amino acid substitutions relative to a CDR set forth in Table 2 or Table 3 can be used in TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide). An amino acid substitution can be made, in some cases, by selecting a substitution that does not differ significantly in its effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at particular sites, or (c) the bulk of the side chain. For example, naturally occurring residues can be divided into groups based on side-chain properties: (1) hydrophobic amino acids (methionine, alanine, valine, leucine, and isoleucine); (2) neutral hydrophilic amino acids (cysteine, serine, and threonine); (3) acidic amino acids (aspartic acid and glutamic acid); (4) basic amino acids (asparagine, glutamine, histidine, lysine, and arginine); (5) amino acids that influence chain orientation (glycine and proline); and (6) aromatic amino acids (tryptophan, tyrosine, and phenylalanine). Substitutions made within these groups can be considered conservative substitutions. Non-limiting examples of conservative substitutions that can be made within a CDR of a TCR provided herein include, without limitation, substitution of valine for alanine, lysine for arginine, glutamine for asparagine, glutamic acid for aspartic acid, serine for cysteine, asparagine for glutamine, aspartic acid for glutamic acid, proline for glycine, arginine for histidine, leucine for isoleucine, isoleucine for leucine, arginine for lysine, leucine for methionine, leucine for phenylalanine, glycine for proline, threonine for serine, serine for threonine, tyrosine for tryptophan, phenylalanine for tyrosine, and/or leucine for valine.

In some cases, a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide) can include an α chain that includes a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44. For example, an α chain that can be included in a TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can include the amino acid sequence set forth in SEQ ID NO:41-44.

In some cases, a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide) can include a β chain that includes a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48. For example, a β chain that can be included in a

TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can include the amino acid sequence set forth in SEQ ID NO:45-48.

In some cases, a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide) can include an α chain that includes a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44, and a β chain that includes a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48. For example, a TCR that can bind to a modified p53 peptide described herein (e.g., a p53 R248L peptide) can include an α chain including the amino acid sequence set forth in SEQ ID NO:41-44 and a β chain including the amino acid sequence set forth in SEQ ID NO:45-48.

This document also provides nucleic acid (e.g., nucleic acid vectors) that can encode a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide). Nucleic acid (e.g., nucleic acid vectors) that can encode a TCR provided herein can be any type of nucleic acid. Nucleic acid can be DNA (e.g., a DNA construct), RNA (e.g., mRNA), or a combination thereof. In some cases, nucleic acid that can encode a TCR provided herein can be a vector (e.g., an expression vector or a viral vector).

In some cases, nucleic acid that can encode a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide) can also include one or more regulatory elements (e.g., to regulate expression of the amino acid chain). Examples of regulatory elements that can be included in nucleic acid that can encode a TCR provided herein include, without limitation, promoters (e.g., constitutive promoters, tissue/cell-specific promoters, and inducible promoters such as chemically-activated promoters and light-activated promoters), and enhancers.

This document also provides cells (e.g., host cells) expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide). A cell expressing one or more TCRs provided herein can be any appropriate type of cell. In some cases, a cell expressing one or more TCRs provided herein can be a T cell (e.g., a CD4⁺ T cell or a CD8⁺ T cell). A cell expressing one or more TCRs provided herein can be obtained from any type of animal. In some cases, a cell expressing one or more TCRs provided herein can be obtained from a human or a non-human mammal such as a mouse. When using a cell expressing one or more TCRs provided herein to treat a

mammal having a cancer (e.g., a cancer containing one or more cancer cells expressing a modified p53 peptide such as a p53 R248L peptide), the cell can be obtained from the mammal to be treated or from another source.

This document also provides methods for using TCRs (e.g., T cells expressing one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide). For example, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) that can target (e.g., bind to) cancer cells expressing the modified p53 peptide. In some cases, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal having a cancer (e.g., a cancer containing cancer cells expressing a modified p53 peptide such as a p53 R248L peptide) to treat the mammal. Administration of T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) to a mammal (e.g., human) having a cancer can be effective to treat the mammal.

In some cases, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal (e.g., a human) in need thereof (e.g., a human having cancer such as a cancer containing cancer cells that express a p53 R248L peptide) to reduce or eliminate the number of cancer cells present within a mammal. For example, the materials and methods described herein can be used to reduce the number of cancer cells present within a mammal having cancer by, for example, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or more percent. For example, the materials and methods described herein can be used to reduce the size (e.g., volume) of one or more tumors present within a mammal having cancer by, for example, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or more percent.

In some cases, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal (e.g., a human) in need thereof (e.g., a human having cancer such as a cancer containing cancer cells that express a p53 R248L peptide) to improve survival of the mammal. For example, disease-free survival (e.g., relapse-free survival) can be improved

using the materials and methods described herein. For example, progression-free survival can be improved using the materials and methods described herein. In some cases, the materials and methods described herein can be used to improve the survival of a mammal having cancer by, for example, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or more percent.

5 In some cases, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal (e.g., a human) in need thereof (e.g., a human having cancer such as a cancer containing cancer cells that express a p53 R248L peptide) to increase the number of tumor-infiltrating lymphocytes (e.g., T cells present in within the tumor microenvironment
10 of a cancer) within the mammal. For example, the materials and methods described herein can be used to increase the number of tumor-infiltrating lymphocytes within a mammal having cancer by, for example, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, or more percent.

Any type of mammal can be treated as described herein. Examples of mammals that can be treated as described herein include, without limitation, primates (e.g., humans and
15 non-human primates such as chimpanzees, baboons, or monkeys), dogs, cats, pigs, sheep, rabbits, mice, and rats. In some cases, a mammal can be a human.

A mammal can be treated for any appropriate cancer. In some cases, a cancer can include one or more cancers cells expressing one or more modified peptides (e.g., one or more MANAs) described herein (e.g., a modified p53 peptide such as a p53 R248L peptide).
20 A cancer can be a primary cancer. A cancer can be a metastatic cancer. A cancer can include one or more solid tumors. A cancer can include one or more non-solid tumors. Examples of cancers that can be treated as described herein (e.g., by administering T cells expressing one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) include, without limitation, lung cancers (e.g., non-small cell lung cancers (NSCLCs)), colon
25 adenocarcinomas, rectal adenocarcinomas, head and neck squamous cell carcinomas, pancreatic adenocarcinomas, melanomas, urothelial carcinomas, uterine corpus endometrial carcinomas, and uterine carcinomas.

In some cases, the methods described herein also can include identifying a mammal as having cancer. Examples of methods for identifying a mammal as having cancer include,
30 without limitation, physical examination, laboratory tests (e.g., blood and/or urine), biopsy,

imaging tests (e.g., X-ray, PET/CT, MRI, and/or ultrasound), nuclear medicine scans (e.g., bone scans), endoscopy, and/or genetic tests. Once identified as having cancer, a mammal can be administered or instructed to self-administer T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide).

When treating a mammal having cancer, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal having cancer to treat the mammal. In some cases, a mammal can have a cancer that includes one or more cancer cells expressing one or more modified peptides described herein. For example, T cells expressing one or more TCRs provided herein can be administered to a mammal having a cancer that includes one or more cancer cells expressing that modified peptide to treat the mammal. For example.

In some cases, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal having cancer (e.g., a cancer containing one or more cancer cells expressing a modified p53 peptide such as a p53 R248L peptide) once.

In some cases, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal having cancer (e.g., a cancer containing one or more cancer cells expressing a modified p53 peptide such as a p53 R248L peptide) multiple times (e.g., over a period of time ranging from days to weeks to months).

In some cases, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be formulated into a composition (e.g., a pharmaceutically acceptable composition) for administration to a mammal having a cancer (e.g., a cancer containing one or more cancer cells expressing a p53 R248L peptide). For example, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be formulated together with one or more pharmaceutically acceptable carriers (additives), excipients, and/or diluents. In some cases, a pharmaceutically acceptable carrier, excipient, or diluent can be a naturally occurring pharmaceutically

acceptable carrier, excipient, or diluent. In some cases, a pharmaceutically acceptable carrier, excipient, or diluent can be a non-naturally occurring (*e.g.*, an artificial or synthetic) pharmaceutically acceptable carrier, excipient, or diluent. Examples of pharmaceutically acceptable carriers, excipients, and diluents that can be used in a composition described

5 herein include, without limitation, sucrose, lactose, starch (*e.g.*, starch glycolate), cellulose, cellulose derivatives (*e.g.*, modified celluloses such as microcrystalline cellulose and cellulose ethers like hydroxypropyl cellulose (HPC) and cellulose ether hydroxypropyl methylcellulose (HPMC)), xylitol, sorbitol, mannitol, gelatin, polymers (*e.g.*, polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), crosslinked polyvinylpyrrolidone

10 (crospovidone), carboxymethyl cellulose, polyethylene-polyoxypropylene-block polymers, and crosslinked sodium carboxymethyl cellulose (croscarmellose sodium)), titanium oxide, azo dyes, silica gel, fumed silica, talc, magnesium carbonate, vegetable stearin, magnesium stearate, aluminum stearate, stearic acid, antioxidants (*e.g.*, vitamin A, vitamin E, vitamin C, retinyl palmitate, and selenium), citric acid, sodium citrate, benzyl alcohol, lysine

15 hydrochloride, trehalose dihydrate, sodium hydroxide, parabens (*e.g.*, methyl paraben and propyl paraben), petrolatum, dimethyl sulfoxide, mineral oil, serum proteins (*e.g.*, human serum albumin), glycine, sorbic acid, potassium sorbate, water, salts or electrolytes (*e.g.*, saline, protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, and zinc salts), colloidal silica, magnesium trisilicate, polyacrylates, waxes,

20 wool fat, lecithin, and corn oil. In some cases, a pharmaceutically acceptable carrier, excipient, or diluent can be an antiadherent, a binder, a colorant, a disintegrant, a flavor (*e.g.*, a natural flavor such as a fruit extract or an artificial flavor), a glidant, a lubricant, a preservative, a sorbent, and/or a sweetener.

A composition (*e.g.*, a pharmaceutical composition) containing T cells expressing one

25 or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be formulated into any appropriate dosage form. Examples of dosage forms include liquid forms including, without limitation, suspensions, solutions (*e.g.*, sterile solutions), sustained-release formulations, and delayed-release formulations.

A composition containing T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be designed for oral, parenteral (including subcutaneous, intramuscular, intravenous, and intradermal), or intratumoral administration. Compositions suitable for parenteral
5 administration include aqueous and non-aqueous sterile injection solutions that can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient. The formulations can be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example,
10 water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets.

A composition containing T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered using any appropriate technique and to any appropriate location. A
15 composition including T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered locally or systemically. For example, a composition provided herein can be administered locally by intratumoral administration (e.g., injection into tumors) or by administration into biological spaces infiltrated by tumors (e.g. intraspinal administration,
20 intracerebellar administration, intraperitoneal administration and/or pleural administration). For example, a composition provided herein can be administered systemically by oral administration or by intravenous administration (e.g., injection or infusion) to a mammal (e.g., a human).

Effective doses can vary depending on the risk and/or the severity of the cancer, the
25 route of administration, the age and general health condition of the subject, excipient usage, the possibility of co-usage with other therapeutic treatments such as use of other agents, and the judgment of the treating physician. An effective amount of T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be any amount that treats a cancer present within the
30 subject without producing significant toxicity to the subject. If a particular subject fails to

respond to a particular amount, then the amount of one or more molecules including one or more antigen-binding domains (*e.g.*, scFvs) that can bind to a modified peptide described herein can be increased (*e.g.*, by two-fold, three-fold, four-fold, or more). After receiving this higher amount, the mammal can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments made accordingly. The effective amount can remain constant or can be adjusted as a sliding scale or variable dose depending on the subject's response to treatment. Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple treatment agents, route of administration, and severity of the condition (*e.g.*, cancer) may require an increase or decrease in the actual effective amount administered.

The frequency of administration of T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be any frequency that effectively treats a mammal having a cancer without producing significant toxicity to the mammal. For example, the frequency of administration of T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be from about two to about three times a week to about two to about three times a year. In some cases, a mammal having cancer can receive a single administration of T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide). The frequency of administration of T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can remain constant or can be variable during the duration of treatment. A course of treatment with T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can include rest periods. For example, T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered every other month over a two-year period followed by a six-month rest period, and such a regimen can be repeated multiple times. As with the effective amount, various factors can influence the actual frequency of administration used for a particular application. For example, the effective amount, duration of treatment, use of multiple treatment agents,

route of administration, and severity of the condition (*e.g.*, cancer) may require an increase or decrease in administration frequency.

An effective duration for administering T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be any duration that effectively treats a cancer present within the mammal without producing significant toxicity to the mammal. In some cases, the effective duration can vary from several months to several years. In general, the effective duration for treating a mammal having a cancer can range in duration from about one or two months to five or more years. Multiple factors can influence the actual effective duration used for a particular treatment. For example, an effective duration can vary with the frequency of administration, effective amount, use of multiple treatment agents, route of administration, and severity of the condition being treated.

In certain instances, a cancer within a mammal can be monitored to evaluate the effectiveness of the cancer treatment. Any appropriate method can be used to determine whether or not a mammal having cancer is treated. For example, imaging techniques or laboratory assays can be used to assess the number of cancer cells and/or the size of a tumor present within a mammal. For example, imaging techniques or laboratory assays can be used to assess the location of cancer cells and/or a tumor present within a mammal.

In some cases, T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal having a cancer as a combination therapy with one or more co-stimulatory molecules. In some cases, a co-stimulatory molecule can be an agonist of one or more co-stimulatory receptors. Examples of co-stimulatory molecules that can be administered to mammal having cancer together with T cells expressing one or more TCRs provided herein include, without limitation, anti-GITR antibodies, anti-CD27 antibodies, anti-4-1BB antibodies, anti-OX40 antibodies, anti-ICOS antibodies, and anti-CD40 antibodies.

In some cases, T cells expressing one or more TCRs provided herein (*e.g.*, one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered to a mammal having a cancer as a combination therapy with one or more

additional cancer treatments. A cancer treatment can include any appropriate cancer treatments. For example, a cancer treatment can include surgery. For example, a cancer treatment can include radiation therapy. For example, a cancer treatment can include administration of one or more therapeutic agents (e.g., one or more anti-cancer agents). In some cases, an anti-cancer agent can be an immunotherapy (e.g., a checkpoint inhibitor). Examples of anti-cancer agents that can be administered together with T cells expressing one or more TCRs provided herein include, without limitation, anti-CTLA-4 antibodies, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-LAG3 antibodies, anti-Tim3 antibodies, anti-TIGIT antibodies, anti-CD39 antibodies, anti-VISTA antibodies, anti-CD47 antibodies, anti-SIRPalpha antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-neuritin antibodies, anti-neuropilin antibodies, anti-IL-35 antibodies, inhibitors of IDO, inhibitors of A2AR, inhibitors of arginase, and inhibitors of glutaminase. In cases where an immunotherapy is administered to mammal having cancer together with T cells expressing one or more TCRs provided herein, the mammal also can be administered one or more co-stimulatory molecules (e.g., one or more agonists of one or more co-stimulatory receptors such as anti-GITR antibodies, anti-CD27 antibodies, anti-4-1BB antibodies, anti-OX40 antibodies, anti-ICOS antibodies, and anti-CD40 antibodies).

In cases where T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) are used in combination with one or more additional cancer treatments, the one or more additional cancer treatments can be administered at the same time or independently. For example, T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) can be administered first, and the one or more additional cancer treatments administered second, or vice versa. In cases, where T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) and one or more anti-cancer agents are administered at the same time, the T cells expressing one or more TCRs provided herein and the one or more anti-cancer agents can be formulated into a single composition.

Also provided herein are kits that include one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide)

and/or nucleic acid that can encode a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide). For example, a kit can include one or more vectors that can encode a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide) and can be used to generate T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide). In some cases, a kit can include instructions for generating T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide). For example a kit can include one or more vectors that can encode a TCR provided herein (e.g., a TCR that can bind to a modified p53 peptide such as a p53 R248L peptide) and can be used to generate T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) and can include T cells. In some cases, a kit also can include instructions for generating T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) and for using the generated T cells (e.g., for performing any of the methods described herein). In some cases, a kit can provide a means (e.g., a syringe) for administering T cells expressing one or more TCRs provided herein (e.g., one or more TCRs that can bind to a modified p53 peptide such as a p53 R248L peptide) to a mammal.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Distinct transcriptional programs characterize neoantigen-specific T cells in lung cancers treated with neoadjuvant PD-1 blockade

TP53 is the most commonly mutated cancer driver gene, but despite extensive efforts, no drug targeting mutant *TP53* has been approved for treatment of the large number of patients whose tumor contain p53 mutations.

This Example describes the identification of MANA specific T cell clones and their function in the tumor microenvironment.

METHODS

Patients and biospecimens

All biospecimens were obtained from patients with stage I-IIIa NSCLC who were enrolled to a phase II clinical trial evaluating the safety and feasibility of administering two doses of anti-PD-1 (nivolumab) prior to surgical resection. Pathological response assessments of primary tumors were as reported elsewhere (see, e.g., Forde et al., *N. Engl. J. Med.*, 378:1976-1986 (2018); and Cottrell et al., *Ann. Oncol.*, 29:1853-1860 (2018)). Tumors with no more than 10% residual viable tumor cells were considered to have a major pathologic response.

Single cell TCRseq/RNAseq

Cryobanked T cells were thawed and washed twice with pre-warmed RPMI with 20% FBS and gentamicin. Cells were resuspended in PBS and stained with a viability marker (LIVE/DEAD™ Fixable Near-IR; ThermoFisher) for 15 minutes at room temperature (RT) in the dark. Cells were incubated with FC block for 15 minutes on ice and stained with antibody against CD3 (BV605, clone SK7) for 30 minutes on ice. After staining, highly viable CD3⁺ T cells were sorted into 0.04% BSA in PBS using a BD FACSAria II Cell Sorter. Sorted cells were manually counted using a hemocytometer and prepared at the desired cell concentration (1000 cells/μL), when possible. The Single Cell 5' V(D)J and 5' DGE kits (10X Genomics) were used to capture immune repertoire information and gene expression from the same cell in an emulsion-based protocol at the single cell level. Cells and barcoded gel beads were partitioned into nanoliter scale droplets using the 10X Genomics Chromium platform to partition up to 10,000 cells per sample followed by RNA capture and cell-barcoded cDNA synthesis using the manufacturer's standard protocols. Libraries were generated and sequenced on an Illumina HiSeq or NovaSeq instrument using 2x150bp paired end sequencing. 5' VDJ libraries were sequenced to a depth of ~5,000 reads per cell, for a total of 5 million to 25 million reads. The 5' DGE libraries were sequenced to a target depth of ~5,000 reads per cell. The 5' DGE libraries were sequenced to a target depth of ~50,000 reads per cell.

Single cell VDJ and DGE data processing

Cell Ranger v3.1.0 was used to demultiplex the FASTQ reads, align them to the GRCh38 human transcriptome, and extract their “cell” and “UMI” barcodes. The output of this pipeline is a digital gene expression (DGE) matrix for each sample, which records the number of UMIs for each gene that are associated with each cell barcode. Quality of cells were then assessed based on (1) the number of detected genes per cell and (2) the proportion of mitochondrial gene/ribosomal gene counts. Low-quality cells were filtered if the number of detected genes was below 250 or above the medians of all cells plus $3 \times$ the median absolute deviation. Cells were filtered out if the proportion of mitochondrial gene counts was higher than 10% or the percent of ribosomal genes was less than 10%. For single-cell VDJ sequencing, only cells with full-length sequences were retained. The SAVER algorithm was used to impute dropouts and adjust unreliable gene expression quantification caused by sparse data by borrowing information across similar genes and cells. After appropriate transformation (e.g., log2), gene expression values were quantile normalized across samples. Using the normalized single-cell data, cells were projected to a common low-dimensional space (e.g., by UMAP49). The Mutual Nearest Neighbors (MNN) approach was used to align cells so that cells of the same cell type from different samples are matched in an unsupervised fashion. Unsupervised clustering of cells was then performed to systematically identify cell subpopulations, including potential new cell subtypes. The TCR beta chain (at the nucleotide level) was used to match MANAFEST positive T cell clones on the UMAP. A “clonotype” was defined by a unique combination of a TCR alpha and beta chain. Single cell data were pre-processed and normalized separately and UMAPs were generated for each patient.

Whole exome sequencing (WES), mutation calling, and neoantigen prediction

Genomic data for most patients in the study was as reported elsewhere (see, e.g., Forde et al., *N. Engl. J. Med.*, 378:1976-1986 (2018)). Tumor mutational burden and neoantigen predictions for patients MD043-003 and NY016-025 were performed. Whole exome sequencing was performed on pre-treatment tumor for NY016-025 and resected tumor for MD043-003 and matched normal samples. DNA was extracted from patients' tumors and

matched peripheral blood using the Qiagen DNA kit (Qiagen, CA). Fragmented genomic DNA from tumor and normal samples was used for Illumina TruSeq library construction (Illumina, San Diego, CA) and exonic regions were captured in solution using the Agilent SureSelect v.4 kit (Agilent, Santa Clara, CA) according to the manufacturers' instructions.

5 Paired-end sequencing, resulting in 100 bases from each end of the fragments for the exome libraries was performed using Illumina HiSeq 2000/2500 instrumentation (Illumina, San Diego, CA). Somatic mutations, consisting of point mutations, insertions, and deletions across the whole exome were identified using the VariantDx custom software for identifying mutations in matched tumor and normal samples. Somatic mutations, consisting of
10 nonsynonymous single base substitutions, insertions and deletions, were evaluated for putative MHC class I neoantigens using the ImmunoSelect-R pipeline (Personal Genome Diagnostics, Baltimore, MD).

Identification of neoantigen-specific TCR V β CDR3 clonotypes

The MANAFEST (Mutation Associated NeoAntigen Functional Expansion of
15 Specific T-cells) assay was used to evaluate T cell responsiveness to MANA and viral antigens. Briefly, pools of MHC class I-restricted CMV, EBV, and flu peptide epitopes (CEFX, jpt Peptide Technologies), pools representing the matrix protein and nucleoprotein from H1N1 and H3N2 (jpt Peptide Technologies), and putative neoantigenic peptides defined by the ImmunoSelect-R pipeline (jpt Peptide Technologies; Table 6 (Figure 13) and Table 8
20 (Figure 14)) were used to stimulate T cells *in vitro* for 10 days. T cells were also cultured without peptide to use as a reference for non-specific clonotypic expansion. On day 10, T cell receptor sequencing was performed on each individual peptide-stimulated T cell culture by the Sidney Kimmel Comprehensive Cancer Center FEST and TCR Immunogenomics Core (FTIC) facility or Adaptive Biotechnologies. Bioinformatic analysis of productive
25 clones was performed to identify antigen-specific T-cell clonotypes meeting the following criteria: 1) significant expansion (Fisher's exact test with Benjamini-Hochberg correction for FDR, $p < 0.05$) compared to T cells cultured without peptide, 2) significant expansion compared to every other peptide-stimulated culture ($FDR < 0.0001$) except for conditions stimulated with similar neoantigens derived from the same mutation, 3) an odds ratio > 5

compared to the “no peptide” control, and 4) present in at least 10% of the cultured wells to ensure adequate distribution among culture wells. A lower read threshold of 300 was used for assays sequenced by the FTIC and a lower threshold of 30 was used for samples sequenced by Adaptive Biotechnologies. In MANAFEST assays testing less than 10
 5 peptides or peptide pools, cultures were performed in triplicate and reactive clonotypes were defined as being significantly expanded relative to T cells cultured without peptide (FDR<0.05) in two out of three triplicates, and not significantly expanded in any other well tested. When available, TCRseq was also performed on DNA extracted from tumor, normal lung, and lymph node tissue obtained before treatment and at the time of surgical resection,
 10 as well as serial peripheral blood samples.

Peptide affinity and stability measurements

Peptide affinity was measured as described elsewhere (see, e.g., Harndahl et al., *J. Biomol. Screen*, 14:173-180 (2009)). The stability of peptide loaded complexes was measured by refolding MHC with peptide and subsequently challenging complexes with a
 15 titration of urea. The denaturation of MHC was monitored by ELISA.

TCR reconstruction and cloning

Ten MANAFEST+ TCR sequences for which the TCR α chain could be enumerated (>3 cells in single cell data with the same α/β pair) and MANA score^{hi} TCRs were selected for cloning. Relevant TCRs were analyzed with the IMGT/V-Quest database
 20 (imgt.org/IMGT). The database allows us to identify the TRAV and TRBV families with the highest likelihood to contain the identified segments which match the sequencing data. To generate the TCRs, the identified TCRA V-J region sequences were fused to the human TRA constant chain, and the TCRB V-D-J regions to the human TRB constant chain. The full-length TCRA and TCRB chains were then synthesized as individual gene blocks (IDT) and
 25 cloned into the pCI mammalian expression vector, containing a CMV promoter, and transformed into competent E. coli cells according to manufacturer's instructions (NEBuilder HiFi DNA Assembly, NEB). Post transformation and plasmid miniprep, the plasmids were sent for Sanger sequencing to ensure no mutations were introduced (Genewiz).

T cell transfection, transient TCR expression, and MANA recognition assays

To generate a Jurkat reporter cell which could transfer the TCRs of interest, the endogenous T cell receptor (TCR) α and β chains were knocked out of a specific Jurkat line that contains a luciferase reporter driven by an NFAT-response element (Promega) using the Alt-R CRISPR system (Integrated DNA Technologies, IDT). Two sequential rounds of CRISPR knockout were performed using crDNA targeting the TCR α constant region (AGAGTCTCTCAGCTGGTACA; SEQ ID NO:54) and the TCR β constant region (AGAAGGTGGCCGAGACCCTC; SEQ ID NO:55). Limiting dilution was then used to acquire single cell clones and clones with both TCR α and TCR β knocked out, as confirmed by Sanger sequencing and restoration of CD3 expression only by the co-transfection of TCR α or TCR β chains, were chosen. CD8 α and CD8 β chains were then transduced into the TCR α / β Jurkat reporter cells using the MSCV retroviral expression system (Clontech). Jurkat reporter cells were then co-electroporated with the pCI vector encoding the TCRB and TCRA gene blocks, respectively, using ECM830 Square wave electroporation system (BTX) at 275volts for 10 ms in OptiMem media in a 4 mm cuvette. Post electroporation, cells were rested overnight by incubating in RPMI 10% FBS at 37°C, 5% CO₂. TCR expression was confirmed by flow cytometric staining for CD3 on a BD FACSCelesta. Reactivity of the TCR transduced Jurkat T cells was assessed by co-culturing the cells with autologous EBV-transformed B cells or autologous PBMC, loaded with titrating concentrations of MANA peptides, viral peptide pools, or negative controls. After overnight incubation, activation of the NFAT reporter gene was measured by the Bio-Glo Luciferase Assay per manufacturer's instructions (Promega).

in vitro short-term TIL stimulation with IL-7

Cryopreserved patient TIL were thawed, counted and stained with viability marker, LIVE/DEAD™ Fixable Aqua (ThermoFisher), and surface markers, CD3 (PE, clone SK1) and CD8 (BV786, clone RPA-T8). 30 thousand CD8⁺ T cells per each TIL population were sorted on a BD FACS Aria II Cell Sorter into a 96-well plate. Autologous peripheral blood mononuclear cells (PBMC) were added as antigen presenting cells (APC) at 1:1 ratio. The

cells were stimulated with respective antigen and recombinant human IL-7 (Miltenyi) for 12 hours in a round-bottomed 96-well plate.

Gene expression analysis of IL-7 stimulated TIL

Following 12 hours of antigen and IL-7 stimulation, cells were spun down, counted
5 and re-suspended in 1% BSA at desired concentration. Single-cell RNA seq and VDJ libraries were prepared using 10x Chromium single cell platform using 5' DGE library preparation reagents and kits according to manufacturer's protocols (10x Genomics, Pleasanton, CA) and as described above.

COS-7 transfection with HLA allele and p53 plasmids

10 gBlocks (IDT) encoding HLA A*6801, p53 R248L and p53 WT were cloned into pcDNA3.4 vector (Thermo Fisher Scientific, A14697). COS-7 cells were transfected with plasmids at 70-80% confluency using Lipofectamine 3000 (Thermo Fisher Scientific, L3000015) and incubated at 37°C overnight in T75 flasks. A total of 30 µg plasmid (1:1 ratio of HLA plasmid/target protein plasmid in co-transfections) was used. Post transfection,
15 COS-7 cells were plated with TCRαβ transfected Jurkat cells containing NFAT reporter gene at a 1:1 ratio. After overnight incubation, activation of the NFAT reporter gene was measured by the Bio-Glo Luciferase Assay per manufacturer's instructions (Promega).

Single cell data preprocessing and quality control

Cell Ranger v3.1.0 was used to demultiplex the FASTQ reads, align them to the
20 GRCh38 human transcriptome, and extract their "cell" and "UMI" barcodes. The output of this pipeline is a digital gene expression (DGE) matrix for each sample, which records the number of UMIs for each gene that are associated with each cell barcode. The quality of cells was then assessed based on (1) the number of genes detected per cell and (2) the proportion of mitochondrial gene/ribosomal gene counts. Low-quality cells were filtered if
25 the number of detected genes was below 250 or above 3× the median absolute deviation away from the median gene number of all cells. Cells were filtered out if the proportion of mitochondrial gene counts was higher than 10% or the proportion of ribosomal genes was less than 10%. For single-cell VDJ sequencing, only cells with full-length sequences were

retained. Dissociation/stress associated genes, mitochondrial genes (annotated with the prefix “MT-“), high abundance lincRNA genes, genes linked with poorly supported transcriptional models (annotated with the prefix “RP-“), and TCR (TR) genes (TRA/TRB/TRD/TRG) were removed from further analysis. In addition, genes that were expressed in less than five cells
5 were excluded.

Single cell data integration and clustering

Seurat (3.1.5) was used to normalize the raw count data, identify highly variable features, scale features, and integrate samples. Principal component analysis (PCA) was performed based on the 3,000 most variable features identified using the vst method
10 implemented in Seurat. Gene features associated with type I Interferon (IFN) response, immunoglobulin genes and specific mitochondrial related genes were excluded from clustering to avoid cell subsets driven by the above genes. Dimension reduction was done using the RunUMAP function. Cell markers were identified by using a Wilcoxon test. Genes with adjusted p.value < 0.05 were retained. Clusters were labeled based on the
15 expression of the top differential gene in each cluster as well as canonical immune cell markers. Global clustering on all CD3 T cells and refined clustering on CD8 T cells were performed using same procedure. To select for CD8⁺ T cells, SAVER was used to impute dropouts by borrowing information across similar genes and cells. A density curve was fitted to the log2-transformed SAVER imputed CD8A expression values (using ‘density’ function
20 in R) of all cells from all samples. A cutoff is determined as the trough of the bimodal density curve (i.e., the first location where the first derivative is zero and the second derivative is positive). All cells with log2-transformed SAVER imputed CD8A expression larger than the cutoff are defined as CD8⁺ T cells. TRB amino acid (aa) sequences were used as a biological barcode to match MANA/EBV/Influenza A specific T cell clonotypes
25 identified from the FEST assay with single-cell VDJ profile and were projected onto CD8⁺ T cell refined UMAP.

Single cell subset pseudobulk gene expression analysis

PCA was performed on a standardized pseudobulk gene expression profile, where each feature was standardized to have a mean of zero and unit variance. In the global

clustering analysis, counts were aggregated at the sample level for each cell cluster and normalized by library size. Combat function in the "sva" R package was applied to address potential batch effects on the normalized pseudobulk profile. Highly variable genes (HVGs) were selected for each cell cluster by fitting a locally weighted scatterplot smoothing (LOESS) regression of standard deviation against the mean for each gene and identifying genes with positive residuals. All cell clusters were then concatenated by retaining cluster-specific HVGs to construct a pseudobulk gene expression matrix. Canonical correlation between the first two PCs (i.e., PC1 and PC2) and a covariate of interest (i.e., tissue type or response status) was calculated. Permutation test was used to assess the significance by randomly permuting the sample labels 10,000 times.

Differential expression tests and antigen-specific T cell marker genes

Differential expression (DE) tests were performed using FindAllMarkers functions in Seurat with Wilcoxon Rank Sum test on SAVER imputed expression values. Genes with > 0.25 log2-fold changes, at least 25% expressed in tested groups, and Bonferroni-corrected p values < 0.05 were regarded as significantly differentially expressed genes (DEGs). Antigen-specific (MANA vs flu vs EBV) T cell marker genes were identified by applying the DE tests for upregulated genes between cells of one antigen specificity to all other antigen specific-T cells in the dataset. Top ranked genes (by log-fold changes) with a log2-fold changes > 0.6 from each antigen-specificity type of interest were extracted for further visualization in heatmap using pheatmap package. Saver imputed expression values of selective marker genes (transcriptional regulators/memory markers/tissue resident markers/T cell checkpoints/effector/activation markers) were plotted using the RidgePlot function in Seurat.

Gene expression analysis of IL-7 stimulated MANA/flu-specific TIL

MANA/flu-specific T cell clonotypes from single-cell dataset were identified by using TRB aa sequences as a biological barcode. SAVER imputed gene expression was scaled and centered using "ScaleData" function in Seurat. A composite score for IL7 upregulated gene set expression was computed using the AddModuleScore function and subsequently visualized using ridgeplot. Mean $\pm$ standard error was used to show dose

response curve of IL7 upregulated gene set score by antigen-specific T cells+peptide stimulation groups.

Immune checkpoint score generation and highly correlated genes

To characterize dysfunctional CD8 MANA TIL, 6 best characterized (and clinically targeted) checkpoints: CTLA4, PDCD1, LAG3, HAVCR2, TIGIT and ENTPD1, were used to compute the T cell checkpoint score using AddModuleScore function in Seurat. Applying T cell checkpoint score as an anchor, genes that were maximally correlated to the score were identified using linear correlation in MANA-specific TIL from MPR and non-MPR, respectively. Top 30 genes with the highest correlation coefficients were plotted using barplot. The difference of the above genes was additionally computed between MPR and non-MPR and visualized using waterfall plot.

RESULTS

The efficacy of immune checkpoint blockade (ICB) agents, such as anti-PD(L)-1, is predicated upon CD8 T cell-mediated anti-tumor immunity (see, e.g., Tumeh et al., *Nature*, 515:568-571 (2014)). The association of improved anti-PD(L)-1 clinical responses with high mutational burden tumors (see, e.g., Le et al., *Science*, 357:409-413 (2017); Snyder et al., *N. Engl. J. Med.*, 371:2189-2199 (2014); Van Allen et al., *Science*, 350:207-211 (2015); Rizvi et al., *Science*, 348:124-128 (2015)) strongly suggests that MANA are important targets of anti-tumor immunity induced by PD-1 blockade (see, e.g., Rizvi et al., *Science*, 348:124-128 (2015); Schumacher et al., *Science* 348:69-74 (2015); and Ward et al., *Adv Immunol* 130:25-74 (2016)).

Improving ICB response rate will require an understanding of the functional state of tumor-specific T cells, particularly in the tumor microenvironment. A fundamental limitation of the understanding of the T cell functional programs underpinning response to ICB has been the absence of transcriptional profiling of true MANA-specific TIL. A related problem is the paucity of information regarding the differences between MANA-specific TIL in ICB responsive vs resistant tumors. Indeed, MANA-specific T cells represent a small fraction of total TIL, highlighting the challenges confronting characterization of the cells responsible for the activity of T cell-targeting immunotherapies.

For the present study, peripheral blood and surgical resection specimens obtained from the first-in-human clinical trial of neoadjuvant anti-PD-1 (nivolumab) in resectable non-small cell lung cancer NSCLC (NCT02259621) were utilized. After 4 weeks of nivolumab (Figure 1A, top), 45% of the patients had a major pathologic response (MPR) at time of resection, defined as $\leq 10\%$ viable tumor at the time of surgery. To identify MANA-specific CD8 T cells after PD-1 blockade, candidate MANA peptides, derived from application of an MHC I binding prediction algorithm to whole exome tumor sequencing, were tested for peripheral blood CD8 T cell recognition using a recently developed high throughput TCRseq-based platform, MANAFEST (Mutation Associated NeoAntigen Functional Expansion of Specific T cells, Figure 1A, bottom). In parallel, simultaneous coupled single cell (sc) RNAseq/scTCRseq analysis of purified T cells from tumor, adjacent NL, and tumor-draining lymph nodes (TDLN), when available, were performed. The TCR β CDR3 was then used as a barcode to identify MANA-specific CD8 T cells among TIL, adjacent NL, and TDLN. With these single cell data, the paired TCR α for MANA-specific TCR β clonotypes were identified which in turn enabled validation of MANA recognition by individual clones via transfer of both TCR genes into an engineered Jurkat reporter cell line. Influenza A (flu)- and EBV-specific clones were also identified among TIL that were expanded in the assay in response to pools of viral peptides (ViraFEST) and further validated by matching with a public database. In all, MANAFEST was performed on 9 patients and scTCRseq/scRNAseq was performed on 16 patients in the trial, including TIL (n=15) together with T cells from paired adjacent normal lung (NL, n=12), and T cells from tumor-draining lymph nodes (TDLN, n=3) which we could sort MANA-specific T cells (Table 4 and Table 5). A total of 560,916 T cells passed quality control (Figure 1B and Table 5) and were carried forward in the analyses.

Table 4. Clinical and histopathological features of patients included in this study.*

Study ID	Age at diagnosis (years)	Gender	Smoking history	Pack Years	Histology	Pre-Treatment Clinical Tumor Stage	% PD-L1 (pre)	% PD-L1 (resection)	% residual tumor	MPR status	HLA haplotype
MD001-005	61	M	Current Smoker	45	Squamous Cell Carcinoma	T3N0	NA	17	0	MPR	HLA-A*25:01; HLA-A*30:01; HLA-B*39:01; HLA-B*38:01; HLA-C*12:03
MD001-004	67	M	Former Smoker	20	Adenosquamous	T4N1	1	65	40	non-MPR	HLA-A*31:01; HLA-A*68:01; HLA-B*35:08; HLA-B*51:01; HLA-C*15:02; HLA-C*04:01
MD0043-008	72	F	Former Smoker	50	Squamous Cell Carcinoma	T1bN0	0	0	10	MPR	HLA-A*02:01; HLA-A*29:02; HLA-B*07:02; HLA-B*44:03; HLA-C*07:02; HLA-C*16:01
MD0043-011	55	M	Former Smoker	40	Adenocarcinoma	T2aN1	NA	0	75	non-MPR	HLA-A*24:02; HLA-A*23:01; HLA-B*40:01; HLA-B*44:03; HLA-C*03:04; HLA-C*02:02
MD001-019	70	M	Former Smoker	50	Adenocarcinoma	T2aN0	0	NA	95	non-MPR	HLA-A*02:01; HLA-A*30:01; HLA-B*13:02; HLA-C*06:02
MD0043-003	62	M	Former Smoker	40	Adenocarcinoma	T2aN0	NA	2	5	MPR	HLA-A*02:01; HLA-A*01:01; HLA-B*40:01; HLA-B*35:01; HLA-C*03:04; HLA-C*07:01
MD0043-006	69	M	Former Smoker	90	Squamous Cell Carcinoma	T2AN1	20	70	50	non-MPR	HLA-A*24:03; HLA-B*35:08; HLA-B*18:01; HLA-C*12:03; HLA-C*04:01
MD001-024	70	F	Never Smoker	0	Adenocarcinoma	T1AN0	NA	NA	100	non-MPR	HLA-A*68:02; HLA-A*68:01; HLA-B*40:01; HLA-B*53:01; HLA-C*03:19; HLA-C*04:01
MD001-010	78	F	Former Smoker	20	Adenocarcinoma	T3N0	NA	NA	5	MPR	HLA-A*02:01; HLA-A*11:01; HLA-B*07:02; HLA-B*55:01; HLA-C*07:02; HLA-C*03:03

NY016-007	68	F	Former Smoker	10	Squamous Cell Carcinoma	T2aN1	0	2	60	non-MPR	HLA-A*01:01; HLA-B*08:01; HLA-C*07:01
NY016-014	58	F	Never Smoker	0	Adenocarcinoma	T2N2	60	25	95	non-MPR	HLA-A*02:01; HLA-A*11:01; HLA-B*51:01; HLA-B*35:01; HLA-C*05:01; HLA-C*04:01
NY016-016	79	F	Current Smoker	20	Adenocarcinoma	T1bN1	NA	NA	0	MPR	HLA-A*29:02; HLA-A*01:01; HLA-B*07:02; HLA-B*08:01; HLA-C*07:02; HLA-C*07:01
NY016-015	58	F	Former Smoker	10	Adenocarcinoma	T2bN1	NA	NA	80	non-MPR	HLA-A*02:01; HLA-A*32:01; HLA-B*27:07; HLA-C*15:02
NY016-021	74	M	Former Smoker	50	Adenocarcinoma	T3N0	NA	NA	100	non-MPR	HLA-A*24:02; HLA-A*31:01; HLA-B*18:01; HLA-B*35:08; HLA-C*04:01; HLA-C*07:01
NY016-022	66	F	Former Smoker	20	Adenocarcinoma	T2bN0	AN	NA	5	MPR	HLA-A*02:01; HLA-A*68:02; HLA-B*37:01; HLA-B*44:02; HLA-C*06:02
NY016-025	74	F	Never Smoker	0	Adenosquamous	T3N1	NA	NA	0	MPR	HLA-A*11:01; HLA-A*26:01; HLA-B*38:01; HLA-B*40:02; HLA-C*03:04; HLA-C*12:03

*Treated as part of a clinical trial described in Forde et al., *N. Engl. J. Med.*, 378:1976-1986 (2018)

Table 5. Single cell TCRseq/RNAseq sequencing information and metrics

Patient Study ID	Sample Source	No. cells sequenced by DGE	No. cells after QC	No. cells with matching VDJ
MD01-024	Tumor	3611	3197	2153
MD01-010	Normal	3502	3271	1707
MD01-010	Tumor	6327	4627	3537

MD01-004	Lymph Node	24659	21965	15151	
MD01-004	Tumor	3210	3082	1995	
MD043-011	Lymph Node	17515	10963	9107	
MD043-011	Normal	39648	33824	25370	
MD043-011	Tumor	29338	22196	18940	
MD043-011	Metastatic Tumor	37106	25696	21329	
MD01-019	Normal	12855	11631	8366	
MD01-019	Tumor	52021	41684	31314	
NY016-007	Normal	5416	3645	2435	
NY016-007	Tumor	20652	16136	13146	
NY016-014	Tumor	35655	29482	23947	
NY016-015	Normal	6042	4467	3580	
NY016-015	Tumor	35807	24126	19971	
NY016-016	Normal	10646	9093	7423	
NY016-021	Tumor	3675	1407	785	
NY016-022	Normal	3395	2974	1585	
NY016-022	Tumor	46348	40089	31402	
NY016-025	Normal	11609	9376	4786	
NY016-025	Tumor	55786	48541	27268	
MD043-008	Normal	2854	2321	1653	

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MID043-008	Tumor	2115	1897	1347	
MID043-003	Normal	10014	8541	6749	
MID043-003	Tumor	34007	29051	23712	
MID01-005	Lymph Node	42570	32874	23404	
MID01-005	Normal	34248	28379	21804	
MID01-005	Tumor	75705	68181	53719	
MID043-006	Normal	10316	7787	6354	
MID043-006	Tumor	11958	10413	8686	

A uniform manifold approximation and projection (UMAP) of filtered and normalized transcript counts for the aggregated T cells from tumor and adjacent NL from all 16 patients defined 15 unique T cell clusters (Figure 1B). At this resolution, multiple CD8 effector T cell (Teff) subsets, CD4 T helper cell (Th) subsets and tissue resident memory (TRM) subsets were evident. Top expressed genes for each cluster were visualized in Figure 1C. Interestingly, a previously undescribed lincRNA, LINC02246, was selectively expressed in all TRM clusters, though at differing levels for different TRM subsets. Expression of T cell subset defining markers (CD8A, CD4, and FOXP3), T cell subset selective genes (GZMK – Teff cells; TCF7 – stem-like/memory cells, which could be resolved into CD4 and CD8 subsets; ZNF683 (HOBIT) – TRM cells; CXCL13 – Tfh cells; SLC4A10 – MAIT cells; and MKI67 – proliferating cells) and major T cell checkpoints being targeted clinically (PDCD1, HAVCR2, TIGIT, ENTPD1, LAG3, and CTLA4) were visualized in red-scale on the UMAP (Figure 1D). Principal component analysis (PCA) of pseudo-bulk gene expression (obtained by first computing the average gene expression vector of each T cell subset and then concatenating these vectors from all cell subsets into one long vector) distinguished adjacent NL T cells from TIL (Figure 1E), but did not separate MPR from non-MPR, indicating that gene expression profiling of total TIL has limited sensitivity in distinguishing pathologic response to PD-1 blockade.

To define the prevalence of MANA-specific CD8 T cells in our cohort, MANAFEST was performed on nine patients treated in the clinical trial, consisting of four MPR and five non-MPR (results from one patient were as described in Forde et al., *N. Engl. J. Med.*, 378:1976-1986 (2018)). Putative MANA, peptide pools representing flu matrix and nucleoproteins, and a pool of MHC class I-restricted CMV, EBV, and flu epitopes were queried for CD8⁺ T cell reactivity (Table 6 (Figure 13) and Table 7). Among seven (three MPR and four non-MPR) of the nine patients, 72 total unique MANA-specific TCRs were identified (Figure 2A, Figure 4, Table 8 (Figure 14), and Table 9). Ten clonotypes for which the TCR α could be confidently identified from the single cell analysis were selected for validation of MANA recognition using the TCR cloning and Jurkat/NFAT luciferase reporter system. 70% of tested clonotypes were validated as MANA-specific (Figure 2B, Figure 4, and Figures 5A, 5B, and 5C). Furthermore, binding assays on MANA validated in MD01-

005 and MD01-004 displayed high MHC class I affinity and stability (Figure 5A and 5B). Pathologic response was not associated with the prevalence or frequency of T cells recognizing MANA (Table 9) or intratumoral representation (Figure 6), suggesting the mere frequency of MANA-specific CD8⁺ T cells did not determine pathologic responsiveness. In fact, more MANA-specific TILs were observed in non-MPR TIL than MPR TIL. An example of a MANAFEST assay output is shown in Figure 2A (MD01-004, non-MPR) in which 41 neoantigen-specific and 2 CMV/EBV/flu (CEF)-specific TCR β CDR3 clonotypes were identified. Four of these clones were specific for the hotspot p53 R248L-derived MANA (MD01-004-MANA12), whose specificities were validated by TCR cloning into the Jurkat/NFAT-luciferase system (Figure 2B). Peptide dose-response curves were comparable to the positive control EBV-specific TCR, suggesting these TCRs were capable of strong ligand-dependent signaling (sometimes referred to as functional avidity). Endogenous processing and HLA A*68:01-restricted presentation of MD01-004-MANA12 were confirmed by transfection of HLA A*68:01 and R248L-mutated p53 into a COS-7 cell line and co-culture with a MD01-004-MANA12-reactive TCR (Figure 5D). Additionally, clones specific for p53 R248L-derived MANA were found at appreciable frequency in the pre- and post-treatment tumor (Figure 2B), despite the tumor not attaining MPR. They were also observed at lower frequency in adjacent NL, TDLN, and peripheral blood (Figure 3). Notably, these MANA-specific clones were detected at very low frequency (median: 0.001%, range: 0-0.038%) in the peripheral blood across all available timepoints, thereby highlighting the sensitivity of the MANAFEST assay.

Table 7. MANAFEST TCR sequencing summary statistics

Patient study ID	Assay timepoint (relative to surgery)	MANA tested in culture	SEQ ID NO	MANA ID	# of unique clonotypes from TCRseq
MD01-005	D+44	HVIENIYF	56	MD01-005_2	8412
MD01-005	D+44	DVA AHLQPL	57	MD01-005_3	4845
MD01-005	D+44	ETPNLDLM	58	MD01-005_4	7879
MD01-005	D+44	SVFNTWNPM	59	MD01-005_5	5261
MD01-005	D+44	EVQQFLRY	60	MD01-005_6	9514

MD01-005	D+44	EVIVPLSGW	49	MD01-005_7	9367
MD01-005	D+44	ETMQCSELY	61	MD01-005_8	7471
MD01-005	D+44	ETMQCSELYHM	62	MD01-005_9	6763
MD01-005	D+44	ETMQCSEL	63	MD01-005_10	6034
MD01-005	D+44	ITRTVSANTV	64	MD01-005_18	6819
MD01-005	D+44	ATKNNKVIMA	65	MD01-005_19	6720
MD01-005	D+44	VAHFQLQMLK	66	MD01-005_20	8012
MD01-005	D+44	EEDTFSYLI	67	MD01-005_23	7548
MD01-005	D+44	AHFQLQML	68	MD01-005_24	6886
MD01-005	D+44	LHAMIQAAAGKL	69	MD01-005_25	6281
MD01-005	D+44	LHEAQPWFEFL	70	MD01-005_26	6596
MD01-005	D+44	LHEAQPWFEF	71	MD01-005_27	5957
MD01-005	D+44	EHLSCPDNFL	72	MD01-005_28	6477
MD01-005	D+44	NHARIDAAKV	73	MD01-005_29	7700
MD01-005	D+44	QHQPVPFEV	74	MD01-005_30	5520
MD01-005	D+44	TQLEKEAL	75	MD01-005_33	5944
MD01-005	D+44	TRARNEYLLSL	76	MD01-005_34	5718
MD01-005	D+44	NPMWVLL	77	MD01-005_35	6131
MD01-005	D+44	KHILVWAL	78	MD01-005_36	7272
MD01-005	D+44	SQSDYIPM	79	MD01-005_37	6470
MD01-005	D+44	VHDYFSVI	80	MD01-005_38	5333
MD01-005	D+44	IYFPAAQTM	81	MD01-005_43	5195
MD01-005	D+44	FSYLIWSNPRY	82	MD01-005_44	5740
MD01-005	D+44	YSWSAQRQAL	83	MD01-005_45	5599
MD01-005	D+44	FAVWTLAETI	84	MD01-005_46	5925
MD01-005	D+44	FASLALARRYL	85	MD01-005_47	8051
MD01-005	D+44	DVIQQDELDSY	86	MD01-005_48	6985
MD01-005	D+44	KNRSSGTVSA	87	MD01-005_49	6349
MD01-005	D+44	KLKRFNLSA	88	MD01-005_50	5819
MD01-005	D+44	KSFAVWTLA	89	MD01-005_51	5444
MD01-005	D+44	KWRLSLCTV	90	MD01-005_52	7218
MD01-005	D+44	RSRPVAATAK	91	MD01-005_53	5256
MD01-005	D+44	RSRPVAATA	92	MD01-005_54	5610
MD01-005	D+44	TAKQAHLTTLK	93	MD01-005_55	4997
MD01-005	D+44	SHCPSAMGI	94	MD01-005_56	5048
MD01-005	D+44	FHASEGWL	95	MD01-005_57	5819
MD01-005	D+44	THEVIVPL	96	MD01-005_58	5842
MD01-005	D+44	SRHCLQPL	97	MD01-005_59	3327

MD01-005	D+44	FASLALARRY	98	MD01-005_60	913
MD01-005	D+44	SVFNTWNPWW	99	MD01-005_61	2546
MD01-005	D+44	LTHEVIVPL	100	MD01-005_62	3832
MD01-005	D+44	YTVMARKSPV	101	MD01-005_63	2207
MD043-003	D+121	LSEKGIEDY	102	MD043-003_1	2372
MD043-003	D+121	MSDVRTVF	103	MD043-003_2	11614
MD043-003	D+121	NSDEPVNLTF	104	MD043-003_3	9178
MD043-003	D+121	MSDVRTVFL	105	MD043-003_4	6417
MD043-003	D+121	ANDVNDALGY	106	MD043-003_6	2332
MD043-003	D+121	LLASVAPRY	107	MD043-003_7	8335
MD043-003	D+121	ALMAVIVLV	108	MD043-003_11	1429
MD043-003	D+121	ALMAVIVLVAL	109	MD043-003_12	7235
MD043-003	D+121	FLNGLEETAGV	110	MD043-003_13	10252
MD043-003	D+121	ALMAVIVL	111	MD043-003_14	3718
MD043-003	D+121	LMAVIVLV	112	MD043-003_15	8100
MD043-003	D+121	VLVALMAV	113	MD043-003_17	7404
MD043-003	D+121	MLAACAGEV	114	MD043-003_19	12755
MD043-003	D+121	YPMCSGEKAY	115	MD043-003_21	10220
MD043-003	D+121	MPSNIQNF	116	MD043-003_22	9067
MD043-003	D+121	LPVAVLVALM	117	MD043-003_25	10244
MD043-003	D+121	MTSGVYAF	118	MD043-003_26	9261
MD043-003	D+121	LPTPTYPL	119	MD043-003_28	12076
MD043-003	D+121	YSMSDVRTVF	120	MD043-003_29	2552
MD043-003	D+121	DANDVNDALGY	121	MD043-003_30	10469
MD043-003	D+121	AEAGAEAASL	122	MD043-003_31	11061
MD043-003	D+121	AEAASLNASL	123	MD043-003_32	1822
MD043-003	D+121	LENCAEVMRL	124	MD043-003_34	3373
MD043-003	D+121	AETQSRFQLL	125	MD043-003_35	7423
MD043-003	D+121	LENCAEVM	126	MD043-003_36	8204
MD043-003	D+121	LENCAEVMRL	127	MD043-003_37	11116
MD043-003	D+121	SENSDEPVNL	128	MD043-003_38	12313
MD043-003	D+121	TEDEIYSRCL	129	MD043-003_39	11559
MD043-003	D+121	AESEAHDRSM	130	MD043-003_40	10305
MD043-003	D+121	YSMSDVRTVFL	131	MD043-003_41	9418
MD043-003	D+121	YSMSDVRTV	132	MD043-003_42	10733
MD043-003	D+121	FASGADVQV	133	MD043-003_43	7321
MD043-003	D+121	MPRQPSCPL	134	MD043-003_63	10902
MD043-003	D+121	TPLCQHIAL	135	MD043-003_64	8635

MD043-008	D-14	SLHEFHLV	136	MD043-008_1	7247
MD043-008	D-14	HLVDLSRRFLV	137	MD043-008_2	7043
MD043-008	D-14	RLSDETLIDIV	138	MD043-008_3	6674
MD043-008	D-14	VLFDTDQDPL	139	MD043-008_4	5953
MD043-008	D-14	VLFDTDQDPLNA	140	MD043-008_5	3903
MD043-008	D-14	LMSAAAIYTV	141	MD043-008_6	5919
MD043-008	D-14	CLMSAAAIYTV	142	MD043-008_7	6544
MD043-008	D-14	ILAGLCLMSA	143	MD043-008_8	5887
MD043-008	D-14	LLLPELCSA	144	MD043-008_9	6801
MD043-008	D-14	KMALLQYL	145	MD043-008_10	5656
MD043-008	D-14	LMSAAAIY	146	MD043-008_11	4685
MD043-008	D-14	CLMSAAAIY	147	MD043-008_12	3594
MD043-008	D-14	LLLPELCSAFY	148	MD043-008_13	5631
MD043-008	D-14	HLGKPGHLSY	149	MD043-008_14	6244
MD043-008	D-14	MSLCVLLY	150	MD043-008_15	5317
MD043-008	D-14	DMSLCVLLY	151	MD043-008_16	4583
MD043-008	D-14	LDMSLCVLLY	152	MD043-008_17	4754
MD043-008	D-14	SLDMSLCVLLY	153	MD043-008_18	5434
MD043-008	D-14	TTYRDWLGLDY	154	MD043-008_19	6571
MD043-008	D-14	LMCQKFLARY	155	MD043-008_20	4641
MD043-008	D-14	APTGNFCPQPL	156	MD043-008_21	6725
MD043-008	D-14	CPQPLLNSSM	157	MD043-008_22	3735
MD043-008	D-14	TPNYSVSML	158	MD043-008_23	4387
MD043-008	D-14	TPNYSVSM	159	MD043-008_24	5374
MD043-008	D-14	YPNGTSSL	160	MD043-008_25	5005
MD043-008	D-14	KPGHLSYAL	161	MD043-008_26	3580
MD043-008	D-14	FTRKLLGSAL	162	MD043-008_27	5003
MD043-008	D-14	GPASYPIPV	163	MD043-008_28	6553
MD043-008	D-14	LPTESPHSSL	164	MD043-008_29	4529
MD043-008	D-14	IPHFTATSDAF	165	MD043-008_30	6195
MD043-008	D-14	VEIKAVPEGF	166	MD043-008_31	4343
MD043-008	D-14	DEVSATETCY	167	MD043-008_32	4327
MD043-008	D-14	GELDNQLTTY	168	MD043-008_33	4151
MD043-008	D-14	AELGQVLIY	169	MD043-008_34	3953
MD043-008	D-14	AELGQVLI	170	MD043-008_35	5558
MD043-008	D-14	AELGQVLIYL	171	MD043-008_36	4401
MD043-008	D-14	PELCSAFY	172	MD043-008_37	4800
MD043-008	D-14	EEFLNHASKAW	173	MD043-008_38	7314

MD043-008	D-14	TEEFLNHASKAW	174	MD043-008_39	6529
MD043-008	D-14	IENLHDDSCY	175	MD043-008_40	4882
MD043-008	D-14	SRMHRGGLRL	176	MD043-008_41	4702
MD043-008	D-14	MYHSRMHRGGL	177	MD043-008_42	5150
MD043-008	D-14	FFFTRKLL	178	MD043-008_43	5196
MD043-008	D-14	IRLQILRQVSL	179	MD043-008_44	4077
MD043-008	D-14	FYYTGVGM	180	MD043-008_45	4947
MD043-008	D-14	FYYTGVGML	181	MD043-008_46	3735
MD043-008	D-14	FYYTGVGMLI	182	MD043-008_47	5955
MD043-008	D-14	YYTGVGML	183	MD043-008_48	4042
MD043-008	D-14	YYTGVGMLI	184	MD043-008_49	5595
MD043-008	D-14	FYPATFGIL	185	MD043-008_50	5992
MD043-008	D-14	YAPPQDGPASY	186	MD043-008_51	5474
MD043-008	D-14	FTATSDAF	187	MD043-008_52	4821
MD043-008	D-14	FQMDDYSLCVL	188	MD043-008_53	6371
MD043-008	D-14	MTFSNPPDWL	189	MD043-008_54	5041
MD043-008	D-14	YAAHLLDIAM	190	MD043-008_55	4947
MD043-008	D-14	FFYAAHLL	191	MD043-008_56	4234
MD043-008	D-14	FYAAHLLDIAM	192	MD043-008_57	7088
MD043-008	D-14	FMDSCTMRF	193	MD043-008_58	4652
MD043-008	D-14	KSMERDCATF	194	MD043-008_59	5787
MD01-004	D+21	RTWRRTTR	195	MD01-004_01	10375
MD01-004	D+21	RTWRRTTRGR	196	MD01-004_02	9922
MD01-004	D+21	RTWRRTTRGRR	197	MD01-004_03	11966
MD01-004	D+21	RTRRGRRSSR	198	MD01-004_04	9713
MD01-004	D+21	RSSRTLRSR	199	MD01-004_05	9031
MD01-004	D+21	VMYDGFVSQR	200	MD01-004_06	8929
MD01-004	D+21	CVKVCAYIR	201	MD01-004_07	8981
MD01-004	D+21	KSTSISTAMR	202	MD01-004_08	7928
MD01-004	D+21	GASSIWYR	203	MD01-004_09	9723
MD01-004	D+21	AGASSIWYR	204	MD01-004_10	7655
MD01-004	D+21	YVMYDGFVSQR	205	MD01-004_11	13002
MD01-004	D+21	NSSCMGGMNLR	1	MD01-004_12	9183
MD01-004	D+21	ELFLVKAKIHK	206	MD01-004_13	11302
MD01-004	D+21	STSISTAMR	207	MD01-004_14	10062
MD01-004	D+21	TSISTAMR	208	MD01-004_15	10850
MD01-004	D+21	FIFTSIAGIR	209	MD01-004_16	8630
MD01-004	D+21	FTSIAGIR	210	MD01-004_17	6833

MD01-004	D+21	FTNRKVPYCFK	211	MD01-004_18	6093
MD01-004	D+21	EAFHQSCFR	212	MD01-004_19	8912
MD01-004	D+21	HPNVILNSLY	213	MD01-004_20	8245
MD01-004	D+21	FPNVVSGL	214	MD01-004_21	9899
MD01-004	D+21	MAENTEGDLNF	215	MD01-004_22	9485
MD01-004	D+21	MLVELTPPY	216	MD01-004_23	7595
MD01-004	D+21	EPSDVTETLM	217	MD01-004_24	6746
MD01-004	D+21	EPSDVTETL	218	MD01-004_25	8694
MD01-004	D+21	SPAMTSTSFFF	219	MD01-004_26	7945
MD01-004	D+21	MTSTSFFF	220	MD01-004_27	6631
MD01-004	D+21	MAIEDILF	221	MD01-004_28	6022
MD01-004	D+21	IPEELEYF	222	MD01-004_29	4958
MD01-004	D+21	MPICPTYNEV	223	MD01-004_30	4557
MD01-004	D+21	CAYIRKQVEKI	223	MD01-004_31	5540
MD01-004	D+21	LAQEGTTVI	224	MD01-004_32	7055
MD01-004	D+21	HPNVILNSLYV	225	MD01-004_33	11278
MD01-004	D+21	LPDHFGLGPV	226	MD01-004_34	8888
MD01-004	D+21	MAIEDILFV	227	MD01-004_35	10310
MD01-004	D+21	IPEELEYFI	228	MD01-004_36	5052
MD01-004	D+21	EPQNFIDSLI	229	MD01-004_37	9296
MD01-004	D+21	CPTYNEVHL	230	MD01-004_38	9098
MD01-004	D+21	MYDGFSVQRL	231	MD01-004_39	11006
MD01-004	D+21	MYDGFSVQRLV	232	MD01-004_40	8422
MD01-004	D+21	AYDASTFRGL	233	MD01-004_41	7408
MD01-004	D+21	FTDCGRPPL	234	MD01-004_42	6671
MD01-004	D+21	KFDLFARL	235	MD01-004_43	4954
MD01-004	D+21	STYLIAQSI	236	MD01-004_44	6837
MD01-004	D+21	KSTSISTAMRL	237	MD01-004_45	8566
MD01-004	D+21	QTFGKMFFV	238	MD01-004_46	6488
MD01-004	D+21	WAYDASTFRGL	239	MD01-004_47	6516
MD01-004	D+21	STHPPGASL	240	MD01-004_48	7184
MD01-004	D+21	RADPRAGPSV	241	MD01-004_49	8889
MD01-004	D+21	MTSTSFFFTL	242	MD01-004_50	7303
MD01-004	D+21	RSAEPQNFI	243	MD01-004_51	7542
MD01-004	D+21	LTSSDDLLI	244	MD01-004_52	7889
MD043-011	D-14	KYMLNSVLENF	245	MD043-011_01	7977
MD043-011	D-14	YMLNSVLENF	246	MD043-011_02	8241
MD043-011	D-14	GYACAEPFSF	247	MD043-011_03	4740

MD043-011	D-14	FFAAQAGAWKI	248	MD043-011_04	10389
MD043-011	D-14	SFFAAQAGAW	249	MD043-011_05	6864
MD043-011	D-14	YMLKAKSQF	250	MD043-011_06	9612
MD043-011	D-14	RYFVPKML	251	MD043-011_07	10540
MD043-011	D-14	ATLNGRMYF	252	MD043-011_08	11654
MD043-011	D-14	YTISFLFW	253	MD043-011_09	4760
MD043-011	D-14	DYTISFLFW	254	MD043-011_10	7551
MD043-011	D-14	KYMLNSVL	255	MD043-011_11	7219
MD043-011	D-14	RYPKAVTL	256	MD043-011_12	10782
MD043-011	D-14	FFAAQAGAW	257	MD043-011_13	3924
MD043-011	D-14	EYMLKAKSQF	258	MD043-011_14	7099
MD043-011	D-14	KESFGPQAL	259	MD043-011_15	9032
MD043-011	D-14	CEVAPNNVV	260	MD043-011_16	5433
MD043-011	D-14	KEMHPNKLNAV	261	MD043-011_17	1852
MD043-011	D-14	CEVAPNNV	262	MD043-011_18	3343
MD043-011	D-14	KQFFYNII	263	MD043-011_19	3030
MD043-011	D-14	SQLQGLQL	264	MD043-011_20	1106
MD043-011	D-14	TEYKLVVVGAC	265	MD043-011_21	2022
MD043-011	D-14	FEDGPYAV	266	MD043-011_22	623
MD043-011	D-14	AQAGAWKI	267	MD043-011_23	1990
MD043-011	D-14	AQAGAWKIYAV	51	MD043-011_24	2723
MD043-011	D-14	AERLVGPGY	268	MD043-011_25	2709
MD043-011	D-14	IEYMLKAKSQF	269	MD043-011_26	2317
MD043-011	D-14	SDYTISFLFW	270	MD043-011_27	10837
MD043-011	D-14	GELGWENPNQW	271	MD043-011_28	2829
MD043-011	D-14	SEMTAVTQKI	272	MD043-011_29	3030
MD043-011	D-14	SEMTAVTQKIV	273	MD043-011_30	3215
MD043-011	D-14	AQAGAWKIY	52	MD043-011_31	7000
MD043-011	D-14	NKMDMNQW	274	MD043-011_32	12195
MD043-011	D-14	YTSSEVSTV	275	MD043-011_33	5938
MD043-011	D-14	YTSSEVSTVEL	276	MD043-011_34	5611
MD043-011	D-14	YSPDILPTV	277	MD043-011_35	3833
MD043-011	D-14	FAAQAGAWKIY	53	MD043-011_36	5945
MD043-011	D-14	FAAQAGAWKI	278	MD043-011_37	8014
MD043-011	D-14	FAAQAGAW	279	MD043-011_38	4933
MD043-011	D-14	KTATLNGRMYF	280	MD043-011_39	8230
MD043-011	D-14	KTATLNGRM	281	MD043-011_40	5929
MD043-011	D-14	YTISFLFWIL	282	MD043-011_41	3883

MD043-011	D-14	YTISFLFWI	283	MD043-011_42	13198
MD043-011	D-14	IALRPSGTM	284	MD043-011_43	7339
MD043-011	D-14	IALRPSGTML	285	MD043-011_44	5416
MD043-011	D-14	FAVEAHQCI	286	MD043-011_45	2936
MD043-011	D-14	MSSLPCPL	287	MD043-011_46	3147
MD043-011	D-14	YACAEPSF	288	MD043-011_47	10633
MD043-011	D-14	IVDPDPVL	289	MD043-011_48	2971
MD043-011	D-14	RALKEKAQPL	290	MD043-011_49	6372
MD01-019	D+38	YLNSRQFPM	291	MD01-019_01	4547
MD01-019	D+38	SIMALSTSI	292	MD01-019_02	3219
MD01-019	D+38	SLTDISTL	293	MD01-019_03	4704
MD01-019	D+38	FLISYWSEI	294	MD01-019_04	4375
MD01-019	D+38	SMLSLPRV	295	MD01-019_05	5301
MD01-019	D+38	AVASVLPLWPA	296	MD01-019_06	5247
MD01-019	D+38	MLLVIIIVSVGI	297	MD01-019_07	2920
MD01-019	D+38	SQHQVLFFL	298	MD01-019_10	5055
MD01-019	D+38	RLRTDLFSK	299	MD01-019_11	4252
MD01-019	D+38	KQRTSSEK	300	MD01-019_12	10161
MD01-019	D+38	RLKYNLQGYK	301	MD01-019_13	6999
MD01-019	D+38	RSRRSTTA	302	MD01-019_14	4772
MD01-019	D+38	RMRAMATA	303	MD01-019_15	4574
NY016-007	D+30	LTSPIVCF	304	NY016-007_1	4236
NY016-007	D+30	LARASPALASL	305	NY016-007_2	4820
NY016-007	D+30	LRNGALTSPi	306	NY016-007_3	4793
NY016-007	D+30	VLRNGALTSPi	307	NY016-007_4	3590
NY016-007	D+30	LRNGALTSPiV	308	NY016-007_5	4288
NY016-007	D+30	LARASPAL	309	NY016-007_6	4906
NY016-007	D+30	SLARASPAL	310	NY016-007_7	4390
NY016-007	D+30	LRLSLTFSLV	311	NY016-007_8	3722
NY016-007	D+30	SAITSKVSTV	312	NY016-007_9	6575
NY016-007	D+30	AITSKVSTV	313	NY016-007_10	4669
NY016-007	D+30	SAITSKVSTV	314	NY016-007_11	5002
NY016-007	D+30	ASLARASPA	315	NY016-007_12	4526
NY016-007	D+30	SLARASPA	316	NY016-007_13	4351
NY016-007	D+30	ASLARASPAL	317	NY016-007_14	3413
NY016-007	D+30	QASLARASPA	318	NY016-007_15	3735
NY016-007	D+30	LARASPALA	319	NY016-007_16	5266
NY016-007	D+30	KLRLSLTFSLV	320	NY016-007_17	2321

NY016-014	D+30	LLADATVEL	321	NY016-014_1	3258
NY016-014	D+30	LLADATVELSL	322	NY016-014_2	3250
NY016-014	D+30	HMAFSPAV	323	NY016-014_3	3886
NY016-014	D+30	YLDIVFL	324	NY016-014_4	3682
NY016-014	D+30	FLEDLSPL	325	NY016-014_5	3939
NY016-014	D+30	YLDIVFLEDL	326	NY016-014_6	3374
NY016-014	D+30	FLEDLSPLEA	327	NY016-014_7	3012
NY016-014	D+30	LLHGAEPKL	328	NY016-014_8	2973
NY016-014	D+30	TLIDVPKV	329	NY016-014_9	3612
NY016-014	D+30	TMACINLA	330	NY016-014_10	2921
NY016-014	D+30	LSKDIMFHFK	331	NY016-014_11	4462
NY016-014	D+30	VTMACINLASK	332	NY016-014_12	4308
NY016-014	D+30	TMACINLASK	333	NY016-014_13	4043
NY016-014	D+30	MSYDNNLFK	334	NY016-014_14	2860
NY016-014	D+30	KTWKEKTLK	335	NY016-014_15	5046
NY016-014	D+30	VTLIDVPK	336	NY016-014_16	5284
NY016-014	D+30	MPLVHMAF	337	NY016-014_21	5254
NY016-014	D+30	MPLVHMAFSPA	338	NY016-014_22	5131
NY016-014	D+30	YPDYLDIVF	339	NY016-014_23	4721
NY016-014	D+30	YPDYLDIVFL	340	NY016-014_24	4822
NY016-014	D+30	MSYDNNLF	341	NY016-014_25	4269
NY016-014	D+30	MPLVHMAF	342	NY016-014_31	4186
NY016-014	D+30	MAFSPAVDV	343	NY016-014_32	5182
NY016-025	D-3	AVQWLRPK	344	NY016-025_01	6087
NY016-025	D-3	HVMPDTPDILK	345	NY016-025_02	6160
NY016-025	D-3	KVMYILFY	346	NY016-025_03	6036
NY016-025	D-3	VQNAVQWLRPK	347	NY016-025_04	5922
NY016-025	D-3	VQNAVQWLR	348	NY016-025_05	6275
NY016-025	D-3	TLFQIYDNLR	349	NY016-025_06	6535
NY016-025	D-3	CLASLHPR	350	NY016-025_07	5801
NY016-025	D-3	RSLGCLASLH	351	NY016-025_08	5860
NY016-025	D-3	KLLHEYWMSLR	352	NY016-025_09	5149
NY016-025	D-3	LLHEYWMSLR	353	NY016-025_10	6186
NY016-025	D-3	EVKEEDEPF	354	NY016-025_11	5870
NY016-025	D-3	QVNKVMYILFY	355	NY016-025_12	6117
NY016-025	D-3	EVQNAVQWL	356	NY016-025_13	5838
NY016-025	D-3	LHEYWMSL	357	NY016-025_14	5991
NY016-025	D-3	YKLLHEYWMSL	358	NY016-025_15	5071

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NY016-025	D-3	MESNNSTL	359	NY016-025_16	5662
NY016-025	D-3	MESNNSTLFI	360	NY016-025_17	5565

Table 9. MANAFEST assay results summary

Study ID	Histologic subtype	% residual tumor	No. of nonsynonymous mutations per exome	No. of predicted nonredundant MANAs	MANAFEST Timepoint ^c	No. of putative MANAs tested	No. of positive MANAs	% of MANAs positive	No. of MANA-specific TCR β clones identified	Jurkat/NFAT validation of MANA specific clonotypes
NY016-025	Adenosquamous	0	27	75	D-3	17	4	23.5%	4	NA
MD01-005 ^{a,b}	Squamous	0	256	158	D+44	47	3	6.3%	4	2
MD043-003 ^a	Adeno	5	66	297	D+121	34	2	5.9%	2	NA
MD043-008*	Adeno	10	310	213	D-14	59	0	0.0%	0	NA
MD01-004*	Adenosquamous	40	99	63	D+21	52	27	51.9%	41	4
NY016-007*	Squamous	60	5	1	D+30	17	2	11.8%	2	NA
MD043-011*	Adeno	75	75	46	D-14	49	3	6.1%	3	1
MD01-019*	Adeno	95	105	59	D-14	13	0	0.0%	0	NA
NY016-014*	Adeno	95	26	19	D+30	23	12	52.1%	16	NA

* WES and predicted neoantigens previously reported in Forde et al., *N. Engl. J. Med.*, 378:1976-1986 (2018))^a No pre-treatment biopsy available for WES. WES performed on resected tumor^b MANAFEST results reported in Forde et al., *N. Engl. J. Med.*, 378:1976-1986 (2018)) and Danilova et al., *Can. Immunol. Res.*, 6:888-899 (2018)^c Relative to surgical resection

Additionally, viral-specific TCRs, identified by culture with CEF (positive control in the MANAFEST assay) or influenza peptide pools, were detected in 5 of the 9 patients tested (Figure 4, Figure 7, and Table 8 (Figure 14)). A total of 88 unique viral-specific TCRs were identified; 54 of these were specific for flu and 34 of these were CEF-specific T cell clones (of which 6 could be mapped to public EBV-reactive TCR β clonotypes, 7 to public flu-reactive TCR β clonotypes). No CMV-reactive TCRs were mapped from our viral-specific TCRs. No consistent pattern was observed for the frequency of viral T cells in the tissue or peripheral blood (Figure 8 and Figure 9).

The transcriptional programming of neoantigen- and viral-specific CD8⁺ T cells was next evaluated. To do this, a more refined clustering of all CD8⁺ T cells (n=235,851) was performed and 15 unique clusters were identified, 3 of which with gene expression programs consistent with T_{eff} cells and 2 additional clusters co-expressing CD4 and CD8 and 6 with gene expression programs associated with TRM T cells, characterized by HOBIT expression, LINC02246 expression, and high CD103 expression (Figure 2C and Figure 10). Selective genes were visualized by UMAP (Figure 10). Among all patients tested, a total of 28 MANA-specific clonotypes (1,350 total cells from 3 MPR and 3 non-MPR) were found in the CD8 single cell analysis; 21 of these (890 cells) were in the tumor (Table 8 (Figure 14)). Of the viral-specific T cell clonotypes, 28 flu-specific (1,009 cells) and 2 EBV-specific (281 cells) clones were found in the CD8 single cell analysis.

Overlay of these clonotypes onto the CD8⁺ T cell UMAP demonstrated a striking distinction between the clonotypes with different antigen specificities. EBV-reactive T cells primarily resided in T_{eff} clusters, whereas flu- and MANA-specific T cells largely occupied distinct TRM clusters. This is notable considering that influenza is a respiratory virus and thus, flu-specific T cells are the quintessential lung-resident memory T cells. None of the patients in this study were symptomatic for influenza in the 6 weeks preceding surgery. It is thus not surprising that flu-specific CD8 cells were TRM rather than T_{eff}. While flu-specific cells were most numerous in normal lung, MANA-specific CD8 cells were more common in the tumor (Figure 2E), likely owing to their exposure to more tumor antigen in the tumor

microenvironment than in normal lung. Indeed, there were significantly more MANA-specific CD8 cells among the proliferating subset of TIL than in adjacent NL.

Surprisingly there were significant shared gene expression programs between MANA- and EBV-specific T cells, in particular genes encoding T cell activation and CTL activity, such as HLA-DR, GZMH, and NKG7 (Figures 2F and 2G). However, genes encoding other cytolytic granule molecules, such as GZMK, were almost absent in MANA-specific TIL. Also, transcription factors critical to CTL activity, Eomes and TBX21 (Tbet), were present in EBV-specific CD8 cells but virtually absent in most of the MANA-specific cells. These findings demonstrate that MANA-specific T cells in the tumor have a partial but incomplete effector program, possibly down-modulated among MANA-specific CD8 cells by higher levels of checkpoint molecules, such as PD-1, CTLA-4, HAVCR2 (Tim3), LAG3, TIGIT, and ENTPD1 (CD39). In fact, each of these checkpoints was more highly expressed among MANA-specific CD8 cells than either flu- or EBV-specific CD8 cells, with CD39 being the most highly differentially expressed (Figure 2G). MANA-specific cells express higher levels of PDRM1, which encodes Blimp-1 and has been reported to participate in coordinated transcriptional activation of multiple of these checkpoint genes, including PD-1, LAG3, TIGIT and HAVCR2. Tox, a chromatin modifier important for exhaustion/anergy programs of chronic virus-specific and tumor-specific T cells, was only marginally increased in MANA-specific cells but its homolog, Tox2, which has also been reported to drive T cell anergy/exhaustion, showed much greater differential expression between MANA-specific and EBV-specific CD8 cells. ZNF683 (HOBIT), whose expression must be turned off in order for TRM to differentiate to T_{eff} upon antigen encounter, was also upregulated in MANA-specific TIL, even relative to flu-specific TRM. Additionally, flu-specific TRM were distinguished from MANA-specific TRM by extremely low levels of both activation (including MHC II) and effector CTL programs and multiple checkpoint molecules such as ENTPD1, TNFRSF9, and CTLA-4, but had the highest levels of genes encoding stem/memory molecules, such as TCF7 and IL7R (Figure 2H). Neither of these molecules are expressed at significant levels in MANA-specific T cells (Figures 2F and 2G) and represent a significant element of the differential gene expression that separates flu- and MANA-specific T cells into distinct TRM clusters. Culture with titrating concentrations of

IL7 *in vitro* induce much higher levels of IL7R-regulated genes in flu-specific TIL relative to MANA-specific TIL (Figure 2I).

Critical to the understanding of ICB sensitivity vs resistance is the expression profiling of MPR vs non-MPR CD8 TIL. The neoadjuvant clinical trial format allowed us to make this distinction pathologically, which has been reported to be more sensitive than classical radiologic assessment, which has been reported to underestimate therapeutically relevant responses. Profiling of MANA-specific CD8+ T cells demonstrated significant differences between pathologic MPR vs. non-MPR tumors (Figure 3). Unsupervised clustering of 6 clones (26 transcriptomes total) of MANA-specific TIL from 3 MPR patients vs 15 clones (864 transcriptomes total) of those from 3 non-MPR demonstrated 100% segregation of MPR transcriptomes from non-MPR transcriptomes (Figure 3A). IL7R is higher in MPR than non-MPR CD8 MANA-specific clones (Figure 3B). A composite checkpoint score was created consisting of 6 of the best characterized (and clinically targeted) checkpoints: CTLA4, PDCD1, LAG3, HAVCR2, TIGIT and ENTPD1. The checkpoint score was significantly higher in non-MPR MANA-specific TIL than MPR MANA-specific TIL (Figure 3C). In non-MPR MANA-specific TIL, all six checkpoints comprising the checkpoint score are within the top 12 genes correlated with the checkpoint score (Figures 3D and 3E), as compared to only one – ENTPD1 – in the top 30 associated genes in MPR TIL. This finding emphasizes the strong coordinate up-regulation of checkpoints in non-MPR. In one non-MPR, MANA-specific cells were identified upon single cell profiling of CD8 TIL from a resected brain metastasis arising 24 months after the resection of the primary tumor (Figure 11A). Relative to the primary tumor, even higher levels of three checkpoints – LAG3, TIGIT and HAVCR2 – were expressed on MANA-specific CD8 TIL from the metastasis and shared with TIL from the primary tumor (Figure 11A). CXCL13 is the most highly expressed checkpoint-associated gene in non-MPR MANA-specific TIL, as was also found to be highly expressed in MANA-specific cells relative to virus-specific cells among CD8 TIL (Figures 2F and 2H). While CXCL13 is known as a Tfh chemokine that attracts B cells to follicles, it is also part of the genetic program in chronic virus-induced CD8 exhaustion, though its role in this process is unknown.

A number of genes encoding T cell inhibitory molecules are also more highly expressed among MANA-specific TIL from non-MPR vs MPR (Figure 3E). These include the killer inhibitory receptor, KIR2DL4, and a subunit of the HLA-E binding receptor – KLRD1 (CD94) – which has been shown to inhibit CD8 activity. While these inhibitory receptors are well studied in NK cells, they also inhibit CD8 T cell activity. Additional inhibitors of T cell activation highly up-regulated in non-MPR were DTX1 (Deltex1), AKAP5, LAYN (Laylin), and ADGRG1. DTX1 has been shown to be an NFAT target that, together with EGR2, drives T cell anergy. AKAPs direct protein kinase A (PKA) to subcellular locations. In T cells, PKA inhibits T cell activation via C-terminal Src kinase (Csk) activation, which in turn inhibits lymphocyte-specific protein tyrosine kinase (Lck) activity through phosphorylation at Y505. Laylin is a membrane protein on T_{reg} and CD8 T cells that appears to inhibit CTL activation, though by as yet unknown mechanisms. ADGRG1 is a HOBIT-induced gene that has been shown to inhibit NK cell activity but has not been previously reported in CD8 T cells.

In pathologic complete responder MD01-005 (no viable tumor in the resection specimen), MANA-specific T cell transcriptional programming in tumor, adjacent NL, TDLN, and peripheral blood was able to be characterized. All MANA-specific clones in the tumor fell into TRM clusters, whereas a significant proportion of these were in T_{eff} clusters in the TDLN and adjacent NL (Figure 3F, top). This clone was enriched via FACS sorting of peripheral blood at different time points after initiation of anti-PD-1 (by sorting with Mab specific for its V β) and found an intriguing pattern. Two weeks after initiation of anti-PD-1, almost all MANA-specific T cells in the peripheral blood fell into a tight TRM cluster, while at 4 weeks (time of surgery) a third of these cells were in T_{eff} clusters. By 3 months after resection they were below limits of detection in the blood (Figure 3F, bottom). While all these tissue compartments were only available for one MPR, these findings are consistent with our hypothesis that successful neoadjuvant PD-1 blockade results in enhanced activation of MANA-specific T cells in TDLN. Without being bound by theory, it's hypothesized that, upon activation, functional effector MANA-specific T cells enter the blood and traffic into tissues, including normal lung, in search of micro-metastatic tumor. Indeed, analysis of

TDLN from two non-MPR patients failed to demonstrate any MANA-specific CD8 cells in a Teff population (Figures 11A and 11B).

Overall, it was found that global T cell gene expression programs are poorly associated with pathologic response to PD-1 blockade. However, transcriptomic analysis of validated MANA-specific TIL demonstrated clear differences associated with response, with TIL from non-responding tumors displaying higher levels of checkpoints and additional inhibitory molecules such as Deltex1, APK5, and ADGRG1, and multiple killer inhibitory receptors.

Thus, together these results demonstrate that T cell targeting of MANA can be used to improve the outcome of ICB and can overcome resistance to ICB.

Example 2: Identification of MANAbody Clones Specific for a p53 R248L Neoantigen

TP53 is the most commonly mutated cancer driver gene, but despite extensive efforts, no drug targeting mutant *TP53* has been approved for treatment of the large number of patients whose tumor contain p53 mutations.

This Example describes the identification of antibodies highly specific to a R248L *TP53* mutation.

The MANAFEST (Mutation Associated NeoAntigen Functional Expansion of Specific T-cells) assay was used to evaluate T cell responsiveness to MANA and viral antigens. Briefly, pools of MHC class I-restricted CMV, EBV, and flu peptide epitopes (CEFX, jpt Peptide Technologies), pools representing the matrix protein and nucleoprotein from H1N1 and H3N2 (jpt Peptide Technologies), and putative neoantigenic peptides defined by the ImmunoSelect-R pipeline were used to stimulate 250,000 T cells *in vitro* for 10 days as previously described. T cells were also cultured without peptide to use as a reference for non-specific clonotypic expansion. On day 10, T cell receptor sequencing was performed on each individual peptide-stimulated T cell culture by the Sidney Kimmel Comprehensive Cancer Center FEST and TCR Immunogenomics Core (FTIC) facility or Adaptive Biotechnologies. Bioinformatic analysis of productive clones was performed to identify antigen-specific T-cell clonotypes meeting the following criteria: 1) significant expansion (Fisher's exact test with Benjamini-Hochberg correction for FDR, $p < 0.05$) compared to T

cells cultured without peptide, 2) significant expansion compared to every other peptide-stimulated culture ($FDR < 0.0001$) except for conditions stimulated with similar neoantigens derived from the same mutation, 3) an odds ratio > 5 compared to the “no peptide” control, and 4) present in at least 10% of the cultured wells to ensure adequate distribution among culture wells.

Example 3: Treating ICB Resistant Cancers

T cells expressing one or more TCRs that can bind to a p53 R248L peptide are administered to a human having an ICB resistant cancer. The administered T cells can infiltrate the tumor microenvironment to target (e.g., target and destroy) cancer cells expressing the p53 R248L peptide.

Example 4: ICB Resistant Cancers

Nuclei acid that encode a TCR that can bind to a p53 R248L peptide is introduced into T cells such that the T cells encode the TCR and the TCR is presented on the surface of the T cells.

The T cells expressing the TCR that can bind to a p53 R248L peptide are administered to a human having an ICB resistant cancer. The administered T cells can infiltrate the tumor microenvironment to target (e.g., target and destroy) cancer cells expressing the p53 R248L peptide.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A T cell receptor (TCR) that can bind to a modified p53 polypeptide comprising a R to L substitution at amino acid residue 248 (R248L).
2. The TCR of claim 1, wherein said modified p53 polypeptide comprises a p53 R248L peptide comprising or consisting essentially of the amino acid sequence set forth in any one of SEQ ID NOs:1-40.
3. The TCR of claim 1, wherein said TCR comprises an alpha (α) chain comprising a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44.
4. The TCR of claim 1, wherein said TCR comprises a beta (β) chain comprising a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48.
5. The TCR of claim 1, wherein said TCR comprises:
an α chain that includes a TCR α -CDR3 set forth in any one of SEQ ID NOs:41-44,
and
a β chain that includes a TCR β -CDR3 set forth in any one of SEQ ID NOs:45-48.
6. A T cell comprising the TRC of any one of claims 1-5.
7. The T cell of claim 6, wherein said T cell is a human T cell.
8. The T cell of claim 6, wherein said T cell is a non-human T cell.
9. A nucleic acid encoding the TRC of any one of claims 1-5.
10. The nucleic acid of claim 9, wherein said nucleic acid is in the form of a vector.

11. The nucleic acid of claim 10, wherein said vector is an expression vector.
12. The nucleic acid of claim 10, wherein said vector is a viral vector.
13. A T cell comprising the nucleic acid of any one of claims 9-12, wherein said nucleic acid encodes said TCR.
14. The T cell of claim 13, wherein said T cell is a human T cell.
15. The T cell of claim 13, wherein said T cell is a non-human T cell.
16. A method for treating a mammal having cancer, said method comprising administering to said mammal the T cell of any one of claims 6-8 or the T cell of any one of claims 13-15, wherein said cancer comprises a cancer cell expressing said modified p53 polypeptide.
17. The method of claim 16, wherein said cancer cell expressing said modified p53 polypeptide presents a p53 R248L peptide in a peptide-HLA complex.
18. The method of claim 17, wherein said p53 R248L peptide comprising or consisting essentially of the amino acid sequence set forth in any one of SEQ ID NOs:1-40.
19. The method of any one of claims 16-18, wherein said mammal is a human.
20. The method of any one of claims 16-19, wherein said cancer is selected from the group consisting of a non-small cell lung cancer (NSCLC), a colon adenocarcinoma, a rectal adenocarcinoma, a head and neck squamous cell carcinoma, a pancreatic adenocarcinoma, melanomas, a urothelial carcinoma, a uterine corpus endometrial carcinoma, and a uterine carcinoma.

21. The method of any one of claims 16-20, wherein said method further comprises administering to said mammal a checkpoint inhibitor.

22. The method of claim 21, wherein said checkpoint inhibitor is selected from the group consisting of an anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) antibody, an anti-PD-1 (programmed death 1) antibody, an anti-PD-L1 (programmed death 1 ligand) antibody, an anti-LAG3 (lymphocyte activation gene 3) antibody, an anti-Tim3 (T cell immunoglobulin and mucin domain-containing protein 3) antibody, an anti-TIGIT (T cell immunoreceptor with Ig and ITIM domains) antibody, an anti-VISTA (V-domain Ig suppressor of T cell activation) antibody, an anti-CD47 (cluster of differentiation 47) antibody, an anti-SIRPalpha (signal regulatory protein alpha) antibody, an anti-B7-H3 (B7 homolog 3) antibody, an anti-B7-H4 (B7 homolog 4) antibody, an anti-neuritin antibody, an anti-neuropilin antibody, an anti-IL-35 (interleukin 35), an IDO (indoleamine-pyrrole 2,3-dioxygenase) inhibitor, an A2AR (adenosine A2A receptor) inhibitor, an arginase inhibitor, and a glutaminase inhibitor.

23. The method of any one of claims 16-22, wherein said method further comprises administering to said mammal a co-stimulatory molecule.

24. The method of claim 23, wherein said co-stimulator molecule is an agonist of a co-stimulatory receptor.

25. The method of claim 24, wherein said agonist of a co-stimulatory receptor is selected from the group consisting of an anti-GITR (glucocorticoid-induced TNFR-related) antibody, an anti-CD27 (cluster of differentiation 27) antibodies antibody, an anti-4-1BB (CD137; cluster of differentiation 137) antibody, an anti-OX40 (CD134; cluster of differentiation 134) antibody, an anti-ICOS (inducible T-cell costimulator) antibody, and an anti-CD40 (cluster of differentiation 40) antibody.

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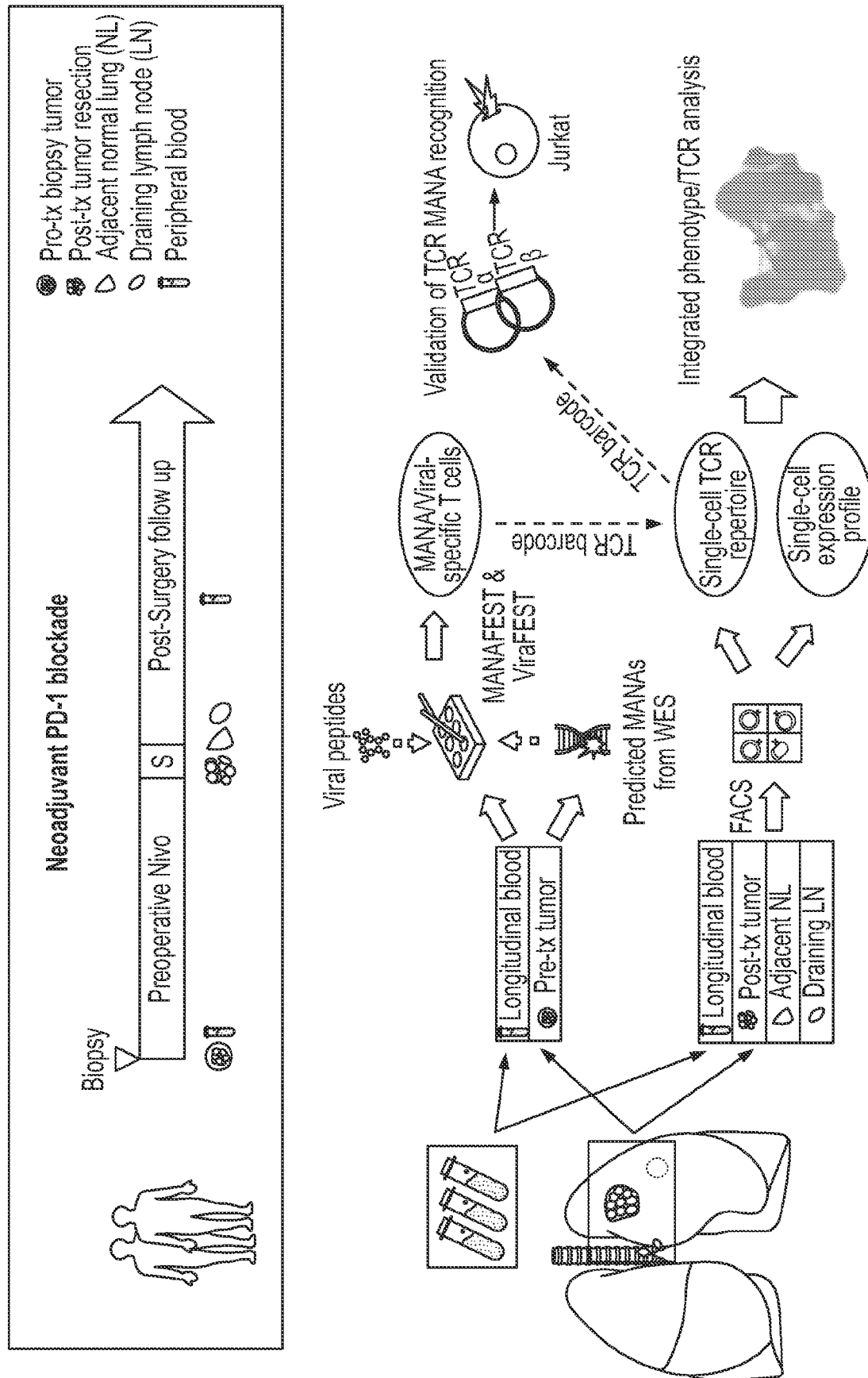


FIG. 1A

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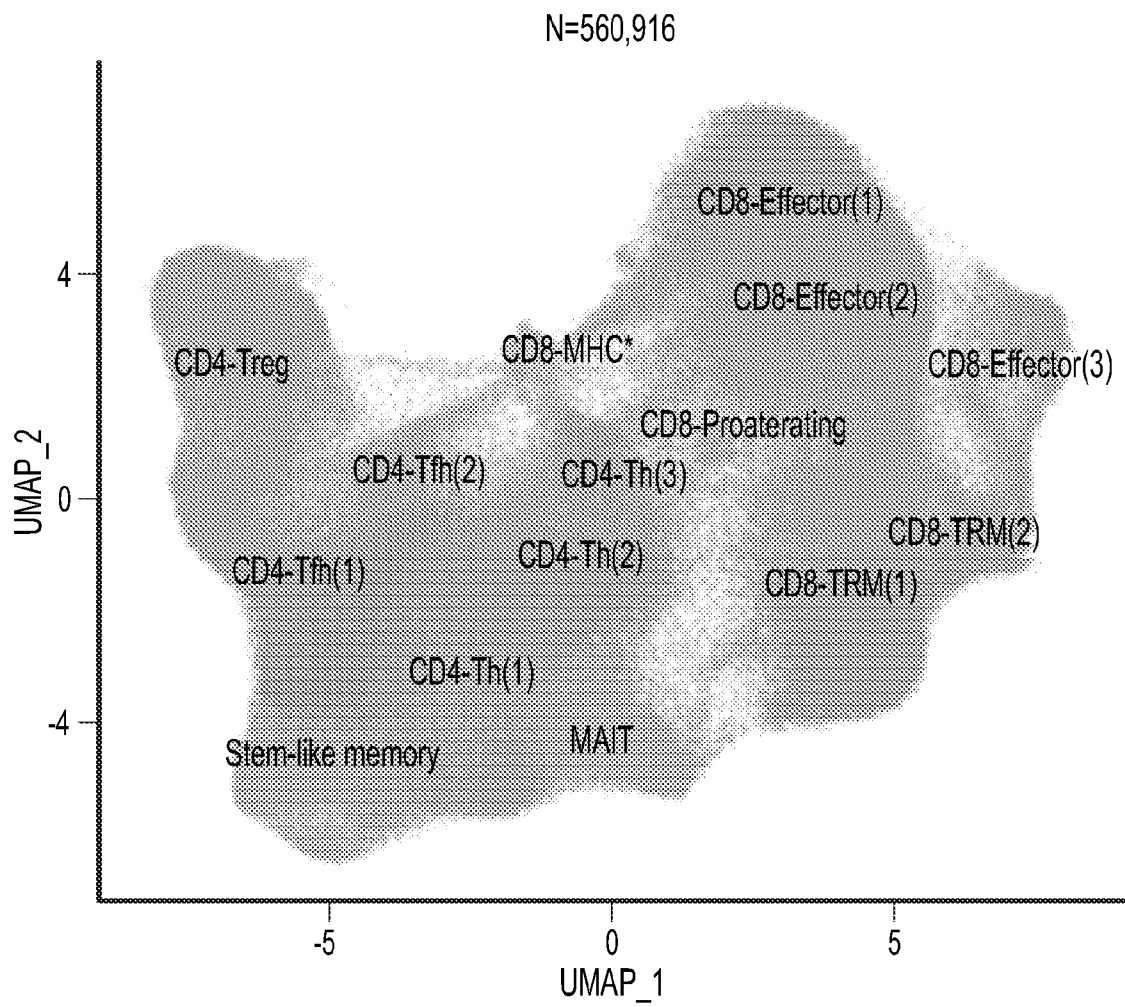


FIG. 1B

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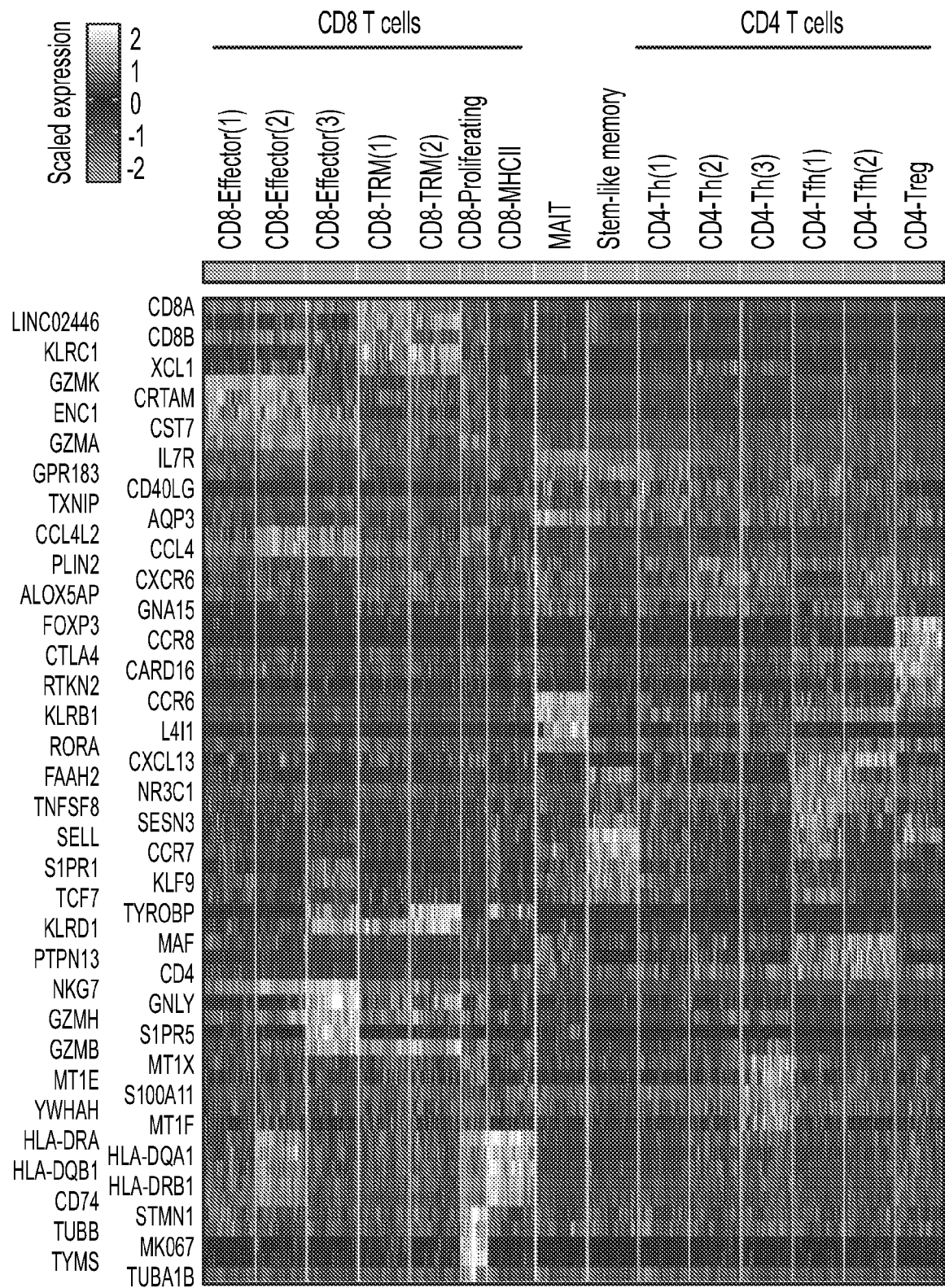


FIG. 1C

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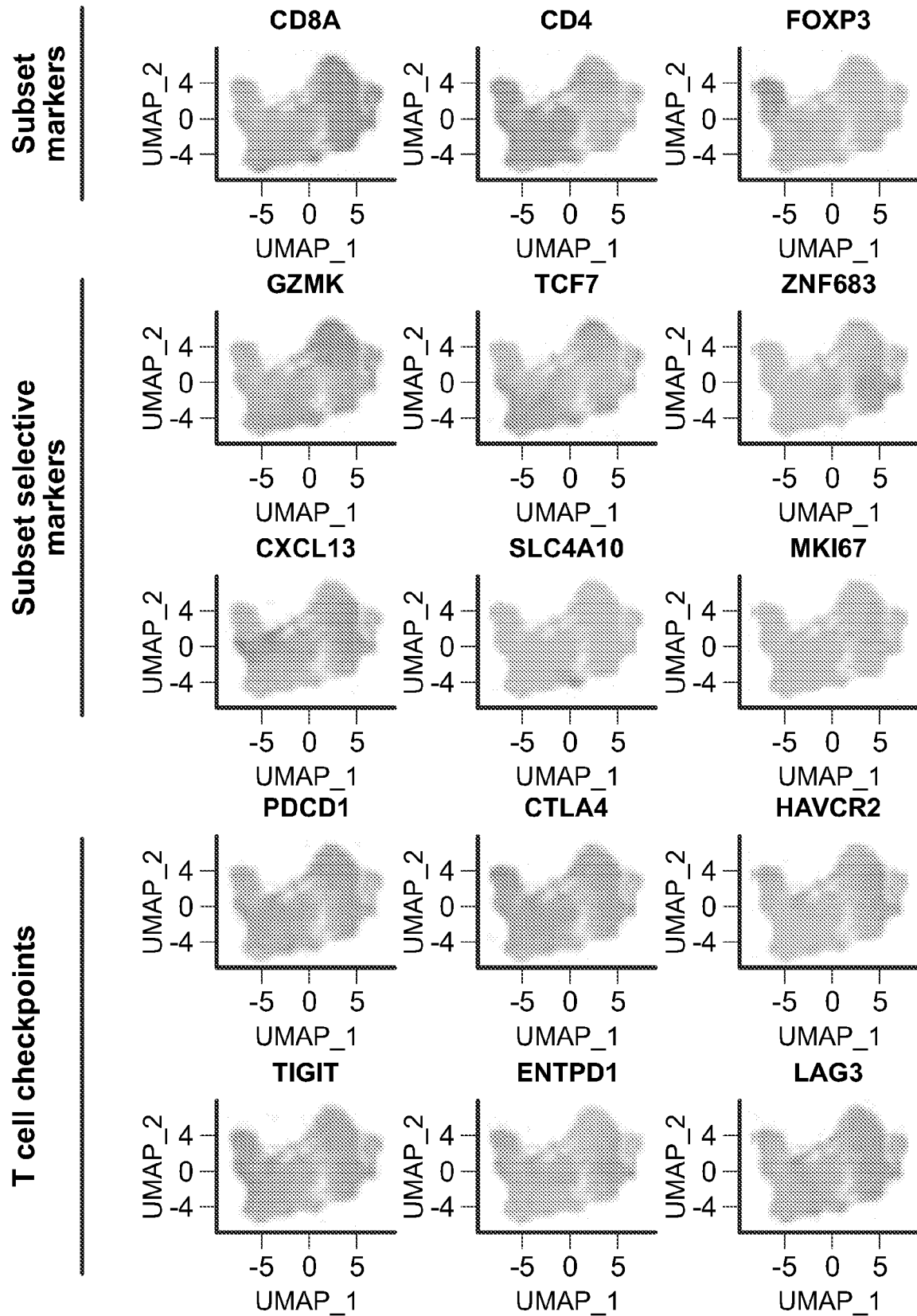


FIG. 1D

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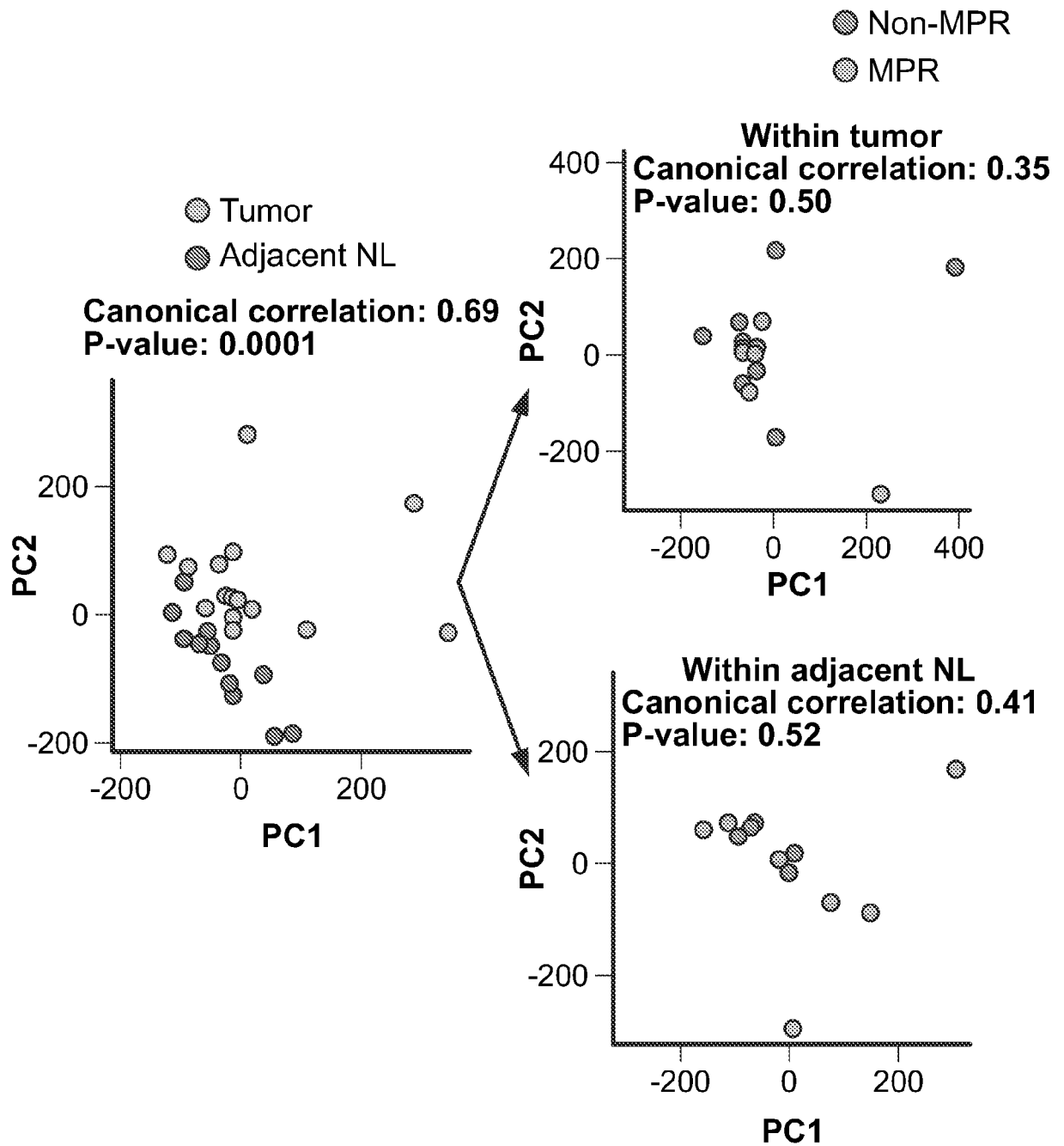
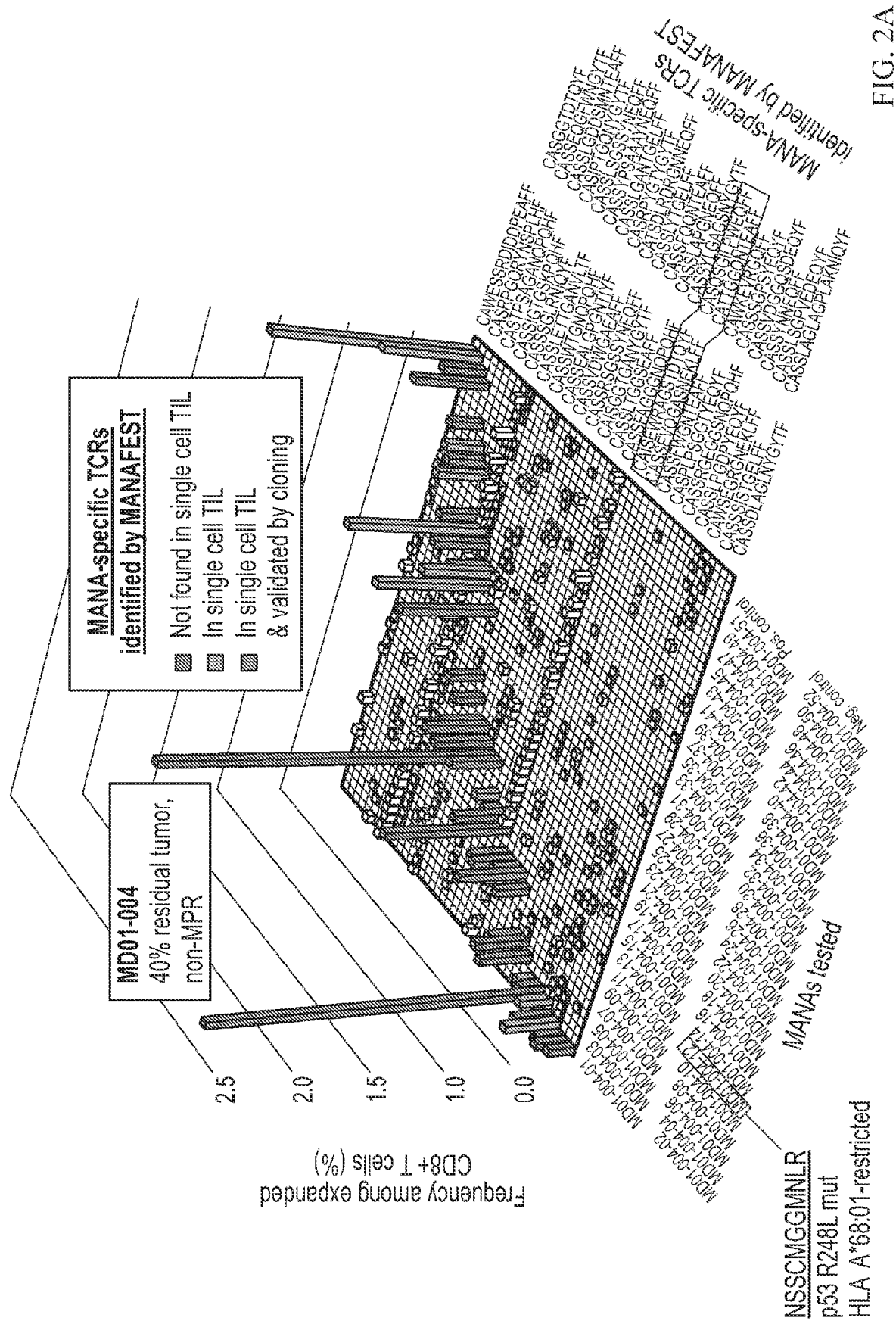


FIG. 1E



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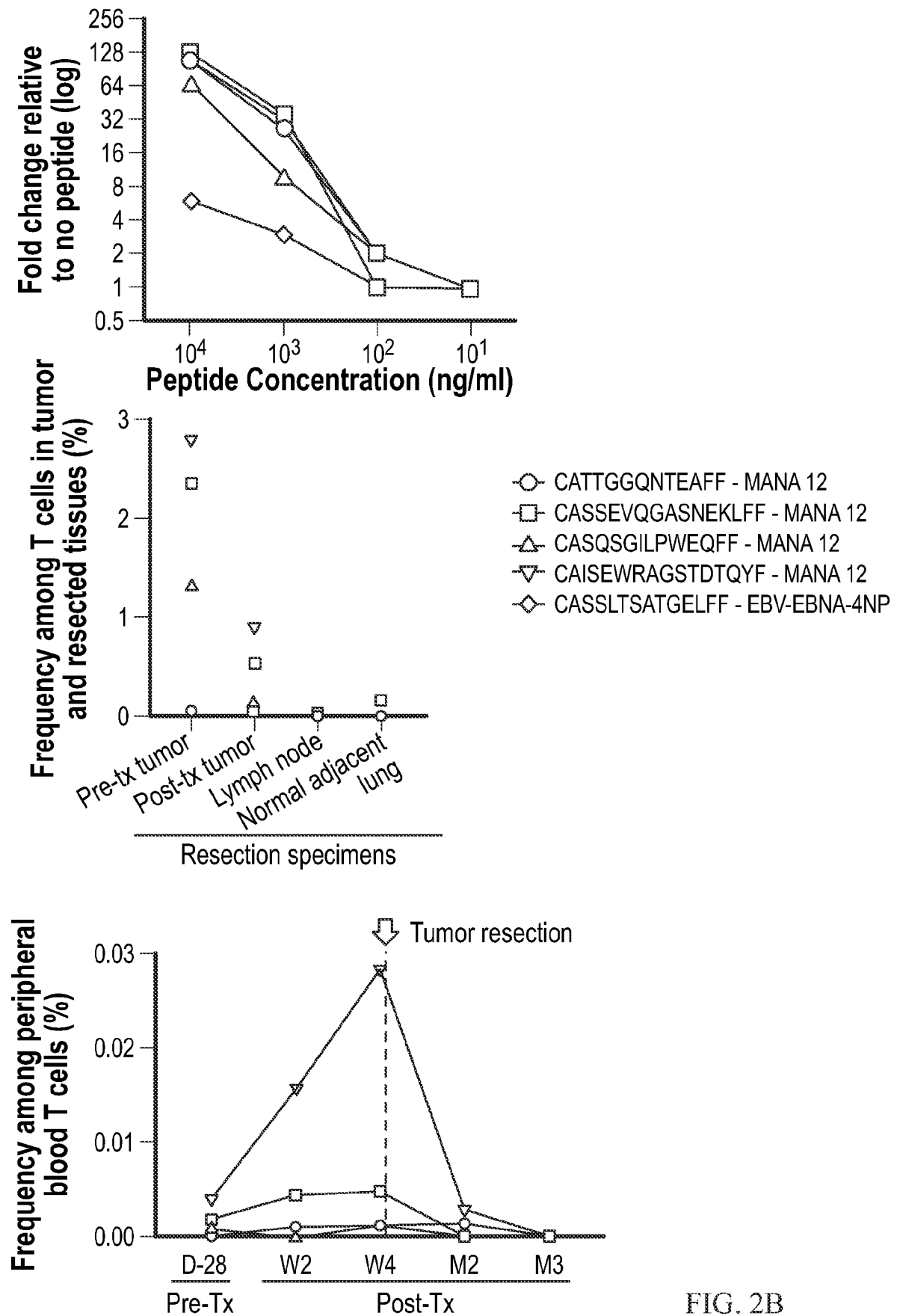


FIG. 2B

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N=235,851

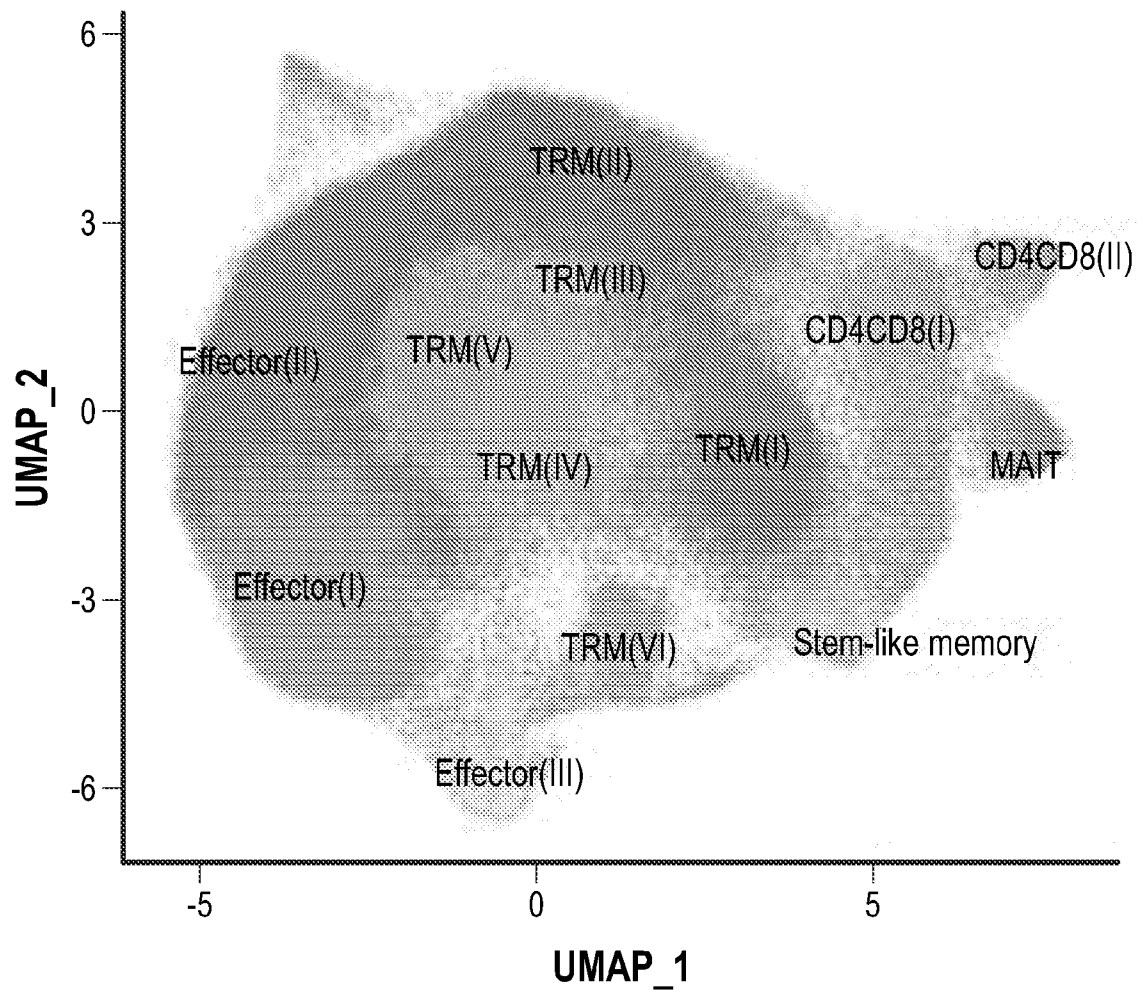


FIG. 2C

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- MANA specific T cell
- Influenza A specific T cell
- EBV specific T cell

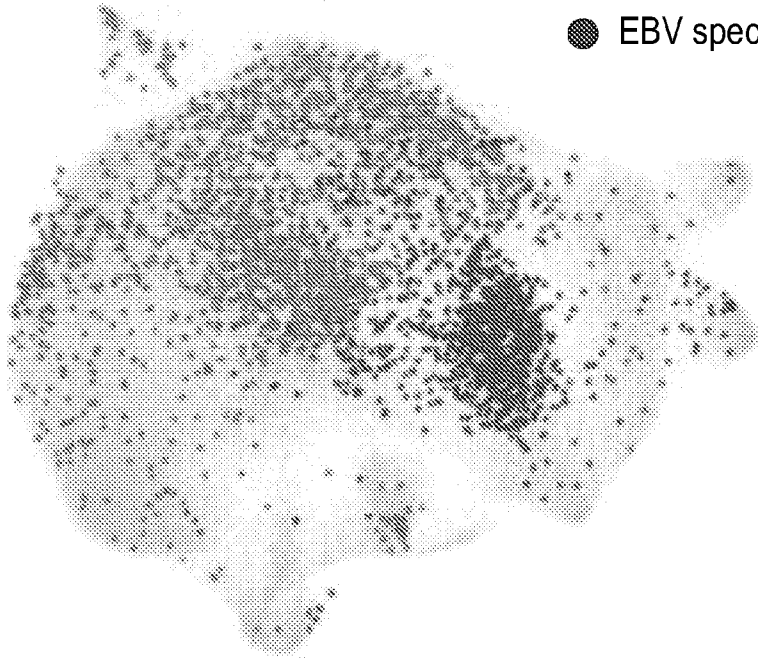


FIG. 2D

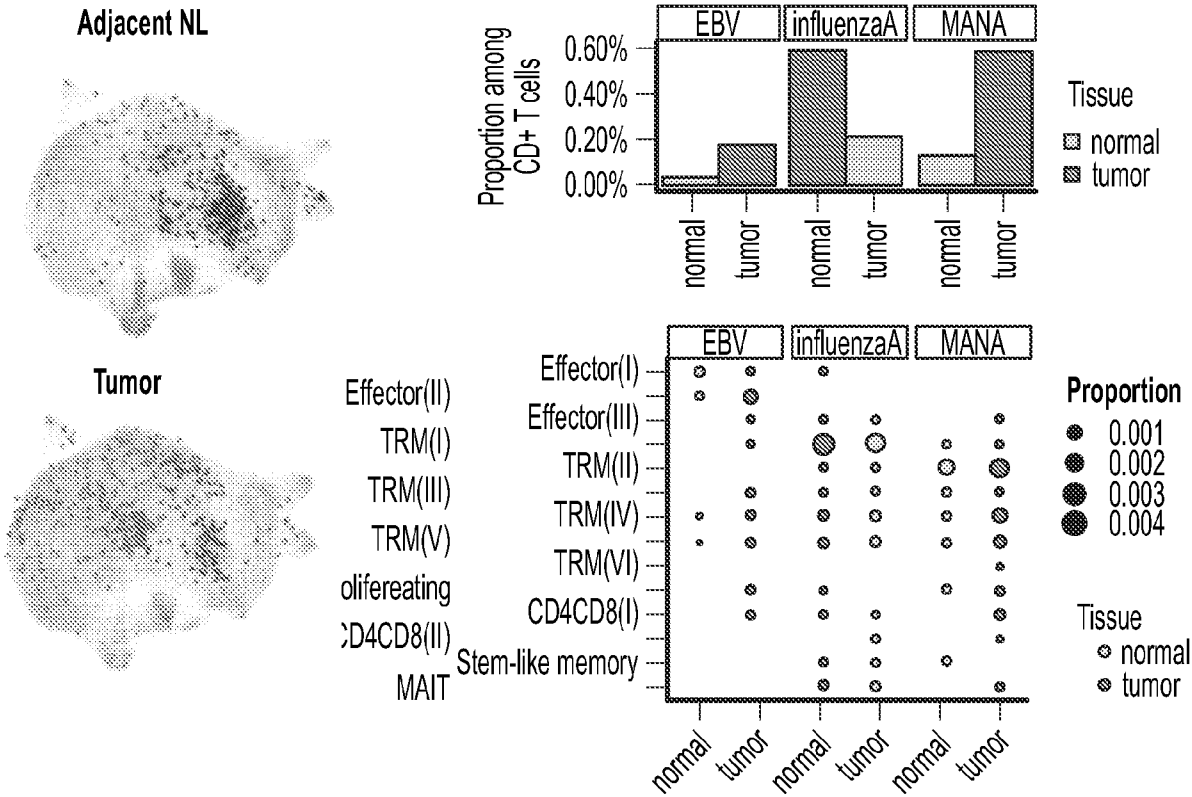


FIG. 2E

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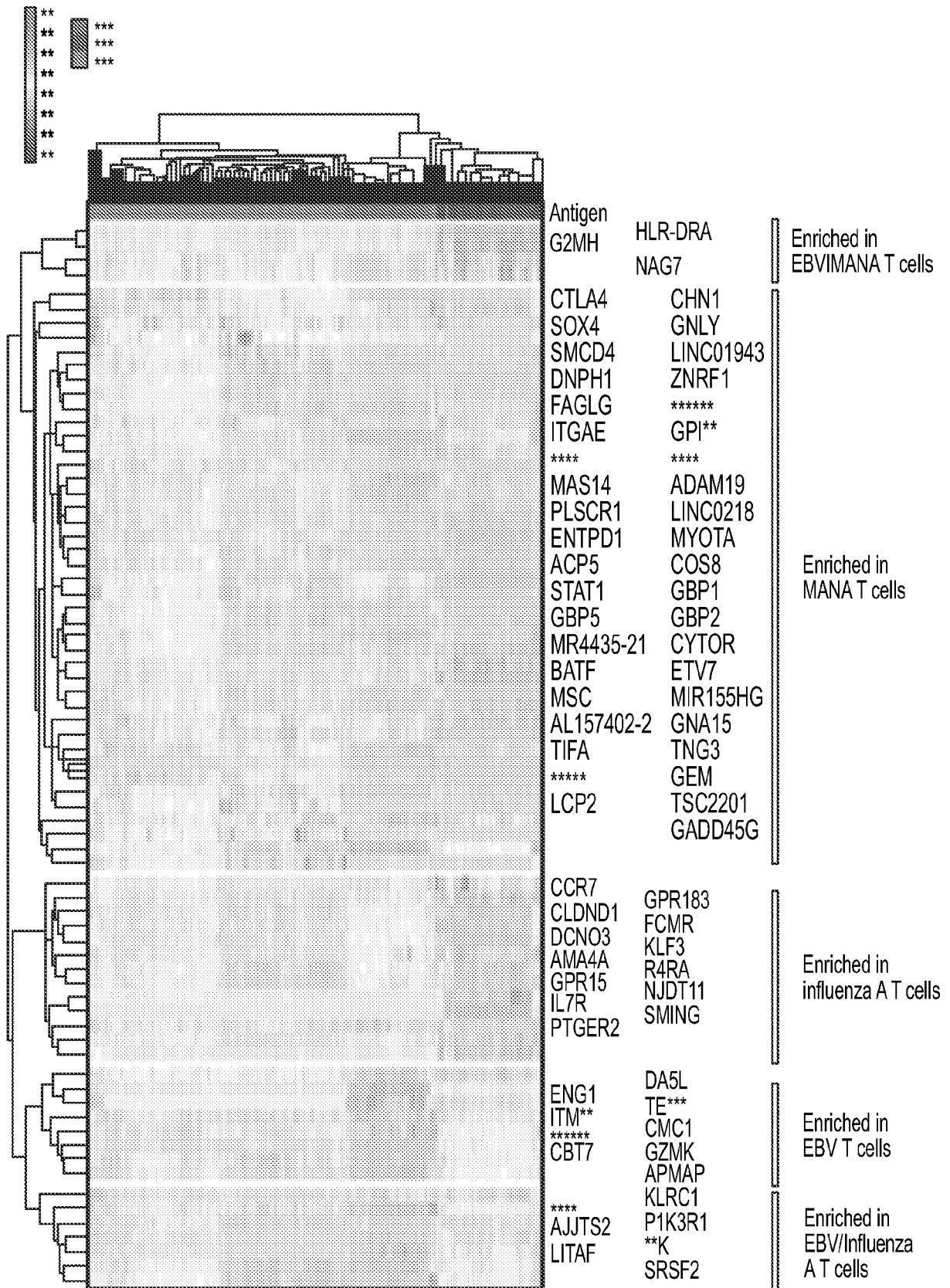
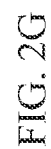
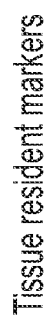
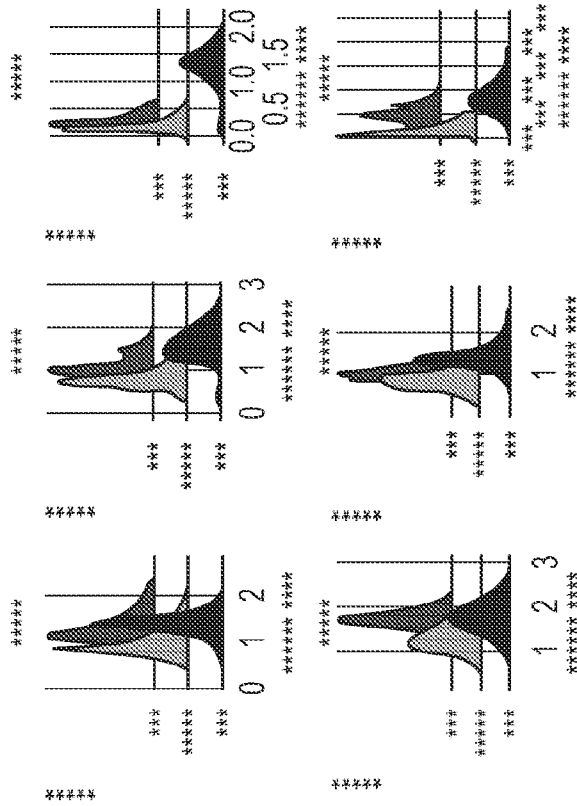


FIG. 2F



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T cell checkpoints



Effector/activation marker

FIG. 2G (Cont.)

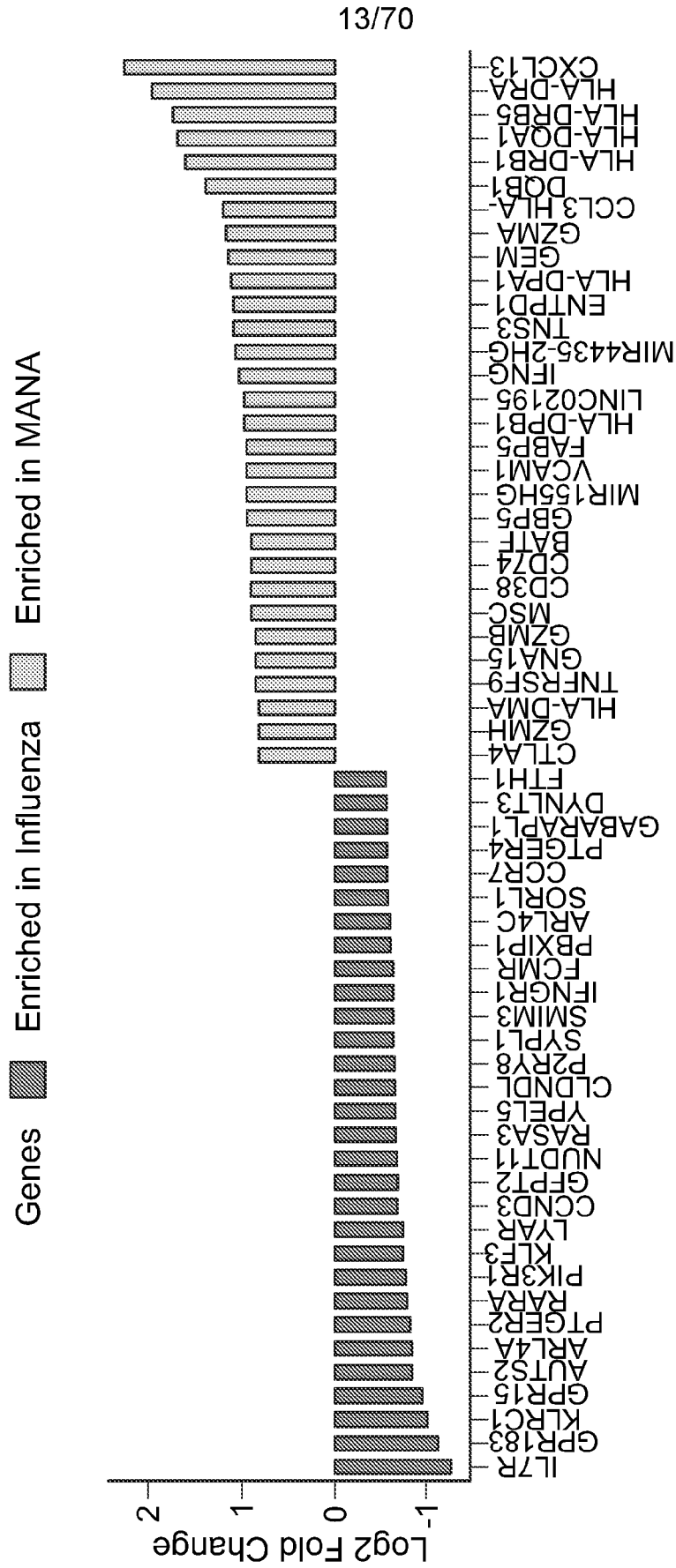


FIG. 2H

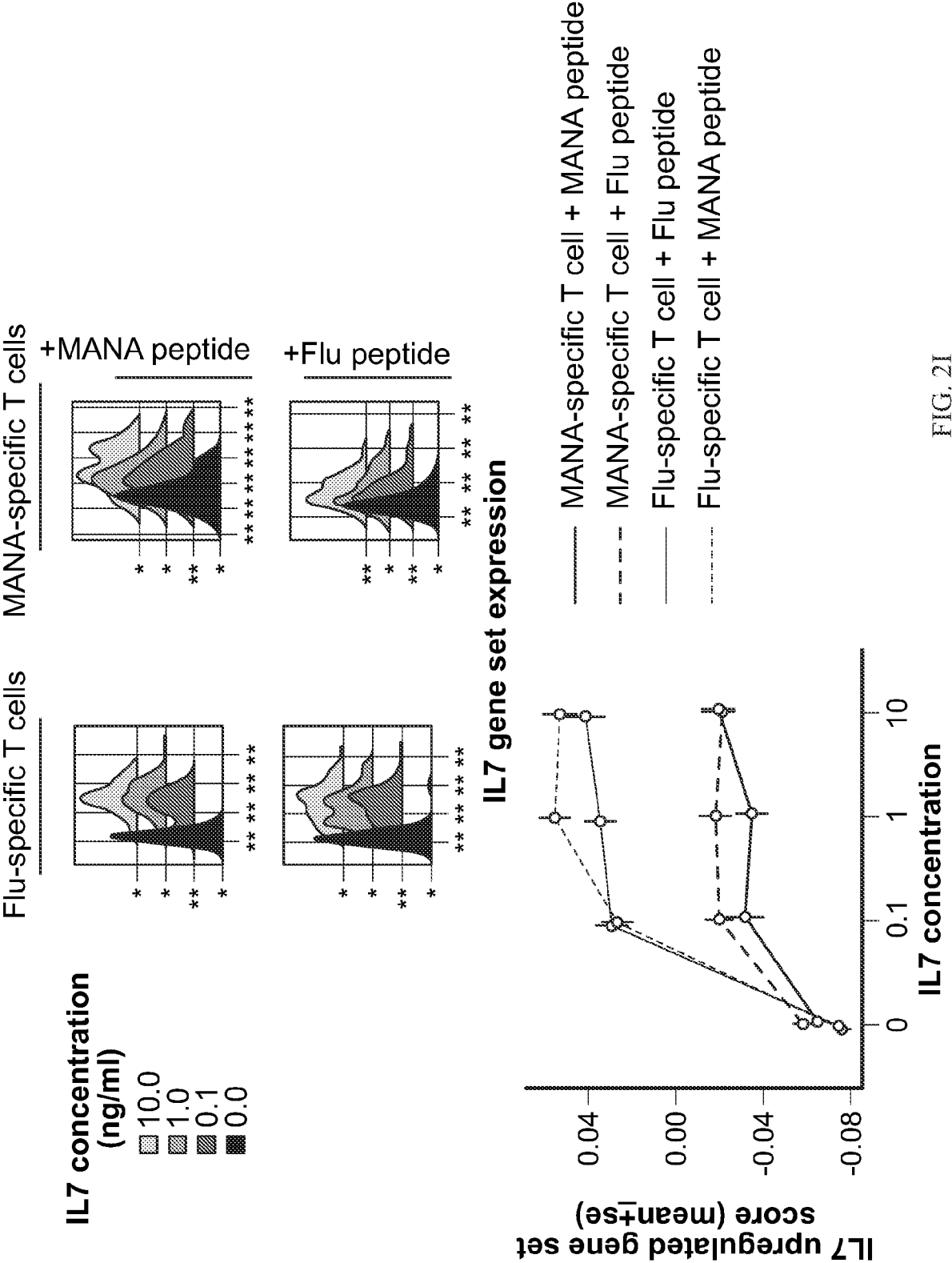


FIG. 2I

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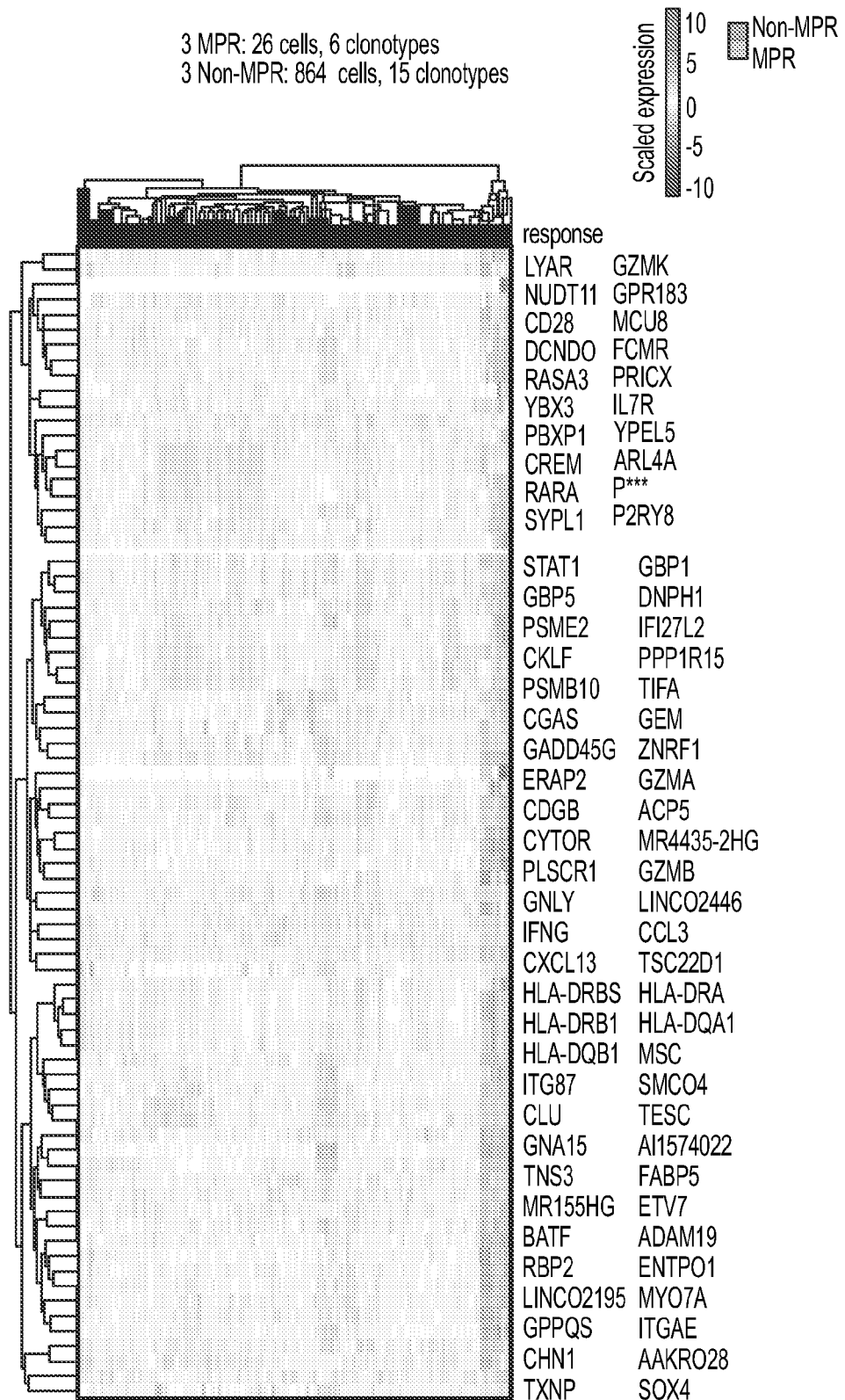


FIG. 3A

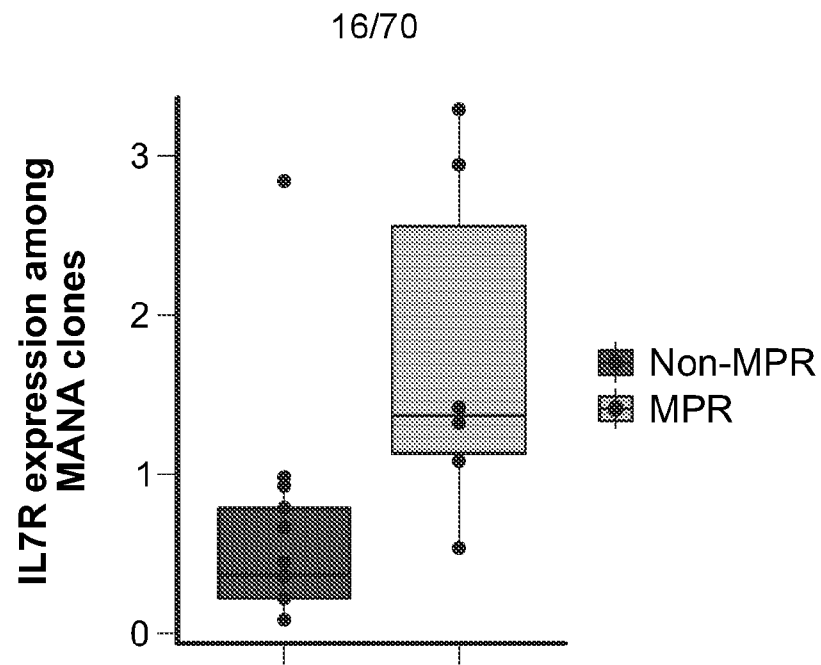


FIG. 3B

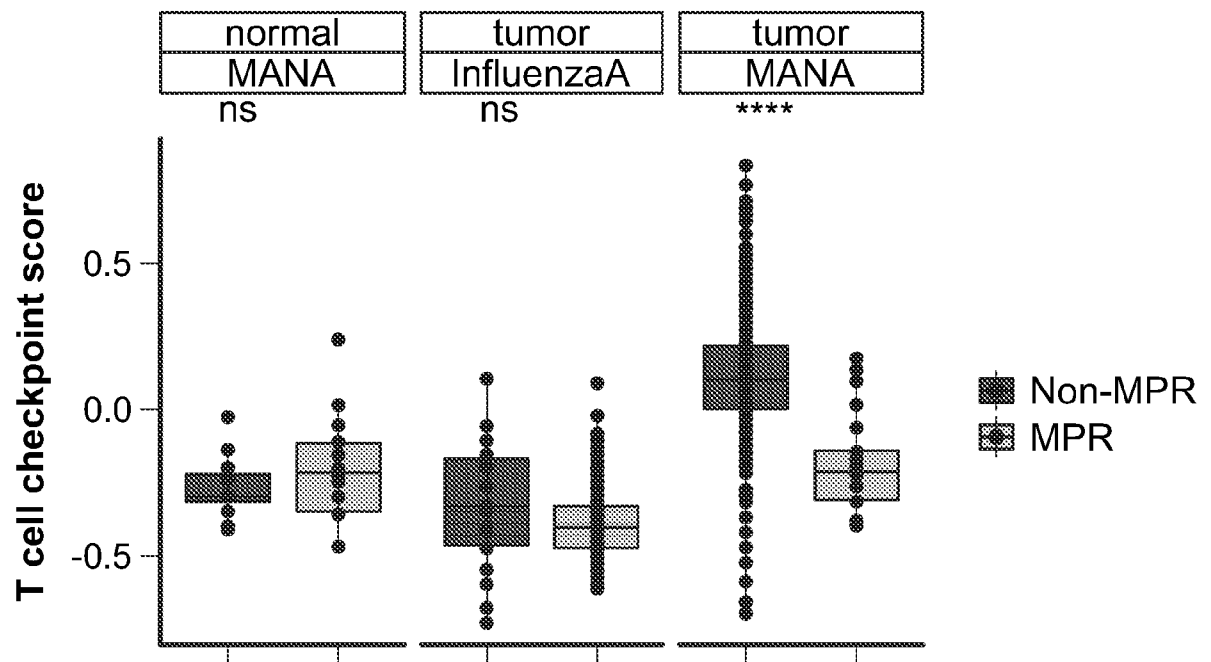


FIG. 3C

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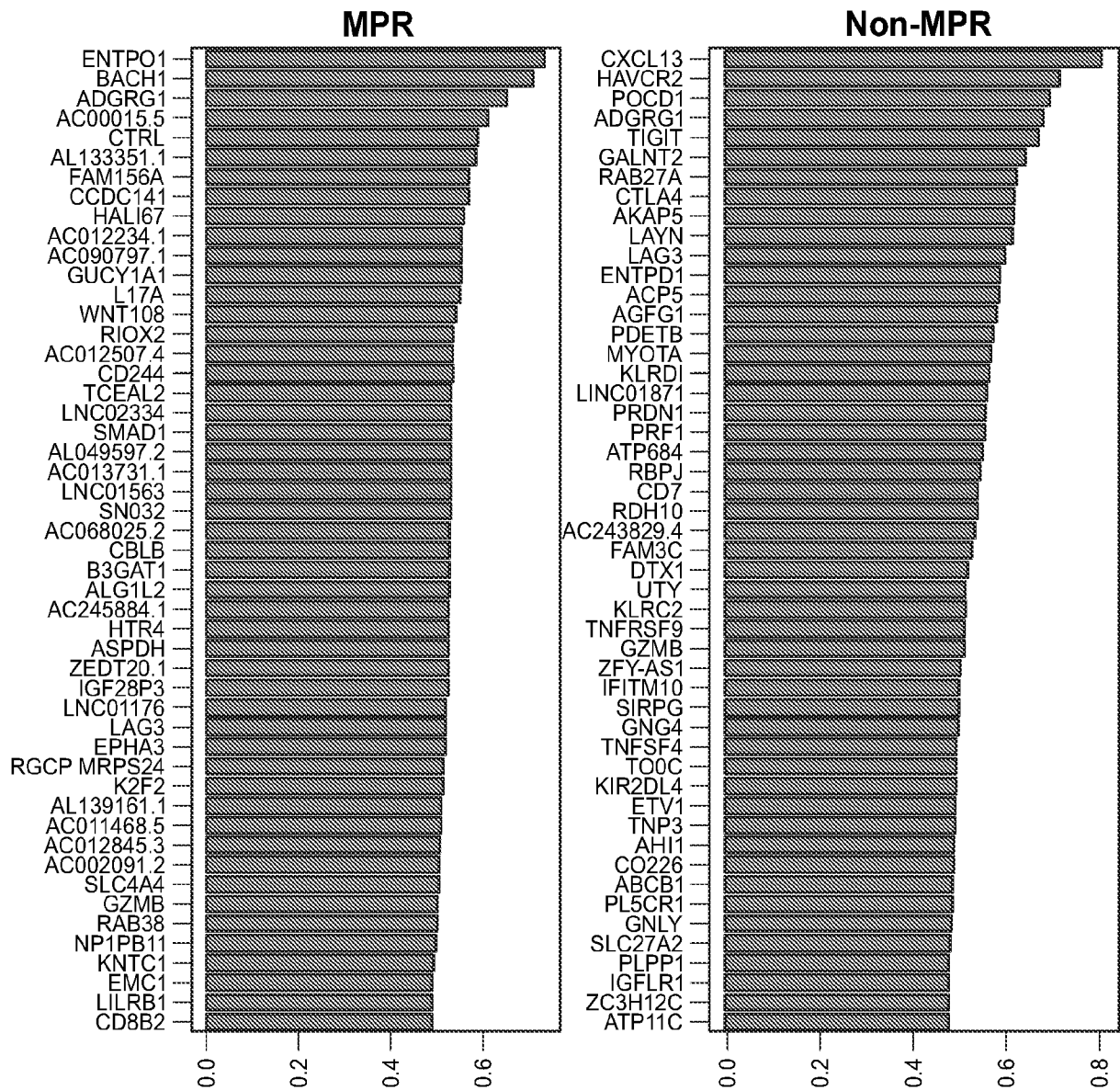


FIG. 3D

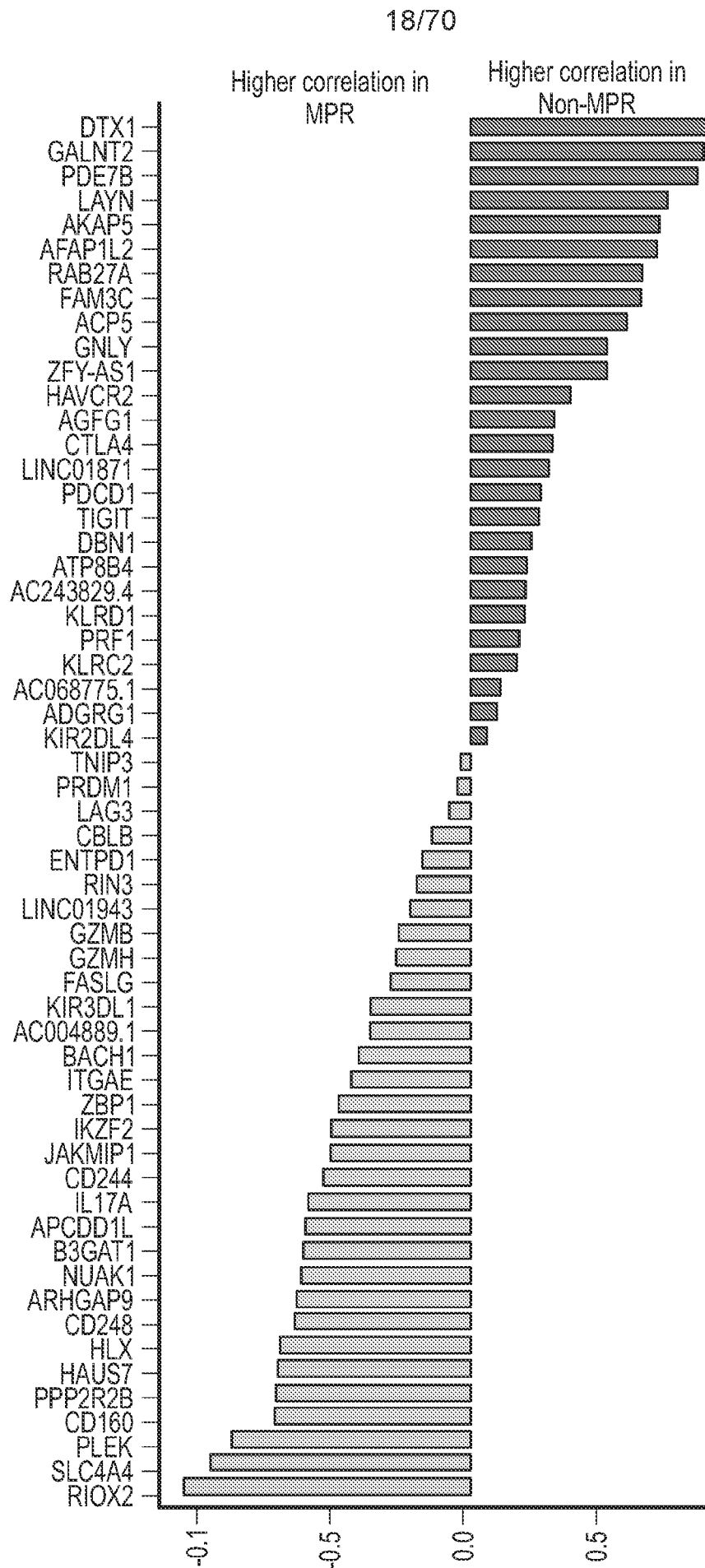
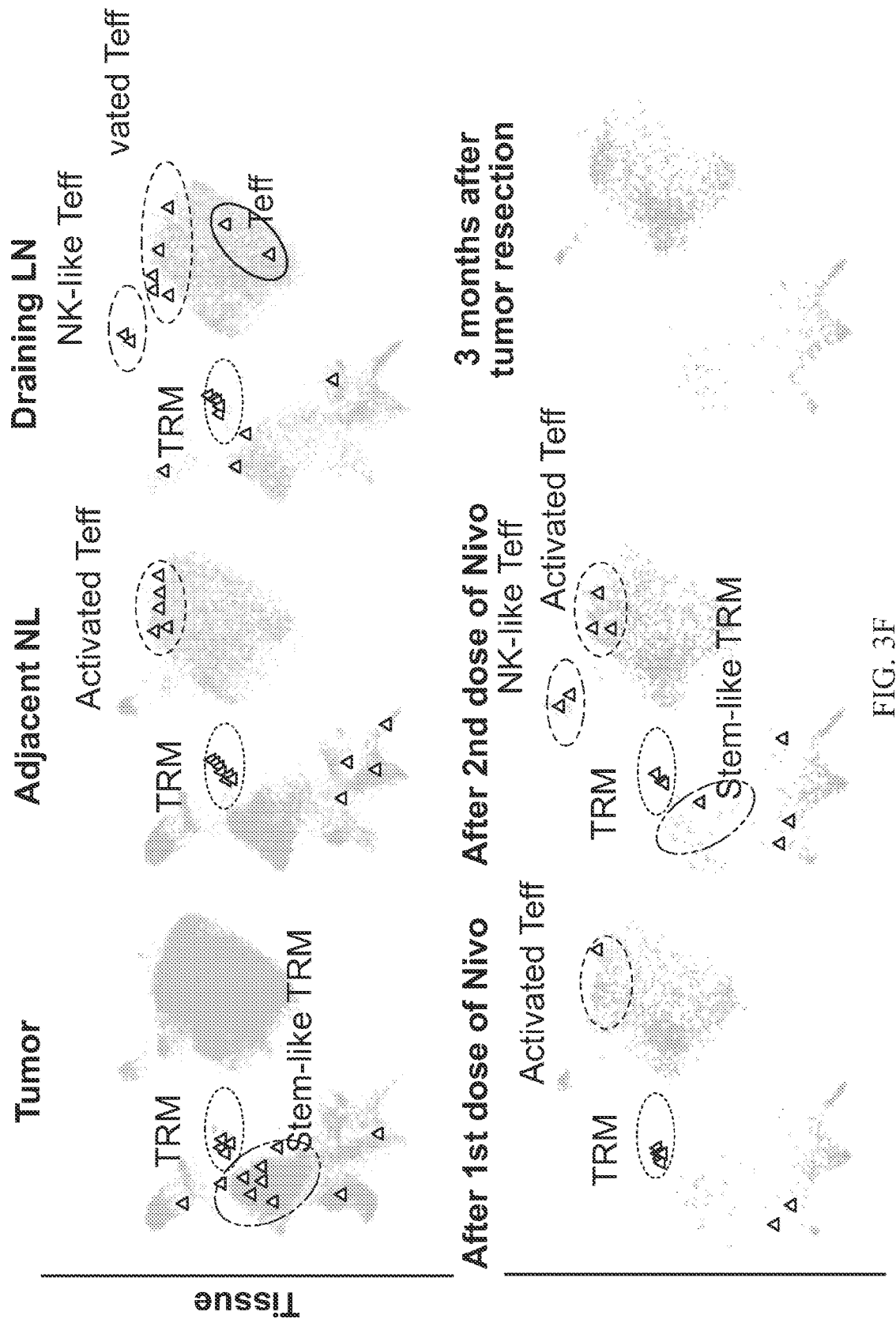


FIG. 3E

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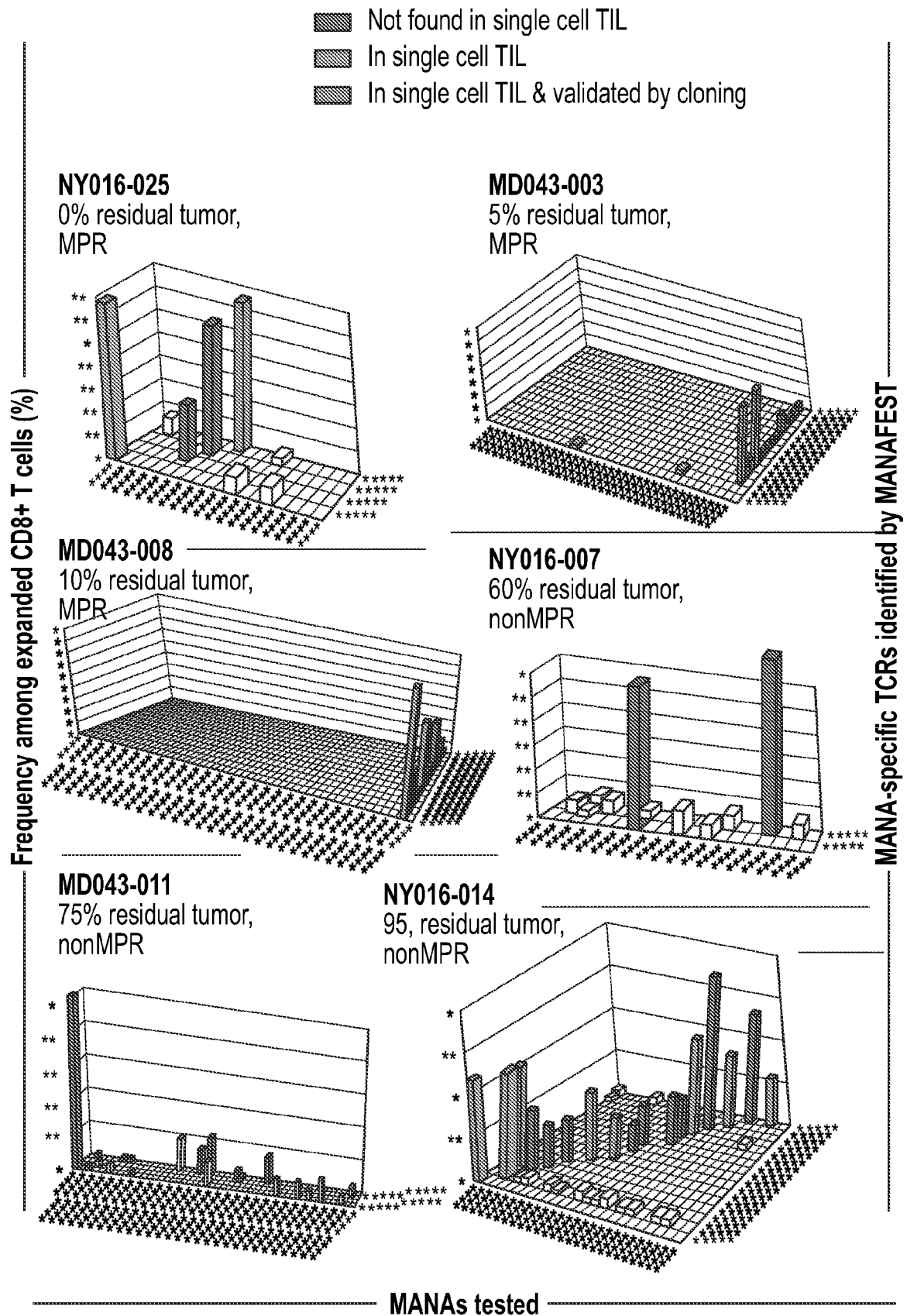


FIG. 4

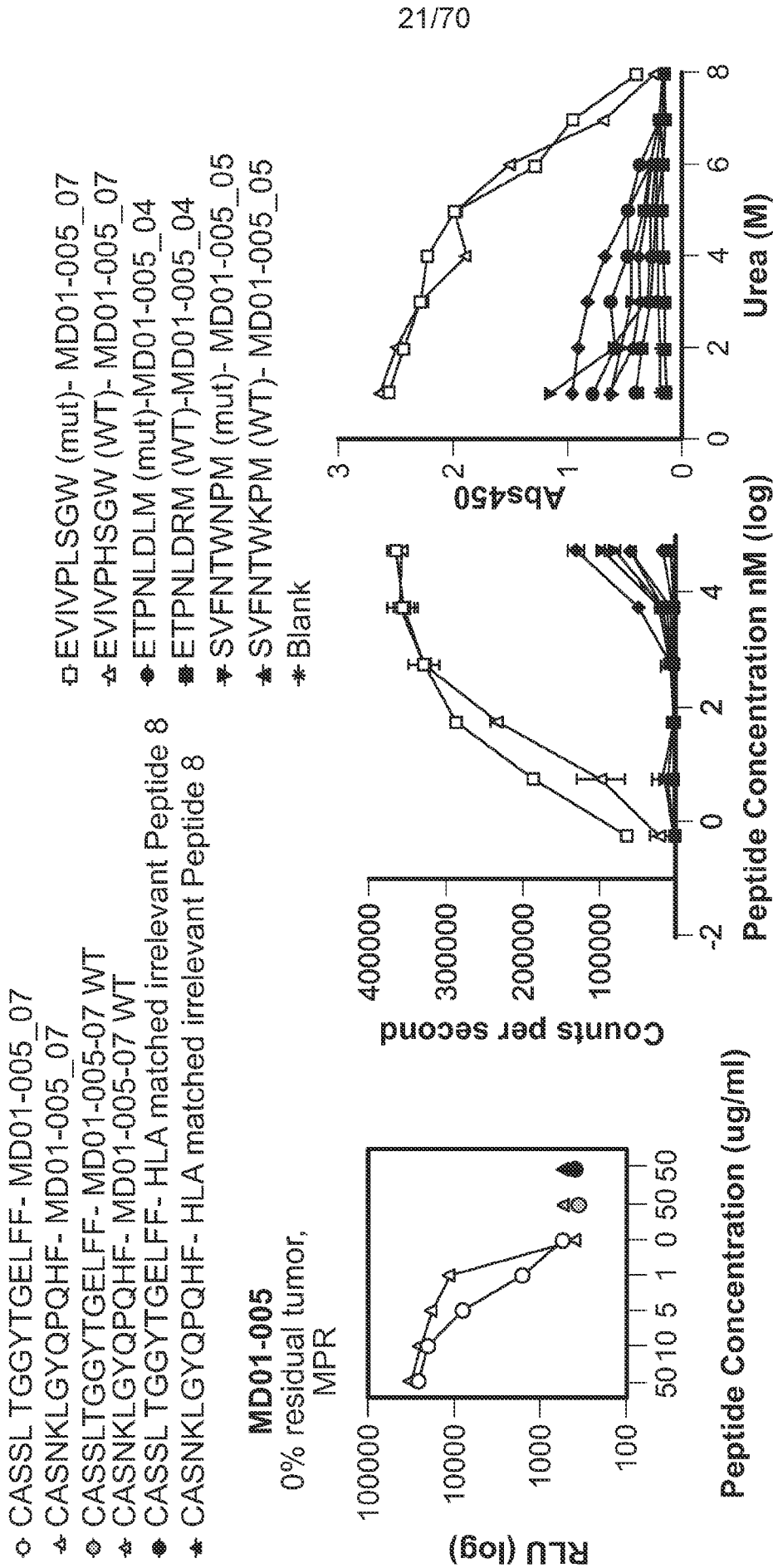


FIG. 5A

MD01-004
40% residual tumor,
nonMPR

- ◇ CATTGGQNTAEFF - MANA 12

□ CASQSGILPWEQFF - MANA 12

△ CAISEWRAGSTDQYF - MANA 12

✱ CASSEVQGASNEKLFF MANA 12

◆ CATTGGQNTAEFF - MANA 12 WT

■ CASQSGILPWEQFF - MANA 12 WT

✱ CAISEWRAGSTDQYF - MANA 12 WT

✱ CASSEVQGASNEKLFF MANA 12 WT
- ◇ NSSCMGGMNLR (mut)- MDOI-004_12

□ SCMGGMNLR (mut)- not tested by MANAFEST

✱ NSSCMGGMNWR (mut)- not tested by MANAFEST

✱ NSSCMGGMNRR (WT)- not tested by MANAFEST

◆ A*68.01 positive control I

◆ A*68.01 positive control II

■ Blank

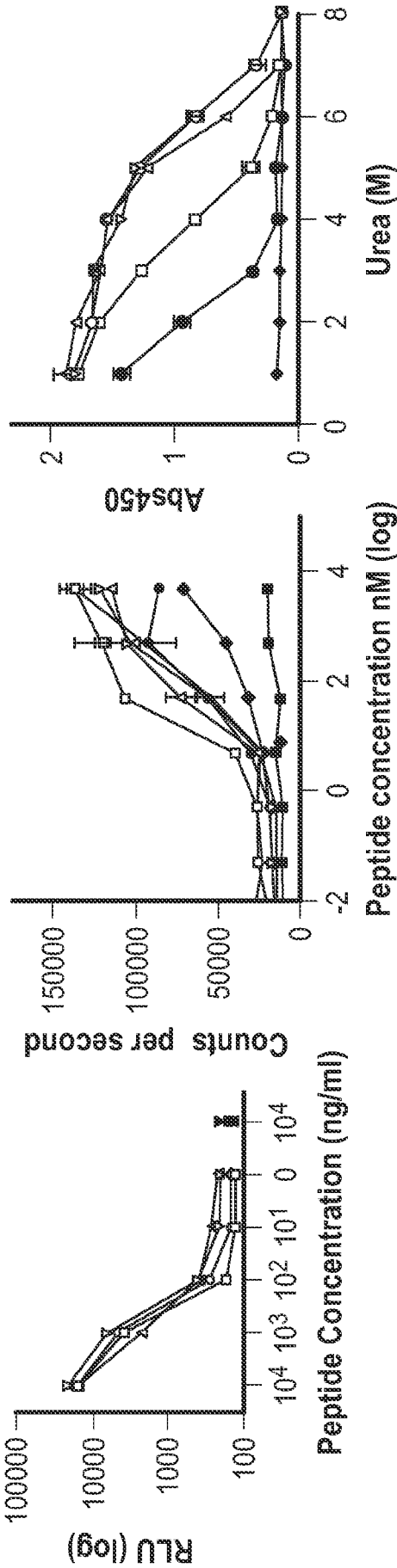


FIG. 5B

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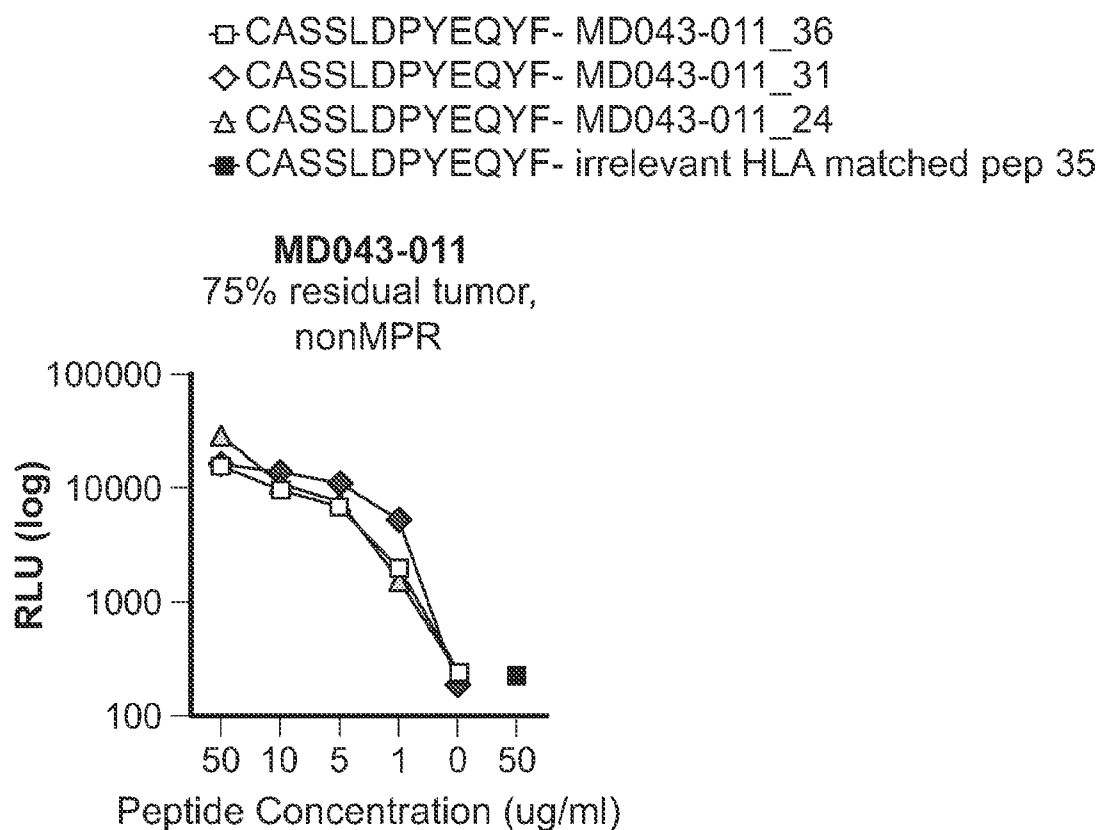


FIG. 5C

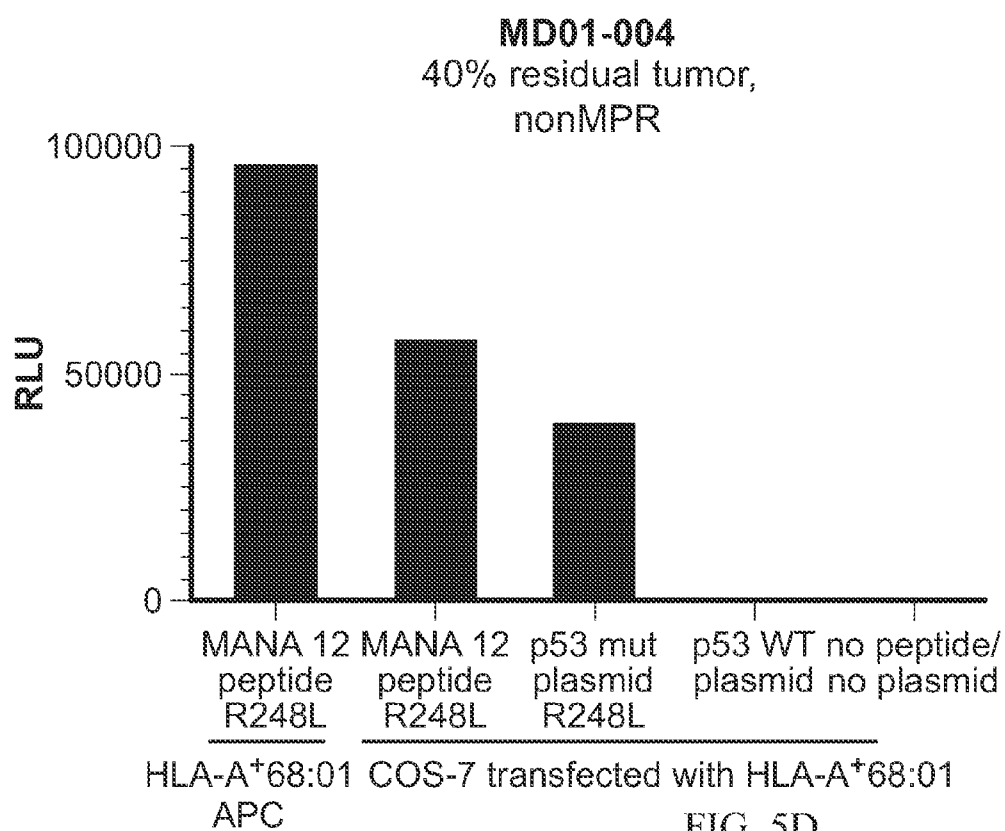


FIG. 5D

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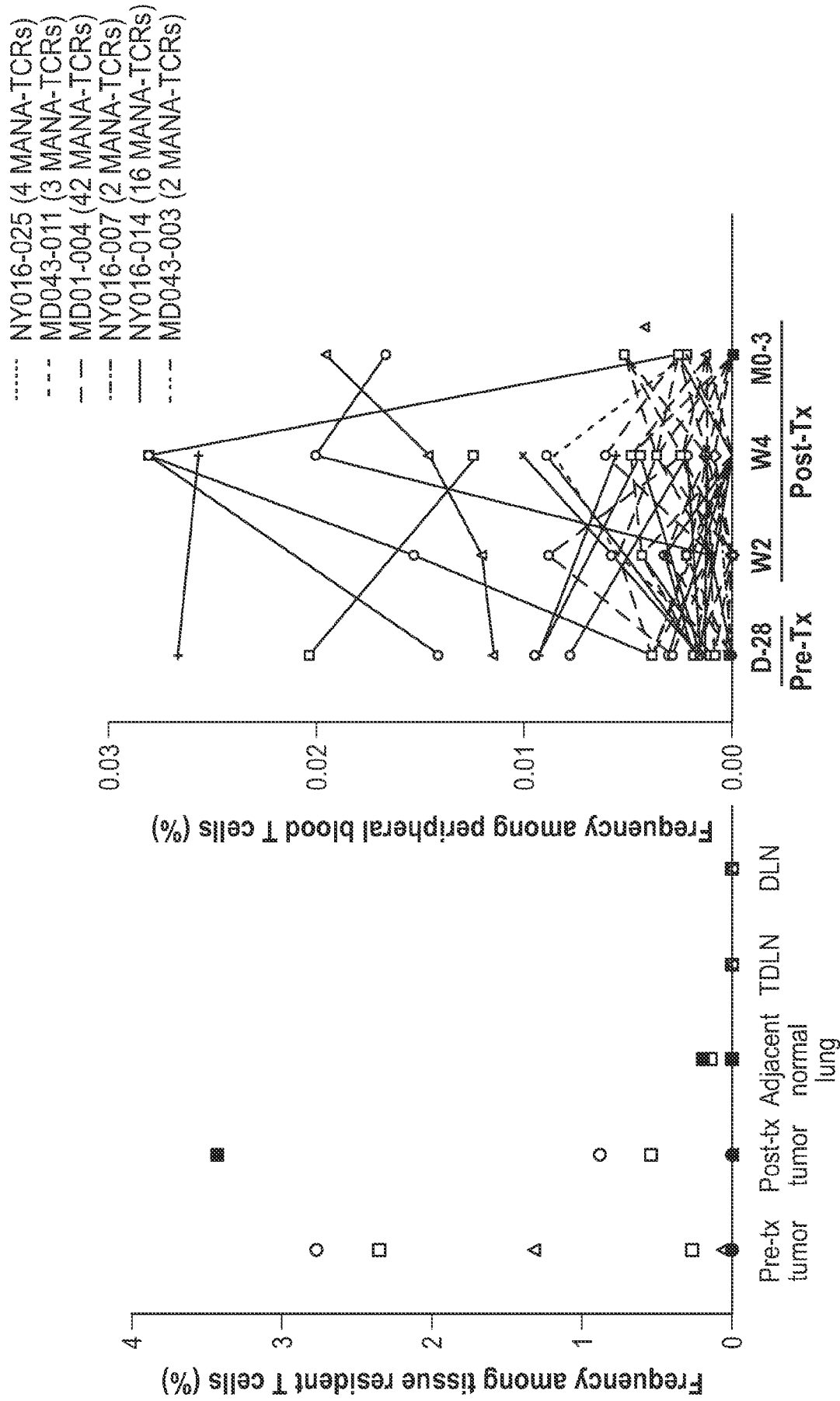


FIG. 6

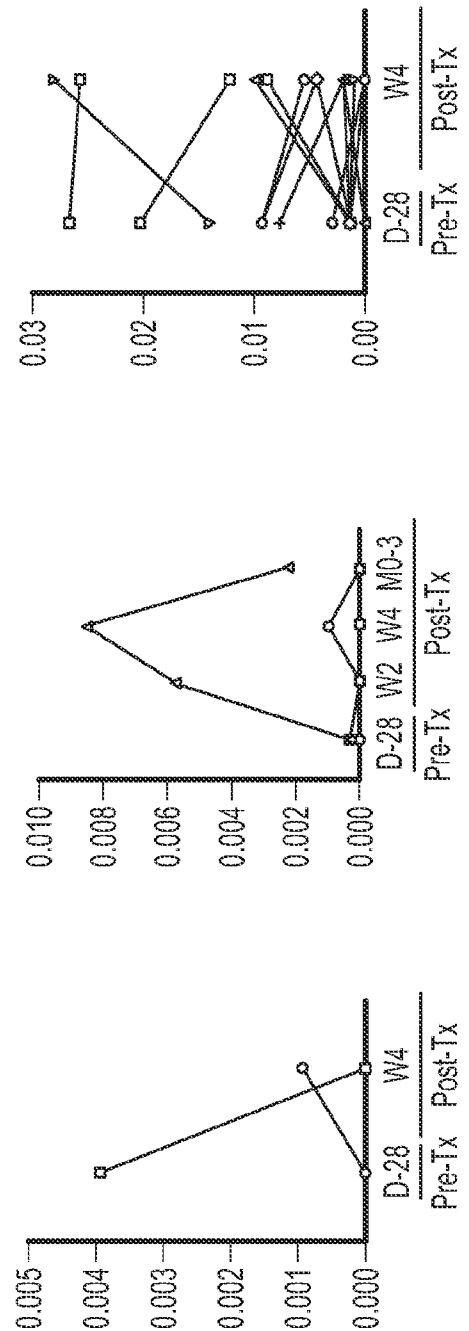
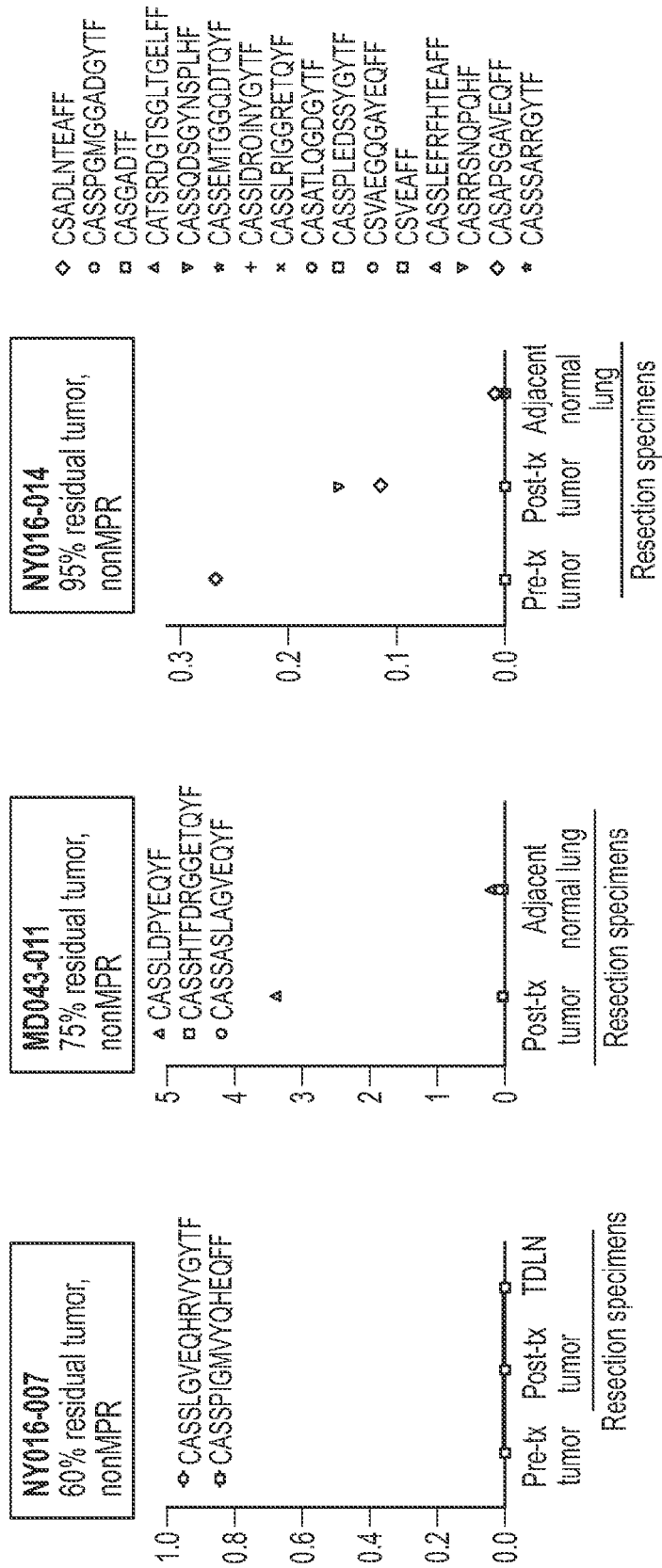
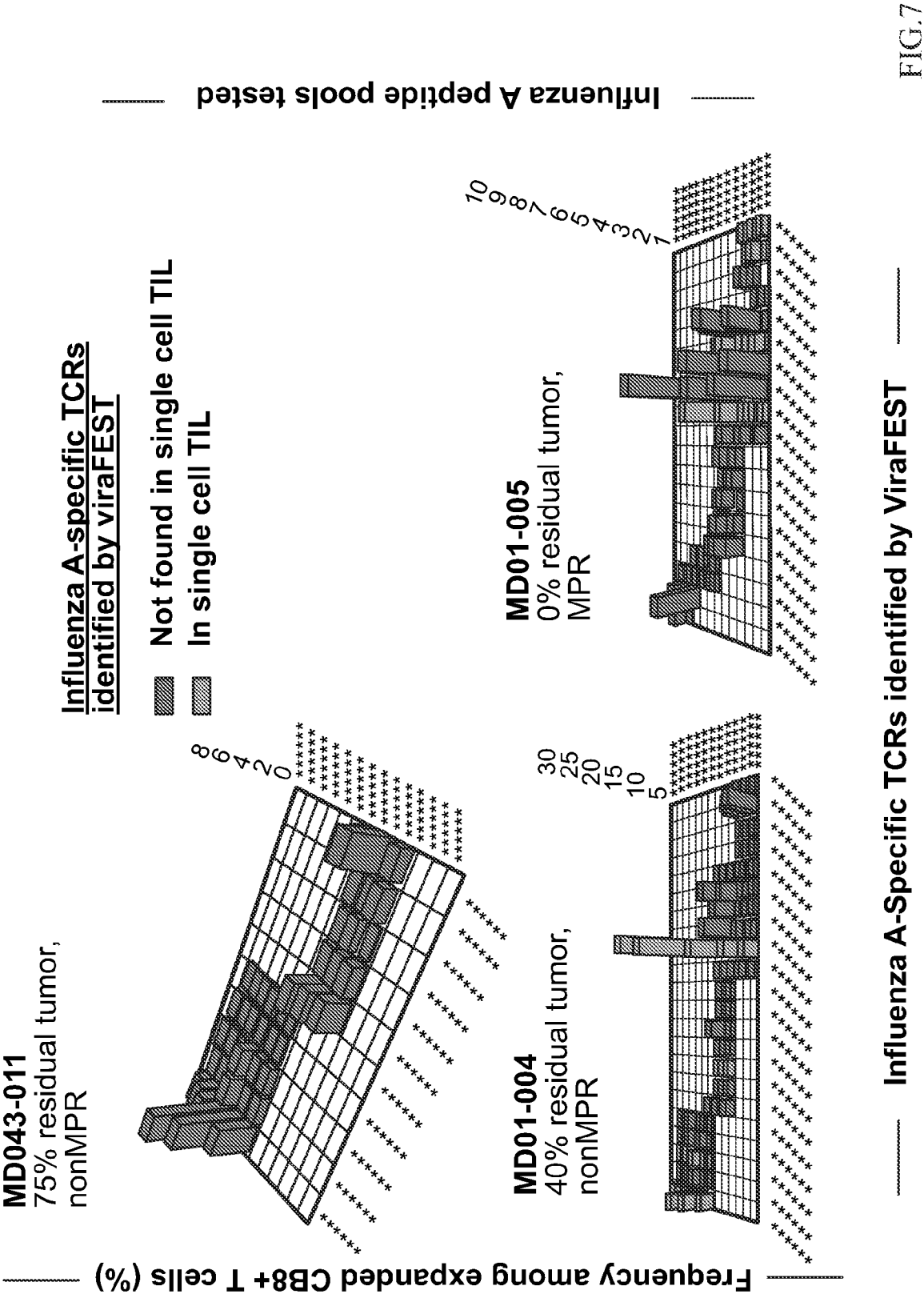


FIG. 6 (cont.)



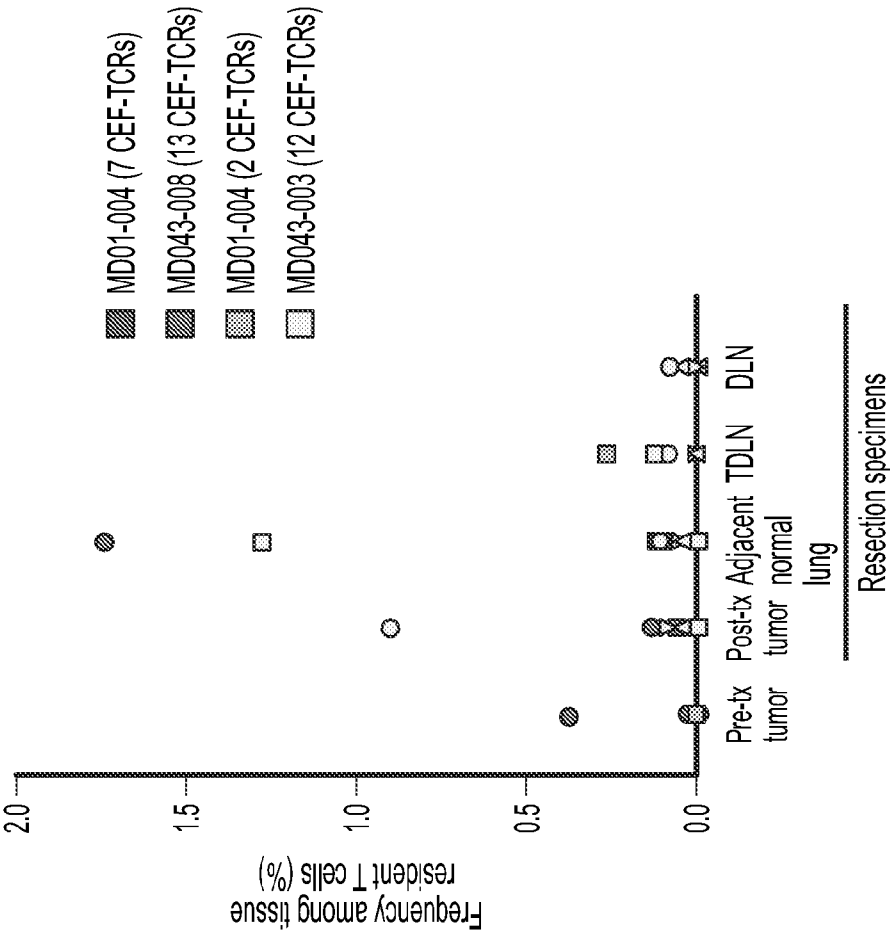


FIG. 8

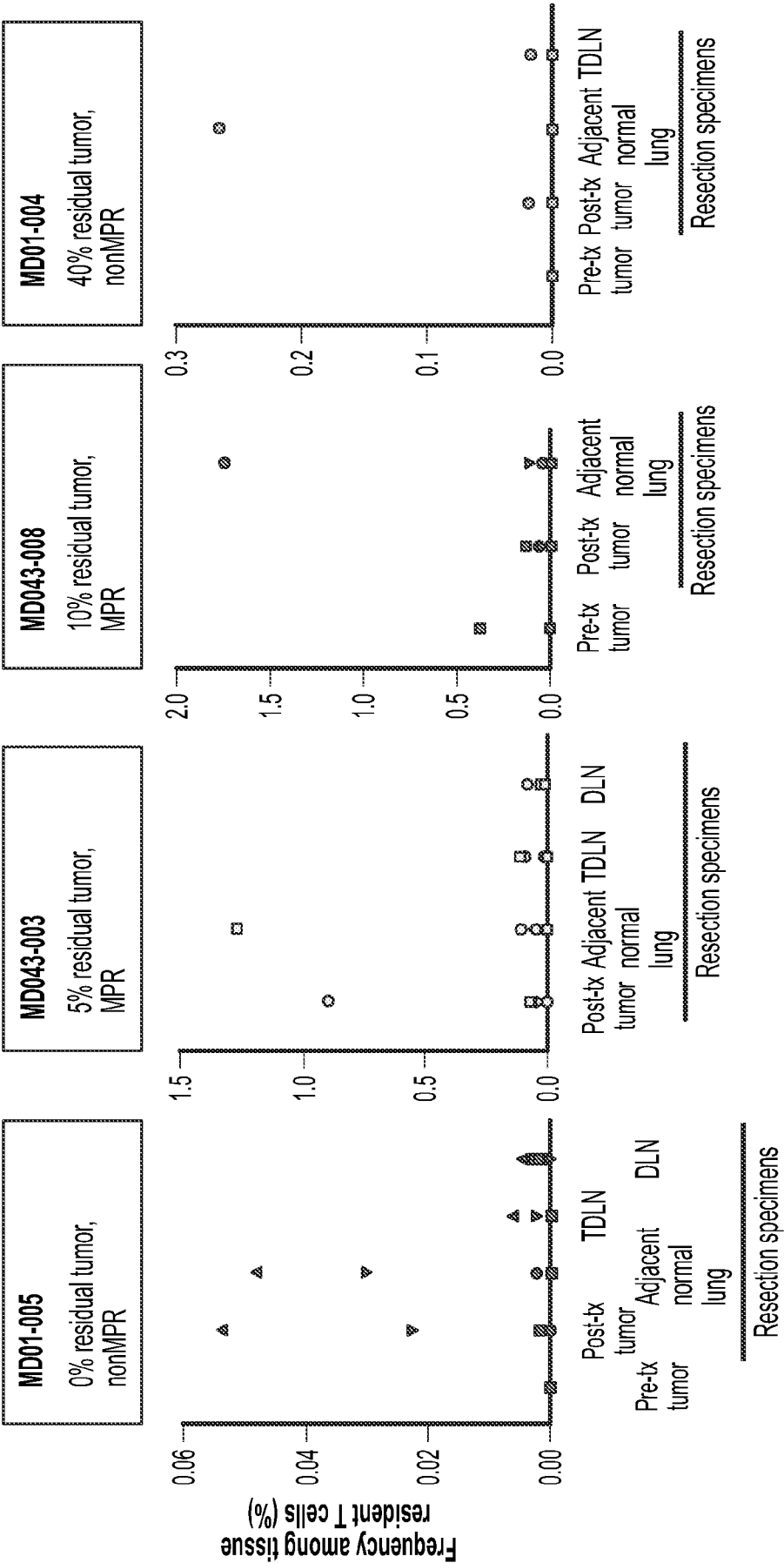


FIG. 8 (cont.)







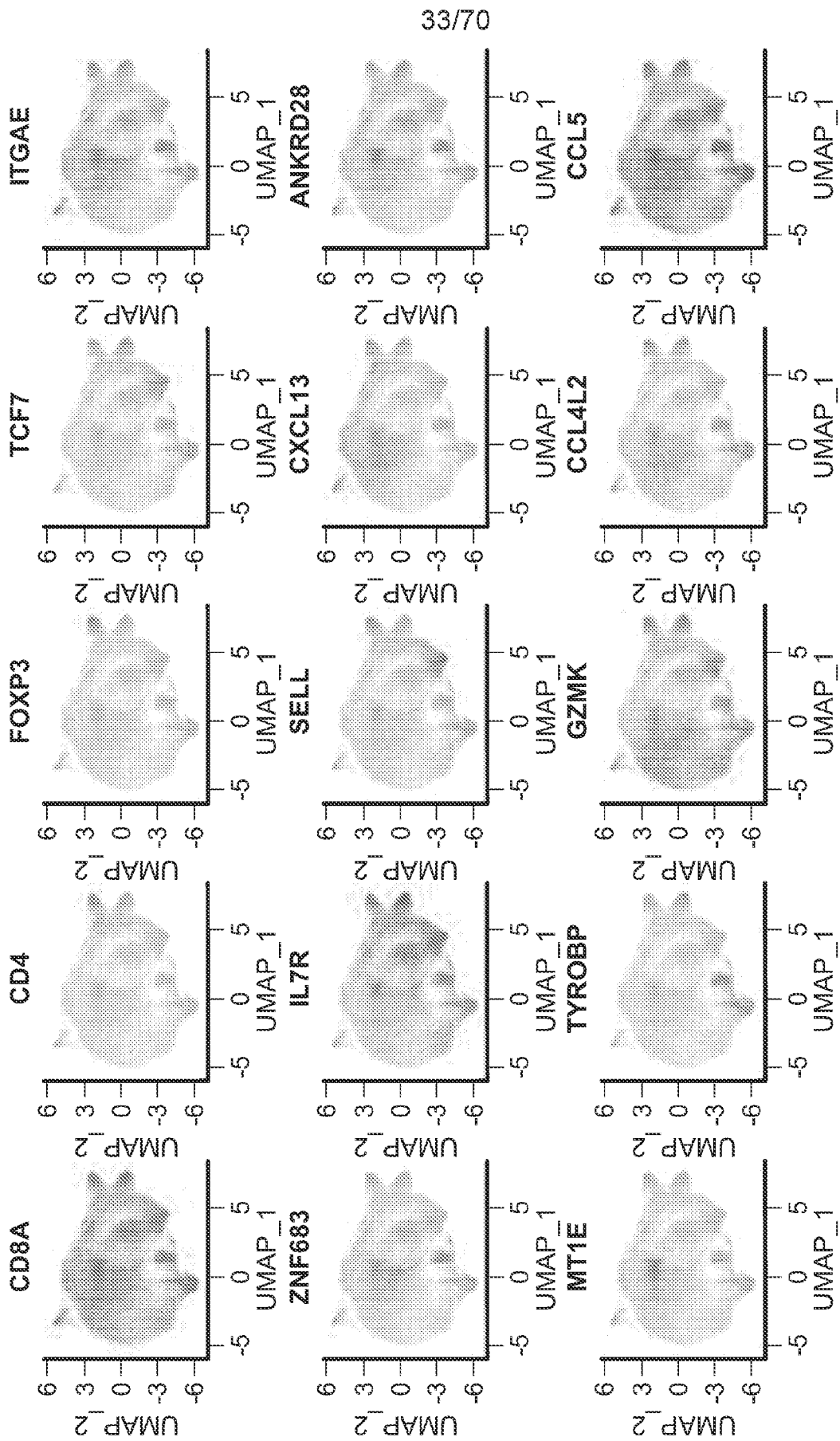


FIG. 10

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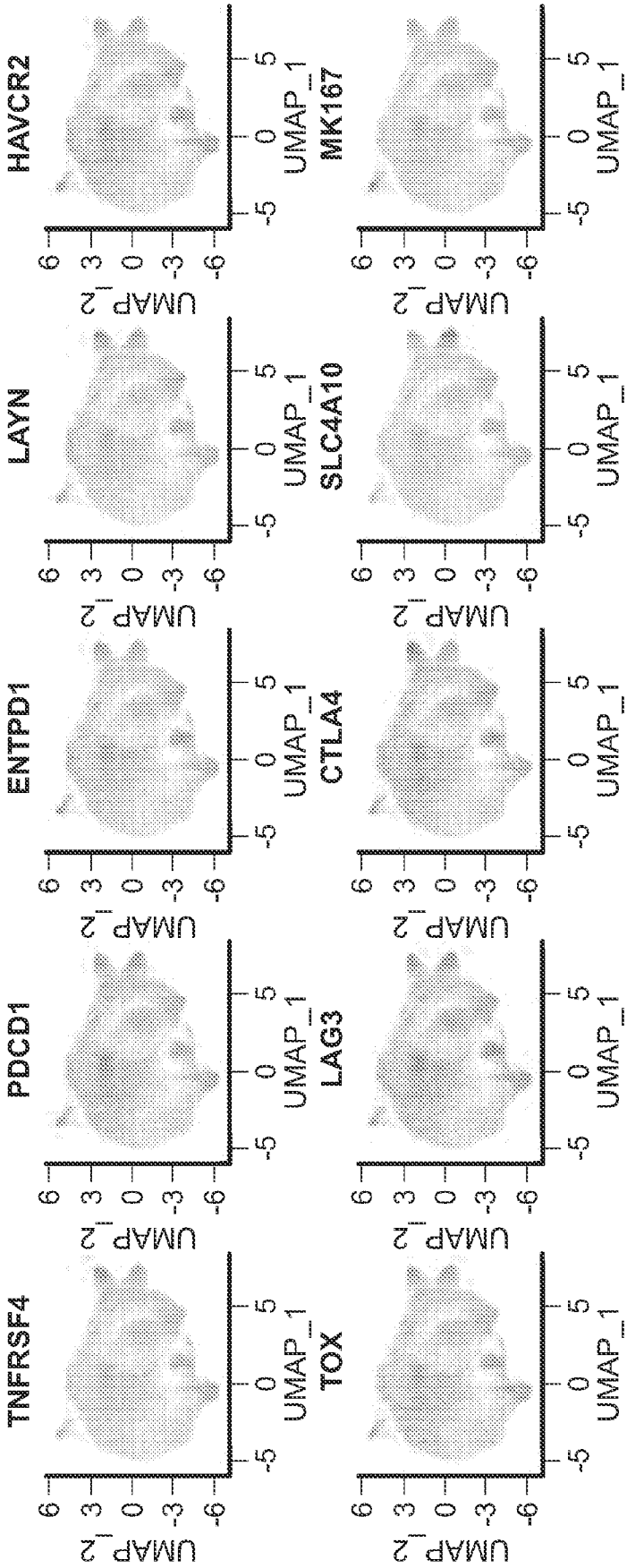


FIG. 10 (Cont.)

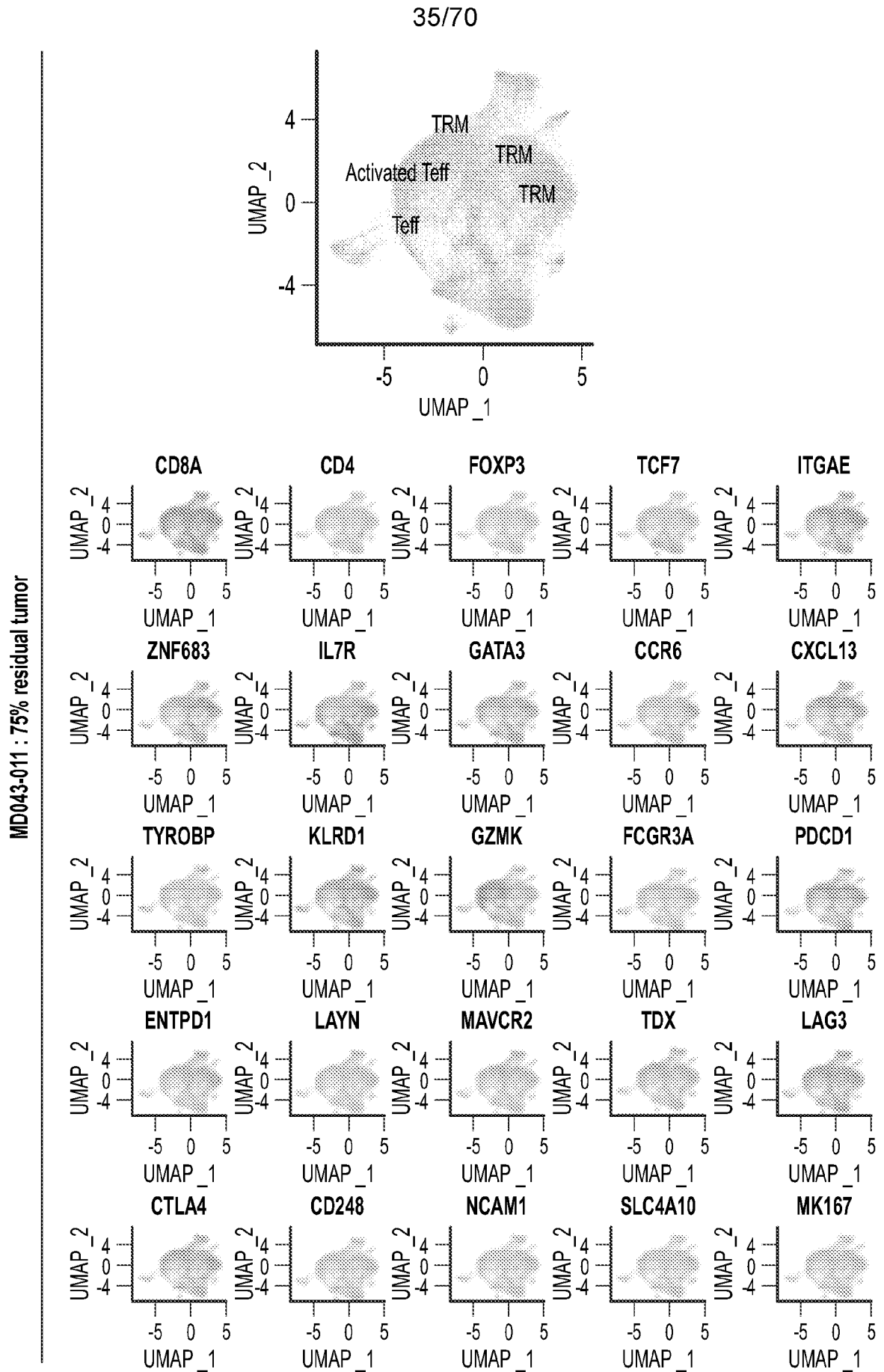


FIG. 11A

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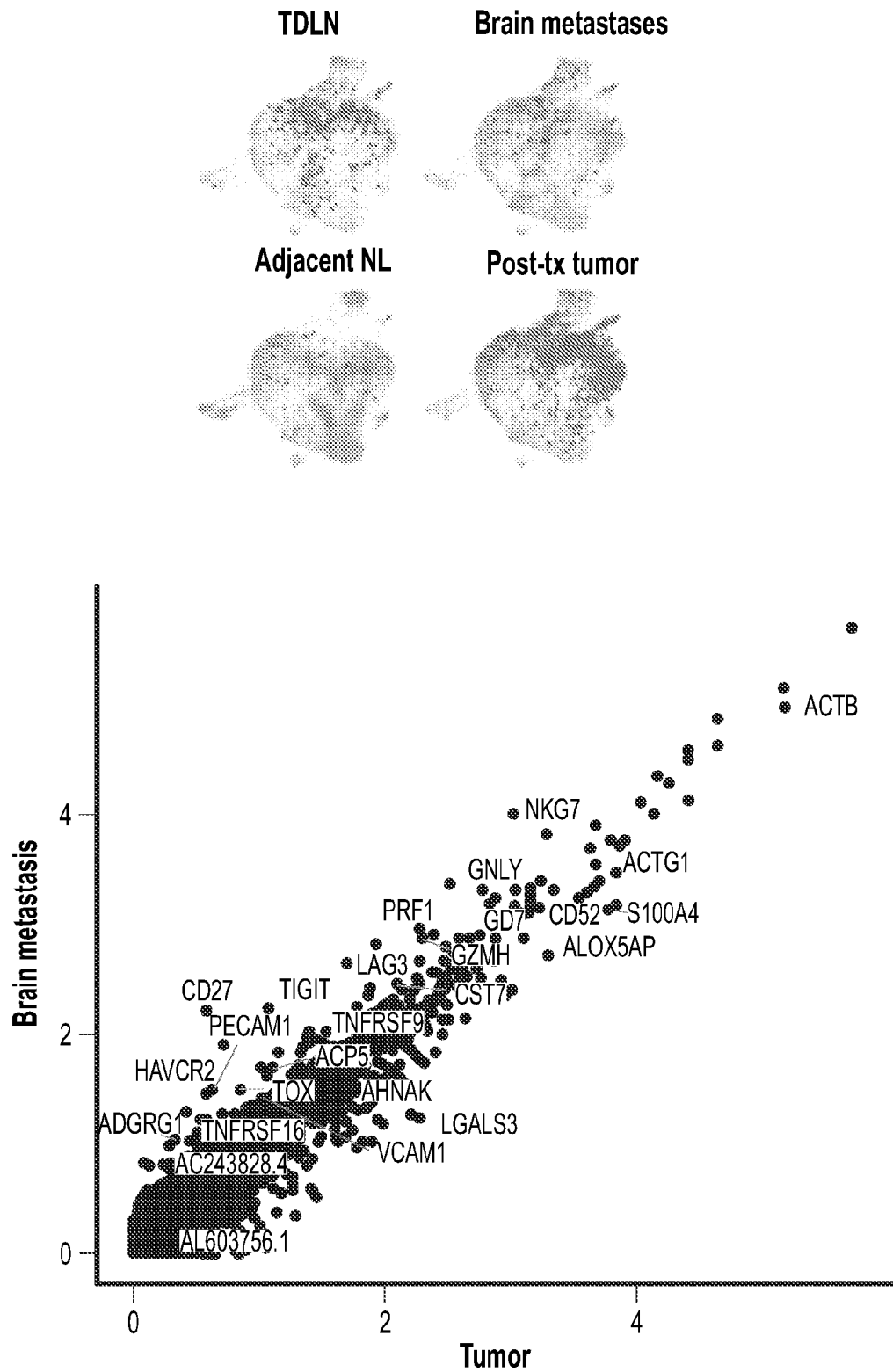


FIG. 11A (Cont.)

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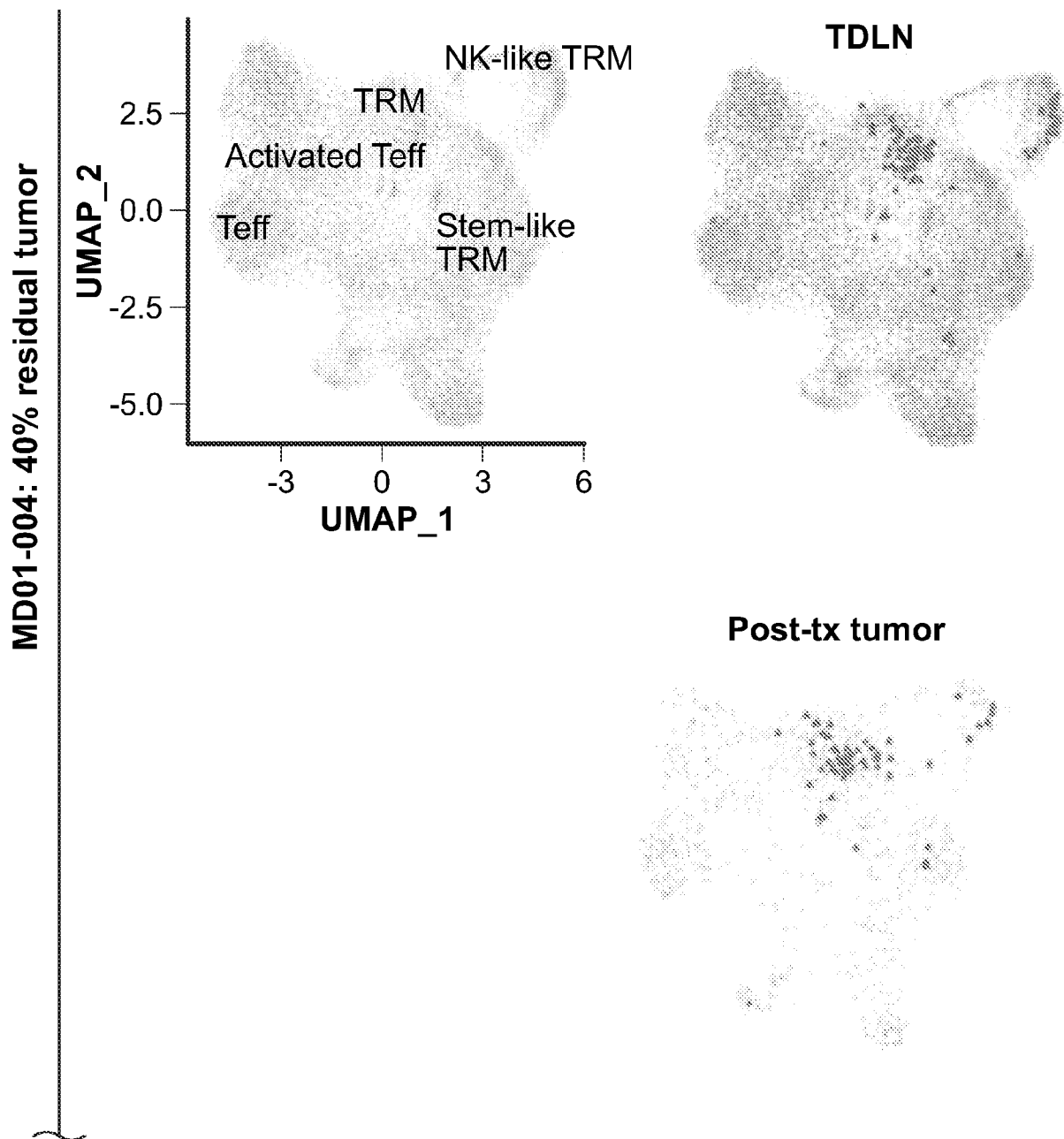


FIG. 11B

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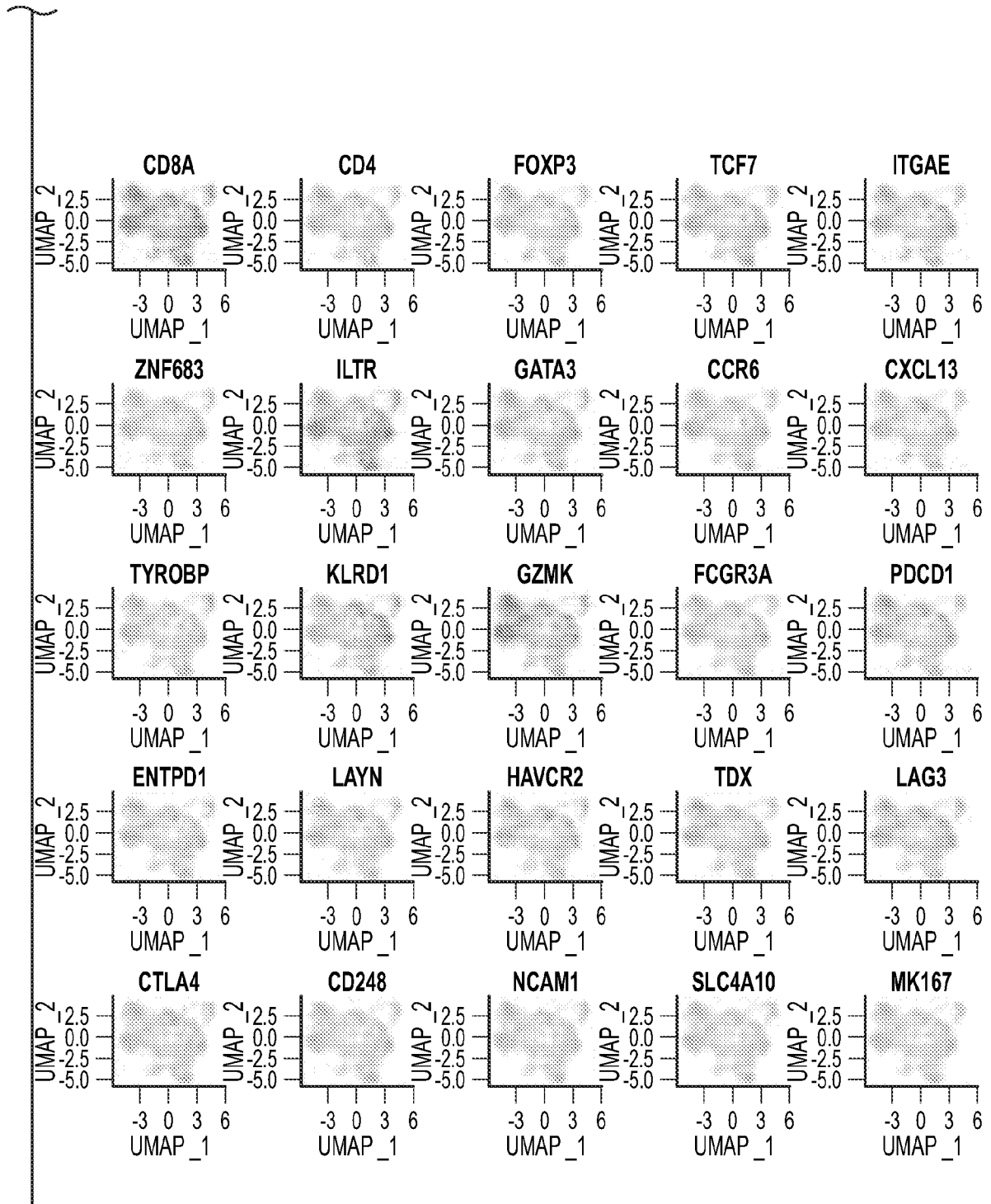


FIG. 11B (Cont.)



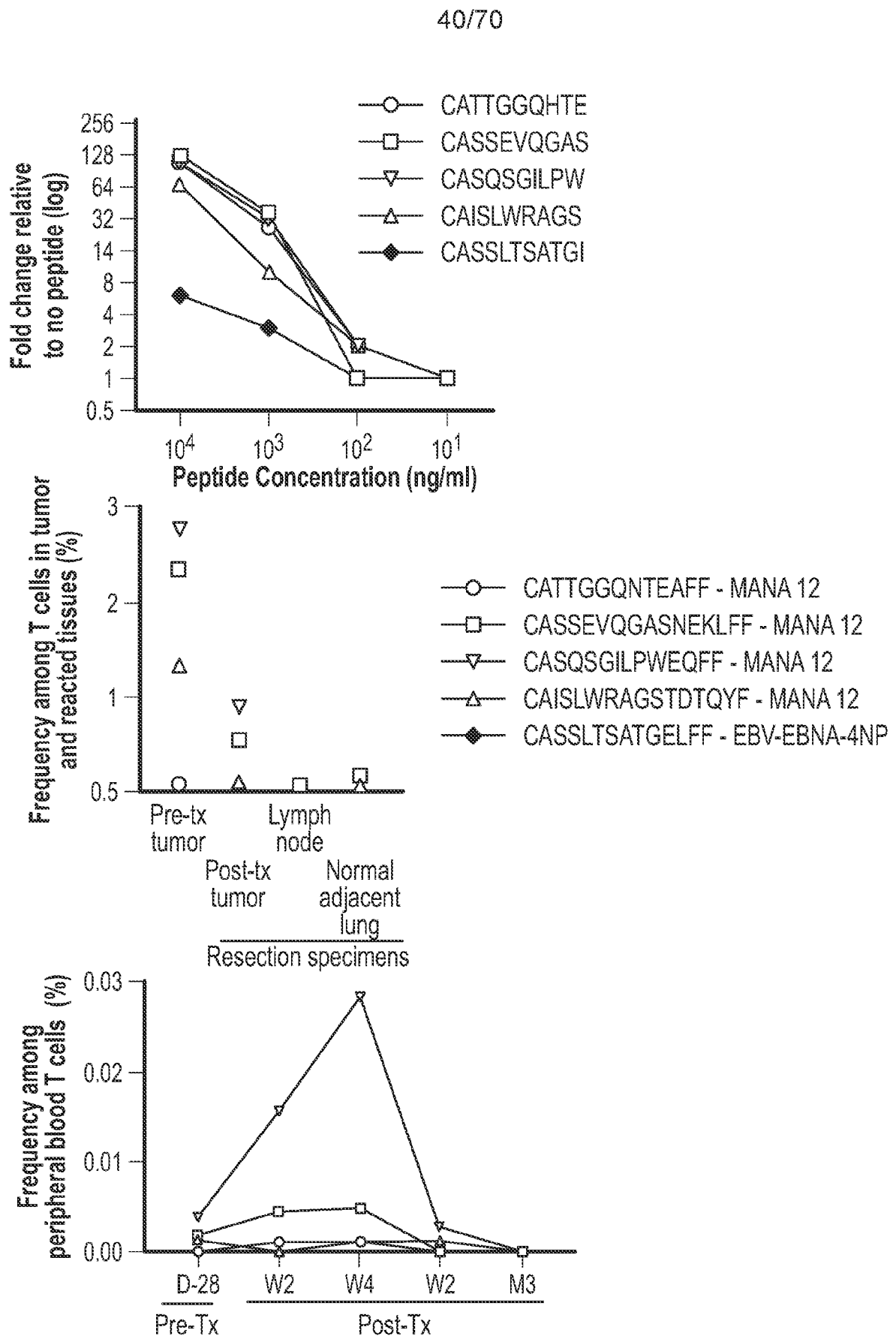


FIG. 12B

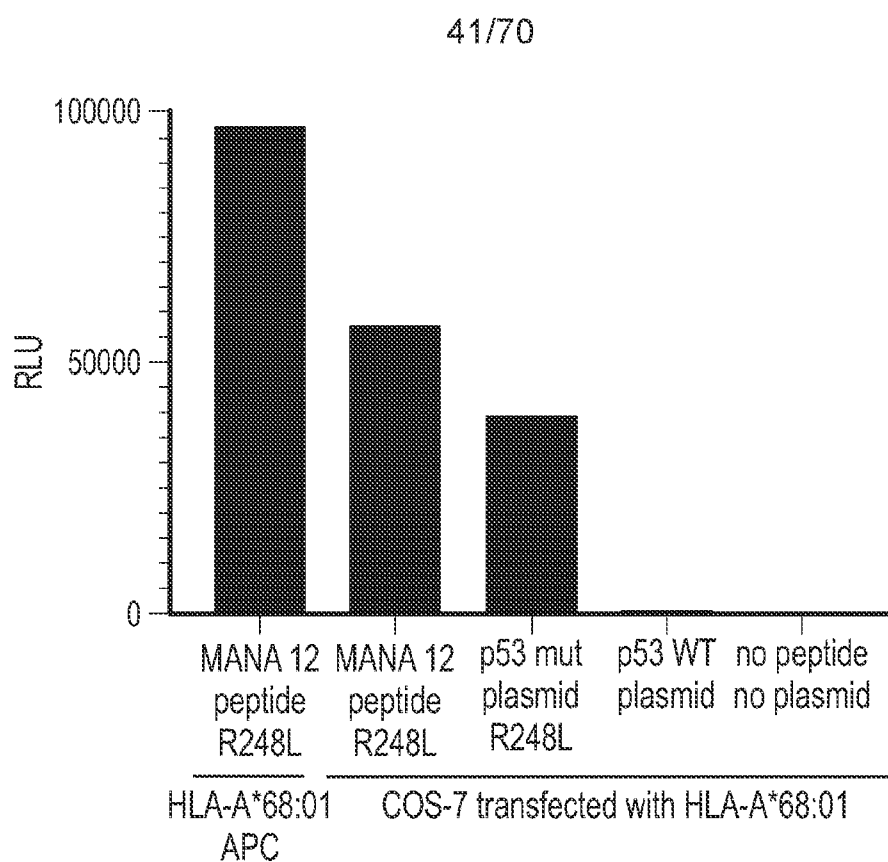


FIG. 12C

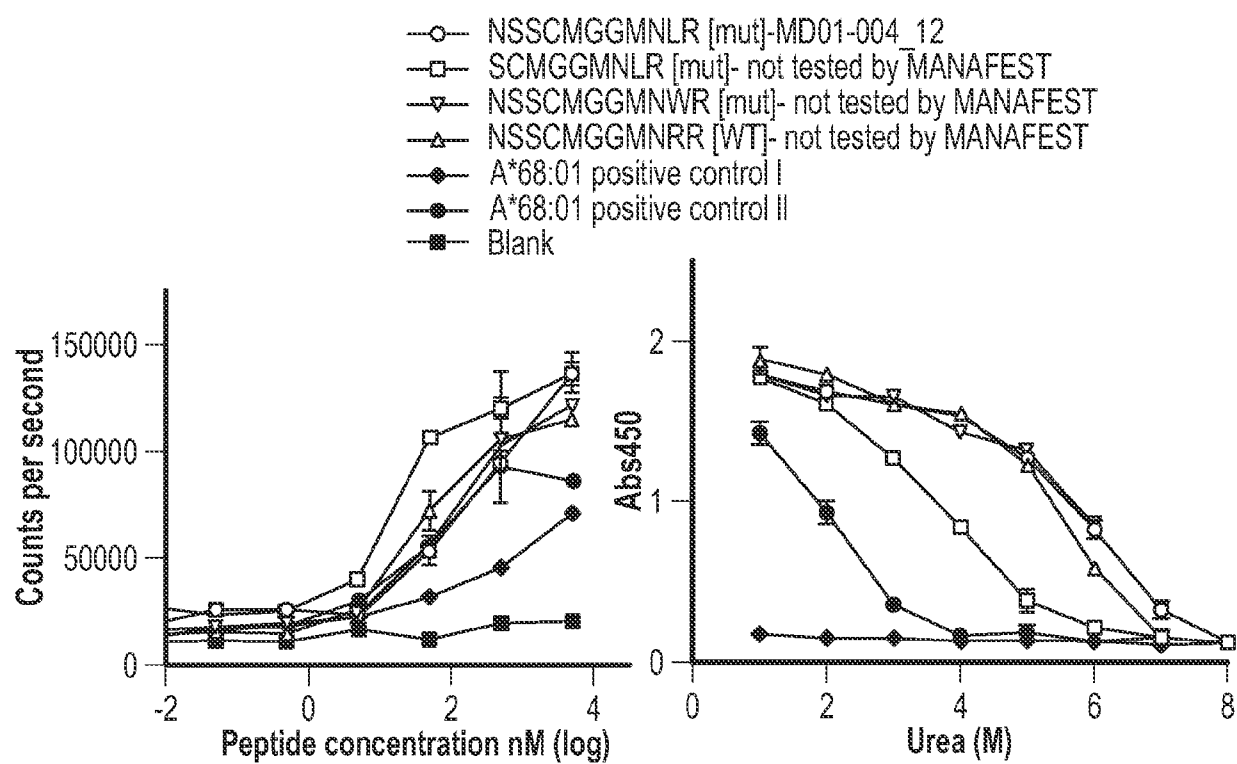


FIG. 12D

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Table 6. MANAs tested by MANAFEST

Gene	Mutation	Variant allele frequency (%)	Predicted MANA sequence	SEQ ID NO	Predicted MANA HLA restriction	Predicted MHC class I affinity (nM)	WT sequence	SEQ ID NO	Predicted WT MHC class I affinity (nM)	TCGA Expression
OPAI	675L>F	46%	HVIENTYF		HLA-A*25:01	171.5	HVIENTYL		686.2	2671.6
HMGXB4	530L>P	35%	DVA AHLQL		HLA-A*25:01	185	DVA AHLQLL		551.1	857.6
GALNS	61R>L	73%	ETPNLDM		HLA-A*25:01	1085.7	ETPNLDRM		1796.9	779.5
PLEK	22K>N	49%	SVENTWNPM		HLA-A*25:01/ C*12:03	738/21.6	SVENTWKPM		2722/56.3	516.3
LARGE	510A>V	38%	EVQQLRY		HLA-A*25:01	665.3	EAQQFLRY		3442.6	365.8
ARVCF	497H>L	39%	EVIVPLSGW		HLA-A*25:01	339.7	EVIVPHSGW		1530.2	253.7
KLHL29	445A>T	79%	ETMQCSELY		HLA-A*25:01	85.2	EAMQCSELY		900.2	207.2
KLHL29	445A>T	79%	ETMQCSELYHM		HLA-A*25:01	208.9	EAMQCSELYHM		2362.4	207.2
KLHL29	445A>T	79%	ETMQCSEL		HLA-A*25:01	773.2	EAMQCSEL		5586.5	207.2
TNC	1847G>A	40%	ITRTVSANTV		HLA-A*30:01	43.4	ITRTVSGNTV		69.9	6942.8
PSMC5	289I>N	36%	ATKNNKVIMA		HLA-A*30:01	28.4	ATKNIKVIMA		23.2	2784.6
SLC4A2	631R>L	25%	VAHFQLQMLK		HLA-A*30:01	49.5	VAHFQRQMLK		28.7	1906.6
ANKLE2	327D>Y	44%	EEDTFSYLI		HLA-B*38:01	1421.3	EEDTFSDLI		2976.8	1946.6
SLC4A2	631R>L	25%	AHFQLQML		HLA-B*38:01/ B*39:01	628.5/83.9	AHFQRQML		1933/129.8	1906.6
CHD6	776Q>H	34%	LHAMIAAGKL		HLA-B*38:01	777.4	LQAMIAAGKL		8269.3	1380.9
SPG11	1031H>Q	31%	LHEAQPWFEL		HLA-B*38:01	517.6	LHEAHFWFEFL		448.7	1306
SPG11	1031H>Q	31%	LHEAQPWFEEF		HLA-B*38:01	822.9	LHEAHPWFEEF		626.8	1306
ATG5	260Y>C	33%	EHLSCPDNFL		HLA-B*38:01	583.5	EHLSPDNFL		465.8	998
CDKN2A	E149Kfs*6	67%	NHARIDAAGV		HLA-B*38:01	1323.2	NHARIDAABG		17959.6	576.8
SGK223	1261L>Q	54%	QHQPWFEEF		HLA-B*38:01	978.5	LHQPWFEEF		745.4	542.1
NIN	1794Q>L	70%	TQLEKEAL		HLA-B*39:01	106	TQQEKEAL		162.7	1078.6
ARHGAP4	281K>R	23%	TRARWEYLLSL		HLA-B*39:01	56.4	TKARNEYLLSL		142	538.9
PLEK	22K>N	49%	NPMWVLL		HLA-B*39:01	60.8	KPMWVLL		334.1	516.3
C22orf59	124P>L	14%	KHILVWAL		HLA-B*39:01/ B*38:01	81.4/406.1	KHILVWAP		10929.7/19	441.3
									040.8	

FIG. 13

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GAB2	475V>D	39%	SQSDYIPM	HLA-B*39:01	44.4	SQSVYIPM	127.9	375.9
SACS	1955D>Y	14%	VHDFYSVI	HLA-B*39:01	114.7	VHDFSVI	177.5	358.2
OPAI	675L>F	46%	IYFPAAQTM	HLA-C*12:03	18.6	IYLPAAQTM	174.5	2671.6
ANKLE2	327D>Y	44%	FSYLIWSNPRY	HLA-C*12:03	32.8	FSDLIWSNPRY	833	1946.6
GAK	211R>Q	18%	YWSAQRQAL	HLA-C*12:03	39.2	YWSAQRREAL	30.5	1492.1
IPO4	880G>W	39%	FAVWTLAETI	HLA-C*12:03	17.4	FAVGTLAETI	24.7	1293.3
TMEM64	140V>A	45%	FASLALARRYL	HLA-C*12:03	17.1	FASLALVRRYL	19.2	653
PVRL3	458L>I	39%	DVIQQDELDY	HLA-A*25:01	151	DVLQQDELDY	3171.1	93.3
HBPI	421H>R	24%	KNRSSGTVSA	HLA-A*30:01	27.5	KNHSSGTVSA	180.6	1549.7
APEH	132S>R	48%	KLKRFNLSA	HLA-A*30:01	8.6	KLKSFNLSA	9.3	1448.6
IPO4	880G>W	39%	KSEFAVWTLA	HLA-A*30:01	23.6	KSEFAVGTLA	57	1293.3
C3orf17	491H>L	44%	KWRLSLCTV	HLA-A*30:01	31.6	KWRLSHCTV	22.8	1213.3
IQCB1	548K>T	45%	RSRPVAATAK	HLA-A*30:01	3	RSRPVAAKAK	4.1	722.9
IQCB1	548K>T	45%	RSRPVAATA	HLA-A*30:01	4.8	RSRPVAAKA	5.3	722.9
IQCB1	548K>T	45%	TAKQAHLTTLK	HLA-A*30:01	33.6	KAKQAHLTTLK	4.8	722.9
NFKBIL1	123R>H	47%	SHCPSAMGI	HLA-B*38:01	925.8	SRCPSAMGI	15334.5	400.2
CENPBD1	139Q>E	75%	PHASEGWL	HLA-B*39:01	54.5	PHASQGWL	31.9	292.7
ARVCF	497H>L	39%	THEVIVPL	HLA-B*39:01	42.5	THEVIVPH	9995.6	253.7
FRS3	205G>S	43%	SRHCLQPL	HLA-B*39:01	58.5	GRHCLQPL	365.5	120.4
TMEM64	140V>A	45%	FASLALARRY	HLA-C*12:03	41.7	FASLALVRRY	32.8	653
PLEK	22K>N	49%	SVENTWNEPMWV	HLA-C*12:03	32.8	SVENTWKPMMWV	71	516.3
ARVCF	497H>L	39%	LTHEVIVPL	HLA-C*12:03	36.7	LTHEVIVPH	747.9	253.7
RAD54L2	V850Yfs*21	74%	YTVMARKSPV	HLA-C*12:03	34.5	VYRYGQKKEPC	38060.3	172.4
CPPED1	306D>Y	11%	LSEKGIETY	HLA-A*01:01	151.6	LSEKGIEDD	34029.5	472
SEMA3A	350R>T	16%	MSDVRTVF	HLA-A*01:01	234.9	MSDVRRVF	1103.4	186.2
ZEB2	860L>V	12%	NSDEPVLTF	HLA-A*01:01	313.3	NSDEPLNLTFF	204.5	766.3
SEMA3A	350R>T	16%	MSDVRTVFL	HLA-A*01:01	317.4	MSDVRRVFL	1188.1	186.2
COL6A1	914T>N	11%	ANDVNDALGY	HLA-A*01:01	396.2	ATDVNDALGY	6.3	4934.2
ITGAL	423G>V	13%	LLASVAPRY	HLA-A*01:01	602	LLASGAPRY	552.1	720.7
SPN	265L>M	11%	ALMAVIVLV	HLA-A*02:01	3.3	ALLAVIVLV	8.3	390.4
SPN	265L>M	11%	ALMAVIVLV	HLA-A*02:01	7.3	ALLAVIVLV	19.3	390.4
HLA-DPB1	163Q>L	12%	FLNGLTEETAGV	HLA-A*02:01	7.9	FLNGLTEETAGV	10	6246.2
SPN	265L>M	11%	ALMAVIVL	HLA-A*02:01	10.8	ALLAVIVL	31.1	390.4
SPN	265L>M	11%	LMMAVIVLV	HLA-A*02:01	13.7	LLAVIVLV	18	390.4

FIG. 13 (CONT)

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SPN	265L>M	11%	VLVALMAV	HLA-A*02:01	14.6	VLVALAV	16.1	390.4
SLC25A26	96S>C	14%	MIAACAGEV	HLA-A*02:01	37.2	MLAASAGEVV	55.5	322.3
RETSAT	170Y>C	11%	YPMCSGEKAY	HLA-B*35:01	2.4	YPMYSGEKAY	2.4	1439.4
SKP2	G370Rfs*16	13%	MPSNIQNF	HLA-B*35:01	7.3	CPQTYRTS	10300.4	132.4
SPN	265L>M	11%	LPVAVILVALM	HLA-B*35:01	9.6	LPVAVILVALL	50.2	390.4
NPR3	283D>V	14%	MTSGVYAF	HLA-B*35:01	11.9	MTSGDYAF	12.5	52.6
DCKZ	957S>Y	17%	LPTPTYPEL	HLA-B*35:01	14	LPTPTSPL	15.8	737.2
SEMA3A	350R>T	16%	YSMSDVRTVF	HLA-B*35:01/	7.9	YSMSDVRRVF	16.1	186.2
				HLA-C*03:04				
COL6A1	914T>N	11%	DANDVNDALGY	HLA-A*801:01/	18.3	DATDVNDALGY	41.4	4934.2
				HLA-B*35:01				
MMRN2	722G>E	14%	AEAGAEAAASL	HLA-B*40:01	0.8	AEAGAGAASL	5.9	446.8
MMRN2	722G>E	14%	AEAASLNASL	HLA-B*40:01	0.79	AGAASLNASL	18021.4	446.8
KLHL15	136I>V	11%	LENCAEVMRL	HLA-B*40:01	0.72	LENCAEIMRL	20.7	227.8
SIGLEC8	94G>S	40%	AETQSRFQLL	HLA-B*40:01	25.1	AETQGRFQLL	20.1	31.1
KLHL15	136I>V	11%	LENCAEVM	HLA-B*40:01	32.1	LENCAEIM	42.4	227.8
KLHL15	136I>V	11%	LENCAEVMRL	HLA-B*40:01	33.9	LENCAEIMRL	31	227.8
ZEB2	860I>V	12%	SENSDEPVNL	HLA-B*40:01	63.2	SENSDEPLNL	81.5	766.3
DUSP9	179D>H	15%	AESEAHDRSM	HLA-C*03:04/	72.8	AESEADRDMS	142.7	11.6
				HLA-C*07:01				
SEMA3A	350R>T	16%	YSMSDVRTVFL	HLA-C*03:04/	9	YSMSDVRRVFL	15.3	186.2
				HLA-C*07:01				
SEMA3A	350R>T	16%	YSMSDVRTV	HLA-C*03:04	15.3	YSMSDVRRV	88.6	186.2
KIAA0430	789N>S	10%	FASGADVQV	HLA-C*03:04	25.1	FANGADVQV	30.4	1864.1
RASSF4	89R>Q	10%	MPRQPSCL	HLA-B*35:01	26.1	MPRRPSCL	53.3	1191.1
DHX29	1070G>C	16%	TPLCQHLLAL	HLA-B*35:01	38.8	TPLGQHLLAL	36	838
HSPG2	581Q>H	13%	SLHEFLV	HLA-A*02:01	8.7	SLHEFQLV	11.2	7194.9
HSPG2	581Q>H	13%	HLVDLSRRFLV	HLA-A*02:01	39.1	QLVDLSRRFLV	56.1	7194.9
HK1	44M>V	15%	RLSDETLIDIV	HLA-A*02:01	30.6	RLSDETLIDIM	559.2	4602.4
MED14	877A>D	14%	VLFDTQDFL	HLA-A*02:01	24.8	VLFDTQAPL	13.1	1436.7
MED14	877A>D	14%	VLFDTQDFLNA	HLA-A*02:01	38.5	VLFDTQAPLNA	28.3	1436.7
PMP22	110V>L	26%	LMSAAAIYTV	HLA-A*02:01	4.6	VMSAAAIYTV	5.3	1074.1
PMP22	110V>L	26%	CLMSAAAIYTV	HLA-A*02:01	6.3	CVMSAAAIYTV	34.5	1074.1
PMP22	110V>L	26%	ILAGLCIMSA	HLA-A*02:01	33.4	ILAGLCVMSA	41.3	1074.1

FIG. 13 (CONT)

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CALCOCO1	625G>C	18%	LLPELCSA	HLA-A*02:01	14	LLPELCSA	63.3	1034.9
ARMC8	655Q>L	17%	KMALQQYL	HLA-A*02:01	13	KMALQQYL	33.4	936.9
PMP22	110V>L	26%	IMSAAAIY	HLA-A*29:02	14.9	VMSAAAIY	16.9	1074.1
PMP22	110V>L	26%	CLMSAAAIY	HLA-A*29:02	16.5	CVMSAAAIY	17.3	1074.1
CALCOCO1	625G>C	18%	LLPELCSAFY	HLA-A*29:02	22	LLPELCSAFY	18.3	1034.9
ATXN1	424R>L	14%	HLGKPGHLSY	HLA-A*29:02	38.3	HLGKPGHRSY	341.3	927.4
QSOX2	666V>L	35%	MSLCVLLY	HLA-A*29:02	8.3	MSLCVVLY	17.1	655.6
QSOX2	666V>L	35%	DMSLCVLLY	HLA-A*29:02	10.3	DMSLCVVLY	34.1	655.6
QSOX2	666V>L	35%	LDMSLCVLLY	HLA-A*29:02	24.2	LDMSLCVVLY	81.2	655.6
QSOX2	666V>L	35%	SLDMSLCVLLY	HLA-A*29:02	38.3	SLDMSLCVVLY	75.1	655.6
MF12	691G>D	17%	TTYRDWLGLDY	HLA-A*29:02	6.5	TTYRGWLGLDY	4.8	546.5
E2F7	150L>M	16%	LMCQKFLARY	HLA-A*29:02	21.6	LLCQKFLARY	54	337.6
ATRN	1037Y>C	12%	APTGNFCFQPL	HLA-B*07:02	14.9	APTGNFYQPL	14.6	2328.2
ATRN	1037Y>C	12%	CPQPLINSSM	HLA-B*07:02	26.4	YPQPLINSSM	9.4	2328.2
PREP	488R>M	20%	TPNYSVSM	HLA-B*07:02	31.9	TPNYSVSRL	50.7	1204.3
PREP	488R>M	20%	TPNYSVSM	HLA-B*07:02	37.6	TPNYSVSR	16548.4	1204.3
PIK3C2A	357D>Y	12%	YFNGTSSL	HLA-B*07:02	6.2	DFNGTSSL	301.6	1021.1
ATXN1	424R>L	14%	KPGHLSYAL	HLA-B*07:02	11.6	KPGHRSYAL	5.9	927.4
CYBB	41Y>F	11%	FTRKLLGSAL	HLA-B*07:02	52.7	YTRKLLGSAL	37	907
TULP4	246A>S	14%	GPASYPIPV	HLA-B*07:02	53	GPAAYPIPV	50.1	877.7
LPHN2	1104V>L	16%	LPTESPHSSL	HLA-B*07:02	21.2	LPTESPHSSV	127.2	791
PDIA5	440A>S	26%	IPHFTATSDAF	HLA-B*07:02	26.1	IPHFTATADAE	33.3	783
NAMPT	1141>V	15%	VEIKAVPEGF	HLA-B*44:03	124	IEIKAVPEGF	96	4317.4
IGJ	117D>V	14%	DEVSATETCY	HLA-B*44:03	236.5	DEDSATEICY	1152.6	3523.6
UTRN	1358K>N	15%	GELDNQLTTY	HLA-B*44:03	35.1	GELDKQLTTY	64.7	1319.1
GALNT7	292K>E	19%	AELGQVLIY	HLA-B*44:03	13.9	AKLGQVLIY	7428.6	1089.6
GALNT7	292K>E	19%	AELGQVLI	HLA-B*44:03	101.8	AKLGQVLI	20288.9	1089.6
GALNT7	292K>E	19%	AELGQVLIYL	HLA-B*44:03	220.5	AKLGQVLIYL	25339.6	1089.6
CALCOCO1	625G>C	18%	PELCSAFY	HLA-B*44:03	195.1	PELCSAFY	239.7	1034.9
HMG20A	232R>W	31%	EEFLNHSKAW	HLA-B*44:03	32.6	EEFLNHSKAP	13080.5	777.6
HMG20A	232R>W	31%	TEEFNHSKAW	HLA-B*44:03	139.4	TEEFNHSKAP	18868.4	777.6
OSBPL3	709K>E	15%	IENLHDDSCY	HLA-B*44:03	162.4	IKNLHDDSCY	18987.4	763.6
IER2	24Q>H	24%	SRMRGGRL	HLA-C*07:02	82.8	SRMRGGRL	70.2	1925.4
IER2	24Q>H	24%	MYHSRMHRGGL	HLA-C*07:02	98.1	MYHSRMQRGGL	142.8	1925.4

FIG. 13 (CONT)

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CYBB	41Y>F	11%	FFTRKLL	HLA-C*07:02	89.2	FFYTRKLL	67.2	907
ZFP64	538S>I	15%	IRLQILRQVSL	HLA-C*07:02	172.7	SRLQILRQVSL	110.1	756.7
MBTPS2	256V>M	14%	FYYTGVGM	HLA-C*07:02	30.7	FYYTGVGV	119.1	555.8
MBTPS2	256V>M	14%	FYYTGVGML	HLA-C*07:02	50.9	FYYTGVGVLI	35.5	555.8
MBTPS2	256V>M	14%	FYYTGVGMLI	HLA-C*07:02	66.4	FYYTGVGVLI	98.9	555.8
MBTPS2	256V>M	14%	YYTGVGML	HLA-C*07:02	152.9	YYTGVGVL	111.6	555.8
MBTPS2	256V>M	14%	YYTGVGMLI	HLA-C*07:02	175.9	YYTGVGVLI	398.2	555.8
ACTR8	401V>L	15%	FYPATFGIL	HLA-C*07:02	80.4	FYPATFGIV	239.6	329.5
TULP4	246A>S	14%	YAPPQDGPASY	HLA-C*16:01	43.3	YAPPQDGPAAV	41.3	877.7
PDIA5	440A>S	26%	FTATSDAF	HLA-C*16:01	40.2	FTATADAF	39.8	783
VWA5A	664G>V	20%	FQMDYSLCVL	HLA-C*16:01	79.9	FQMDYSLCGL	215.8	463.4
SCN8A	158S>L	11%	MTFSNPPDWL	HLA-C*16:01	51.4	MTFSNPPDWS	5389.1	40.6
RYR2	2607L>V	31%	YAAHLLDIAM	HLA-C*16:01	23.6	FAAHLLDIAM	29.6	27.8
RYR2	2607L>V	31%	FFYAHL	HLA-C*16:01	33.5	FFFAAHL	29.8	27.8
RYR2	2607L>V	31%	FYAHLLDIAM	HLA-C*16:01	38.1	FFAHLLDIAM	42.5	27.8
RNF112	539Y>C	14%	FMDSCTRMF	HLA-C*16:01	60.6	FMDSYTRMF	53.5	10.6
CUX1	643E>K	14%	KSMERDCATF	HLA-C*16:01	211.7	ESMERDCATF	2419.4	1916.4
FLNA	D25EfS*32	57%	RTWRRTRR	HLA-A*31:01	5.1	EKDLAEDA	48482.1	21631.7
FLNA	D25EfS*32	57%	RTWRRTRRGR	HLA-A*31:01	6.5	EKDLAEDAPW	46177.9	21631.7
FLNA	D25EfS*32	57%	RTWRRTRRGR	HLA-A*31:01	7.7	EKDLAEDAPWK	42603.5	21631.7
FLNA	D25EfS*32	57%	RTRRGRRSSR	HLA-A*31:01	14.7	AEDAPWKKTQ	46660	21631.7
FLNA	D25EfS*32	57%	RSSRTLSR	HLA-A*31:01	25.9	KKIQQNTF	31516.6	21631.7
GJB2	163M>V	24%	VMYDGFVSQR	HLA-A*31:01	11.2	VMYDGFVSQR	11.4	397.1
EXOC5	583V>I	26%	CVKVCAYIR	HLA-A*31:01	25.7	CVKVCAYVR	18.5	1210.4
POLE	1058K>M	27%	KSTSISTAMR	HLA-A*31:01	26.1	KSTSISTAKR	30.5	767.6
FAM135A	470T>R	39%	GASSIWYR	HLA-A*31:01/ A*68:01	12.4	GASSIWYT	16498.1	408.7
FAM135A	470T>R	39%	AGASSIWYR	HLA-A*31:01	17.9	AGASSIWYT	16608.6	408.7
GJB2	163M>V	24%	VMYDGFVSQR	HLA-A*68:01	6.6	YVMYDGFVSQR	5.7	397.1
TP53	248R>L	37%	NSSCMGGMNLR	HLA-A*68:01	29.4	NSSCMGGMNPR	70.1	1362.9
NAA15	418Y>H	35%	ELFLVKAKIHK	HLA-A*68:01	38.6	ELFLVKAKIYK	15.8	1031.5
POLE	1058K>M	27%	STSISTAMR	HLA-A*68:01	7.8	STSISTAKR	8.9	767.6
POLE	1058K>M	27%	TSISTAMR	HLA-A*68:01	8.2	TSISTAKR	13.3	767.6
LMERD1	382M>I	24%	FIFTSIAGIR	HLA-A*68:01	8.4	FIFTSMAGIR	10	1128.2

FIG. 13 (CONT)

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LMERD1	382M>I	24%	FTSIAGIR	HLA-A*68:01	22.7	FTSMAGIR	19.7	1128.2
CDC40	379V>F	30%	FTNRKVPYCEK	HLA-A*68:01	11.9	FTNRKVPYCVK	50.1	526.6
FRAS1	714C>F	22%	EAFHQSCFR	HLA-A*68:01	4	EACHQSCFR	14.5	203.5
ZHX2	38P>L	21%	HPNVILNSLY	HLA-B*35:08	24.2	HPNVILNPLY	25.3	727.2
MLLT10	585S>L	33%	FPNVVSGL	HLA-B*35:08/ B*51:01	60.5	FPNVVSGS	1872.9	552.3
PGM2L1	11S>F	23%	MAENTEGDINF	HLA-B*35:08	81	MAENTEGDINS	9873.6	732.3
DQX1	671A>T	50%	MLVELTPPY	HLA-B*35:08	16.9	MLVELAPPY	35.2	3.4
ZSCAN22	159N>D	26%	EPDVTETLM	HLA-B*35:08	29.7	EPSNVTEILM	45.2	114.7
ZSCAN22	159N>D	26%	EPDVTETL	HLA-B*35:08	54.1	EPSNVTEIL	103.9	114.7
MUC16	8577S>F	45%	SPAMTSTSTFFF	HLA-B*35:08	13.3	SPAMTSTSTFFS	3242.9	541.8
MUC16	8577S>F	45%	MTSTSTFFF	HLA-B*35:08	70.8	MTSTSTFFS	9450.1	541.8
CCR6	91A>E	15%	MAIEDILF	HLA-B*35:08	28.1	MAIADILF	24.9	100
RYR2	2716K>E	24%	IPEEELEYF	HLA-B*35:08	87.9	IPEKLEYF	175.3	38.9
ZNFX1	339Y>C	14%	MPICPTYNEV	HLA-B*51:01	158.7	MPIYPTYNEV	174.2	1992.4
EXOC5	583V>I	26%	CAYIRKQVEKI	HLA-B*51:01	1750.8	CAYVRKQVEKI	1849	1210.4
BTBD7	389E>G	38%	LAQEGTTVI	HLA-B*51:01	1356.5	LAQETTTVI	2337	827.3
ZHX2	38P>L	21%	HPNVILNSLYV	HLA-B*51:01	1165.5	HPNVILNPLYV	1377.8	727.2
SCML2	385P>L	63%	LPDHFGLGPV	HLA-B*51:01	1382.8	LPDHFPGPV	1712.8	17.1
CCR6	91A>E	15%	MAIEDILFV	HLA-B*51:01/ C*15:02	84.9	MAIADILFV	74.8	100
RYR2	2716K>E	24%	IPEEELEYFI	HLA-B*51:01	1049.1	IPEKLEYFI	2207	38.9
IQCD	180A>P	21%	EPQNFIDSLI	HLA-B*51:01	1549.6	EAQNFIDSLI	4408.4	71.5
ZNFX1	339Y>C	14%	CPTYNEVHL	HLA-B*51:01	671	YPTYNEVHL	112.6	1992.4
GJB2	163M>V	24%	MYDGFSVQRL	HLA-C*04:01	1470.1	MYDGFSMQRL	1384.6	397.1
GJB2	163M>V	24%	MYDGFSVQRLV	HLA-C*04:01	1757.7	MYDGFSMQRLV	1694.2	397.1
FAM173B	217R>L	11%	AYDASTFRGL	HLA-C*04:01	1961	AYDASTFRGR	18001.9	266.2
CDH23	860V>F	24%	ETDCGRPPL	HLA-C*04:01	3091.6	VTDCGRPPL	6315.1	58
MYOM2	E1123Nfs*80	29%	KFDLFARL	HLA-C*04:01	4490.1	EVRLVCKV	34250.4	17.2
TACC2	1895D>Y	24%	STYLIAQSI	HLA-C*15:02	104.5	STDLIAQSI	252.5	1821.2
POLE	1058K>M	27%	KSTSIISTAMRL	HLA-C*15:02	129	KSTSIISTAKRL	425.6	767.6
GUCY1B3	69M>T	35%	QTFFCKMFFV	HLA-C*15:02	90.4	QMEGKMEFFV	2344.4	316.8
FAM173B	217R>L	11%	WAYDASTFRGL	HLA-C*15:02	95.5	WAYDASTFRGR	9861	266.2
KIAA1244	P1950Hfs*28	14%	STHPPGASL	HLA-C*15:02	89.9	STPSTGGFS	21991.1	454.3

FIG. 13 (CONT)

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KIF26A	1537A>D	42%	RADPRAGPSV	HLA-C*15:02	82.2	RAAPRAGPSV	47.3	54.6
MUC16	8577S>F	45%	MTSTSTFFFTL	HLA-C*15:02	84.9	MTSTSTFFFTL	92.1	541.8
IQCD	180A>P	21%	RSAAEPQNF	HLA-C*15:02	46.3	RSAAEQNFI	60.1	71.5
COPB2	121M>L	28%	LTSSDDLLI	HLA-C*15:02	463	LTSSDDMLI	753.4	3920.2
TEAD4	386M>L	12%	KYMLNSVLENF	HLA-A*23:01/ A*24:02	5.7	KYMMNSVLENF	5.3	391.8
TEAD4	386M>L	12%	YMLNSVLENF	HLA-A*23:01/ A*24:02	17.4	YMMNSVLENF	13.5	391.8
ATP5G2	23H>Y	25%	GYACAEPSE	HLA-A*23:01/ A*24:02	122.5	GHACAEPSE	9285.5	4420.3
CARM1	208R>W	14%	FFAAQAQAGAWKI	HLA-A*23:01/ A*24:02	259.6	FFAAQAQAGARKI	2433.7	1617
CARM1	208R>W	14%	SFFAAQAQAGAW	HLA-A*23:01/ A*24:02	401.1	SFFAAQAQAGAR	16677.3	1617
AP1M1	307I>L	14%	YMLKAKSQF	HLA-A*23:01/ A*24:02	81.8	YMIKAKSQF	107.7	1056.4
SCFD1	570D>V	16%	RYFVFKML	HLA-A*23:01	154.9	RYFDPKML	287.4	882.8
KLHL2I	471I>R	11%	ATLNGRMVF	HLA-A*23:01	438.1	ATLNGLMVF	156.5	1519.7
COL14A1	1293R>W	17%	YTISFLFW	HLA-A*23:01	84.9	YTISFLER	8098.8	371
COL14A1	1293R>W	17%	DYTISFLFW	HLA-A*23:01	147.8	DYTISFLER	13351	371
TEAD4	386M>L	12%	KYMLNSVL	HLA-A*24:02	89.9	KYMMNSVL	78.3	391.8
TTN	1393SL>V	14%	RYPAKVTL	HLA-A*24:02	89.7	RYPAKLTL	63.4	124.4
CARM1	208R>W	14%	FFAAQAQAGAW	HLA-A*24:02	1097.1	FFAAQAQAGAR	24577.3	1617
AP1M1	307I>L	14%	EYMLKAKSQF	HLA-A*24:02	648	EYMIKAKSQF	658.6	1056.4
VCAN	1301A>S	20%	KESFGPQAL	HLA-B*40:01/ B*44:03	17.4	KEAFGPQAL	16.7	3206.8
LINS	701D>N	18%	CEVAPNNVV	HLA-B*40:01/ B*44:03	119.8	CEVAPNDVV	80.3	NA
FSIP2	1668K>N	10%	KEMHPNKLNAV	HLA-B*40:01	135.5	KEMHPNKLKAV	295.3	NA
LINS	701D>N	18%	CEVAPNNV	HLA-B*40:01	705.7	CEVAPNDV	914.3	NA
PIEZO2	2178L>I	11%	KQFFYNII	HLA-B*40:01	553.6	KQFFYNLI	164.4	NA
CARF	560T>S	16%	SQIQGLQL	HLA-B*40:01	1256.8	TQLQGLQL	1784.5	NA
KRAS	12G>C	24%	TEYKLVVVGC	HLA-B*40:01	1201	TEYKLVVVGCAG	3696.9	1428
ITGA6	238E>A	10%	FEDGPIYAV	HLA-B*40:01	79.7	FEDGPIYEV	186.2	6874.6

FIG. 13 (CONT)

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CARM1	208R>W	14%	AQAGAWKI	HLA-B*40:01	1547.4	AQAGARKI	6008.1	1617
CARM1	208R>W	14%	AQAGAWKIYAV	HLA-B*40:01	1874.4	AQAGARKIYAV	2982.5	1617
ATP5G2	23H>Y	25%	AERLVGPGY	HLA-B*44:03	90.9	AERLVGPGH	2742.6	4420.3
APIM1	307I>L	14%	IEYMIKAKSQF	HLA-B*44:03	683.3	IEYMIKAKSQF	665.8	1056.4
COL14A1	1293R>W	17%	SDYTISFLFW	HLA-B*44:03	103.6	SDYTISFLFR	24366.4	371
TMEM74	146E>Q	18%	GELGWENPNQW	HLA-B*44:03	35.4	GELGWENPNW	36.2	3.1
ADAM18	205M>T	33%	SEMTAVTQKI	HLA-B*44:03	19.3	SEMTAVTQKI	25.8	0
ADAM18	205M>T	33%	SEMTAVTQKIV	HLA-B*44:03	223.1	SEMTAVTQKIV	304.4	0
CARM1	208R>W	14%	AQAGAWKIY	HLA-B*44:03	1621.1	AQAGARKIY	4575.2	1617
SATL1	273Q>K	30%	NKMDMNQW	HLA-B*44:03	1791.7	NQMDMNQW	223.1	16.4
FOXP2	39D>Y	14%	YTSSEVSTV	HLA-C*02:02	107	DTSSEVSTV	7091.4	51.8
FOXP2	39D>Y	14%	YTSSEVSTVEL	HLA-C*02:02/	111.2	DTSSEVSTVEL	8436.8	51.8
				C*03:04				
DMGDH	381M>T	28%	YSPDILPTV	HLA-C*02:02	98	YSPDILEMV	155.1	4.1
CARM1	208R>W	14%	FAAQAGAWKIY	HLA-C*02:02	475.9	FAAQAGARKIY	546.8	1617
CARM1	208R>W	14%	FAAQAGAWKI	HLA-C*02:02/	441.5	FAAQAGARKI	413.6	1617
				C*03:04				
CARM1	208R>W	14%	FAAQAGAW	HLA-C*02:02/	1172.2	FAAQAGAR	8245.8	1617
				C*03:04				
KLHL21	471L>R	11%	KTATLNGRMVF	HLA-C*02:02	511.2	KTATLNGLMVF	388.1	1519.7
KLHL21	471L>R	11%	KTATLNGRM	HLA-C*02:02	759.5	KTATLNGLM	302.4	1519.7
COL14A1	1293R>W	17%	YTISFLFWIL	HLA-C*02:02	700.6	YTISFLFRIL	314.9	371
COL14A1	1293R>W	17%	YTISFLFWI	HLA-C*02:02	563	YTISFLFRI	489.6	371
KIF21B	1421S>R	35%	IALRPSGTM	HLA-C*03:04	31.2	IALSPSGTM	16.7	131.3
KIF21B	1421S>R	35%	IALRPSGTM	HLA-C*03:04	34.8	IALSPSGTML	18.5	131.3
ZNF688	231W>C	13%	FAVEAHQCI	HLA-C*03:04	12.2	FAVEAHQWI	17.1	175.4
DLX4	2T>S	23%	MSSLPCPL	HLA-C*03:04	36.2	MTSLPCPL	45.5	42.8
ATP5G2	23H>Y	25%	YACAEPSF	HLA-C*03:04	56.7	HACAEPSF	801.4	4420.3
CREBBP	1655L>V	26%	IVDPDPVL	HLA-C*03:04	249.5	IVDPDELL	608.5	2325.8
MKI67	2669P>L	14%	RALKEKAQPL	HLA-C*03:04	129.5	RAPKEKAQPL	352.6	2611
CLSTN1	615T>M	11%	YINSRQFFM	HLA-A*02:01	10.9	YINSRQFFP	24.3	4867.2
UBR4	514Q>L	17%	SIMALSTSI	HLA-A*02:01	41.3	SIMAQSTSI	134.2	3605.3
SORT1	D391Tfs*17	38%	SLTDISTL	HLA-A*02:01	40.7	SLDRHLYT	703.7	2796.6

FIG. 13 (CONT)

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TCIRG1	225G>S	29%	FLISYWSEQI	HLA-A*02:01/ HLA-B*03:01	4.7	FLISYWGEQI	10.8	2313.5
RBM10	T371Sfs*113	53%	SMLSIPRV	HLA-A*02:01	12.4	TINVEFAK	32,500.10	1280.4
RBM10	T371Sfs*113	53%	AVASVLPWPAP	HLA-A*02:01	30.2	EGSRISAASVA	39,890.40	1280.4
NEO1	I114V>I	22%	MLLVITIVSVGI	HLA-A*02:01	35.1	MLLVITIVSVGV	10	1069.8
ZNF644	I77L>F	21%	SHQVLFEL	HLA-A*02:01/ HLA-B*13:02	46.1	SHQVLFLL	118.4	940.5
SRGN	76P>S	28%	RLRTDLFSK	HLA-A*30:01	7.7	RLRTDLFPK	8	2510.5
ATRX	I175K>E	41%	KQRTSSEK	HLA-A*30:01	6.6	KQRTSSKK	7.6	1486.3
TBC1D2B	491D>Y	42%	RLKYNLQGYK	HLA-A*30:01	12.8	RLKDNLQGYK	12.5	1392.9
RBM10	T371Sfs*113	53%	RSRRSTTA	HLA-A*30:01	4.4	SEEPFVDY	41,955.60	1280.4
RBM10	T371Sfs*113	53%	RMRAMATA	HLA-A*30:01	5.6	QQDEGYGN	44,477.20	1280.4
SON	7248delA	50%	LTSPIVCF	HLA-A*01:01	4544.3	LTRPNCMF	13977.2	4388.1
TMPRSS13	257C>T	46%	LARASPALASL	HLA-B*08:01	398.9	PARASPALASL	7493.2	588.2
SON	7248delA	50%	LRNGALTSPF	HLA-C*07:01	733	LRNGALTFRN	24120.2	4388.1
SON	7248delA	50%	VLNGALTSPF	HLA-B*08:01	767.5	VLNGALTFRN	17375.2	4388.1
SON	7248delA	50%	LRNGALTSPFV	HLA-C*07:01	1272.1	LRNGALTFRNC	22442.5	4388.1
TMPRSS13	257C>T	46%	LARASPAL	HLA-B*08:01	573.4	PARASPAL	9228	588.2
TMPRSS13	257C>T	46%	SLARASPAL	HLA-B*08:01	766.6	SPARASPAL	2010.1	588.2
CARD14	A282V	11%	LRSLTFSLV	HLA-C*07:01	1038.9	LRSLTFSLA	9959	131.5
MUC4	I428V	11%	SAITSKVSTV	HLA-B*08:01	2553.5	SAITSKVSTI	2799.1	843.1
MUC4	I428V	11%	AITSKVSTV	HLA-B*08:01	4405.1	AITSKVSTI	4405.3	843.1
MUC4	I428V	11%	SAITSKVSTV	HLA-C*07:01	4584.2	SAITSKVSTI	7272.5	843.1
TMPRSS13	257C>T	46%	ASLARASPA	HLA-B*08:01	1703.3	ASPARASPA	8888.1	588.2
TMPRSS13	257C>T	46%	SLARASPA	HLA-B*08:01	1840.5	SPARASPA	1841.9	588.2
TMPRSS13	257C>T	46%	ASLARASPAL	HLA-B*08:01	1849.8	ASPARASPAL	8597.9	588.2
TMPRSS13	257C>T	46%	QASLARASPA	HLA-B*08:01	4802.8	QASPARASPA	6796.9	588.2
TMPRSS13	257C>T	46%	LARASPALA	HLA-B*08:01	4987.6	PARASEPALA	22618.8	588.2
CARD14	A282V	11%	KLRSLTFSLV	HLA-B*08:01	2331.1	KLRSLTFSLA	3232	131.5
SNX1	89F>L	16%	LLADATVEL	HLA-A*02:01	5	LFADATVEL	2158.6	2235.9
SNX1	89F>L	16%	LLADATVELSL	HLA-A*02:01	11.3	LFADATVELSL	4419	2235.9
ZNF692	8S>F	14%	HMAFSPAV	HLA-A*02:01	22.3	HMASSPAV	26.4	469.8
CMKLR1	27V>F	25%	YLDISIVFL	HLA-A*02:01	3.6	YLDISIVVL	8.8	377.7
CMKLR1	27V>F	25%	FLEDLSPL	HLA-A*02:01	9.3	VLEDLSPL	82.5	377.7

FIG. 13 (CONT)

FIG. 13 (cont.)

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CMKLR1	27V>F	25%	YLDIVFLEDL	HLA-A*02:01	13.3	YLDIVFLEDL	17.2	377.7
CMKLR1	27V>F	25%	FLEDLSPLEA	HLA-A*02:01	48.7	VLEDLSPLEA	489.8	377.7
CDKN2A	N71Kfs*49	23%	LLHGAEPKL	HLA-A*02:01	41.2	LLHGAEPNC	2313.4	122.9
C2orf16	502I>T	20%	TLIDVPKV	HLA-A*02:01	11.6	TLIDVPKV	6.8	40.6
CCNL1	129A>T	12%	TMACINLA	HLA-A*02:01	139.8	AMACINLA	55.1	1147
CUL4A	506V>F	19%	LSKDIMFHK	HLA-A*11:01	27.4	LSKDIMVHK	38	1718.3
CCNL1	129A>T	12%	VTMACINLASK	HLA-A*11:01	7	VAMACINLASK	16.8	1147
CCNL1	129A>T	12%	TMACINLASK	HLA-A*11:01	21.5	AMACINLASK	22.2	1147
COL11A1	1748P>L	17%	MSYDNNLFIK	HLA-A*11:01	6.8	MSYDNNPFIK	8.3	369.8
CENPE	2590R>K	23%	KTWKERTLK	HLA-A*11:01	49.4	KTWKERTLK	25.1	191.1
C2orf16	502I>T	20%	VTLIDVPK	HLA-A*11:01	32.2	VILIDVPK	69.8	40.6
ZNF692	8S>F	14%	MPLVHMAF	HLA-B*35:01	3	MPLVHMAS	131.3	469.8
ZNF692	8S>F	14%	MPLVHMAFSPA	HLA-B*35:01	29.2	MPLVHMAFSPA	22.9	469.8
CMKLR1	27V>F	25%	YPDYLDIVF	HLA-B*35:01	3	YPDYLDIVV	41.5	377.7
CMKLR1	27V>F	25%	YPDYLDIVFL	HLA-B*35:01	31.8	YPDYLDIVVL	13.4	377.7
COL11A1	1748P>L	17%	MSYDNNLF	HLA-B*35:01	27.4	MSYDNNF	8.9	369.8
ZNF692	8S>F	14%	MPLVHMAF	HLA-B*51:01	1135.2	MPLVHMAS	7497.6	469.8
ZNF692	8S>F	14%	MAFSPAADV	HLA-B*51:01	1401.7	MASPAADV	3342.1	469.8
COL6A3	3901C>T	11%	AVQWLRPK	HLA-A*11:01	34.4	AVQWLRPK	79.4	12151.9
NEB	6775C>A	17%	HVMPDTPDILK	HLA-A*11:01	14.8	HVMPDTPDILQ	10312.6	27.3
NIF3	44_45delCCinsAT	13%	KVMYILFY	HLA-A*11:01	17.8	KVMSILFY	11.3	6.8
COL6A3	3901C>T	11%	VQNAVQWLRPK	HLA-A*11:01	76.8	VQNAVQRLPK	248.7	12151.9
COL6A3	3901C>T	11%	VQNAVQWLR	HLA-A*11:01	277.7	VQNAVQRLR	2353.8	12151.9
ARMCX2	1723G>C	12%	TLFQIYYDNL	HLA-A*11:01	268.4	TLFEIYYDNL	224.4	519.7
ZNF219	1343C>G	11%	CLASLHPR	HLA-A*11:01	286.9	SLASLHR	195.8	442
ZNF219	1343C>G	11%	RSLGCLASLH	HLA-A*11:01	488	RSLGSLASLH	538.2	442
SHPRH	4030G>T	12%	KLLHEYWMSLR	HLA-A*11:01	367.6	KLLHEYWMALR	415.3	202.4
SHPRH	4030G>T	12%	LLHEYWMSLR	HLA-A*11:01	476.4	LLHEYWMALR	537.1	202.4
EZH1	433A>C	22%	EVKEEDEFF	HLA-A*26:01	322.5	EVKEEDEFF	1015.1	665.6
NIF3	44_45delCCinsAT	13%	QVKNVMIIFY	HLA-A*26:01	255.2	QVKNVMSILFY	186.9	6.8
COL6A3	3901C>T	11%	EVQNAVQWL	HLA-A*26:01	2065.9	EVQNAVQRL	2812.6	12151.9
SHPRH	4030G>T	12%	LHEYWMSL	HLA-B*38:01	410.9	LHEYWMAL	348.1	202.4
SHPRH	4030G>T	12%	YKLLHEYWMSL	HLA-B*38:01	1136.7	YKLLHEYWMAL	1085.3	202.4
OR4P4	7A>G	36%	MEESNNSTL	HLA-B*38:01	454.9	MEKSNNSTL	4717.9	0
OR4P4	7A>G	36%	MEESNNSTLFI	HLA-B*38:01	923.6	MEKSNNSTLFI	5149.5	0

FIG. 13 (CONT)

Table 8. Antigen-specific TCR clonotypes identified by the MANAFEST and viraFEST assays

TCR clonotype (AA)	SEQ		TCR clonotype (nt)	SEQ		Antigen	Epitope	SEQ		Antigen specificity matched to VDJdb	No. of cells in single cell data										
	ID NO	TCR clonotype (nt)		ID NO	Type of antigen			ID NO	Antigen		Lymph node	Metastatic tumor	Adjacent normal lung	Peripheral blood	Tumor	Flu peptide (IL-7 Expt)	MANA peptide (IL-7)				
CAISEWRAGSTDTQYF		TGTGCCATCAGTGA		MANA TP53		NSSCMGGMNLR		NA	15	NA	NA	NA	5	58	43						
		GTGGCGAGCGGCA																			
		GCACAGATACGCAG																			
		TATTTTGGCCCA																			
		TGTGCCATCAGTGG		MANA FRAS1		EAFHQSCFR		NA	0	NA	NA	NA	0	0	0						
CAISGGTGANYGYTF		GGGGACAGGGGCTA																			
		ACTATGGCTACACC																			
		TTCGGTTTCG																			
		TGTGCCAGCATCCC		MANA MYOM2		KFDLEARL		NA	6	NA	NA	NA	1	8	2						
		GACAGGGCAGAACT																			
CASQSGILPWEQFF		ATGGCTACACCTTC																			
		GGTTTCG																			
		TGTGTATTTCGTGG		MANA TP53		NSSCMGGMNLR		NA	15	NA	NA	NA	32	156	67						
		CCAGCCAAAGCGGA																			
		ATACTCCCATGGGA																			
CASRPGQRNYSPLHF		GCAGTCTCTCGGGC																			
		CA																			
		TGTGCCAGCAGACC		MANA COPB2		LTSSDDLLI		NA	0	NA	NA	NA	0	0	0						
		CGGACAGCGGTATA																			
		ATTACACCCCTCCAC																			
CASRPYGYGYTF		TTTGGGAAC																			
		TGTGCCAGCAGGCC		MANA ZSCAN22		EPSDVTETL		NA	0	NA	NA	NA	0	0	0						
		TTATGGGACCTATG																			
		GCTACACCTTCGGT																			
		TCG																			

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FIG. 14

CASSDIAGLNYGYTF	TGTGCCAGCAGTGA TTTAGCAGGGCTCA ACTATGGCTACACC TTCGGTTCCG	MANA FLNA	PTWRRTRR	NA	0	NA	NA	NA	0	0	0
CASSEALGPNTIYF	TGTGCCAGCAGTGA AGCGTCGGTCCAG GAAACACCATATAT TTTGGAGAG	MANA BTBD7	LAQEGTTVI	NA	0	NA	NA	NA	0	0	0
CASSEQGFWNGYF	TGTGCCAGCAGCGA ACAGGGGTTTTGGA ATGGCTACACCTTC GGTTCG	MANA IQCD	RSAEPQNF	NA	0	NA	NA	NA	0	0	0
CASSEVQASNEKLFF	TGTGCCAGCAGTGA GGTACAGGGGGCGT CCAATGAAAAAAGT TTTTTTGGCAGT	MANA TP53	NSSCMGCMNLR	NA	26	NA	NA	NA	17	41	44
CASSEFAGGNQPQHF	TGTGCCAGCAGTTT CGCAGGGGGAGGCA ATCAGCCCCAGCAT TTTGGTGAT	MANA POLE	TSISTAMR	NA	0	NA	NA	NA	0	0	0
CASSFQONTEAFF	TGTGCCAGCAGTTT TCAGCAGAACACTG AAGCTTCTTTTGA CAA	MANA FRAS1	EAFHQSCFR	NA	0	NA	NA	NA	0	0	0
CASSFWGNTEAFF	TGTGCCAGCAGCTT TTGGGCAACACTG AAGCTTCTTTTGA CAA	MANA FAM13A	AGASSIWYR	NA	0	NA	NA	NA	0	0	0
CASSIGTGSKPQHF	TGTGCCAGTAGTAT AGGACAGGGAGTA AGCCCCAGCATTTT GGTGAT	MANA TACC2	STYLIAQSI	NA	0	NA	NA	NA	0	0	0

FIG. 14 (CONT)

CASSLAGLAGPLAKNIQYF	TGTGCCAGCAGCTT	MANA FLNA	RTWRRTRR	NA	0	NA	NA	NA	0	0	0
	AGCGGGACTAGCGG										
	GACCCCTAGCCAAA										
	AACATTCACTACTT										
CASSLAPGNEQFF	CGGCGCC	MANA POLE	TSISTAMR	NA	0	NA	NA	NA	0	1	0
	TGTGCCAGCAGCTT										
	AGCGCCAGGCAATG										
	AGCAGTTCTTCGGG										
CASSLETTCANVLTF	CCA	MANA IQCD	EPQNFIDSLI	NA	0	NA	NA	NA	0	0	0
	TGTGCCAGCAGCTT										
	AGAGACAACTGGGG										
	CCAACGTCCTGACT										
CASSLGANTGELFF	TTCGGGGCC	MANA SCML2	LPDHFGGLGPV	NA	1	NA	NA	NA	0	0	1
	TGTGCCAGCAGCTT										
	GGGTGCGAACACCG										
	GGGAGCTGTTTTT										
CASSLGDDSMNTEAFF	GCAGAA	MANA POLE	KSTSISTAMRL	NA	2	NA	NA	NA	1	0	1
	TGTGCCAGCAGCCT										
	CGGGACGATTCTA										
	TGAACACTGAAGCT										
CASSLPGDPYEQYF	TTCCTTTGGACAA	MANA FLNA	RTWRRTRRGR	NA	0	NA	NA	NA	0	0	0
	TGTGCCAGCAGCTT										
	ACCAGGGGACCCCT										
	ACGAGCAGTACTTC										
CASSLSGFEVEQYF	GGGCGG	MANA FLNA	RTWRRTRRGR	NA	1	NA	NA	NA	0	0	0
	TGTGCCAGCAGCTT										
	ATCAGGTCGGTCTG										
	AGGACGAGCAGTAC										
CASSLSGSSSYNEQFF	TTCGGGCGG	MANA ZNFXI	CPTYNEVHL	NA	2	NA	NA	NA	0	1	1
	TGTGCCAGCAGCCT										
	AAGCGGCGAGCTCCT										
	ACAATGASCAGTTC										
	TTCGGGCGCA										

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FIG. 14 (CONT)

CASSLTGGSEAFF	TGTGCCACGAGTTT	MANA LMBRD1	FIFTSIAGIR	NA	2	NA	NA	NA	0	0	1
	GACAGGGGCTCTG										
	AAGCTTCTTTTGA										
	CAA										
CASSLYTGELEFF	TGCGCCAGAGCTT	MANA ZHX2	HENVILNSLY	NA	0	NA	NA	NA	0	1	0
	GTACACCGGGGAGC										
	TGTTTTTGGAGAA										
	TGTGCCAGCAGTCC	MANA GJB2	VMYDGFVSQR	NA	0	NA	NA	NA	0	1	0
CASSPLPSGGTYEQYF	ACTTCCTAGCGGG										
	GGACCTACGAGCAG										
	TACTTCGGGCGG										
	TGCGCCAGCAGCCG	MANA FLNA	RTWRTRRGRR	NA	1	NA	NA	NA	0	0	1
CASSRCEGGSNQPOHF	CGGGAGGGGGGA										
	GCAATCAGCCCCAG										
	CATTTTGGTGAT										
	TGTGCCAGTAGTCG	MANA MLLT10	FPNVVSGL	NA	0	NA	NA	NA	0	0	0
CASSRSGSSYNEQFF	ATCGGGAGCTCCT										
	ACAATGAGCASTTC										
	TTGGGGCCA										
	TGTGCCAGCAGTTC	MANA GJB2	MYDGFVSQRL	NA	0	NA	NA	NA	0	0	2
CASSSGLRNIQYF	GGGACTTCGAAACA										
	TTCAGTACTTCGGC										
	GCC										
	TGTGCCAGCAGCTC	MANA FLNA	RSSFILSR	NA	0	NA	NA	NA	0	0	0
CASSGSPSYEQYF	GGGACCAAGCTACG										
	AGCAGTACTTCGGG										
	CCG										
	TGTGCCAGCAGTTC	MANA FLNA	RTWRTRPR	NA	0	NA	NA	NA	0	0	0
CASSISTGELFF	CATATCTACCGGG										
	AGCTGTTTTTTTGA										
	GAA										

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FIG. 14 (CONT)

CASSVDGGQSDEQYF	TGTGCCAGCAGCGT	MANA FLNA	RTWRTRRGRR	NA	0	NA	NA	NA	0	0	0
	AGATGGGGGGCAGA										
	GCGACGAGCAGTAC										
	TTCCGGGCGG										
CASSVDWGAEAFF	TGTGCCAGCAGCGT	MANA DQXI	MLVELTFPY	NA	0	NA	NA	NA	0	0	0
	AGATTGGGTGCTG										
	AAGCTTTCTTTGGA										
	CAA										
CASSWTGNQPQHF	TGCGCCAGCAGCTG	MANA CCR6	MAEDILFV	NA	1	NA	NA	NA	0	0	0
	GACAGGCAATCAGC										
	CCGAGCATTTTGGT										
	GAT										
CASSYLVGAGSNYGYTF	TGTGCCACAGCTTA	MANA POLE	STSLSTAMR	NA	0	NA	NA	NA	0	1	1
	CCTCGTTGGGGCAG										
	GGTCTAACTATGGC										
	TACACCTTCGGTTC										
CASSYNNEQFF	G										
	TGTGCCAGCAGCTTA	MANA FLNA	RTWRTRRGRR	NA	16	NA	NA	NA	5	31	13
	TAACAATGAGCAGT										
	TCTTCGGGCCA										
CASSYPSAAAYNEQFF	TGTGCCAGCAGCTTA	MANA CCR6	MAEDILFV	NA	0	NA	NA	NA	0	0	0
	CCCGTCAGCGGCCG										
	CTTACAATGAGCAG										
	TTCTTCGGGCCA										
CASTPSAGANQPQHF	TGTGCCAGCACCCC	MANA FAM173B	WAYDASTERGL	NA	0	NA	NA	NA	0	0	0
	CTCGGCAGGGGCTA										
	ATCAGCCCCAGCAT										
	TTTGGTGAT										
CATSDLRDRGNNEQFF	TGTGCCACAGTGA	MANA MLT10	FPNVVSGL	NA	0	NA	NA	NA	0	0	0
	TCTCCGGGACAGGG										
	GGAACAATGAGCAG										
	TTCTTCGGGCCA										

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FIG. 14 (CONT)

CATTGGQNTAEFF	TGTGTATTTCTGGG CCACCACAGGGGGC CAAAACACTGAAGC TTTCTTTGGACAA TGTGCTTGGAAAGA GGTACGGGGGCGAGT TCTTCGGGGCCA TGTGCTCTGGAGTGA GGGGCGGGGGAATG AAAAACTGTTTTTT GGCAGT TGTGCCAGCGGGGG AACAGATACGCAGT ATTTTGGCCCCA TGTGCTCTGGGAATC CTCCCGGGACATTG ATGATCCTGAAGCT TTCFTTGGACAA TGTGCCAGCAGTTT CGGCGGAAATGAGC AGTCTTTC TGCGCCAGCGGGGA GGGCAATCAGCCCC AGCATTTT TGTGCCAGCAGTTT ATCGTCGACAGTT TGCAGCCCCAGCAT TTT TGTGCCAGCAGTCC AAACCGGGACACCT ATCGGGGCTACACC TTC	MANA TP53	NSSCMGGMNLR	NA	0	NA	NA	NA	1	12	5
CAWKEVRGQFF	TGTGCTTGGAAAGA GGTACGGGGGCGAGT TCTTCGGGGCCA	MANA GJB2	VMYDGFVSQVR	NA	0	NA	NA	NA	0	0	1
CAWSEGRGNEXLEFF	TGTGCTCTGGAGTGA GGGGCGGGGGAATG AAAAACTGTTTTTT GGCAGT	MANA FLNA	RTWRRTRRGR	NA	0	NA	NA	NA	0	0	0
CASGGTDTQYF	TGTGCCAGCGGGGG AACAGATACGCAGT ATTTTGGCCCCA	Viral unknown (CMV, EBV, Influenza A)	unknown	NA	6	NA	NA	NA	0	4	4
CAWESSRDIDDEAFF	TGTGCTCTGGGAATC CTCCCGGGACATTG ATGATCCTGAAGCT TTCFTTGGACAA	Viral unknown (CMV, EBV, Influenza A)	unknown	NA	30	NA	NA	NA	0	10	9
CASFEQNEQFF	TGTGCCAGCAGTTT CGGCGGAAATGAGC AGTCTTTC	Viral MPI_H1N (Influenza)	unknown 1/H3N2	NA	205	NA	NA	NA	12	183	186
CASGEGNQPHF	TGCGCCAGCGGGGA GGGCAATCAGCCCC AGCATTTT	Viral MPI_H1N (Influenza)	unknown 1/H3N2	NA	11	NA	NA	NA	0	2	2
CASSLSSTGLQPHF	TGTGCCAGCAGTTT ATCGTCGACAGTT TGCAGCCCCAGCAT TTT	Viral NP_H3N2 (Influenza)	unknown	NA	2	NA	NA	NA	0	0	1
CASSENRTYRGYTF	TGTGCCAGCAGTCC AAACCGGGACACCT ATCGGGGCTACACC TTC	Viral MPI_H1N (Influenza)	unknown 1/H3N2	NA	2	NA	NA	NA	0	1	1

FIG. 14 (CONT)

CATSDLNQPNQPQHF	TGTGCCACCACTGA TTTGAACCAAGCCGA ATCAGCCCCAGCAT TTT	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	0	NA	NA	0	0	0
CASSVGLGGELEF	TGTGCCAGACGCGT AGGTCAGGCGGG AGCTGTTTTT	Viral MP1_H1N1 unknown (Influenza) 1/H3N2	NA	64	NA	NA	3	117	99
CASSWEDRDADTQYF	TGCGCCAGCAGCTG GGAAGACAGGACG CAGATACGCAGTAT TTT	Viral NP_H1N1 unknown (Influenza)	NA	26	NA	NA	3	27	24
CAWESSRDIDDPEAFF	TGTGCTGGGAATC CTCCCGGACATTG ATGATCCTGAAGCT TTCTTT	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	30	NA	NA	0	10	9
CASSMDGTLLEYQYF	TGTGCCAGTAGTAT GGATGGGACTCTTC TCTACGAGCAGTAC TTC	Viral NP_H3N2 unknown (Influenza)	NA	4	NA	NA	0	0	0
CASSLSTGGPRVNSFLHF	TGTGCCAGCAGCTT ATCTACGGGAGGGC CTAGGGTTAATTCA CCCCGCCACTTT	Viral MP1_H1N1 unknown (Influenza) 1/H3N2	NA	3	NA	NA	0	2	1
CASSLTGGSTEAF	TGCGCCAGCAGCTT AACAGGGGGGAA CTGAAGCTTCTTT	Viral MP1_H1N1 unknown (Influenza) 1/H3N2	NA	18	NA	NA	0	11	13
CASSLPQDTHLETQYF	TGTGCCAGCAGCTT ACCGGACAGGATA CGCATCTGGACAC CAGTACTTC	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	0	NA	NA	0	2	1
CASSVAGVTDTQYF	TGTGCCAGCAGCGT AGCGGGAGTCACAG ATACGCAGTATTTT	Viral MP1_H1N1 unknown (Influenza) 1/H3N2	NA	12	NA	NA	1	31	35

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FIG. 14 (CONT)

CASSINSEAFF	TGTGCCAGCAGTAT TAACAGCTTTGAAG CTTTCCTTT	Viral NP_H1N1/ (Influenza) H3N2	NA	11	NA	NA	NA	0	9	7
CASSFVIAISGNTIYF	TGTGCCAGCAGTTT CGTGTCTGCCATCT CTGGAACACACCATA TATTTT	Viral NP_H3N2 (Influenza) unknown	NA	0	NA	NA	NA	0	0	0
CASSLEGGGLSINTEAFF	TGTGCCAGCAGCTT AGAAGCGGGTTGG GCTCCCTGAACACT GAAGCTTCTTTT	Viral NP_H1N1/ (Influenza) H3N2	NA	2	NA	NA	NA	0	2	2
CASSELAGDTYSYEQYF	TGTGCCAGCAGTGA ACTGGCGGGGACAA CATACTCCTACGAG CAGTACTTC	Viral NP_H3N2 (Influenza) unknown	NA	0	NA	NA	NA	0	1	1
CAAGKDWSEGLFF	TGTGCCCGGGGGAA GGATTGGTCCGGGG AGCTGTTTTT	Viral NP_H1N1/ (Influenza) H3N2	NA	37	NA	NA	NA	1	8	12
CSALDRGNEKLFF	TGCAGTCCCTTGA CAGCGTAATCAAA AACTGTTTTT	Viral NP_H1N1/ (Influenza) H3N2	NA	2	NA	NA	NA	0	0	4
CSAQKNTGGGEKLFF	TGCAGTCCCCAGAA AAATACAGGGGAG GCGAAAAACTGTTT TTT	Viral NP_H1N1 (Influenza) unknown	NA	1	NA	NA	NA	0	0	0
CSARDRSDEKLFF	TGCAGTGTCTAGAGA TCGAAGCGATCAAA AACTGTTTTT	Viral NP_H1N1/ (Influenza) H3N2	NA	1	NA	NA	NA	1	0	1
CSARDSGYTF	TGCAGTGTCTAGAGA TTCGGGTACACCT TC	Viral MP1_H1N (Influenza) 1/H3N2	NA	3	NA	NA	NA	0	1	3

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FIG. 14 (CONT)

CSARYRDRGQGGSTDTQYF	TGCAGTCCCGTTA CCGGACAGGGGC AGGCGGGAGCACC GATACGCAGTATTT T	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	6	NA	NA	NA	0	3	2
CASGGQGAGNTIYF	TGTGCCAGCGGGG ACAGGGGGCTGGAA ACACCATATATTTT	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	0	NA	0	NA	0	NA	NA
CASKTGGANTEAFF	TGTGCCAGCAGCCC AAGTGGGGGGGACA CCGGGGAGCTGTTT TTT	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	0	NA	0	NA	0	NA	NA
CASNAWGGNQPHF	TGTGCCAGCAATGC ATGGGGGGGCAATC AGCCCCAGCATTTT	Viral NP_H3N2 unknown (Influenza)	NA	0	NA	0	NA	0	NA	NA
CASNGEAEQYF	TGTGTATTTCTGTG CCAGCAACGGGGAG GCGGAGACCCAGTA CTTCGGGCCA	MANA CDKN2A NHARIDAAKV	NA	0	NA	0	NA	0	NA	NA
CASSILENQPHF*	TGTGCCAGCAGCTT GCTGGAGAAATCAGC CCAGCATTTTGGT GAT	MANA ARVCF EVIVPLSGW	NA	4	NA	0	2	3	NA	NA
CASNKLGYQPQHF	TGTGCCAGCAACAA ACTGGGGTATCAGC CCAGCATTTTGGT GAT	MANA ARVCF EVIVPLSGW	NA	13	NA	1	25	0	NA	NA
CASSLTGGYTGELEF	TGTGCCAGCAGCTT AACGGGAGGCTACA CCGGGGAGCTGTTT TTTGAGAA	MANA ARVCF EVIVPLSGW	NA	9	NA	24	NA	17	NA	NA
CASRLGQTSYEQYF	TGTGCCAGCAGGCT TGGACAGACCTCCT ACGAGCAGTACTTC	Viral unknown (CMV, EBV, Influenza A)	NA	0	NA	1	NA	0	NA	NA

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FIG. 14 (CONT)

CASSFDLNTAEFF	TGTGCCAGCAGCTT TGATCTGAACACTG AAGCTTTCCTTT	Viral (CMV, EBV, influenza A)	unknown	unknown	NA	0	NA	0	4	1	NA	NA
CASSLDLNTAEFF	TGTGCCAGCAGCTT GGACCTTAACACTG AAGCTTTCCTTT	Viral (CMV, EBV, influenza A)	unknown	unknown	NA	0	NA	15	9	10	NA	NA
CASSLDVAVEQYF	TGTGCCAGCAGCTT GGACGTGGCTGTGG AGCAGTACTTC	Viral (CMV, EBV, influenza A)	unknown	unknown	NA	4	NA	12	9	23	NA	NA
CASSLDVNTEAFF	TGTGCCAGCAGCTT GGACGTGAACACTG AAGCTTTCCTTT	Viral (CMV, EBV, influenza A)	unknown	unknown	NA	0	NA	0	NA	0	NA	NA
CASSPDDLGYTF	TGCGCCAGCAGCCC GGACGATCTATATG GCTACACCTTC	Viral (CMV, EBV, influenza A)	unknown	unknown	NA	0	NA	0	NA	0	NA	NA
CASSPFRSPDPLHF	TGTGCCAGTAGCCC CTTCAGGAGCCCTG ATTCACCCCTCCAC TTT	Viral (CMV, EBV, influenza A)	unknown	unknown	NA	0	NA	0	NA	0	NA	NA
CASRLGQTSYEQYF	TGTGCCAGCAGGCT TGGACAGACCTCCT ACGAGCAGTACTTC	Viral (influenza)	NP_H1N1/ H3N2	unknown	NA	0	NA	1	NA	0	NA	NA
CASRTQNTAEFF	TGTGCCAGCAGGAC ACAGGGGAACACTG AAGCTTTCCTTT	Viral (influenza)	NP_H1N1/ H3N2	unknown	NA	0	NA	0	NA	0	NA	NA
CASSDNVQIGGYTF	TGTGCCAGCAGCGA TAATGTCCAGGGGA TTGGCGGCTACACC TTC	Viral (influenza)	MP1_H1N 1/H3N2	unknown	NA	0	NA	0	NA	0	NA	NA
CASSDPDGGDTGELFF	TGTGCCAGCAGCGA CCGGACGGGGCG ACACGGGGAGCTG TTTTTT	Viral (influenza)	NP_H3N2	unknown	NA	7	NA	19	NA	10	NA	NA

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FIG. 14 (CONT)

CASSKGNRRSYGYTF	TGTGCCAGCACCAA AGGGAACAGGCGAT CCTATGGCTACACC TTC	Viral MP1_H1N1 unknown (Influenza) 1	NA	0	NA	0	NA	0	NA	0	NA	NA
CASSLDVAVEQYF	TGTGCCAGCAGCTT GGACGTGGCTGTGG AGCAGTACTTC	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	4	NA	12	9	23	NA	NA	NA	NA
CASSLGGSFYGYTF	TGTGCCAGCAGCTT GGGGGGGAGTTTTT ATGGCTACACCTTC	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	0	NA	0	NA	0	NA	0	NA	NA
CASSLLGQGSYGYTF	TGTGCCAGCAGCTT ATTGGGACAGGCT CCTATGGCTACACC TTC	Viral NP_H1N1 unknown (Influenza)	NA	0	NA	0	NA	0	NA	0	NA	NA
CASSLVGDTYNEQFF	TGTGCCAGCAGCTT AGTGGGGGACACCT ACAAATGAGCAGTTC TTC	Viral NP_H3N2 unknown (Influenza)	NA	0	NA	0	NA	0	NA	0	NA	NA
CASSPDDLIGYTF	TGCGCCAGCAGCCC GGACGATCTATATG GCTACACCTTC	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	0	NA	0	NA	0	NA	0	NA	NA
CASSPDVGRVYF	TGTGCCAGCAGCCC TGACGTTGGTCGGG TATATTTT	Viral NP_H1N1 unknown (Influenza)	NA	0	NA	0	NA	0	NA	0	NA	NA
CASSPSGGTIGELFF	TGTGCCAGCAGCCC AAGTGGGGGGGACA CCGGGGAGCTGTTT TTT	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	1	NA	1	NA	0	NA	0	NA	NA
CASSQTGGGGGNRAFF	TGTGCCAGCAGCCA AACCGGGGGGGAC AGGAAATCGAGCT TTCITT	Viral NP_H3N2 unknown (Influenza)	NA	0	NA	0	NA	0	NA	0	NA	NA

FIG. 14 (CONT)

CASSSDRAVGDTTEAFF	TGTGCCAGTAGTTC CGACAGAGCAGTGG GGGACACTGAAGCT TTCTTT	Viral MPI_H1N unknown (Influenza) I/H3N2	NA	0	NA	1	NA	0	NA	NA
CASSSPQGDYEQYF	TGTGCCAGCAGCTC GCCACAGGGTGATT ACGAGCAGTACTTC	Viral NP_H1N1/ unknown (Influenza) H3N2	NA	6	NA	14	NA	21	NA	NA
CASSWSTGGDSPLHF	TGTGCCAGCAGTTG GAGTACAGGGGGG ATTCACCCCTCCAC TTT	Viral NP_H3N2 unknown (Influenza)	NA	6	NA	119	NA	203	NA	NA
CATSAGSETQYF	TGTGCCACATCGGC GGGTGCGGAGACCC AGTACTTC	Viral MPI_H1N unknown (Influenza) I/H3N2	NA	0	NA	0	NA	0	NA	NA
CAMEGGPYGYTF	TGTGCCTGGGAAGG ACAGGGCCCTTACT ATGGCTACACCTTC	Viral MPI_H1N unknown (Influenza) I	NA	0	NA	0	NA	0	NA	NA
CASSVGGEAYEQYF	TGTGCCAGCAGCGT TGGGGGGAGGCCCT ACGAGCAGTACTTC GGGCCG	MANA ZNF615 TEDEIYSRIOL	NA	NA	NA	0	NA	1	NA	NA
CASSVGSVGEAFF	TGTGCCAGCAGCGT AGGAAGCGTGGTG AAGCTTTCCTTGG CAA	MANA SKP2 MPSNIQNF	NA	NA	NA	1	NA	1	NA	NA
CASSEGAKAFGEQYF	TGTGCCAGCAGTGA AGGGCGGAAGGCTC CTGGGGAGCAGTAC TTGGGGCCG	Viral unknown unknown (CMV, EBV, Influenza A)	NA	NA	NA	0	NA	0	NA	NA
CASSESSGTTDTQYF	TGTGCCAGCAGCTT CTCTAGCGGTACCA CAGATACGCAGTAT TTTGGCCCCA	Viral unknown unknown (CMV, EBV, Influenza A)	EBV	NA	NA	11	NA	262	NA	NA

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FIG. 14 (CONT)

CASSIGSHGYTF	TGTGCCAGTAGTAT	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	NA	NA	NA	NA
	AGGAGCCATGGCT										
	ACACCTTCGGTTCG										
CASSLAVVKSNTIYF	TGTGCCACGACCTT	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	NA	NA	NA	NA
	GGCCGTGGTCAAAT										
	CTGGAAACACCATATA										
CASSRSAYEQYF	TATTTTGGAGAG	Viral (CMV, EBV, Influenza A)	unknown	unknown	InfluenzaA	NA	NA	5	NA	2	NA
	TGTGCCAGTAGTAC										
	TCGTTCCGCCTACG										
CASSVRSYEQYF	AGCAGTACTTCGGG	Viral (CMV, EBV, Influenza A)	unknown	unknown	InfluenzaA	NA	NA	62	NA	30	NA
	CCG										
	TGTGCCAGTAGTGT										
CSARDETNGYTF	TAGGAGCTCCTACG	Viral (CMV, EBV, Influenza A)	unknown	unknown	EBV	NA	NA	0	NA	0	NA
	AGCAGTACTTCGGG										
	CCG										
CSARDTGNGYTF	TGCAGTGCTAGAGA	Viral (CMV, EBV, Influenza A)	unknown	unknown	EBV	NA	NA	3	NA	5	NA
	TGAGACAGGGAATG										
	GCTACACCTTCGGT										
CSARDTQGAQVYEQYF	TCG	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	0	NA
	TGCTAGGACGGGA										
	CAGGCAATGGCTAC										
CSARDREYGNNTIYF	ACCTTCGGTTCG	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	0	NA
	TGCTAGACATATAC										
	AGGGGGCGCAGGTC										
CSARDTVGNNTIYF	TACGAGCACTACTT	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	0	NA
	CGGGCCG										
	TGCAGTGCCCGGA										
CSARDTQGAQVYEQYF	CAGGGAGTACGGAA	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	1	NA	10	NA
	ACACCATATATTTT										
	GGAGAG										
CSARDTVGNNTIYF	TGCAGTGCTAGAGA	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	1	NA	10	NA
	TACCGTTGGAAACA										
	CCATATATTTTGA										
CSARDTVGNNTIYF	GAG	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	1	NA	10	NA

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FIG. 14 (CONT)

CSARGGQTEVGNIIYF	TGCAGTGCTAGGGG	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	1	NA	NA	NA
	TGGACAGACGGAAG												
	TTGGAAACACCAATA												
	TATTTTGGAGAG												
CASSDGRVAPGELEFF	TGTGCCAGCAGTGA	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	0	NA	NA	NA
	CGGCAGGGTGGCCC												
	CCGGGGAGCTGTTT												
	TTTGAGAGAA												
CASSGGQVAPSEQYF	TGTGCCAGCAGTGG	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	4	NA	2	NA	NA	NA
	TGSTCAGGTCGCCC												
	CCTCCGAGCAGTAC												
	TTCGGGCCG												
CASSIRSSYEQYF	TGTGCCAGTAGTAT	Viral (CMV, EBV, Influenza A)	unknown	unknown	InfluenzaA	NA	NA	30	NA	0	NA	NA	NA
	TAGGAGCTCCTACG												
	AGCAGTACTTCGGG												
	CCG												
CASSLSSYTEAEFF	TGTGCCAGCAGTTTT	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	0	NA	NA	NA
	ATCGTCCTACACTG												
	AAGCTTTCCTTGGG												
	CAA												
CASSQPDKAAYNQEFF	TGTGCCAGCAGCCA	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	0	NA	NA	NA
	ACCCGACAAGGCAG												
	CCTACAATGAGCAG												
	TTCTTCGGGCCA												
CASSSPGIDYGYTF	TGTGCCAGCAGCTC	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	3	NA	2	NA	NA	NA
	CCCTGGTATCGACT												
	ATGGCTACACCTTC												
	GGTTCG												
CASSSPGINYGYTF	TGTGCCAGCAGCTC	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	1	NA	0	NA	NA	NA
	TCCGGGGATAAACT												
	ATGGCTACACCTTC												
	GGTTCG												

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FIG. 14 (CONT)

CASSVVGGDEQYF	TGTGCCAGCAGCGT AGTGGGGGCGACG AGCAGTACTTCGGG CCG	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	1	NA	1	NA	NA
CSARDSTCNGYF	TGCAGTCTAGAGA CTCTACAGGGAATG GCTACACCTTCGGT TCG	Viral (CMV, EBV, Influenza A)	unknown	unknown	EBV	NA	NA	0	NA	0	NA	NA
CSSQEGGYGYF	TGCAGCTCACAGGA GGGGGATATGGCT ACACCTTCGGTTCG	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	0	NA	0	NA	NA
CSVGGRGDRDQPQHF	TGCAGCGTGGGGG CAGGGAGATCGAG ACCAGCCCCAGCAT TTTGGTGAT	Viral (CMV, EBV, Influenza A)	unknown	unknown	NA	NA	NA	1	NA	0	NA	NA
CSVGQGGTNEKLEF	TGCAGCGTTGGCCA GGGGGAACATAATG AAAAACTGTTTTT GGCAGT	Viral (CMV, EBV, Influenza A)	unknown	unknown	EBV	NA	NA	0	NA	0	NA	NA
CSVGTGGTNEKLEF	TGCAGCGTTGGGAC AGGGGGAACATAATG AAAAACTGTTTTT GGCAGT	Viral (CMV, EBV, Influenza A)	unknown	unknown	EBV	NA	NA	0	NA	0	NA	NA
CASSASLAGVEQYF	TGTGCCAGCAGCGC GAGCTTAGCGGGAG TCGACAGTACTTC GGCCG	MANA TEAD4	YMLNSVLENF	NA	NA	0	0	0	NA	0	NA	NA
CASSHTFDRGETQYF	TGCGCCAGCAGCCA TACGTTGACAGGG GAGGGAGACCCAG TACTTCGGGCCA	MANA COL14A1	SDYTISFLFW	NA	NA	0	0	0	NA	0	NA	NA
CASSIDPYEQYF	TGTGCCAGCAGCTT GGACCCCTACGAGC AGTACTTCGGGCCG	MANA CARM1	FAAQAGAWKIY	NA	NA	234	67	30	NA	782	NA	NA

FIG. 14 (CONT)

CASSIFSRTQYF	TGTGCCAGTAGTAT CTTTCTCGGATA CGCAGTATTTT	Viral MPI_HIN unknown (influenza) 1/H3N2	NA	0	0	0	NA	0	NA	NA
CASSIRSVEQYF	TGTGCCAGTAGTAT TCGGAGCTCCTACG AGCAGTACTTC	Viral MPI_HIN unknown (influenza) 1/H3N2	InfluenzaA	0	0	0	NA	0	NA	NA
CASRLTGGGLAYEQYF	TGTGCCAGCAGATT AACAGGGGGAGGGC TGGCCTACGAGCAG TACTTC	Viral NP_HIN1 unknown (influenza)	NA	0	0	0	NA	0	NA	NA
CASSPDGADEQFF	TGCGCCAGCAGCCC CGACGGGGCGGATG AGCAGTTCCTC	Viral NP_HIN1 unknown (influenza)	NA	0	0	0	NA	0	NA	NA
CASSPRSTDTQYF	TGTGCCAGTAGCCC CAGTCCACAGATA CGCAGTATTTT	Viral MPI_HIN unknown (influenza) 1	InfluenzaA	0	0	0	NA	0	NA	NA
CASSRSAYEQYF	TGTGCCAGTAGTAC TCGTTCCGGCTACG AGCAGTACTTC	Viral MPI_HIN unknown (influenza) 1/H3N2	InfluenzaA	0	0	0	NA	0	NA	NA
CASSVRSVEQYF	TGTGCCAGTAGTGT CAGAAGCTCCTACG AGCAGTACTTC	Viral MPI_HIN unknown (influenza) 1/H3N2	InfluenzaA	0	0	0	NA	0	NA	NA
CASFLAPNAAEAF	TGTGCCAGCACCTT CCTTGCCCCAAATG CCGCTGAAGCTTTC TTT	Viral NP_HIN1 unknown (influenza)	NA	0	0	0	NA	0	NA	NA
CATGKSPEETQYF	TGTGCCACCGGAA AAGCCCTGAAGAGA CCCAGTACTTC	Viral NP_HIN1/ unknown (influenza) H3N2	NA	0	0	0	NA	0	NA	NA
CTSSRKVQETQYF	TGCACCCAGCAGCCG CAAAGTCCAAGAGA CCCAGTACTTC	Viral NP_HIN1/ unknown (influenza) H3N2	NA	0	0	0	NA	0	NA	NA

FIG. 14 (CONT)

CASSLGVEDHVRVGYTF	TGTGCCAGCAGCTT	MANA	TMPRSS1	LARASPALA	NA	NA	NA	0	NA	0	NA	NA	NA
	AGGTGTAGAGATC		3										
	ACAGGGTGTATGGC												
	TACACCTTC												
CASSPIGMWNEQFF	TGTGCCAGCAGTCC	MANA	CARD14	LRLTFFSLV	NA	NA	NA	0	NA	0	NA	NA	NA
	CATTGGAATGTGGG												
	GAAATGAGCAGTTC												
	TTC												
CASATLQGDGYTF	TGTGCCAGCGCCAC	MANA	CCNL1	VTMACINLASK	NA	NA	NA	0	NA	0	NA	NA	NA
	ACTCCAGGGGATG												
	GCTACACCTTC												
	TGTGCTTCGGGGC	MANA	CMKLRI	YPDYLDLSVF	NA	NA	NA	0	NA	1	NA	NA	NA
CASGADTF	AGACACCTTC												
	TGTGCCAGCAGACG	MANA	CMKLRI	FLEDLSPL	NA	NA	NA	0	NA	2	NA	NA	NA
	TAGCAATCAGCCCC												
	AGCATTTT												
CASSEMFGGDTQYF	TGCGCCAGCAGTCA	MANA	CENPE	KTWKTKLK	NA	NA	NA	0	NA	0	NA	NA	NA
	GATGACGGGGGGC												
	AAGATACGCAGTAT												
	TTT												
CASSIDRGINYGYTF	TGTGCCAGTAGTAT	MANA	C2orf16	VTLLIDVEK	NA	NA	NA	0	NA	1	NA	NA	NA
	CGACAGGGGGATTA												
	ACTATGGCTACACC												
	TTC												
CASSLEPRPHTAFF	TGTGCCAGCAGCTT	MANA	CENPE	KTWKTKLK	NA	NA	NA	0	NA	0	NA	NA	NA
	AGAACCCGCCCTC												
	ATACTGAAGCTTTC												
	TTT												
CASSLRIGGRETQYF	TGTGCCAGCAGCTT	MANA	COL11A1	MSYDNNLF	NA	NA	NA	0	NA	0	NA	NA	NA
	AAGGATAGGGGGC												
	GAGAGACCCAGTAC												
	TTC												

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FIG. 14 (CONT)

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CASSPMGGADGYTF	TGTGCCAGCAGCCC CGGATGGGGGCG CGGATGGCTACACC TTC	MANA ZNF692	MPLVHMAF	NA	NA	NA	0	NA	0	NA	NA
CASSPLEDSSYGYTF	TGTGCCAGCAGCCC TTTAGAGGACAGTT CCTATGGCTACACC TTC	MANA SNX1	LLADATVEL	NA	NA	NA	0	NA	1	NA	NA
CASSQDSGYNPLHF	TGCGCCAGCAGCCA AGATAGTGGTTATA ATTCAACCCCTCCAC TTT	MANA CCNLI	VTMACINLASK	NA	NA	NA	0	NA	0	NA	NA
CASSSARRGYTF	TGTGCCAGCAGTTC AGCCAGGCGTGGCT ACACCTTC	MANA CMKLRI	YLDIVFLEDL	NA	NA	NA	0	NA	0	NA	NA
CATSRDGTSLTGELEFF	TGTGCCACCAGCAG AGATGGGACTAGCG GGCTAACCGGGGAG CTGTTTTT	MANA ZNF692	MAFSPAVDV	NA	NA	NA	0	NA	2	NA	NA
CSADLNTAEFF	TGCAGCGCAGATCT GAACACTGAAGCTT TCTTT	MANA C2orf16	TLIDVPKV	NA	NA	NA	0	NA	18	NA	NA
CSASPSCAVEQFF	TGCAGTGTAGCCC CTCGGGAGCAGTTG AGCAGTCTTC	MANA CMKLRI	FLEDLSPL	NA	NA	NA	0	NA	0	NA	NA
CSVAEGQGA YEQFF	TGCAGTGTCCAGA AGGACAGGGTGCTT ATGAGCAGTCTTC	MANA CMKLRI	FLEDLSPLEA	NA	NA	NA	0	NA	0	NA	NA
CSVEAFF	TGCAGCGTTGAAGC TTTCTTT	MANA CUL4A	LSKDIMFHK	NA	NA	NA	0	NA	1	NA	NA
CAISEVVGLOPQHF	TGTGCCATCAGTGA AGTCGTCGGACTGC AGCCCCAGCATTTT	MANA SHPRH	LLHEYWMSLR	NA	NA	NA	0	NA	3	NA	NA

FIG. 14 (CONT)

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CASSAGRYSTYEQYF	TGTGCCAGCAGTGC CGGCAGGTATTCGA CCTACGAGCAGTAC TTC	MANA ZNF219	PSLGCLASLH	NA	NA	NA	0	NA	0	NA	NA
CASSPTQGAVEQYF	TGTGCCAGCAGCCC GACCCAGGGGGCAG TAGAGCAGTACTTC	MANA COL6A3	AVQWLRPK	NA	NA	NA	0	NA	1	NA	NA
CASSWGGNTEAFF	TGTGCCAGCAGTTG GGCGGCAACACTG AAGCTTCTTTT	MANA ZNF219	CLASLHPR	NA	NA	NA	0	NA	0	NA	NA

*This rearrangement is the result of incomplete allelic exclusion at the TCRβ locus and was consistently detected in the same cell as the CASNKLGYQPQHF TCRβ by scTCRseq/RNAseq

FIG. 14 (CONT)